European Commission

Combined Draft Renewal Assessment Report prepared according to

Regulation (EC) N° 1107/2009

and

Proposal for Harmonised Classification and Labelling (CLH Report)
according to Regulation (EC) N° 1272/2008

Glyphosate

Volume 3 – B.8 (AS)

Rapporteur Member State: Assessment Group on Glyphosate (AGG) consisting of FR, HU, NL and SE

Version History

When	What
2021/06	Initial RAR

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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B.8. ENVIRONMENTAL FATE AND BEHAVIOUR

In this document, information is provided with respect to the fate and behaviour of glyphosate in soil, water, and air. In agreement with recommendations from the EFSA administrative guidance (2019)[1], all available studies regarding the fate and behaviour of glyphosate in the environment are assessed in this document. This includes re-assessment of the old studies previously considered in the first DAR 2001 or in RAR 2015 (AIR II), and the new studies specifically submitted for the current renewal (AIR V).

According to Regulation 1107/2009, scientific peer-reviewed open literature have been considered also. Publications which were classified as relevant or of unclear relevance following the literature review analysis are summarised and assessed in the respective data requirement sections for which they are considered relevant. Please refer to B.8.6 for further information.

The studies concerning the fate and behaviour of glyphosate in the environment were conducted using either glyphosate or glyphosate-trimesium. For studies performed with glyphosate-trimesium, only the results for the glyphosate (PMG) anion are considered relevant and have been presented for evaluation and further assessment. Results for the cation (TMS) are not presented.

For each section, the regulatory studies are presented in order of acceptability, with acceptable studies first, followed by supportive studies and finally non acceptable studies, in order to ease the reading.

B.8.1. FATE AND BEHAVIOUR IN SOIL

B.8.1.1. Route and rate of degradation in soil

B.8.1.1.1. *Route of degradation in soil*

B.8.1.1.1.1. Aerobic degradation

Laboratory studies

The fate of glyphosate in soil under aerobic laboratory conditions was investigated in 17 existing studies. No new study was provided in this renewal dossier.
Route and rate of degradation studies were all summarized below since even rate of degradation studies can provide useful information on the route of degradation of glyphosate in soil.

Table 8.1.1.1-1: List of existing studies on route and rate of degradation – laboratory aerobic - glyphosate

Study	Soil (Origin)	Soil texture (USDA)	Incubation conditions	pH (medium*)	Max. occurrence of AMPA (% AR)**	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021
[redacted], 2010a CA 7.1.1.1/001 Route and rate of degradation study	Gartenacker CH	Loam	pF 2.5 20°C	7.1 (H2O)	14.7	Accepted	Acceptable
[redacted], 2010b CA 7.1.2.1.1/002 Rate of degradation study	Drusenheim	Loam	pF 2.5 20°C	7.4 (H2O)	21.2	Accepted	Acceptable
	Pappelacker	Sandy loam	pF 2.5 20°C	7.0 (H2O)	29	Accepted	Acceptable
	18-Acres	Clay loam	pF 2.5 20°C	5.7 (H2O)	13.3	Accepted	Acceptable
[redacted], 1996 CA 7.1.1.1/003 Route and rate of degradation study	Soil A Japan	Loam	75% of 1/3 bar 25°C	5.9 (H2O)	-	Not accepted	Not acceptable
	Soil B Japan	Sandy Loam	75% of 1/3 bar 25°C	6.7 (H2O)	21.0	Accepted	Acceptable
[redacted], 1995 CA 7.1.1.1/005 Route and rate of degradation study	Arrow UK	Sandy loam	40% MWHC 20°C	5.9 (CaCl2)	27.3	Accepted	Acceptable

[1] European Food Safety Authority, 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612. 49 pp. doi:10.2903/sp.efsa.2019.EN-1612

Study	Soil (Origin)	Soil texture (USDA)	Incubation conditions	pH (medium*)	Max. occurrence of AMPA (% AR)**	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021
[redacted] 1993 CA 7.1.1.1/006 Route and rate of degradation study	Les Evouettes CH	Silt loam	40% MWHC 20°C	6.1 (ukn)	29.3	Accepted	Acceptable
[redacted] 1993 CA 7.1.2.1.1/003 Addendum: [redacted], 2002 CA 7.1.2.1.1/004 Rate of degradation study	Speyer 2.1 GE	Sand	40% MWHC 20°C	6.1 (ukn)	41.2	Accepted	Acceptable (for route only)
	Speyer 2.2 GE	Sand	40% MWHC 20°C	6.0 (ukn)	42.4	Accepted	Acceptable
	Speyer 2.3 GE	Loamy Sand	40% MWHC 20°C	6.9 (ukn)	25.1	Accepted	Acceptable
[redacted] 1992 CA 7.1.2.1.1/005 Rate of degradation study	Speyer 2.1 GE	Sand	40% MWHC 20°C	6.9 (H2O)	31.8	Accepted	Acceptable
	Speyer 2.1 GE	Sand	20% MWHC 20°C	6.9 (H2O)	27.55	Accepted	Acceptable (for route only)
	Speyer 2.1 GE	Sand	40% MWHC 8°C	6.9 (H2O)	23.19	Accepted	Acceptable (for route only)
	Speyer 2.1 GE	Sand	40% MWHC 20°C - sterile	6.3 (H2O)	20.35	Accepted	Acceptable (for route only)
	Speyer 2.1 GE	Sand	40% MWHC 20°C low rate	6.9 (H2O)	31.42	Accepted	Acceptable (for route only)
	Beedon manor UK	Clay Loam	40% MWHC 20°C	7.8 (H2O)	-	Accepted	Not acceptable
[redacted], 1996 CA 7.1.1.1/002 Route and rate of degradation study	Visalia CA, USA	Sandy loam	75% of 1/3 bar 25°C	8.3 (ukn)	-	Not accepted	Not acceptable
[redacted] 1996 CA 7.1.1.1/004 Route and rate of degradation study	Speyer 2.1 GE	Sand	45% MWHC 20°C	5.9 (CaCl2)	-	Accepted	Not acceptable
	Speyer 2.2 GE	Loamy Sand	45% MWHC 20°C	5.6 (CaCl2)	-	Accepted	Not acceptable
	Speyer 2.3 GE	Loamy Sand	45% MWHC 20°C	6.4 (CaCl2)	-	Accepted	Not acceptable
	Speyer 2.3 GE	Loamy Sand	45% MWHC 10°C	6.4 (CaCl2)	-	Accepted	Not acceptable
[redacted], 1993 CA 7.1.1.1/007 Route and rate of degradation study	Droevendaal, NL	sand	1/3 bar 20°C	5.2 (KCl)	-	Accepted	Not acceptable
	Maasdijk NL	Sandy loam	1/3 bar 20°C	7.5 (KCl)	-	Accepted	Not acceptable
	Lisse, NL	Sand	1/3 bar 20°C	7.2 (KCl)	-	Accepted	Not acceptable
[redacted], 1991 CA 7.1.1.1/008 Honegger, 1992	Kickapoo, KT, USA	Sandy loam	75% FC 25°C	7.3 (ukn)	-	Accepted	Not acceptable

Study	Soil (Origin)	Soil texture (USDA)	Incubation conditions	pH (medium*)	Max. occurrence of AMPA (% AR)**	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021
CA 7.1.1.1/009 Route and rate of degradation study	Dupo IL, USA	Silt loam	75% FC 25°C	7.5 (ukn)	-	Accepted	Not acceptable
[redacted], 1985 CA 7.1.1.1/010 [redacted], 1985 Route and rate of degradation study	Sorrento IT	Loam	FC 18 – 26.7 °C	6.8 (ukn)	-	Not accepted	Not acceptable
[redacted], 1972 CA 7.1.1.1/011 Route and rate of degradation study	Ray	Silt loam	30°C	6.5 (ukn)	-	Not accepted	Not acceptable
	Drummer	Sitly clay loam	30°C	7.0 (ukn)	-	Not accepted	Not acceptable
	Lintonia	sandy loam	30°C	6.0 (ukn)	-	Not accepted	Not acceptable
	Norfolk	sandy loam	30°C	5.7 (ukn)	-	Not accepted	Not acceptable
[redacted]., 1972 CA 7.1.2.1.1/009 Rate of degradation study	Ray	Silt loam	32°C	6.5 (ukn)	-	Not accepted	Not acceptable
	Drummer	Sitly clay loam	32°C	7.0 (ukn)	-	Not accepted	Not acceptable
	Norfolk	sandy loam	32°C	5.7 (ukn)	-	Not accepted	Not acceptable
[redacted], 1991 CA 7.1.2.1.1/006 Rate of degradation study	Speyer 2.1, GE	Loamy sand	40% MWHC 20°C	6.6 (H_2O)	-	Not accepted	Not acceptable
	Speyer 2.2, GE	Loamy sand	40% MWHC 20°C	6.0 (H_2O)	-	Not accepted	Not acceptable
	East Jubilee, UK	Sandy loam	40% MWHC 20°C	5.7 (H_2O)	-	Not accepted	Not acceptable
	18 Acres, EN	Sandy clay loam	40% MWHC 20°C	6.2 (H_2O)	-	Not accepted	Not acceptable
[redacted], 1991 CA 7.1.2.1.1/007 Rate of degradation study	LUFA F1, GE	Sand	22-26°C	5.7 (H_2O)	-	Not accepted	Not acceptable
	LUFA F2, GE	Sand	22-26°C	6.4 (H_2O)	-	Not accepted	Not acceptable
	LUFA 2.2, GE	Loamy sand	22-26°C	5.6 (H_2O)	-	Not accepted	Not acceptable
	Eigenboden, GE	Sand/loamy sand	22-26°C	5.7/6.4 (H_2O)	-	Not accepted	Not acceptable
[redacted], 1980 CA 7.1.2.1.1/008 Rate of degradation study	Drummer	Silty clay loam	25-30°C	6.2 (ukn)	-	Not accepted	Not acceptable
	Spinks	Sandy loam	25-30°C	4.7 (ukn)	-	Not accepted	Not acceptable

* ukn: unknown

** Value reported only for soils considered acceptable to describe route of degradation of glyphosate in RAR 2021

Before presenting the summaries of laboratory studies, some information about the analytical methods is presented below. Indeed, RMS noted that the following was reported in RAR 2015.

"Since the first EU review of glyphosate (2001), the Glyphosate Task Force (GTF) has conducted a new aerobic soil route study ([redacted], 2010, BVL no 2310242) in order to comply with current guidelines according to the requirements of the new OECD guideline no. 307 (OECD, 2002) for aerobic transformation in soil. The GTF members concluded that a new aerobic soil study is needed since older

reviewed studies have deficiencies including low mass balance in one study and chromatographic anomalies in the other studies.

In the old studies, one-dimensional Thin Layer Chromatography (TLC) systems were used as the primary analytical tool for quantification of glyphosate soil metabolites. TLC analyses of the soil extracts often showed glyphosate and AMPA in addition to some unidentified radioactive smear that usually started at the origin of the TLC plates and slowly moved as the plate developed in the TLC solvent system utilised in these studies. The study reports characterised these unidentified fractions as glyphosate and AMPA bound to humic or fulvic acids, coextracted by the high pH extraction solvent.

Since 1993, the year that these studies were conducted, this TLC chromatographic anomaly was consistently confirmed in other glyphosate environmental fate studies. To circumvent the chromatographic artefacts, in recent environmental fate studies, small amounts of EDTA in soil extracts have been routinely employed prior to the TLC analysis in order to reverse the binding of glyphosate and AMPA to natural materials".

It is RMS understanding that EDTA would form a complex with the metal ions, freeing any compound that would have been chelated. Therefore the use of EDTA would prevent that the components of interest (glyphosate, AMPA or any other degradation product) are chelated, forming a complex of high molecular weight which would remain unidentified at the origin of the chromatograms. As a consequence, RMS was wondering whether in studies in which EDTA was not used, the quantification of glyphosate, AMPA or other degradation products might be underestimated.

Since this issue relative to EDTA was not reminded by the applicant in the current renewal dossier, further information were requested by RMS for AIR V renewal. In particular, the applicant was asked to:

- provide a more detailed explanation on the effect of EDTA
- comment on the impact on the acceptability of results from studies in which EDTA was not used (in particular, to clarify whether the quantifications of glyphosate and AMPA can be considered reliable in these studies)..

The applicant answered the following:

"The statement about effects of EDTA was given in the previous evaluation when introducing the new route and rate of degradation studies of [redacted] (2010a, KCA 7.1.1.1/001 and 2010b, KCA 7.1.2.1.1/002). However, no details were given about the effect and outcome on results, i.e. influence on detectability and/or quantification of components in aerobic soil degradation testing. It would therefore not allow to generalise conclusions upon the validity of studies not employing such a precautionary method.

Of the studies submitted and still considered valid, only in [redacted] 2010a and [redacted] 2010b 0.01 M aqueous EDTA solution was added to soil extracts prior to the concentration step in order to break down potential chelates which may have been formed from the interaction of glyphosate with metal ions ([redacted] 2010a: only in DAT 55 to 132 samples; [redacted] 2010b: all samples). No significant regions of unidentified radioactivity were observed in these studies. However, as final analysis in these two studies was done with HPLC and not with TLC, the effect of EDTA use can only be hypothesised, and clear conclusions on the impact on TLC analysis cannot be drawn.

In fact, in the other studies submitted and still considered valid, none used EDTA in the way mentioned above, and glyphosate as well as its degradation product AMPA could be reliably identified and quantified. This shows that, the lack of EDTA use has not resulted in significant chromatographic challenges in most cases as also not only complexation could lead to potential analytical issues. Also, in the validated analytical methods for soil analysis submitted in Section 4 of the dossier, EDTA is not used.

Evidence and plausibility indicate that regions of unidentified radioactivity whenever they occurred are likely to be artefacts from material converted by microbial activity as highly transformed residues integrated into natural components/materials, and rather not of distinct components. When applying TLC for investigation of soil extracts, the chances are increased to observe immobile to low mobile radioactivity that is non-assignable radioactivity with components that have certain characteristics like high molecular weight like being parts of soil organic matter, but also radioactive material integrated into microbes. The result is usually an immobile spot at the start and/or a 'smear', especially at later sampling intervals (aged radioactive residues).

EDTA seems to be commonly applied in trace level analysis of glyphosate in drinking and surface water where detection limits below 1 ng/L are required. However, levels of glyphosate in soil extracts of soil degradation studies are significantly higher and no significant effect of EDTA is expected.

Overall, GRG concludes that even if the use of EDTA can be helpful for optimizing analysis of soil extracts, it is not a prerequisite, and residues of glyphosate and AMPA in soil can be identified and quantified reliably without EDTA use. Thus, the quantification of glyphosate and AMPA in the soil degradation studies submitted and still considered valid is considered reliable for use of derivation of endpoints."

RMS opinion:

In addition to the answer from the applicant, RMS also checked the results from the currently accepted studies.

Studies performed without addition of EDTA ([redacted] 1996, [redacted] 1995 and [redacted] 1992) did not show a significant higher amount of radioactivity at "origin" than in [redacted] 2010a-b which used EDTA.

In most of these studies without EDTA, the unassigned radioactivity from TLC remaining at the "origin" was low (≤1.6% AR in [redacted] 1996, ≤4% in [redacted] 1995, ≤4.6-6% in [redacted] 1992 depending on solvent use). It is also noted that in [redacted] 1996, both TLC and HPLC analysis were performed, and results between the 2 methods are quite consistent (in particular, "origin" was ≤1.6% AR by TLC and ≤0.05% by HPLC).

Unassigned radioactivity at "origin" was found in significant amounts in [redacted] 1993 and [redacted] 1993, however some additional explanations are provided by the applicant for these studies (see the detailed respective studies below).

In conclusion, RMS agrees with the conclusion from the applicant that glyphosate and AMPA can be identified and quantified reliably without EDTA.

[redacted] – *2010a*

Data point:	CA 7.1.1.1/001
Report author	[redacted]
Report year	2010
Report title	Rate and route of degradation of [14C]glyphosate in one soil incubated under aerobic conditions
Report No	1923W
Guidelines followed in study	US EPA OPPTS 835.4100 OECD Guideline 307
Deviations from current test guideline	From OECD 307: - the duration of experiment slightly exceeded the recommended period of 120 days (132 days) and microbial biomass is < 1% OC - application rate does not cover the maximum intended application rate
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C-phosphonomethyl]-glyphosate
Lot No.: 53463-3-23
Specific activity: 10.28 MBq/mg (47 mCi/mmol)

Radiochemical purity: 99.8 % (radiochemical purity measured before treatment: 96.3 %)

2. Soil:

The soil was collected freshly in Switzerland, no fertilizers or pesticides have been applied to the soil for 5 years. Following arrival at the testing facility the soil was sieved to ≤ 2 mm and stored refrigerated in the dark in a container with free access to air for less than three months. Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-2: Characteristics of test soil

Parameter		Results
Soil		Gartenacker
Country		Switzerland
Textural Class (USDA)		Loam
Sand (50 µm – 2 mm) (%)		49
Silt (2 µm – 50 µm) (%)		38
Clay (< 2 µm) (%)		13
pH (water)		7.1
Organic carbon (%)		2.0
Organic matter (%)		3.5
Cation exchange capacity (meq/100 g)		13.6
Maximum Water Holding Capacity (%)		52.1
Water Holding Capacity at 0.33 bar (%)		21.4
Water Holding Capacity at 15 bar (%)		6.1
Bulk Density (disturbed) (g/cm^3)		0.91
Microbial biomass (mg C/100g)	Experimental Start (prior to dosing)	3.75 (0.19% OC)
	During Incubation Period	7.17 (0.36% OC)
	Study end (132 DAT)	5.92 (0.30% OC)

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

The individual soil samples were connected to form flow-through test systems, purged with moistened, CO_2 free air. After leaving the test vessels, the air was passed through a trap containing ethylene glycol to trap volatile organic compounds followed by two traps containing 1 N aqueous NaOH to collect carbon dioxide.

Each test vessel consisted of 50 g of sieved soil (dry weight equivalents) and soil moisture was adjusted to 50 %±10 % of the water holding capacity at pF 2.5. The samples were acclimated for one week at test conditions.

The dosing solution was prepared by combining an aliquot (7.76 mg) of [^{12}C]glyphosate standard with an aliquot (8.83 mg) of [^{13}C]glyphosate standard and an aliquot of [14C]glyphosate test substance (4.23 mg, 1.2 mCi) in a 120 mL amber bottle and dissolving in water to a final volume of 54.5 mL. The resulting test concentration was 3.8 mg/kg soil. Considering a 5cm depth and the bulk density of the soil (0.91 g/cm^3), it corresponds to a dose of 1729 g/ha of glyphosate.

Test systems were incubated under aerobic conditions in the dark at 20 °C and 50 % of the water holding capacity at pF 2.5 for 132 days in maximum.

2. Sampling

Duplicate test systems were removed 0, 3, 6, 10, 20, 34, 55, 90, 112 and 132 days after treatment (DAT). All samples were processed the same day. Approximately once every 10-20 days, trapping solutions for all remaining samples were exchanged for fresh ones.

3. Analytical procedures

At each sampling interval, soil samples were extracted 3 to 4 times successively with 0.5 M NH_4OH solution by shaking for one hour. The extracts were pooled and an aliquot removed for radioactivity determination by LSC.

Combined soil extracts were acidified to pH 2 to 3 by adding concentrated phosphoric acid (H_3PO_4) prior to further workup. Soil extracts were concentrated and cleaned up before HPLC analysis: For extracts from 55 to 132 DAT, 0.01 M EDTA was added prior to concentration to breakdown any potential chelates formed from the interaction of glyphosate with metal ions in soil. The average workup-recovery was 99.3 ± 6.1 %. The LOD each for glyphosate and metabolites observed in the HPLC radio chromatograms was 0.003 µg/g soil (3 µg/kg soil).

All samples were extracted at the day of removal from the test system, followed by initial HPLC analysis performed within 7 days of removal. All samples and standard solutions were stored frozen (<0°C) when not in use. Traps from the samplings and monthly trap changes were stored at room temperature.

Identification and quantification of glyphosate residues was done by cation-exchange HPLC analysis. Confirmatory HPLC analysis with anion-exchange HPLC method was carried for representative extracts. Peak assignment for glyphosate was based on co-elution with the reference standard injected with each sample. Peak assignment for AMPA was by comparison of retention time with a [^{14}C]-AMPA reference standard using the corresponding HPLC method.

The non-extractable radioactivity in soil post-extraction was determined by combustion/LSC.

For the two replicates of 90 DAT, NER were fractionated into fulvic acid, humic acid and humins. The extracted soil sample was treated with 0.1 M aqueous NaOH. The extract was acidified with 12 N aqueous hydrochloric acid (HCl). After precipitation overnight, the precipitated humic acid fraction was separated by centrifugation, and the fulvic acid fraction (supernatant) was decanted. The humic acid fraction was re-dissolved in aqueous 0.1 M NaOH. The two fractions were analysed by LSC.

Radioactivity in trapping solutions was determined by LSC. The confirmation of identity of ^{14}C-CO_2 in the NaOH trapping solution traps was performed by precipitation as $Ba^{14}CO_3$.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts as well as results from fractionation of NER are summarised in the tables below.

Table 8.1.1.1-3: Distribution of radioactivity in soil Gartenacker following incubation of [^{14}C]glyphosate under aerobic conditions (expressed as percent of applied radioactivity)

		DAT									
Compound	**Replicate**	**0**	**3**	**6**	**10**	**20**	**34**	**55**	**90**	**112**	**132**
Glyphosate	A	96.6	71.1	58.1	44.4	33.3	17.6	10.5	4.5	3.0	2.3
	B	95.8	69.2	56.6	43.4	29.2	18.0	9.3	4.7	3.4	2.7
	Mean	96.2	70.2	57.4	43.9	31.3	17.8	9.9	4.6	3.2	2.5
AMPA	A	0.6	4.3	7.0	8.2	11.0	11.5	**14.9**	12.1	9.9	8.8
	B	0.6	4.6	7.2	8.0	13.7	12.7	14.5	12.3	10.2	7.8
	Mean	0.6	4.5	7.1	8.1	12.4	12.1	**14.7**	12.2	10.1	8.3
Unknown D-1 [1]	A	0.7	0.9	1.5	1.8	1.2	4.0	1.9	2.2	2.0	1.9
	B	0.4	0.9	1.5	1.7	2.2	3.0	2.1	2.2	2.0	1.9
	Mean	0.6	0.9	1.5	1.8	1.7	3.5	2.0	2.2	2.0	1.9
Other unknowns	A	0.1	0.2	0.7	0.6	0.6	2.3	1.0	0.7	0.6	1.0
	B	0.3	0.1	0.1	0.8	0.5	1.8	1.5	0.5	0.6	1.2
	Mean	0.2	0.2	0.4	0.7	0.6	2.1	1.3	0.6	0.6	1.1
Carbon Dioxide	A	NS	9.5	16.6	22.3	34.0	43.4	48.8	55.4	58.3	60.4
	B	NS	9.5	15.1	23.6	33.7	40.6	46.8	52.9	54.9	59.5
	Mean	NS	9.5	15.9	23.0	33.9	42.0	47.8	54.2	56.6	60.0
Volatile organic compounds	A	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	98.0	76.5	67.3	55.0	46.0	35.4	28.3	19.4	15.6	14.0
	B	97.1	74.8	65.4	54.0	45.6	35.5	27.4	19.7	16.2	13.5
	Mean	97.6	75.7	66.4	54.5	45.8	35.5	27.9	19.6	15.9	13.8
	A	2.1	11.8	13.4	14.2	16.8	18.8	18.1	19.9	19.7	19.1
	B	2.1	11.7	12.9	13.6	17.2	17.2	18.1	20.8	19.7	18.7

Non-extractable Residues	Mean	2.1	11.8	13.2	13.9	17.0	18.0	18.1	20.4	19.7	18.9
Total mass balance	A	100.1	97.8	97.3	91.5	96.8	97.6	95.2	94.7	93.6	93.5
	B	99.2	96.0	93.4	91.2	96.5	93.3	92.3	93.4	90.8	91.7
	Mean	**99.7**	**96.9**	**95.4**	**91.4**	**96.7**	**95.5**	**93.8**	**94.1**	**92.2**	**92.6**

DAT: days after treatment
NS: not sampled
[1] Secondary HPLC analysis of the D-1 isolate showed multiple peaks demonstrating the presence of multiple degradates but none represented >1.8 % applied radioactivity.

Table 8.1.1.1-4: Soil organic matter fractionation of day 90 post extracted soil (in percent of applied radioactivity)

Experiment	Replicate	Fulvic acid	Humic acid	Humin
Gartenacker	A	5.8	5.0	9.1
	B	5.9	5.1	9.8
	Mean	5.9	5.1	9.5

B. MASS BALANCE

The material balance ranged from 91.4 to 99.7 % of applied radioactivity (% AR) for soil Gartenacker (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The radioactivity in the soil decreased from 0 DAT to 132 DAT from 97.6 to 13.8 % AR. Non-extractable residues (NER) increased from 0 DAT to 90 DAT from 2.1 to 20.4 % AR to then slightly decrease to 18.9 % AR at 132 DAT (mean of two replicates).

Following partitioning of NER for extracted 90 DAT samples, the insoluble humin fraction was the largest portion representing 9.5 % AR on average. The fulvic and humic acid fractions represented 5.9 and 5.1 % AR, respectively.

D. VOLATILE RADIOACTIVITY

The maximum radioactivity found as carbon dioxide in traps was 60.0 % AR at study end (132 DAT, mean of two replicates). There were no organic volatiles determined (<0.1% AR) at all sampling points. Results of barium precipitation confirmed the identity of volatile radioactivity as ^{14}C-carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The portion of glyphosate extractable from soil decreased from 0 DAT to 132 DAT from 96.2 to 2.5 % AR. Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was identified to occur at a maximum of 14.7 % AR at 55 DAT to decrease to 8.3 % AR at 132 DAT. No other radioactive components were detected at or beyond 5 % AR at any point in time.

F. KINETICS

The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020a, CA 7.1.2.1.1/001).

III. CONCLUSIONS

An aerobic soil metabolism study was conducted on a loam soil from Switzerland using [^{14}C]glyphosate at a dose equivalent to a field application rate of 1.7 kg/ha at 20 °C for 132 days. The material balance averaged 94.8 ± 2.8 % of the applied dose. Glyphosate degraded rapidly and represented 2.5 % AR at 132 DAT. The main degradate observed in the study was $^{14}CO_2$, with a maximum average of 60.0 % AR at 132 DAT. The metabolite AMPA, which represented a maximum average of 14.7 % AR at 55 DAT, and subsequently declined to 8.3 % AR at 132 DAT. No other metabolites were detected above 1.8 % of the applied glyphosate. Bound residues represented up to 20.4 % AR at 90 DAT.

Assessment and conclusion by applicant:

The study was conducted according to the current guideline. The study duration was 132 days compared to a standard maximum duration of 120 days. This minor deviation is regarded to have no influence on the outcome of the study.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS

The study is overall well performed.

The following deviations from the OECD 307 can be observed.

The treatment rate is below the maximum intended dose (worst GAP for renewal of 3600 g/ha; equivalent treatment dose in the study is 1729 g/ha). From all available data on degradation of glyphosate, there is no evidence of an impact of the application rate on the degradation of glyphosate, therefore no impact on the outcome of the study is expected.

Microbial biomass was measured to be higher during and at the end of the study compared to the beginning. Therefore the slightly longer duration of the study (132d) than the recommended 120d is considered to have no impact. It is noted that microbial biomass is below 1% OC at all measurements. However considering that the soil was freshly collected, stored less than 3 months, and acclimated during 1 week before experiment, RMS considers that there is no reason to consider that it may have influenced the outcome of the study.

The study is acceptable.

[redacted] – *2010b*

Data point:	CA 7.1.2.1.1/002
Report author	[redacted]
Report year	2010
Report title	Rate of degradation of [14C]glyphosate in three soils incubated under aerobic conditions
Report No	1946W-1
Guidelines followed in study	OECD 307 US EPA OPPTS 835.4100
Deviations from current test guideline	From OECD 307: - one soil (Drusenheim) was stored slightly longer than 3 months (111 days) - some measurements of microbial biomass < 1% OC - application rate used does not cover the maximum intended application rate - mass balance < 90% at some sampling dates
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes (except for degradation rates for Drusenheim soil)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C-phosphonomethyl]-glyphosate
Lot No.: 53463-3-23
Specific activity: 10.28 MBq/mg (47 mCi/mmol)
Radiochemical purity: 99.8 % (radiochemical purity measured before treatment: 96.3 %)

2. Soil:

The soils were collected freshly in France, Switzerland and the UK, no fertilizers or pesticides have been applied to the soils for 5 years. The soils were sieved to ≤ 2 mm. Following arrival at the testing facility, the soils were stored refrigerated in the dark in containers with free access to air for less than three months for soils Pappelacker and 18-acres, while soil Drusenheim was stored for 111 days. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-5: Characteristics of test soil

Parameter	**Results**		
Soil	**Drusenheim**	**Pappelacker**	**18-Acres**
Country	France	Switzerland	UK
Textural Class (USDA)	Loam	Loamy Sand	Sandy clay loam
Sand (50 µm – 2 mm) (%)	47	75	51
Silt (2 µm – 50 µm) (%)	28	20	24
Clay (< 2 µm) (%)	25	5	25
pH (water)	7.4	7.0	5.7
Organic carbon (%)	1.7	1.9	2.5
Organic matter (%)	2.9	3.2	4.4
Cation exchange capacity (meq/100 g)	23.6	11.7	18.1
Maximum Water Holding Capacity (%)	34.3	40.7	51.5
Water Holding Capacity at 0.33 bar (%)	17.6	12.4	19.7
Bulk Density (disturbed) (g/cm3)	1.14	0.98	1.03
Microbial biomass (µg C/g)			
Experimental Start (0 DAT)	255.2 (1.5%OC)	164.4 (0.86 %OC)	487.8 (1.95%OC)
During study (91 DAT)	not reported	256.3 (1.35 %OC)	615.7 (2.46 %OC)
Study end	134.8 (0.79 %OC)	157.3 (0.83 %OC)	305.2 (1.22 %OC)

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems, purged with moistened, CO_2-free air were used. After leaving the test vessels, the air was passed through a trap containing ethylene glycol to trap volatile organic compounds and two traps containing 1 N aqueous NaOH to collect carbon dioxide.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel and soil moisture was adjusted to 50 %±10 % of the water holding capacity at pF 2.5 and the test systems were acclimated for one week at test conditions.

A test solution of [^{14}C]-, [^{13}C]- and [^{12}C]-glyphosate was prepared in water. 0.5 mL of this solution were applied to each test system, resulting in a final concentration of 3.8 mg/kg.

Considering a 5cm depth and the bulk density of the soils (1.14, 0.98 and 1.03 g/cm^3, respectively for soils Drusenheim, Pappelacker and 18-Acres), this is equivalent to 2166, 1862 and 1957 g/ha of glyphosate.

Test systems were incubated under aerobic conditions in the dark for up to 120 days at 20 °C and 50 % of the water holding capacity at pF 2.5.

2. Sampling

For all soils, duplicate samples were collected immediately after treatment (time 0) and at 7 to 9 subsequent sampling times up to 120 days after treatment (DAT, soil Drusenheim: 0, 1, 3, 8, 14, 27, 48 and 70 DAT, soil Pappelacker: 0, 1, 3, 8, 14, 27, 48, 70, 91 and 120 DAT, soil 18-Acres: 0, 8, 14, 21, 41, 63, 91 and 120 DAT). Trapping solutions were exchanged at each sampling point.

3. Analytical procedures

At each sampling interval, soil samples were extracted 3 times successively with 100 mL 0.5 M NH4OH solution. The extracts were pooled, and radioactivity was determined by LSC.

The soil extracts were adjusted to pH of 2 to 3 by dropwise adding concentrated phosphoric acid prior to further workup. 0.01 M EDTA was added prior to concentration to breakdown any possible glyphosate-

metal ions chelation. Soil extracts were concentrated under reduced pressure via roto-vac, Savant Speed-Vac or by rotary evaporation followed up by HPLC analysis. The average workup-recoveries were 98.6 ± 8.4 %, 98.2 ± 8.3 % and 95.5 ± 6.5 % for soils Drusenheim, Pappelacker and 18-Acres, respectively. The LOD for glyphosate and metabolites observed in the HPLC radio chromatograms was 3 µg/kg soil.

Identification and quantitation of radioactive glyphosate soil residues was done by cation-exchange HPLC analysis. Confirmatory HPLC analysis with anion-exchange HPLC method was carried for representative extracts. Peak assignment for glyphosate was based on co-elution with the reference standard injected with each sample. Peak assignment for AMPA was by comparison of retention time with a [^{14}C]-AMPA reference standard using the corresponding HPLC method.

The non-extractable radioactivity in post-extracted soil was determined by combustion/LSC.

For the two replicate samples from the last sampling date of all experiments, NER were fractionated into fulvic acid, humic acid and humin fractions. The previously extracted soil sample was extracted with 0.1 M aqueous NaOH. The extract was acidified with aqueous 12 N HCl. After precipitation overnight, the precipitated humic acid fraction was separated by centrifugation, and the fulvic acid fraction (supernatant) was decanted. The humic acid fraction was re-dissolved in aqueous 0.1 M NaOH. The two fractions were analysed by LSC.

Aliquots of the trapping solutions were analyzed by LSC. The identification of CO_2 in the NaOH traps was determined by the addition of BaCl2 to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba1_4CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts as well as results from fractionation of NER are summarised in the tables below.

Table 8.1.1.1-6: Degradation of [^{14}C]glyphosate in soil Drusenheim under aerobic conditions (expressed as percent of applied radioactivity)

		DAT							
Compound	**Replicate**	**0**	**1**	**3**	**8**	**14**	**27**	**48**	**70**
Glyphosate	A	91.4	64.9	43.5	18.3	10.2	4.9	1.6	1.1
	B	90.5	66.2	44.1	18.1	10.8	3.3	1.5	0.9
	Mean	91.0	65.6	43.8	18.2	10.5	4.1	1.6	1.0
AMPA	A	0.5	9.6	15.0	21.2	19.7	17.5	9.5	6.2
	B	0.3	7.7	15.1	21.1	18.9	15.9	9.8	6.1
	Mean	0.4	8.7	15.1	21.2	19.3	16.7	9.7	6.2
Other*	A	1.2	1.4	3.1	2.9	2.9	3.1	2.3	1.9
	B	1.0	1.7	2.0	2.6	4.7	2.9	2.5	2.4
Carbon Dioxide	A	NA	6.7	16.3	31.9	42.1	51.4	60.6	62.1
	B	NA	6.7	16.3	31.9	42.1	51.4	59.8	62.1
	Mean	NA	6.7	16.3	31.9	42.1	51.4	60.2	62.1
Volatile organic compounds	A	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	93.1	75.9	61.6	42.4	32.8	25.5	13.4	9.2
	B	91.8	75.6	61.2	41.8	34.4	22.1	13.8	9.4
	Mean	92.5	75.8	61.4	42.1	33.6	23.8	13.6	9.3
Non-extractable Residues	A	9.1	13.4	14.1	13.4	13.5	14.3	11.9	15.8
	B	9.1	12.5	14.2	13.5	13.8	13.2	13.1	14.6
	Mean	9.1	13.0	14.2	13.5	13.7	13.8	12.5	15.2
Mass balance	A	102.2	96.0	92.0	87.7	88.4	91.2	85.9	87.1
	B	100.9	94.8	91.7	87.2	90.3	86.7	86.7	86.1
	Mean	**101.6**	**95.4**	**91.9**	**87.5**	**89.4**	**89.0**	**86.3**	**86.6**

* Calculated by RMS as % Total Extractable - % glyphosate - % AMPA
DAT: days after treatment; NA: not applicable

Table 8.1.1.1-7: Degradation of [14C]glyphosate in soil Pappelacker under aerobic conditions (expressed as percent of applied radioactivity)

		DAT									
Compound	**Replicate**	**0**	**1**	**3**	**8**	**14**	**27**	**48**	**70**	**91**	**120**
Glyphosate	A	99.4	77.1	59.0	27.4	19.1	10.1	4.5	2.3	2.0	2.0
	B	98.0	77.2	58.1	29.2	29.6	18.2	9.1	2.9	1.8	2.2
	Mean	98.7	77.2	58.6	28.3	24.4	14.2	6.8	2.6	1.9	2.1
AMPA	A	0.4	4.2	7.4	14.5	14.2	13.7	13.6	10.4	10.0	9.1
	B	0.3	3.9	7.9	13.7	12.2	13.2	15.4	11.6	9.5	9.0
	Mean	0.4	4.1	7.7	14.1	13.2	13.5	14.5	11.0	9.8	9.1
Other*	A	0.6	2	2.5	2.6	3.5	4.1	3.6	2.9	2.6	3.2
	B	1.8	1.1	2.3	2.6	4.2	4.7	3.9	3.4	2.5	3.5
Carbon Dioxide	A	NA	4.8	12.1	27.2	36.3	46.0	53.2	49.7	52.0	54.4
	B	NA	4.8	12.1	27.2	36.3	46.0	45.4	49.7	52.0	54.4
	Mean	NA	4.8	12.1	27.2	36.3	46.0	49.3	49.7	52.0	54.4
Volatile organic compounds	A	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	100.4	83.3	68.9	44.5	36.8	27.9	21.7	15.6	14.6	14.3
	B	100.1	82.2	68.3	45.5	46.0	36.1	28.4	17.9	13.8	14.7
	Mean	100.3	82.8	68.6	45.0	41.4	32.0	25.1	16.8	14.2	14.5
Non-extractable Residues	A	1.8	10.1	13.9	14.7	17.6	15.0	15.9	16.0	13.7	18.4
	B	1.9	9.7	14.1	13.6	18.0	16.8	17.3	18.8	13.3	21.9
	Mean	1.9	9.9	14.0	14.2	17.8	15.9	16.6	17.4	13.5	20.2
Mass balance	A	102.2	98.2	94.9	86.4	90.7	88.9	90.8	81.3	80.3	87.1
	B	102.0	96.7	94.5	86.3	100.3	98.9	91.1	86.4	79.1	91.0
	Mean	**102.1**	**97.5**	**94.7**	**86.4**	**95.5**	**93.9**	**91.0**	**83.9**	**79.7**	**89.1**

* Calculated by RMS as % Total Extractable - % glyphosate - % AMPA
DAT: days after treatment; NA: not applicable

Table 8.1.1.1-8: Degradation of [14C]glyphosate in soil 18-Acres under aerobic conditions (expressed as percent of applied radioactivity)

		DAT							
Compound	**Replicate**	**0**	**8**	**14**	**21**	**41**	**63**	**91**	**120**
Glyphosate	A	95.5	73.9	69.4	65.6	55.9	47.0	44.7	42.1
	B	93.3	73.9	73.1	65.3	54.4	49.3	46.7	41.3
	Mean	94.4	73.9	71.3	65.5	55.2	48.2	45.7	41.7
AMPA	A	0.6	3.3	3.9	6.4	9.1	11.7	13.3	14.3
	B	1.0	3.4	2.9	7.2	8.5	12.0	13.2	12.1
	Mean	0.8	3.4	3.4	6.8	8.8	11.9	13.3	13.2
Other*	A	1.4	1.4	1	3.1	3.1	3.1	1.9	2.6
	B	1.4	1.2	0.5	2.8	4.9	3.1	2	4.9
Carbon Dioxide	A	NA	4.0	5.9	7.7	10.6	13.7	15.5	16.9
	B	NA	4.0	5.9	7.7	10.6	13.7	15.5	16.9
	Mean	NA	4.0	5.9	7.7	10.6	13.7	15.5	16.9
Volatile organic compounds	A	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	97.5	78.6	74.3	75.1	68.1	61.8	59.9	59.0
	B	95.7	78.5	76.5	75.3	67.8	64.4	61.9	58.3
	Mean	96.6	78.6	75.4	75.2	68.0	63.1	60.9	58.7
Non-extractable Residues	A	3.8	13.0	14.8	15.4	18.5	16.9	15.6	22.6
	B	3.8	12.8	16.0	15.2	18.2	17.0	17.7	20.6
	Mean	3.8	12.9	15.4	15.3	18.4	17.0	16.7	21.6
Mass balance	A	101.3	95.6	95.0	98.2	97.2	92.4	91.0	98.5
	B	99.5	95.3	98.4	98.2	96.6	95.1	95.1	95.8
	Mean	**100.4**	**95.5**	**96.7**	**98.2**	**96.9**	**93.8**	**93.1**	**97.2**

* Calculated by RMS as % Total Extractable - % glyphosate - % AMPA

DAT: days after treatment; NA: not applicable

Table 8.1.1.1-9: Fractionation post extracted soil from last sampling dates (in percent of applied radioactivity)

Experiment	DAT	Replicate	Fulvic acid	Humic acid	Humins
Drusenheim	70	A	2.5	2.4	10.9
		B	2.1	3.1	9.4
		Mean	2.3	2.8	10.2
Pappelacker	120	A	4.2	3.2	11.0
		B	4.3	2.9	14.7
		Mean	4.3	3.1	12.9
18-Acres	120	A	2.8	10.8	9.0
		B	2.9	10.6	7.1
		Mean	2.9	10.7	8.1

DAT: days after treatment

B. MASS BALANCE

Material balances ranged from 86.3 to 101.6 % of applied radioactivity (% AR) for soil Drusenheim, from 79.7 to 102.1 % AR in soil Pappelacker and from 93.1 to 100.4 % AR in soil 18-Acres (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

In all soils, the amount of radioactivity extractable from soil decreased from 0 DAT to the end of the experiments at 70 DAT (soil Drusenheim) or 120 DAT (soils Pappelacker and 18-Acres) from 92.5 to 9.3 % AR in soil Drusenheim, from 100.3 to 14.2 % AR in soil Pappelacker and from 96.6 to 58.7 % AR in soil 18-Acres. Accordingly, the amount of non-extractable residues (NER) increased from 9.1 to 15.2 % AR in soil Drusenheim, from 1.9 to 20.2 % AR in soil Pappelacker and from 3.8 to 21.6 % AR in soil 18-Acres.

D. VOLATILE RADIOACTIVITY

The maximum amount of carbon dioxide reached at study end was 62.1 % AR at 70 DAT in soil Drusenheim, 54.4 % AR at 120 DAT in soil Pappelacker and 16.9 % AR at 120 DAT in soil 18-Acres (mean of two replicates). There were no organic volatiles determined in all soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in soil extracts decreased from 0 DAT to the end of the experiments at 70 DAT (soil Drusenheim) or 120 DAT (soils Pappelacker and 18-Acres) from 91.0 to 1.0 % AR in soil Drusenheim from 98.7 to 2.1 % AR in soil Pappelacker and from 94.4 to 41.7 % AR in soil 18-Acres. Besides carbon dioxide, major metabolite aminomethylphosphonic acid (AMPA) was detected. In soil Drusenheim, the maximum amount of 21.2 % AR was reached at 8 DAT and then decreased to 6.2 % AR at 70 DAT. In soil Pappelacker, AMPA was detected with a maximum amount of 14.5 % AR at 48 DAT and decreased to 9.1 % AR at 120 DAT. In soil 18-Acres AMPA was detected with a maximum amount of 13.3 % AR at 91 DAT and decreased to 13.2 % AR until the end of the study (120 DAT). No other metabolites were detected above 5 % AR at any time.

NER were further partitioned for the last sampling dates of the experiments. For soils Drusenheim and Pappelacker, the insoluble humin fraction was the largest component representing an average of 10.2 and 12.9 % AR while the fulvic and humic acid fractions represented below 4.3 % AR. For soil 18-Acres, the humic acid and humin fraction represented 10.7 and 8.1 % AR, respectively, while the fulvic acid fractions represented 2.9 % AR.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found under CA7.1.2.1.1/001.

III. CONCLUSIONS

The study on aerobic soil degradation rate was conducted on three soils using [14C]-glyphosate at a dose rate of 3.80 mg/kg at 20 °C for 70 to 120 days. Material balances, averaged 90.9 ± 5.2 % AR for soil Drusenheim, 91.4 ± 7.0 % AR for soil Pappelacker, and 96.5 ± 2.6 % AR for soil 18-Acres. The main degradate observed in the study was 14CO2, with a maximum average of 62.1 % AR for soil Drusenheim, 54.4 % AR for soil Pappelacker and 16.9 % AR for soil 18-Acres at the end of the study. The metabolite AMPA occurred with maximum 21.2 % AR in soil Drusenheim, 14.5 % AR in soil Pappelacker and 13.3 % AR in soil 18-Acres.

Assessment and conclusion by applicant:

The study was conducted according to the current guidelines, showing minor deviations. Storage of soil Drusenheim was for more than 3 months (sampling 15/07/2009; application 03/11/2009, i.e. 111 days). The material balance was below 90 % AR for some samples of soil Drusenheim (min: 86.1 %) and soil Pappelacker (min: 79.1 %). Losses can be attributed to incomplete trapping of CO2 as the container had to be opened at each sampling point, which allowed CO2 to escape. The deviations are considered to have no influence on the overall outcome of the study.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

The following deviations from the OECD 307 guidance can be observed.

The treatment rate is below the maximum intended dose (3600 g/ha). From all available data on degradation of glyphosate, there is no evidence of an impact of the application rate on the degradation of glyphosate, therefore no impact on the outcome of the study is expected.

Drusenheim soil was stored for 111 days before being used. Based on microbial biomass measured at the beginning of the study for this soil, no impact is expected. Microbial biomass was slightly below 1% OC for Drusenheim soil at the end of the study (0.8% OC), but no significant impact on degradation is expected. For Pappelacker soil, it was slightly below 1% OC at beginning and end of the study (around 0.8%), but above 1% OC at 91 days. Therefore no significant impact on the degradation is expected.

Glyphosate still amounted to 41% AR at the end of the study in soil 18Acres and further sampling dates would have been welcome.

The mass balance is observed to fall slightly below 90% AR from DAT 8 in Drusenheim soil and from DAT 70 in Pappelacker soil. The study reports suggests that it could be related to the "rapid formation of $^{14}CO_2$ in the samples, which may have resulted in incomplete trapping of the radiocarbon in the caustic traps". RMS acknowledges that this is a possible explanation. The production of CO_2 is indeed high and rapid in these two soils, compared to the third soil 18 Acres. However it is also highlighted that rapid formation of CO_2 was also observed in [redacted], 2010a in which CO_2 trapping was not an issue. In addition, the high increase of CO_2 between DAT 0 and DAT 70 in Pappelacker soil has been quite well managed. Without more justification, RMS considers that it cannot be confirmed that the loss of radioactivity is due to CO2 loss.

For Drusenheim soil, mass balance is below 90% from day 8. RMS notes that in RAR 2015, this soil was taken forward for kinetic assessment but only the data points where the material balance was 90-110% were reported to be used. When excluding the data points with mean mass balance below 90%, only 3 data points remain, which would not allow for a reliable kinetic assessment. Since mass balance remains very close to 90% AR with consistent extractable radioactivity, in this specific case RMS considers that results can be used for both route and rate of degradation.

For Pappelacker soil, mass balances decrease below 90% AR from DAT 70 but glyphosate was already significantly degraded, representing only 6.8%AR after 48 days with a mass balance of 91%AR.

Therefore the impact of the less good mass balance from DAT 70 is not expected to significantly impact the results.

The study is considered acceptable. Results from the 3 soils can be used for both route and rate of degradation.

[redacted] *1996*

Data point:	CA 7.1.1.1/003
Report author	[redacted]
Report year	1996
Report title	(14C)-Glyphosate: aerobic soil metabolism
Report No	1413/1-1015
Guidelines followed in study	OECD guideline 307 EPA Pesticide Assessment Guidelines, Subdivision N Paragraph 162-1 (October 1982); Japanese MAFF Guidelines (January 1985)
Deviations from current test guideline	From OECD 307: - no information about soil history - Soil A is not representative for European agricultural soils since it is a humus volcanic ash loam soil with a high organic carbon content (6.8 %) - application rate does not cover the maximum intended application rate - Microbial biomass < 1% OC
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015) – kinetics for soil B only
Acceptability/Reliability:	Yes, for soil B only

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C-phosphonomethyl]-glyphosate
Lot No.: CFQ 8910
Specific activity: 55 mCi/mmol
Radiochemical purity: 99.2 %

2. Soil:

The soils were received 76 days before use and stored refrigerated at 4 °C in the dark in loosely tied plastic bags. Soils were sieved to ≤ 2 mm. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-10: Characteristics of test soils

Parameter		**Results**	
Soil		**A (Humus volcanic ash)**	**B (Non-volcanic inorganic)**
Country		Japan	Japan
Textural Class (USDA)		Loam	Sandy loam
Sand (50 µm – 2 mm) (%)		46.0	68.3
Silt (2 µm – 50µm) (%)		44.9	16.6
Clay (< 2 µm) (%)		9.1	15.2
pH (H_2O)		5.9	6.7
pH (KCl)		5.5	6.1
Organic carbon (%)		6.8	0.7
Organic matter (%)		11.7	1.2
Cation exchange capacity (meq/100 g)		65.9	11.7
Water Holding Capacity at 0.33 bar (%)		72.1	14.2
Microbial biomass (mg C/100g)	Study begin	44.2 (0.65 %OC)	21.4 (0.31 %OC)
	Study end	54.6 (0.80 %OC)	22.9 (0.33 %OC)

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of Erlenmeyer flasks filled with soil. The flasks were purged with moist carbon-dioxide free air. After leaving the test vessels the air was passed through a series of traps: an empty trap for security, a trap containing ethanediol for collection of polar organic volatiles, a trap containing 2% paraffin in xylene to collect non-polar organic volatiles and two traps containing 0.1 M sodium hydroxide to trap carbon dioxide.

25 g of sieved soil (dry weight equivalents) were weighed into each test vessel and the test systems were acclimated for 5 days at test conditions.

A test solution of [^{14}C]glyphosate was prepared by dissolving 6.552 mg glyphosate in 12 mL water, and the final concentration of the application solution was determined by LSC. 76.9 µg of glyphosate were applied to each test system, resulting in a final concentration of 3.076 mg/kg dry soil. Considering a 5cm depth and a default bulk density of 1.5 g/cm^3, it corresponds to a dose of 2307 g/ha of glyphosate.

Test systems were incubated under aerobic conditions in the dark for 121 days at 25 °C and 75 % of moisture holding capacity at 0.33 bar.

2. Sampling

Duplicate test vessels were processed and analysed 0, 1, 3, 7, 14, 30, 63, 90 and 121 days after treatment (DAT). The trapping reagents associated with the 0, 1, 3 and 7 day incubation units were removed at these sampling intervals. From the 14 DAT sampling point onwards, the trapping reagents from all remaining test systems were sampled and replenished with fresh reagent at the time of sampling.

3. Analytical procedures

At each sampling interval, soil samples were extracted four times with 0.5 M aqueous ammonia solution and once with acetone. Extracts and soil were separated by centrifugation and decantation. The ammonia and acetone extracts were each analysed by LSC.

The ammonia extracts were directly analysed by HPLC/radio-detection. The acetone extracts contained less than 1.0% of the applied radioactivity and were not analysed further. The limit of detection (LOD) was defined as a signal correlating to 0.05 % AR. The amount of radioactivity in volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Glyphosate and metabolites were identified by radio HPLC and TLC co-chromatography with reference standards. The identity of glyphosate and the major metabolite AMPA was further confirmed by HPLC-MS for selected samples.

The non-extractable residues in the two replicates of each soil system from days 1, 14 and 90 were further fractionated into fulvic acid, humic acid and humin fractions. The previously extracted soil sample was shaken with of 0.5 M NaOH for five hours at 50 °C. The samples were centrifuged and separated into the solids (humin fraction) and the supernatant. The supernatant was adjusted to pH 2 and centrifuged. The precipitate, containing the humic acid fraction was separated and redissolved in 0.1 M NaOH. The supernatant, containing the fulvic acid fraction was partitioned against dichloromethane into organo-soluble and aqueous soluble fractions. Solid subsamples (humic acid fraction) were combusted and analysed by LSC. The solutions (fulvic and humic acid fraction) were analysed by LSC.

The identification of CO_2 in the sodium hydroxide traps was confirmed by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarized below.

Soil extracts were analysed by HPLC and TLC but it is not reported which method was used as primary method. The results of analysis of extractable residues with HPLC and TLC were found to be very similar at each sampling interval. Therefore, further discussion and kinetic evaluation refers to average values of HPLC and TLC analysis.

Table 8.1.1.1-11: Degradation of [14C]glyphosate in volcanic ash soil A under aerobic conditions (expressed as percent of applied radioactivity)

volcanic ash - soil A										
Compound	DAT Replicate	0	1	3	7	14	30	63	90	121
HPLC Results [1]										
Glyphosate	A	30.5	25.3	27.5	26.0	24.0	19.2	18.6	19.0	17.2
	B	33.7	26.7	25.7	25.3	23.8	18.6	19.9	18.7	17.7
	Mean	32.1	26.0	26.6	25.7	23.9	18.9	19.3	18.9	17.5
AMPA	A	3.2	2.3	1.0	1.2	0.7	0.8	1.2	1.1	1.3
	B	2.0	1.2	0.8	1.1	0.7	0.7	1.5	0.9	1.1
	Mean	2.6	1.8	0.9	1.1	0.7	0.8	1.4	1.0	1.2
Unknowns	A	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND
Background	A	0.2	0.8	0.1	0.2	ND	0.2	0.1	ND	0.1
	B	0.1	0.2	0.4	0.2	ND	0.1	0.1	ND	0.1
	Mean	0.2	0.5	0.3	0.2	ND	0.1	0.1	ND	0.1
TLC Results [1]										
Glyphosate	A	32.0	27.1	27.2	25.9	22.8	18.0	18.0	17.9	14.5
	B	33.3	26.6	25.6	24.7	23.0	17.6	19.0	17.2	15.3
	Mean	32.6	26.8	26.4	25.3	22.9	17.8	18.5	17.5	14.9
AMPA	A	0.9	0.5	0.8	0.6	0.8	1.0	0.9	1.2	1.7
	B	1.2	0.6	0.6	0.9	0.6	0.9	1.1	1.2	1.6
	Mean	1.1	0.5	0.7	0.8	0.7	0.9	1.0	1.2	1.6
Unknowns	A	ND	ND	ND	ND	ND	ND	ND	ND	1.7
	B	ND	ND	ND	ND	ND	ND	ND	ND	1.2
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	1.5
Background	A	0.1	0.2	ND	ND	0.1	0.2	0.1	0.1	0.1
	B	ND	0.2	0.2	0.2	0.2	0.1	0.3	0.2	0.1
	Mean	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1
Mean of HPLC and TLC Results[3]										
Glyphosate	A	31.3	26.2	27.4	26.0	23.4	18.6	18.3	18.5	15.9
	B	33.5	26.7	25.7	25.0	23.4	18.1	19.5	18.0	16.5
	Mean	32.4	26.4	26.5	25.5	23.4	18.4	18.9	18.2	16.2
AMPA	A	2.1	1.4	0.9	0.9	0.8	0.9	1.1	1.2	1.5
	B	1.6	0.9	0.7	1.0	0.7	0.8	1.3	1.1	1.4
	Mean	1.8	1.2	0.8	1.0	0.7	0.9	1.2	1.1	1.4
Unknowns	A	ND	ND	ND	ND	ND	ND	ND	ND	0.9
	B	ND	ND	ND	ND	ND	ND	ND	ND	0.6
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	0.7
Background	A	0.2	0.5	0.1	0.2	0.1	0.2	0.1	0.1	0.1
	B	0.1	0.2	0.3	0.2	0.2	0.1	0.2	0.2	0.1
	Mean	0.1	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.1
Recovery										
Carbon Dioxide	A	NA	0.5	0.9	1.5	2.1	3.0	3.7	4.5	4.5
	B	NA	0.4	0.9	2.2	2.2	3.0	4.0	4.4	4.8
	Mean	NA	0.5	0.9	1.9	2.2	3.0	3.9	4.5	4.6
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	NA	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable residues [2, 3]	A	33.9	28.4	28.7	27.5	24.8	20.2	20.0	20.2	18.7
	B	35.9	28.3	27.0	26.7	24.6	19.6	21.6	19.6	18.9
	Mean	34.9	28.4	27.9	27.1	24.7	19.9	20.8	19.9	18.8

Non-extractable Residues	A	65.1	72.2	68.8	69.9	71.7	77.5	72.6	80.3	76.6
	B	63.1	71.1	70.8	72.3	73.5	78.2	74.3	82.6	77.2
	Mean	64.1	71.7	69.8	71.1	72.6	77.8	73.5	81.4	76.9
Mass balance	A	99.0	101.1	98.3	98.8	98.7	100.8	96.3	105.0	99.8
	B	98.9	99.8	98.6	101.3	100.3	100.7	99.8	106.6	100.9
	Mean	**99.0**	**100.4**	**98.5**	**100.0**	**99.5**	**100.8**	**98.0**	**105.8**	**100.3**

1 Analysis of ammonia extracts
2 Total extractable residues were calculated as sum of radioactivity in acetone and ammonia extracts, the maximum amount in acetone extracts was 0.1 % AR
[3] These values were calculated by the applicant in writing this summary.
DAT: days after treatment
NA: Not applicable
ND: Not detected (defined as less than 0.05 % AR)

Table 8.1.1.1-12: Degradation of [^{14}C]glyphosate in Japanese non-volcanic soil B under aerobic conditions (expressed as percent of applied radioactivity)

Soil B										
Compound	DAT Replicate	0	1	3	7	14	30	63	90	121
HPLC results [1]										
Glyphosate	A	92.8	44.7	34.4	18.6	11.4	6.0	13.3	2.9	1.8
	B	92.9	45.6	34.0	18.6	13.5	5.0	2.6	2.6	2.0
	Mean	92.9	45.2	34.2	18.6	12.5	5.5	7.9	2.7	1.9
AMPA	A	3.6	20.6	17.4	22.6	19.7	21.9	7.3	14.4	13.0
	B	2.3	19.1	17.3	22.1	19.7	21.3	16.3	13.9	15.8
	Mean	3.0	19.9	17.3	22.4	19.7	21.6	11.8	14.1	14.4
Unknowns	A	ND	2.7	2.7	1.9	3.1	2.3	ND	1.4	1.6
	B	ND	1.7	2.0	2.3	3.0	2.0	1.3	1.1	1.4
	Mean	ND	2.2	2.3	2.1	3.0	2.2	0.7	1.3	1.5
Background	A	0.4	1.3	0.4	0.2	1.0	0.3	0.2	ND	0.1
	B	0.3	0.5	0.4	0.1	ND	0.5	0.6	0.5	ND
	Mean	0.4	0.9	0.4	0.2	0.5	0.4	0.4	0.3	0.1
TLC results [1]										
Glyphosate	A	93.6	56.2	38.8	21.4	11.2	6.6	2.2	1.3	1.9
	B	92.4	55.3	38.7	20.5	13.6	5.7	2.1	1.5	2.1
	Mean	93.0	55.7	38.7	20.9	12.4	6.1	2.1	1.4	2.0
AMPA	A	2.7	12.7	15.7	18.9	21.2	20.8	15.6	14.5	10.8
	B	2.5	11.6	14.9	19.4	19.5	20.0	16.0	13.3	13.5
	Mean	2.6	12.2	15.3	19.1	20.4	20.4	15.8	13.9	12.1
Unknowns	A	ND	ND	ND	2.1	2.4	2.3	2.0	1.5	3.3
	B	ND	ND	ND	2.6	2.4	2.5	1.9	1.6	3.4
	Mean	ND	ND	ND	2.4	2.4	2.4	2.0	1.5	3.3
Background	A	ND	0.3	0.4	0.2	ND	0.2	0.1	ND	0.3
	B	0.1	0.1	0.1	0.1	0.2	0.1	ND	0.1	0.2
	Mean	0.1	0.2	0.2	0.2	0.1	0.2	ND	0.1	0.2
Mean of HPLC and TLC Results[3]										
Glyphosate	A	93.2	50.5	36.6	20.0	11.3	6.3	7.8	2.1	1.9
	B	92.7	50.5	36.4	19.6	13.6	5.4	2.4	2.1	2.1
	Mean	92.9	50.5	36.5	19.8	12.4	5.8	5.1	2.1	2.0
AMPA	A	3.2	16.7	16.6	20.8	20.5	21.4	11.5	14.5	11.9
	B	2.4	15.4	16.1	20.8	19.6	20.7	16.2	13.6	14.7
	Mean	2.8	16.0	16.3	20.8	20.0	21.0	13.8	14.0	13.3
Unknowns	A	ND	1.4	1.4	2.0	2.8	2.3	1.0	1.5	2.5
	B	ND	0.9	1.0	2.5	2.7	2.3	1.6	1.4	2.4
	Mean	ND	1.1	1.2	2.2	2.7	2.3	1.7	1.4	2.4
Background	A	0.2	0.8	0.4	0.2	0.5	0.3	0.2	ND	0.2
	B	0.2	0.3	0.3	0.1	0.1	0.3	0.3	0.3	0.1
	Mean	0.3	0.6	0.3	0.2	0.3	0.3	0.2	0.3	0.2
Recovery										

Carbon Dioxide	A	NA	19.2	31.6	40.8	51.2	58.9	68.1	70.5	73.4
	B	NA	19.0	30.1	38.8	45.7	60.2	68.7	70.7	67.6
	Mean	NA	19.1	30.9	39.8	48.5	59.6	68.4	70.6	70.5
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	NA	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable residues [2, 3]	A	96.8	70.0	55.0	43.5	35.3	30.6	21.0	18.8	16.7
	B	95.6	68.1	54.0	43.3	36.4	29.1	21.2	18.1	19.4
	Mean	96.2	69.1	54.5	43.4	35.9	29.9	21.1	18.5	18.1
Non-extractable Residues	A	3.5	11.1	14.3	14.6	15.3	13.0	12.6	12.3	11.7
	B	3.6	11.9	14.7	15.1	16.0	12.6	12.1	12.3	13.1
	Mean	3.6	11.5	14.5	14.8	15.6	12.8	12.3	12.3	12.4
Mass balance	A	100.3	100.3	101.0	98.8	101.8	102.5	101.7	101.4	101.9
	B	99.2	98.9	98.8	97.2	98.1	101.9	102.0	101.1	100.3
	Mean	99.8	99.6	99.9	98.0	100.0	102.2	101.8	101.3	101.1

1 Analysis of ammonia extracts
2 Total extractable residues were calculated as sum of radioactivity in acetone and ammonia extracts, the maximum amount in acetone extracts was 1.1 % AR
[3] These values were calculated by the applicant in writing this summary.
DAT: days after treatment
NA: Not applicable
ND: Not detected (defined as less than 0.05 % AR)

Table 8.1.1.1-13: Fractionation of post extracted soil (in percent of applied radioactivity)

	Fulvic acid fraction	**Humic acid fraction**	**Humin fraction**
Soil A			
1 DAT	2.4	29.2	17.8
14 DAT	17.4	22.9	19.1
90 DAT	2.9	21.5	20.2
Soil B			
1 DAT	8.6	0.4	2.5
14 DAT	11.0	0.8	3.8
90 DAT	7.7	0.5	3.5

B. MASS BALANCE

Material balances ranged from 98.0 to 105.8 % of applied radioactivity (% AR) for soil A and from 98.0 to 102.2 % AR for soil B (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 121 DAT from 34.9 to 18.8 % AR in soil A and from 96.2 to 18.1 % AR in soil B (mean of two replicates).

The amount of non-extractable residues (NER) was in the range from 64.1 to 81.4 % AR in soil A for all sampling points. In soil B, it increased from 0 DAT to 14 DAT from 3.6 to 15.6 % AR and then slightly declined to 12.4 % AR until 121 DAT (mean of two replicates).

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (120 DAT) were 4.6 % AR in soil A and 70.5 % AR in soil B (mean of two replicates). No organic volatiles were detected for both soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The results of analysis of extractable residues with HPLC and TLC were found to be very similar at each sampling interval. Therefore, further discussion refers to average values of HPLC and TLC analysis.

In soil A, Glyphosate was recovered with an amount of 32.4 % AR at 0 DAT and decreased to 16.2 % AR at 121 DAT. In soil B, it was detected with an amount of 92.9 % AR at 0 DAT and decreased to 2.0 % AR

at 121 DAT. Besides carbon dioxide, the metabolite AMPA was detected. In soil A, the maximum amount of 1.8 % AR occurred already at 0 DAT, then decreased to around 0.8 % AR at 3 DAT and remained stable until the end of the experiment. In soil B, AMPA was detected with a maximum amount of 21.0 % AR at 30 DAT and decreased to 13.3 % AR at 121 DAT. All values presented are the mean of two replicates, average values of HPLC and TLC analysis. No other metabolites were detected above 5 % AR at any time.

In the fractionation of non-extractable residues of soil A 2.4 to 17.4 % AR were found in the fulvic acid fraction, 21.5 to 29.2 % AR were found in the humic acid fraction and 17.8 to 20.2 % AR were found in the humin fraction. In soil B, 7.7 to 11.0 % AR were found in the fulvic acid fraction, 0.4 to 0.8 % AR were found in the humic acid fraction and 2.5 to 3.8 % AR were found in the humin fraction.

F. KINETICS

The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020a, CA 7.1.2.1.1/001).

III. CONCLUSIONS

Glyphosate is degraded in soil under aerobic conditions in the dark in the laboratory. Formation of carbon dioxide was up to a maximum of 70.6 % AR in soil B. Besides carbon dioxide, the major metabolite AMPA was detected with a maximum amount of 21.0 % AR at 30 DAT in soil B. Formation of non-extractable residues in soil B was up to 15.6 % AR.

Assessment and conclusion by applicant:

The study was conducted consistent with the current guideline, showing minor deviations. These include that no information on the soil history and storage conditions are provided. The deviation is considered to not influence the results and overall outcome of the study.

Therefore the study is considered valid to address the data point.

Results for volcanic ash soil A are excluded from use in risk assessment since the soil is not representative for the EU.

Assessment and conclusion by RMS

RMS agrees with the applicant that soil A is not representative of agricultural soils in the EU. The OECD 307 does not explicitly exclude volcanic soils but the organic carbon content is very high and the nature of such allophane containing soils is also different than of normal agricultural soils. It should not be used to describe the route of degradation of glyphosate nor for deriving degradation endpoints. Please also note that a singular behaviour is observed in this soil: recovery of glyphosate at T0 is only 33% AR and non-extractable residues are already as high as 63%AR at this time.

The following deviations are identified for soil B.

The treatment rate is below the maximum intended dose (3600 g/ha). From all available data on degradation of glyphosate, there is no evidence of an impact of the application rate on the degradation of glyphosate, therefore no impact on the outcome of the study is expected.

The microbial biomass is below 1% OC at all measured times. No information on the soil history was provided and it is not well known whether the soil was freshly collected. However, contrary to what has been indicated by the applicant, information on the storage conditions of the soil before the study is available in the report: "after arrival at CHE the soils were passed through a 2 mm mesh sieve and stored in the dark, at 4 ± 2 ° C, in loosely tied plastic bags in accordance with ISO/DIS 10381-6 guidelines. Soils were stored under these conditions for a period of 76 days prior to dispensing into the study units. Following dispensing, the units were maintained at 25 ± 2 ° C for a further 5 days prior to application of the test compound."

The report indicates that only ammonium extracts were analysed by TLC/HPLC and not the acetone extracts but as these acetone extracts only represented <1% AR, this is considered acceptable.

The applicant proposed to average HPLC and TLC results. This is quite unusual and RMS considers that separated results should be considered. RMS is in favour of relying on the HPLC data which are usually preferred for distribution of radio-activity and identification purposes.

RMS considers that results from soil B provide reliable information on the route of degradation of glyphosate and can be used to derive degradation endpoints. HPLC results are further considered for the determination of rates of degradation.

[redacted] *1995*

Data point:	CA 7.1.1.1/005
Report author	[redacted]
Report year	1995
Report title	HR-001: Aerobic soil metabolism and route of degradation
Report No	SNY-333/951445
Guidelines followed in study	US EPA pesticide Assessment Guidelines, Subdivision N, 162-1; German BBA Guidelines for the Official Testing of Plant Protection Products; Part IV, 4-1, Stage 1; Japanese MAFF, 59 NohSan No. 4200, January 1985; Draft Guidelines Concerning the Inclusion of Active Substances in Annex I to Council Directive 91/414/EEC part 7.1.1.2. OECD guideline 307
Deviations from current test guideline	From OECD 307: - No information about soil history and storage conditions are reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C-phosphonomethyl]-glyphosate
Lot No.: CFQ8432
Specific activity: 327.7 µCi/mg
Radiochemical purity: >99 %

2. Soil:

The soil was sieved to ≤ 2 mm. Characteristics of the test soil are presented in the table below

Table 8.1.1.1-14: Characteristics of test soils

Parameter		**Results**
Soil		**Arrow**
Country		United Kingdom
Textural Class (DIN)		Sandy loam
Sand (>63 µm) (%)		68.61
Silt (2 µm – 63 µm) (%)		19.22
Clay (< 2 µm) (%)		12.18
pH ($CaCl_2$)		5.9
pH (H_2O)[2]		6.4
Organic carbon (%)		2.2
Organic matter (%)[1]		3.8
Cation exchange capacity (meq/100 g)		10.0
Maximum Water Holding Capacity (%)		37.95
Microbial biomass (mg C/100g)	At application	33.7 (1.53 %OC)
	Intermediate (120 DAT)	33.7 (1.53 %OC)

	Study end (217 DAT)	25.6 (1.16 %OC)

DAT = days after treatment

[1] Calculated from organic carbon according to OM = OC x 1.72

[2] calculated by RMS considering the formula pH_{H2O} = 0:982pH_{CaCl2} + 0:648 presented in the EFSA guidance for predicting environmental concentration in soil (2017)[2]

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of glass columns of 10 cm inner diameter where the individual test vessels were stored on a rack. The columns were connected to a set of washing bottles. Air entering the system was passed through a water bottle to moisten incoming air. After leaving the glass column, the air was passed through a polyurethane foam bung to collect neutral, volatile organic compounds followed by a trap system. It consisted of an empty trap to prevent suck back into the system, a trap containing 2-(ethoxyethoxy)ethanol to collect volatile organic compounds, one trap containing 1 M KOH aqueous solution laced with phenolphthalein indicator and another trap containing ethanolamine/2-ethoxyethanol (1/3, v/v), both to collect carbon dioxide.

50 g of sieved soil (dry weight equivalents) with a soil moisture slightly above 40 % of the maximum water holding capacity were weighed into each test vessel and the test systems were acclimated for 7 days at test conditions.

An aqueous application solution containing a mixture of [^{14}C]-labelled and unlabelled glyphosate with a concentration of 1 mg/mL was prepared. 0.450 mL of this solution were applied to each test system, The study application rate was 9.7 mg a.s./kg dry soil. Considering a 5cm depth and a default bulk density of 1.5 g/cm^3, it corresponds to a dose of 7275 g/ha of glyphosate.

Test systems were incubated under aerobic conditions in the dark for 180 days at 20 °C and 40 % MWHC.

2. Sampling

Duplicate samples were processed and analysed 0, 3, 7, 14, 30, 60, 90, 120 and 180 days after treatment (DAT). Samples were extracted on the day of sampling. Extracts were stored at <-15 °C prior to analysis. Extracts were generally analysed within 6 weeks of sampling. The trapping solutions were assayed and changed at each sampling time or at approximately two weeks intervals.

3. Analytical procedures

Duplicate soil samples were analysed separately at each sampling time. Each soil sample was extracted three times with 150 mL of an aqueous solution containing $NH_4(OH)$ (0.25 M) and KH_2PO_4 (0.1 M) by treatment in an ultrasonic bath at ambient temperature for 15 min followed by shaking for 15 min at ambient temperature. A fourth extraction was conducted with 100 mL of the same extraction solution by sonication at 50 °C for 60 minutes and followed by shaking for 15 min at ambient temperature. After each extraction step, the solvent was separated by centrifugation and the radioactivity of the extracts was determined by LSC.

Prior to analysis by HPLC and TLC, the soil extracts were pooled. The combined extracts were cleaned-up by strong cation exchange solid phase extraction. The column was eluted with 0.5 M HCl, the eluate was concentrated to dryness and reconstituted in 5 mM KH_2PO_4 and 4% methanol (v/v) adjusted to pH 2.1 with phosphoric acid. Recovery of radioactivity from this procedure was quantitative.

The extracted soil residue was allowed to air dry and then combusted. The combustion products were analysed by LSC.

The polyurethane foam bungs removed at sampling (0 DAT to 60 DAT inclusive) were individually extracted with an aqueous solution containing $NH_4(OH)$ (0.25 M) and KH2PO4 (0.1 M), and the extracts were analysed by LSC. The volatile trapping solutions were also analysed by LSC.

2 EFSA Journal 2017;15(10):4982, source of the formula: Boesten et al. 2012

Glyphosate and metabolites were identified and quantified by HPLC. The presence of glyphosate and AMPA was confirmed by TLC co-chromatography with reference items.

The identification of CO2 in the KOH traps was determined by the addition of sodium carbonate to aliquots of the trap solution. The mixture was added to saturated barium chloride, the barium carbonate precipitate formed was separated by centrifugation and the supernatant was analysed by LSC.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised below.

Table 8.1.1.1-15: Mass balance and distribution of radioactivity in Arrow soil (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**3**	**7**	**14**	**30**	**60**	**90**	**120**	**180**
Glyphosate	A	92.6	87.0	74.0	64.2	54.0	41.1	32.5	28.1	26.5
	B	91.2	82.2	73.9	69.5	54.6	38.4	35.5	29.0	27.6
	Mean	91.9	84.6	74.0	66.9	54.3	39.8	34.0	28.5	27.1
AMPA	A	1.0	3.9	6.9	10.4	14.4	22.1	27.5	28.0	25.8
	B	1.1	3.1	6.6	8.3	13.7	22.3	25.4	26.6	25.3
	Mean	1.1	3.5	6.8	9.4	14.1	22.2	26.5	**27.3**	25.6
Polar compounds	A	0.6	0.7	1.4	1.8	2.6	3.3	2.7	2.8	4.0
	B	0.8	0.8	1.5	1.2	3.0	3.0	2.3	3.0	3.9
	Mean	0.7	0.8	1.5	1.5	2.8	3.2	2.5	2.9	4.0
Others	A	0.7	0.6	1.1	0.8	1.2	1.4	0.8	0.2	0.8
	B	1.2	0.6	0.9	1.1	1.4	1.4	0.8	0.4	0.6
	Mean	1.0	0.6	1.0	1.0	1.3	1.4	0.8	0.3	0.7
Carbon Dioxide	A	ns	2.3	4.2	7.1	11.7	16.6	19.4	21.3	23.9
	B	ns	2.1	4.3	7.0	11.0	15.8	18.7	20.7	23.3
	Mean	ns	2.2	4.3	7.1	11.4	16.2	19.1	21.0	21.6
Volatile organic compounds	A	ns	nd	nd	nd	nd	ns	ns	ns	ns
	B	ns	nd	nd	nd	nd	ns	ns	ns	ns
Total extractable residues	A	94.9	92.1	83.3	76.7	72.0	67.9	63.5	59.1	57.1
	B	94.2	86.2	82.8	79.4	72.6	64.9	63.9	59.0	57.4
Non-extractable Residues	A	2.7	5.9	9.0	7.6	7.6	9.6	11.3	10.0	9.4
	B	2.4	5.3	8.2	8.4	9.0	9.7	10.5	11.8	9.3
Mass balance	A	97.6	100.3	96.5	91.4	91.3	94.1	94.2	90.4	90.4
	B	96.6	93.6	95.3	94.8	92.6	90.4	93.1	91.5	90.0
	Mean	**97.1**	**97.0**	**95.9**	**93.1**	**92.0**	**92.3**	**93.7**	**91.0**	**90.2**

DAT: days after treatment
nd: below limit of accurate determination (two times background noise)
ns: not sampled

B. MASS BALANCE

Material balances ranged from 90.2 to 97.1 % AR (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of extractable radioactivity decreased from 0 DAT to 180 DAT from 94.6 to 57.3 % AR (mean of two replicates).

The amount of non-extractable residues (NER) increased from 0 DAT to 90 DAT from 2.6 to a maximum level of 10.9 % AR, stayed at a constant level until 120 DAT and slightly decreased to 9.4 % AR at 180 DAT (mean of two replicates).

D. VOLATILE RADIOACTIVITY

The maximum amount of carbon dioxide reached at study end (180 DAT) was 23.6 % AR (mean of two replicates). No organic volatiles were detected at any sampling point. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

Glyphosate residues decreased from 91.9 % AR at 0 DAT to 27.1 % AR at 180 DAT. Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected with a maximum amount of 27.3 % AR at 120 DAT and a further slight decrease to 25.6 % AR by the end of the experiment (mean of two replicates). No other metabolites were detected above 5 % AR at any time.

F. KINETICS

The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020a, CA 7.1.2.1.1/001).

III. CONCLUSIONS

The aerobic degradation of glyphosate in soil Arrow at 20 °C in darkness and 40 % maximum water holding capacity has been studied. Under these conditions, fast degradation of glyphosate occurred in the soil. The two most important degradation products were identified as AMPA (aminomethylphosphonic acid, the only significant soil metabolite) and carbon dioxide due to mineralization.

Assessment and conclusion by applicant:

The study was conducted consistent with the current guideline, showing minor deviations.

The test duration of 180 days was prolonged in comparison to 120 days recommended. No information on the soil history and storage conditions are reported. These deviations are considered to not have influenced the results and overall outcome of the study.

Therefore, it is considered valid to address the data point.

Assessment and conclusion by RMS

The study is overall well performed.

As mentioned by the applicant, the test duration was 180 days but as recommended by the OECD 307 guidelines, the microbial biomass was measured at the end of the study and show no significant decline which could have impacted the degradation of glyphosate.

The history of the field is missing from the report. No indication on whether the soil was freshly collected was reported, nor the storage conditions. However these deviations are not expected to have significantly impacted the outcome of the study in this case, especially as the soil was biologically active throughout the study.

The study is therefore considered as reliable for use for both route and rate of degradation of glyphosate and AMPA.

[redacted], *1993*

Data point:	CA 7.1.1.1/006
Report author	[redacted]
Report year	1993
Report title	Degradation and metabolism of 14C-Glyphosate in soil incubated under aerobic conditions
Report No	246486
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision N: Chemistry: Environmental Fate Section 162-1 (October, 1982); BBA Richtlinie Part IV, 4-1 (December, 1986) OECD guideline 307

Deviations from current test guideline	From OECD 307: - History of treatments not available for the last 5 years - Application rate used does not cover the maximum intended application rate - LOD/LOQ was not reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]-phosphonomethyl-glyphosate
Lot No.: CFA. 745 C4
Specific activity: 11.2 MBq/mg (304 µCi/mg)
Radiochemical purity: 96.6 %

2. Soil:

About one month prior to application, the soil was sampled from outdoor containers, where it was stored after retrieval from the field, and acclimated at room temperature. No pesticides or fertilizers were applied for at least one year. Soils were sieved to ≤ 2 mm. Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-16: Characteristics of test soils

Parameter		**Results**
Soil		**Les Evouettes**
Country		Switzerland
Textural Class (USDA)		Silt loam
Sand (50 µm – 2 mm) (%)		38.0
Silt (2 µm – 50µm) (%)		50.7
Clay (< 2 µm) (%)		11.3
pH (medium not stated)		6.1
Organic carbon (%) [1]		1.40
Organic matter (%) [2]		2.41
Cation exchange capacity (meq/100 g)		15.5
Maximum Water Holding Capacity (MWHC) (%)		55.3
Field Capacity (FC) (%)*		40.2
Bulk Density (40% MWHC) (g/cm^3)		0.913
Microbial biomass (mg C/100g)	Before application	58.5 (4.18 %C)
	Study end (364 DAT)	22.0 (1.57 %C)

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Referring to soil dry weight
[2] Calculated from organic carbon according to OM = OC x 1.72
* It is not indicated in the study report whether it corresponds to pF2 or pF2.5

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of glass jars filled with soil and connected to washing bottles. Air entering the system was moistened by a water-filled gas-washing bottle. After leaving the test vessels, the air was passed through a trap containing 50 mL 2N NaOH aqueous solution to collect carbon dioxide and a trap containing 50 mL of ethylene glycol to trap volatile organic compounds. Airflow was controlled by a flow meter.

100 g of sieved soil (dry weight equivalents) were weighed into each test vessel.

The study application rate corresponded to the anticipated use rate of 1.8 kg a.s./ha. 900 µL of an aqueous test solution, containing a mixture of labelled [^{14}C]glyphosate and unlabelled glyphosate with a specific

activity of 14.8 µCi/mL were applied to each test system, resulting in a final concentration of 240 µg/100 g dry soil. Considering a 5cm depth and the bulk density of the soil (0.913 g/cm^3), it corresponds to a dose of 1095.6 g/ha of glyphosate.

After application, the soil moisture was adjusted to 40 % of the maximum water holding capacity (MWHC, corresponding to 55 % of the field capacity), the test vessels were closed with trap attachments and the airflow was set to 60 mL/minute.

Test systems were incubated under aerobic conditions in the dark for 364 days at 20 °C and 40 % MWHC.

2. Sampling

Duplicate test systems were processed and analysed at 3, 7, 14, 28, 56, 84, 112, 168, 252 and 364 days after treatment (DAT). At 0 DAT one replicate was processed and analysed. All soil samples were processed on the designated sampling day. The ethylene glycol and NaOH traps were assayed and changed at 3 DAT, then on a weekly basis for the first four weeks. After 42 DAT, the ethylene glycol trap was removed and the NaOH trap was changed and analysed every two weeks.

3. Analytical procedures

At each sampling interval, soil samples were extracted four to five times with 0.5 N NH4OH solution for 30 minutes followed by one or two extractions with water. The respective extracts were combined and the radioactivity was measured by liquid scintillation counting (LSC). The day 0 sample was extracted five times with methanol/water (8/2, v/v), three times with water and four times with 0.5 N NH4OH.

Aliquots of the combined extracts were filtered and concentrated by evaporation under reduced pressure. The aqueous phase was further analysed by thin layer chromatography (TLC), and high performance liquid chromatography (HPLC). The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Test item and metabolites were identified by radio-HPLC-UV and TLC co-chromatography with reference items. As well as high voltage electrophoresis (HVE) of selected samples.

The stability of the extracts for at least two years was demonstrated by repeated TLC analysis of selected extracts.

The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium hydroxide solution to aliquots of the trap contents. The presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised below.

Table 8.1.1.1-17: Degradation of [^{14}C]glyphosate in soil Les Evouettes under aerobic conditions (values expressed as percent of applied radioactivity), Results of TLC analysis

Compound	Replicate	DAT										
		0	**3**	**7**	**14**	**28**	**56**	**84**	**112**	**168**	**252**	**364**
Glyphosate	A	78.3	65.6	49.5	48.9	36.7	24.3	19.4	16.3	9.4	8.3	7.4
	B	-*	69.0	58.6	38.7	36.1	25.4	19.6	21.8	10.8	8.4	6.0
	Mean	78.3	67.3	54.0	43.8	36.4	24.8	19.5	19.1	10.1	8.3	6.7
AMPA (M1)	A	4.0	6.2	14.9	12.2	19.7	21.1	28.3	28.3	16.6	17.7	21.2
	B	-*	6.4	11.5	13.5	21.9	22.7	30.4	26.9	21.7	18.8	21.4
	Mean	4.0	6.3	13.2	12.8	20.8	21.9	29.3	27.6	19.2	18.3	21.3
Unknown 1 (M2)	A	9.9	8.5	11.9	9.1	8.7	8.1	3.7	5.4	0.0	0.0	0.0
	B	-*	10.5	13.2	21.2	4.8	6.1	5.0	2.0	0.0	0.0	0.0
	Mean	9.9	9.5	12.6	**15.2**	6.8	7.1	4.4	3.7	0.0	0.0	0.0
Unknown 2 (M3)	A	0.0	0.0	0.0	6.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	-*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unknown 3	A	0.0	0.0	0.0	0.0	0.0	0.0	4.9	0.0	0.0	0.0	0.0

(M4)	B	-*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unknown 4 (M5)	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.0	14.0	7.8
	B	-*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.2	12.5	8.7
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**13.6**	**13.3**	8.3
Total extractable residues	A	92.2	80.3	76.3	77.1	65.1	53.5	56.3	50.0	56.6	53.3	44.7
	B		85.9	83.3	73.4	62.8	54.2	55.0	50.7	42.7	39.7	36.1
Carbon Dioxide	A	ND	5.9	10.5	15.2	22.0	29.2	32.2	34.0	36.6	39.1	41.6
	B	-*	5.7	10.9	15.8	22.2	28.4	32.5	31.3	36.9	38.3	41.6
Volatile organic compounds	Mean	ND	<0.1	<0.1	<0.1	<0.1	ND	ND	ND	ND	ND	ND
Non-extractable Residues	A	6.4	10.5	9.9	13.8	14.6	13.4	13.6	14.1	15.7	13.4	16.1
	B	-*	9.3	10.2	11.3	12.7	14.9	12.1	13.6	18.0	13.0	23.5
Mass balance	A	98.6	96.7	96.7	106.1	101.7	96.1	102.1	98.1	108.9	105.8	102.4
	B	-*	100.9	104.4	100.5	97.7	97.5	99.6	95.6	97.6	91	101.2

DAT: days after treatment
* only one sample analysed at T0
ND: not determined
NA: not applicable

B. MASS BALANCE

Material balances ranged from 91.8 to 103.2 % AR (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of extractable radioactivity decreased from 0 DAT to 364 DAT from 92.2 to 36.3 % AR.

The amount of non-extractable residues (NER) increased from 0 DAT to 364 DAT from 6.4 to 19.8 % AR (all values mean of two replicates). The rather low and stable amount of NER was explained by the high pH value of the extraction medium (0.5 N NH4OH). It was concluded that fulvic and humic acids were extracted at the same time.

D. VOLATILE RADIOACTIVITY

The maximum amount of carbon dioxide reached at study end (364 DAT) was 41.6 % AR. Organic volatiles determined were ≤0.1 % AR at all sampling points (all values mean of two replicates). The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

Glyphosate was detected with 78.3 % AR at 0 DAT and decreased to 6.7 % AR at 364 DAT (mean of two replicates). Glyphosate concentrations were confirmed by HPLC analysis. Besides carbon dioxide, the major metabolite AMPA was detected with a maximum amount of 29.4 % AR at 84 DAT and decreased to 21.3 % AR at 364 DAT (mean of two replicates).

Two highly polar radioactive fractions (M2 and M5) were observed with amounts exceeding 10 % of the applied radioactivity. Fraction 1 (M2) was detected with a maximum amount of 15.2 % AR at 14 DAT and decreased to zero from 168 DAT onwards. Fraction 4 (M5) was detected from 168 DAT onwards with a maximum amount of 13.6 % AR at 168 DAT and decreased to 8.3 % AR at 364 DAT.

Next to parent and AMPA (reference B), another nine reference items were analysed by TLC, however did not overlap with the two fractions above (M2 and M5). These reference items were sarcosine (reference A), N-methyl-AMPA (reference C), N-methyl-glyphosate (reference D), hydroxymethyl phosphonic acid (reference E), methylamine, hydrochloride (reference F), dimethylamine hydrochloride (reference G), N-carboxylmethyl-N-phosphonomethyl)glycine (reference H), methylphosphonic acid (reference I) and N-N-dimethalymino-methylphosphonic acid (reference J). M2 was present at day 0 as TLC start radioactivity in all chromatographic systems. From day 168, the start radioactivity (M2) changed its properties towards a more mobile behaviour and could be differentiated as M5. In conclusion, it is most likely that M2 and M5 represent radiolabelled substances bound to fulvic or humic acids which were co-extracted at the high pH.

Other unknown compounds were below 5 % AR at any time.

F. KINETICS

The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020a, CA 7.1.2.1.1/001).

III. CONCLUSIONS

After incubation of soil Les Evouettes with [14C]-glyphosate, 6.4 % of the radioactivity applied was rapidly and irreversibly bound to the soil. The main metabolic product found in the soil was aminomethyl-phosphonic acid (AMPA).

As expected, the extent of mineralization of glyphosate was significant, i.e. 41.6 % of the radioactivity applied in 364 days.

The non-extractable radioactivity remained low, i.e. 6.4 % to 19.8 % of the radioactivity applied. This was an expected result, because of the high pH value of the extracting solutions. The extraction method applied allowed the relatively easy detection of parent and AMPA, but co-extractions complicated the analyses.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing minor deviations. The study duration is 364 days instead of the proposed 120 days to fulfill US data requirements. No LOD or LOQ is reported. These deviations do not influence the results and outcome of the study.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS

The following deviations from the OECD 307 guidance can be observed.

The treatment rate is below the maximum intended dose (3600 g/ha). From all available data on degradation of glyphosate, there is no evidence of an impact of the application rate on the degradation of glyphosate, therefore no impact on the outcome of the study is expected. It is also noted that history of the field treatments is only mentioned for the last year before collection instead of 5 years.

As mentioned by the applicant, the test duration was 364 days but as recommended by the OECD 307 guidelines, the microbial biomass was measured at the end of the study. It decreased but was still above 1% OC, indicating that the soil is still considered as viable.

LOD and LOQ are not reported.

During the study, two major unknown compounds were observed in the TLC results, M2, from DAT0 and M5 from DAT 168. The study author indicates that "M2 and M5 represent radiolabelled substances bound to fulvic or humic acids which were co-extracted at the high pH".

Additional information was provided by the applicant further to RMS request and is presented below at the end of [redacted] (1993) study. Based on the additional information on [redacted] (1993), RMS agrees with the applicant that the unassigned radioactivity M2 and M5 do not correspond to any additional metabolite, nor to glyphosate/AMPA chelated, please refer to the discussion under [redacted] (1993).

It is noted that in this study, HPLC was only used to confirm the concentration of parent glyphosate. The study report indicates that TLC results were kept since the resolution found was better. Since only TLC results are available for both glyphosate and AMPA, TLC results are used for the kinetic analysis.

This study is acceptable.

[redacted], 1993 + Addendum [redacted] 2003

Data point:	CA 7.1.2.1.1/003
Report author	[redacted]
Report year	1993

Report title	Degradation of 14C-glyphosate in three soils incubated under aerobic conditions
Report No	271618
Guidelines followed in study	BBA Guideline Part IV, 4-1
Deviations from current test guideline	From OECD 307: - no information on soil history prior to arrival at test site - LOD/LOQ was not reported - Only one replicate was analysed per sampling point - mass balance below 90% at some sampling points
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

Data point:	CA 7.1.2.1.1/004
Report author	[redacted]
Report year	2002
Report title	First amendment (addendum) to report - Degradation of 14C-glyphosate in three soils incubated under aerobic conditions
Report No	271618
Guidelines followed in study	BBA Guideline Part IV, 4-1
Deviations from current test guideline	From OECD 307: refer to [redacted]
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C-phosphonomethyl]-glyphosate
Lot No.: Not provided
Specific activity: 11.1 MBq/mg (298.8 µCi/mg)
Radiochemical purity: 99 %
Chemical purity: Not provided

2. Soil:

Soils were sieved to ≤2 mm. The soils arrived at the testing facility about 4 months prior to start of the study and were stored in concrete cylinders under outdoor conditions. The soils have not been subjected to any pesticide or organic nor inorganic fertilizer treatment since their arrival. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-18 Characteristics of test soils

Parameter	**Results**		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Country	Germany	Germany	Germany
Textural Class (BBA)	Sand	Sand	Loamy sand
Sand (> 20 µm) (%)	92.1	89.4	80.5
Silt (2 µm – 20 µm) (%)	4.4	5.6	11.4
Clay (< 2 µm) (%)	3.5	5.1	8.3
pH (medium not stated)	6.1	6.0	6.9
Organic carbon (%)	0.70	2.29	1.34
Cation exchange capacity (meq/100 g soil)	4.9	9.7	9.5
Maximum Water Holding Capacity (%)	31.9	44.3	34.9

Microbial biomass (mg C /100 g soil)	Start	11.7 (1.6% OC)	40.3 (1.8 % OC)	37.2 (2.8 % OC)
	Completion of incubation	7.8 (1.1% OC)	32.8 (1.4 % OC)	26.4 (1.9% OC)

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of test vessels (Petri dishes) filled with soil, which were placed in glass cylinders equipped with air inlets and outlets. Air entering the system was moistened with a water trap. After leaving the cylinders, the air was passed through two traps containing 50 mL of 2 N NaOH to collect carbon dioxide and a trap containing 50 mL of ethylene glycol to trap volatile organic compounds.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel and soil moisture was adjusted to 40 % of the maximum water holding capacity (MWHC).

6.69 ml of the stock solution (4.48 mg glyphosate) were combined with 9.39 mg of the unlabelled compound and diluted with bidistilled water to a total volume of 50 ml. The activity of this application solution was measured by liquid scintillation counting (LSC) and the new specific activity was determined to be 8.65 pCi/ml (96.58 pCi/mg).

The target rate was 3600 g/ha glyphosate. Freshly sieved (2 mm) samples of soil corresponding to 50 g of dry soil were treated with 0.2386 mg glyphosate (target: 0.24 mg/50 g of dry soil, calculated using a soil density of 1.5 g (dry weight basis)/cm3 and a penetration depth of 5 cm; specific radioactivity: 298.8 uCi/mg).

After application, the test vessels (except 0 DAT) were placed in the glass cylinders, and the cylinders were closed with trap attachments.

Test vessels were incubated under aerobic conditions in the dark for 105 days at 20 °C and 40 % MWHC.

2. Sampling

Duplicate samples from each system were taken at 0, 7, 14, 28, 56, 84, and 105 days after treatment (DAT) one replicate per sampling point was processed and analysed. The volatiles traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds.

3. Analytical procedures

At each sampling interval, soil samples were extracted 4-5 times with 0.5 N ammonia solution, and the extract was analysed by LSC.

Extractions with 0.5 N KC1 or bidistilled water (pH 2.0, adjusted with cont. HCl) after the NH, extractions showed that the extracted amount of radioactivity could not be considerably increased. Thus, neither KC1 nor water (pH 2.0) were routinely used.

Extraction was performed on the day of sampling, and extracts were analysed within 4 to 14 days. Extracts were stored at -20 °C.

The soil debris resulting from the extractions were combusted, and the resulting $^{14}CO_2$ was determined by LSC.

Aliquots of the combined extracts were either treated by centrifugation or by ultrafiltration (PM10 membranes, Amicon), and concentrated by evaporation at 50°C on a rotary evaporator. Thin layer chromatography (TLC) analyses were performed as well with supernatants of centrifugation as with ultrafiltrates at sampling intervals 0, 7, 14 and 28 DAT, with ultrafiltrates additionally at the intervals 56, 84 and 105 DAT. Since no differences due to the workup procedure could be detected, the best TLCs were used for the evaluation.

Glyphosate was identified by thin layer chromatography (TLC) co-chromatography with a reference item using two different sets of stationary/ mobile phase. High performance liquid chromatography (HPLC) with fluorescence spectrometry detection was used to confirm glyphosate concentrations derived from TLC analysis.

Sodium hydroxide trapping solutions were mixed with water and analysed by LSC. Ethylene glycol was radioassayed directly. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium hydroxide to aliquots of the trap contents. The presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and its metabolites in soil extracts are summarised in the tables below for the respective soils.

Table 8.1.1.1-19: Degradation of [^{14}C]glyphosate in soil Speyer 2.1 under aerobic conditions (expressed as percent of applied radioactivity)

	DAT						
Fraction	**0**	**7**	**14**	**28**	**56**	**84**	**105**
Glyphosate	86.7	56.0	38.1	22.6	9.7	9.7	8.0
AMPA	1.4	21.7	41.2	32.6	40.0	38.7	23.5
Others*	2.7	0	2.6	3.4	5.5	4.9	16.4
Total extractable radioactivity	90.8	77.7	81.9	58.6	55.2	53.3	47.9
Carbon Dioxide	ND	12.3	15.1	20.5	23.7	25.2	26.1
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable radioactivity	0.3	2.3	2.5	2.0	2.5	1.6	1.6
Mass balance	91.1	92.3	99.6	81.1	81.3	80.1	75.6

* Calculated by RMS as % Total Extractable - % glyphosate - % AMPA
DAT: days after treatment
ND = Not determined

Table 8.1.1.1-20: Degradation of [^{14}C]glyphosate in soil Speyer 2.2 under aerobic conditions (expressed as percent of applied radioactivity)

	DAT						
Fraction	**0**	**7**	**14**	**28**	**56**	**84**	**105**
Glyphosate	91.3	41.4	48.8	39.3	31.3	19.3	13.5
AMPA	0.0	42.4	31.4	33.1	34.6	33.9	35.4
Others*	5.5	0	3.2	11.2	7.1	9.8	13.7
Total extractable radioactivity	96.8	83.8	83.4	77.6	73.0	63.0	62.6
Carbon Dioxide	ND	5.8	9.0	13.9	18.9	20.9	23.5
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable radioactivity	0.8	6.8	7.3	7.1	7.3	4.9	8.6
Mass balance	97.6	96.4	99.7	98.6	99.2	88.8	94.7

* Calculated by RMS as % Total Extractable - % glyphosate - % AMPA
DAT: days after treatment
ND = Not determined

Table 8.1.1.1-21: Degradation of [^{14}C]glyphosate in soil Speyer 2.3 under aerobic conditions (expressed as percent of applied radioactivity)

	DAT						
Fraction	0	7	14	28	56	84	105
Glyphosate	90.9	39.4	19.7	5.5	4.3	3.0	2.5
AMPA	0.0	13.6	25.1	25.1	18.9	18.5	12.1
Others*	0.0	6.0	0.0	2.1	1.4	0.0	3.7
Total extractable radioactivity	90.9	59.0	44.8	32.7	24.6	21.5	18.3
Carbon Dioxide	ND	31.0	39.8	50.3	56.9	58.9	61.4
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable radioactivity	1.4	7.7	7.0	7.0	8.6	6.0	5.0
Mass balance	92.3	97.7	91.6	89.9	90.1	86.4	84.7

* Calculated by RMS as % Total Extractable - % glyphosate - % AMPA
DAT: days after treatment
ND = Not determined

B. MASS BALANCE

Mass balances ranged from 75.6 to 99.6 % of applied radioactivity (% AR) for soil Speyer 2.1, from 88.8 to 99.7 % AR for soil Speyer 2.2, and from 84.7 to 97.7 % AR for soil Speyer 2.3. The partly low mass balances as well as the decrease over time can most likely be explained by the configuration of the test system. $^{14}CO_2$ is supposed to have escaped from the cylinders each time they had to be opened and these losses probably explain the observed recoveries.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from the soil decreased from 0 DAT to 105 DAT from 90.8 to 47.9 % AR in soil Speyer 2.1, from 96.8 to 62.6 % AR in soil Speyer 2.2, and from 90.9 to 18.3 % AR in soil Speyer 2.3.

Non-extractable residues (NER) were <10% in all soils. In soil Speyer 2.1, they increased from 0.3 % AR at 0 DAT to 2.5 % AR at 14 DAT and 56 DAT, and then slightly decreased to 1.6 % AR at 105 DAT. In soil Speyer 2.2, increased from 0.8 % AR at 0 DAT to 8.6 % AR at 105 DAT. In soil Speyer 2.3, NER increased from 1.4 % AR at 0 DAT to 8.6 % AR at 56 DAT, and then slightly decreased to 5.0 % AR at 105 DAT.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (105 DAT) were 26.1, 23.5, and 61.4 % AR in soils Speyer 2.1, Speyer 2.2, and Speyer 2.3, respectively. Organic volatiles determined were ≤0.1 % AR for all soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in soil extracts decreased from 0 DAT to 105 DAT from 86.7 to 8.0 % AR in soil Speyer 2.1, from 91.3 to 13.5 % AR in soil Speyer 2.2 and from 90.9 to 2.5 % AR in soil Speyer 2.3.

The major metabolite formed, aminomethylphosphonic acid (AMPA), was detected with a maximum amount of 41.2 % AR at 14 DAT in soil Speyer 2.1 where it subsequently decreased to 23.5 % AR at 105 DAT. In soil Speyer 2.2, the maximum amount was 42.4 % AR at 7 DAT followed by a decrease to 35.4 % AR at 105 DAT. In soil Speyer 2.3, the maximum amount was 25.1 % AR at 14 DAT followed by a decrease to 12.1 % AR at 105 DAT.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found under CA7.1.2.1.1/001.

III. CONCLUSIONS

The fastest degradation of [^{14}C]-glyphosate was found in soil Speyer 2.3 and the slowest degradation was found in soil Speyer 2.2. The degradation rates could not be correlated to the characteristics of the soil types used. The only metabolite formed was AMPA. The mineralization of glyphosate was rather high and can be considered one of the important pathways of disappearance of [^{14}C]-glyphosate from standard Speyer soils.

Assessment and conclusion by applicant:

The study was conducted in accordance to the current guidelines with minor deviations.

For the two soils showing highest mineralisation, mass balances were below 90% AR for a number of samples. The losses can be assigned to a loss of $^{14}CO_2$ when the test vessels had to be opened for sampling while significant radioactivity was in the gas phase of the test vessels.

The deviations in study conduct are not regarded to influence the results and general outcome of the study.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

No information on the soils history was provided. RMS notes that the sand content of Speyer 2.1 and Speyer 2.2 soils is very high.

As mentioned by the applicant, the OECD 307 guidance recommends ensuring the repeatability of the analysis, whereas only one replicate was analysed per sampling point.

LOD/LOQ for analysis of glyphosate and AMPA is not clearly indicated. However based on the presented results, it is expected that it is 0.1% AR.

RMS considers that these deviations have no impact on the overall outcome of the study.

The mass balance for Soil Speyer 2.1 significantly falls below 90% AR for the larger part of the study (from DAT28). RMS considered that an analytical issue cannot be easily excluded. When excluding the data points with mean mass balance below 90%, only 3 data points remain, which would not allow for a reliable kinetic assessment. In addition, at 14 days (last day with acceptable mass balance), glyphosate still represents 38% AR and it is not clear whether AMPA has reached its maximum. It is therefore proposed that data from this soil provide supportive information regarding the route of degradation of glyphosate but are not used to derive degradation endpoints.

For Speyer 2.2 soil, mass balance is below 90% AR on one sampling point only, but very close to 90% AR. For Speyer 2.3, mass balance slightly fall below 90% AR from day 84. Glyphosate only accounted for 4.3% AR at DAT 56 and AMPA seemed to have already reached its maximum and started to decline. Therefore, results for these 2 soils are considered sufficiently robust for route and rate of degradation.

Glyphosate was identified by HPLC and TLC during the initial study. The addendum from [redacted], 2003 identified AMPA from the TLC samples.

Results presented in the study report seem to indicate that additional compounds may be present beside glyphosate and AMPA: in Speyer 1.1 and Speyer 2.2 soils: the difference between total extractable and glyphosate+AMPA reach 16.4% AR and 13.7% AR respectively at the end of the study. RMS also notes that TLC results show more than 2 peaks. On the other hand, HPLC results only show 2 clear peaks, one of them being glyphosate, but then the identification of AMPA was only performed with the TLC results in the addendum [redacted] 2002. However, considering the additional information provided by the applicant following RMS request and detailed below, RMS considers that the unassigned radioactivity from TLC does not correspond to additional metabolite, nor to glyphosate/AMPA chelated.

The study is considered acceptable. Results from Speyer 2.2 and Speyer 2.3 soils can be used for both route and rate of degradation. Results from Speyer 2.1 soil are considered as supportive for route of degradation and should not be used for rate of degradation.

Additional information provided by the applicant

The applicant was asked to provide further argumentation regarding the unassigned radioactivity from TLC analysis in study [redacted] 1993. The answer from the applicant is reproduced below.

"Additional TLC chromatograms were obtained from the actual study owner, FMC Agricultural solutions. To address the request by AGG, eight exemplary TLC chromatograms for the soils 2.1 and 2.2 were evaluated in detail. Additionally, the raw data scans of the chromatograms as received from the study owner is compiled in a separate document which is submitted together with this response.

The study by [redacted] (1993, KCA 7.1.2.1.1/003) investigated the degradation of ^{14}C-glyphosate under aerobic conditions of the laboratory in the three German 'standard soils' Speyer 2.1, 2.2 and 2.3 for a maximum incubation period of 105 days.

For the last sampling interval at 105 days after treatment (DAT), total extractable radioactivity was reported as 47.9 % AR for soil 2.1 and 62.6 % AR for soil 2.2. The total assigned radioactivity (active

substance and AMPA) was 31.5 % AR for soil 2.1 and 48.9 % AR for soil 2.2. This resulted in 16.4 % AR (soil 2.1) and 13.7 % AR (soil 2.2) of extractable radioactivity not assigned to individual components.

The study report focused on the rate of degradation of the active substance glyphosate and results were based on TLC analysis. The identification of the active substance was supported by HPLC analysis. In HPLC, the active substance showed retention (retention time of about 7.3 to 8.2 min) while no separation was observed for other degradation products. Apart from the active substance, the predominant fraction of radioactivity eluted in the dead volume of the chromatographic column without retention (study report, Figure 19, page 65).

Later, the quantification of metabolite AMPA was reported by an amendment ([redacted], 2002, KCA 7.1.2.1.1/004). The identification and quantification of AMPA was based on the same TLC chromatograms (i.e. two chromatograms per sampling interval and soil) as before in the original report for the quantification of the parent active substance.

For analysis of soil extracts, two contrasting TLC methods were used, i.e. Cellulose- and a Reversed Phase (RP 18)-static phases and their associated mobile phase. The radioactivity per TLC lane was distributed into the various components. Radioactivity distribution in a TLC lane was detected for quantification by a one-dimensional 'linear' Berthold counter. Being state-of the art at the time of the study, this one-dimensional detector counted radioactivity per TLC lane and illustrated the results in the form of peaks rather than as spots with limited spatial resolution as documented by the broadened signals.

Components in radioactive spots detected were identified by co-chromatography with known reference standards, regularly performed for the active substance and metabolite AMPA. Results from counting via the Berthold detector served as quantification of the regions detected in a TLC lane. At each sampling interval, the two values (expressed as % ROI) from the two TLC chromatograms were averaged, and the mean % ROI was then taken as basis for quantification of a given component in the total soil extract (mean value of % AR from two TLC systems).

Besides the mean values for the known components, glyphosate and AMPA, additional radioactivity was present in the soil extracts that could not be assigned to individual components. The exact quantities were not reported in detail. Quantification was performed now for the last two sampling intervals, i.e. 84 DAT and 105 DAT for soils 2.1 and 2.2 based on the additionally available single TLC chromatograms.

Results are discussed exemplarily in more detail in the following for sampling interval 105 DAT (for earlier sampling intervals, the overall distribution of radioactivity on TLC plates was comparable).

For soil 2.1, in total four radioactive regions (Regions 1 to 4) were observed in TLC using method 1 (Cellulose phase) while five regions were observed when using TLC method 2 (RP 18 phase).

> *For TLC method 1, Regions 3 and 4 were assigned to the active substance (7.3 % AR) and AMPA (21.7 % AR). Radioactivity not assigned to known components was in Region 1 (start of lane, 2.6 % AR) and Region 2 (12.3 % AR).*
>
> *For TLC method 2, Regions 4 and 5 were assigned to the active substance (8.7 % AR) and AMPA (25.3 % AR). Radioactivity not assigned to known components was in Region 1 (start of lane, 1.2 % AR), Region 2 (8.7 % AR) and Region 3 (4.0 % AR).*

For soil 2.2, in total three radioactive regions (Regions 1 to 3) were observed in TLC using method 1 (Cellulose phase) while four regions were observed when using TLC method 2 (RP 18 phase).

> *For TLC method 1, Regions 2 and 3 were assigned to the active substance (13.5 % AR) and AMPA (39.4 % AR). Radioactivity not assigned to known components was in Region 1 (start of lane, 9.7% AR).*
>
> *For TLC method 2, Regions 3 and 4 were assigned to the active substance (13.3 % AR) and AMPA (31.4 % AR). Radioactivity not assigned to known components was in Region 1 (start of lane, 15.0 % AR) and Region 2 (2.8 % AR).*

Considering the elution behaviour on the TLC plates, the unknown radioactivity was mostly associated with radioactivity at start and 'next to' start, i.e. the 'polar regions' in the chromatograms while it was possible for the 'defined components' (glyphosate and AMPA) to demonstrate movement on the plates under the chromatographic conditions. While the 'defined components' altered their elution behaviour dependent on

the static phase chosen for TLC, one of the main characteristics observed for the unknown radioactivity is no movement/no elution at all under the various conditions of chromatography.

The quality of separation resulted in variable quantity of the unidentified radioactive regions observed. This observation is not surprising in soil analysis in particular to occur at late sampling intervals - when considering a number of other factors contributing to the overall chromatography pattern in this study. These factors include but are not limited to the influence of soil matrix (soil type) as well as sample preparation (extraction by ammonia as basic extraction solvent and concentration for analysis which results in high salt/matrix load). Variability in the distribution and quantification of unidentified radioactive residues are directly related to a combination of these factors. Thus, the observation of radioactivity extracted and not assigned to individual known components in this study has several reasons:

The extraction of soil samples was performed with 0.5 N aqueous ammonia solution medium strong base (NH_4OH, pH >11 in aqueous media). This solvent readily forms water-soluble ammonium salts of glyphosate which allows exhaustive extraction of glyphosate and its degradates from soil. In general, for soil degradation testing, the use of very alkaline solvents for 'exhaustive' soil extraction is avoided as the soil matrix may be destructed, e.g. by dissolution of the soil organic matter fraction (fulvic and humic acids, and the salt load in the extracts is increased. The enhanced load from salts and organic matter may influence the quality of chromatographic separation and thus identification as well as quantification of components. For this study, the two TLC methods applied were able to clearly separate the active substance and metabolite AMPA from other radioactive components and thus allowed for unambiguous identification and reliable quantification.

While the separation of the unknown radioactivity was overall not sufficient for identification or quantification of single components, it allowed to characterise it as polar fractions by their chromatographic behaviour: The retention behaviour of the known radioactive components glyphosate and AMPA changed dependent on static phase used, i.e. change in elution behaviour resulted in change of order of spots in the TLC lane. While this effect is intended and simply the principle of contrasting analytical methods followed, this behaviour was not observed for the non-assigned radioactivity, i.e. under chromatographic conditions of TLC the fractions showed no movement at all and consequently no significant change in elution behaviour.

The chromatographic behaviour of the non-assigned radioactivity is however typical for fractions related to soil organic matter, i.e. multiple components and higher molecular weight without defined structure (including humic and fulvic acids and radioactive material incorporated into the soil microbial). As mentioned before, the occurrence of these organic matter fractions in soil extracts is very likely due to the basic extraction solvent applied. Thus, it can be concluded that the non-assigned radioactivity most likely consists of radioactivity incorporated into soil organic matter (including living organisms like bacteria and fungi and their organic material from use of the radioactivity applied as nutrient, i.e. as carbon, phosphorous and nitrogen source) and not of defined individual components in amounts relevant for further identification. This conclusion is in line with the observation that more than 26 to 61 % AR was ultimately degraded to ^{14}C-CO_2 (considering also losses in material balance from lack of trapping).

The simple structure of the active substance and its known metabolites also allows for the conclusion that the potential to form other components in the soil metabolic pathway is very limited. For example, HMPA was available as reference compound, but was excluded to occur as residue.

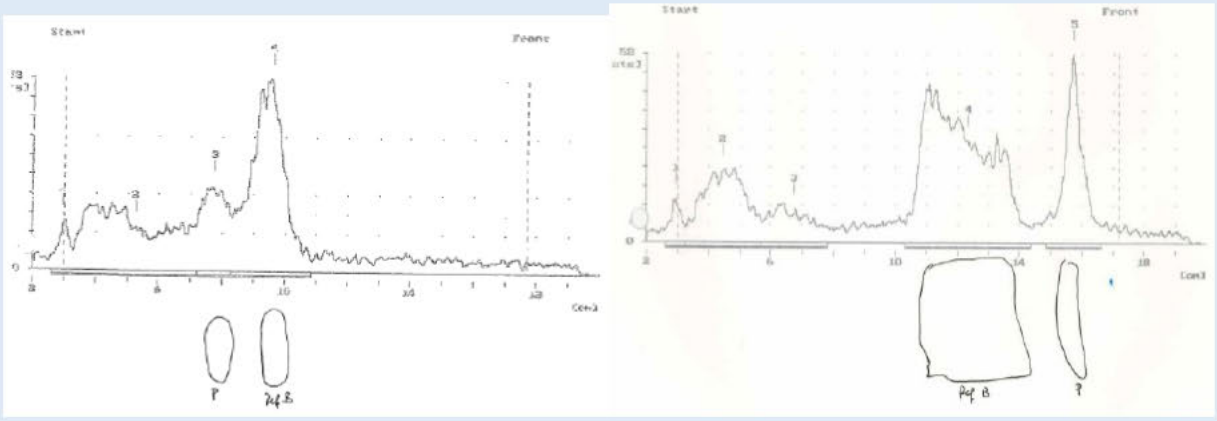
Overall, the degradation of glyphosate resulted in the formation of AMPA and further in the integration of radioactive residues to become part of the carbon and nitrogen constituents of soil. The comparatively harsh extraction at alkaline pH favoured the co-extraction of residues in the form of salts and radioactive residues integrated in soil constituents that could be observed in TLC chromatography as radioactivity preferably at the start and non-assignable to distinct components.

Thus it can be concluded that quantification of glyphosate and AMPA is reliable based on the identification of distinct peaks. Further, the TLC chromatograms conclusively show that the immobile spots at the start of the TLC chromatogram cannot be assigned to distinct components in amounts relevant for further identification."

Assessment and conclusion by RMS

The statement provided by the applicant for [redacted] 1993 also provides relevant information to better interpret results from [redacted] 1993.

RMS checked the TLC chromatograms provided by the applicant (DAT84 and DAT105 for replicate A of soils 2.1 and 2.2, 2 TLC methods). Only the TLC chromatograms of Soil 2.1, DAT105 showed additional radioactive peaks between start and front of the plates.



Method 1 (cellulose) Method 2 (reversed phase)

For all other chromatograms, the undetermined radioactivity was a peak at "origin".

RMS understands the applicant argumentation regarding the lack of mobility of the compounds at the start from one method or the other. This is particularly noteworthy as both TLC and reversed phase TLC were used. In normal phase chromatography, the stationary phase (here cellulose) is more polar than the mobile phase; in reverse phase, the stationary phase is less polar than the mobile phase. Retention times of the different compounds would therefore be impacted. A very polar compound would be retained at origin in a normal TLC analysis while it would migrate rapidly in a reversed phase TLC. In the case of the two studies [redacted] 1993 and [redacted] 1993, this radioactivity does not move whichever TLC plate / eluent / approach is used.

RMS therefore agrees with the applicant explanation that this unassigned radioactivity does not represent any additional metabolite, nor glyphosate/AMPA chelated.

Regarding the origin of this unassigned radioactivity, the applicant's explanation was that the extraction was harsh and that bound radioactivity to soil organic matter would have been extracted. RMS observed that the method of extraction was very similar for all the acceptable studies presented under point B.8.1.1.1 ([redacted], 2010 a and b, [redacted], [redacted]), with NH_4OH used 3-4 times f, and centrifugation or shaking inbetween the extractions. No significant radioactivity (max 4% AR, please refer also to discussion on EDTA) was observed at the start of the TLC plate or HPLC results in the other studies.

In conclusion, while no sound explanation was provided on the high amount of radioactivity at origin, the applicant's argumentation is sufficient in RMS opinion to exclude any potential additional metabolite or loss of glyphosate or AMPA (complexed with metal ions or other soil components).

[redacted], *1992*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.1.1/005
Report author	[redacted]
Report year	1992
Report title	Glyphosate-Trimesium: Soil dissipation study (incl. addendum to final report)
Report No	7043-38/165
Guidelines followed in study	Not stated

Deviations from current test guideline	From OECD 307: - No history is reported for soil Beedon Manor - No analysis was conducted for volatiles - Determination of non-extractable residues was only performed for day 0, hence full material balance is only available for that sampling interval
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes – except for soil Beedon Manor

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate-trimesium, [^{14}C]-methylene labelled in the glyphosate anion moiety
Lot No.: 88-J30 and 91-J19
Specific activity: 2.07 GBq mmol-1 (228 µCi/mg)
Radiochemical purity: 95.1 and 98.2 % (for two batches)
Chemical purity: Not provided

The experiments with [^{14}C]glyphosate-trimesium, radiolabelled in the trimethylsulphonium cation (TMS) are not presented in this summary.

2. Soil:

Soil Speyer 2.1 (initial study and addendum) was stored in a storage plot and shipped to the test site eight days before the start of the experiment. The soil had received no pesticide treatment prior to the study. Soil Beedon Manor was collected from [redacted] (UK). Upon receipt, the soil was stored for about two weeks covered under outdoor conditions at the test site. Soils were sieved to ≤2 mm. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-22: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1		Beedon Manor
Country	Germany		UK
Textural Class (USDA)	Sand		Clay loam
Incubation groups	A, B, C and E	D	
Sand (50 µm – 2 mm) (%)	88	96	33
Silt (2 µm – 50 µm) (%)	6	1	33
Clay (< 2 µm) (%)	6	3	34
pH (water)	6.9	6.3	7.8
Organic matter (%)	0.9	0.8	3.7
Organic carbon [1] (%)	0.5	0.5	2.1
Cation exchange capacity (meq/100 g)	2.7	2.6	20.9
Maximum Water Holding Capacity (%)	32.95	31.31	57.94
Water Holding Capacity at 0.33 bar (%)	4.5	14.2	23.4
Water Holding Capacity at 15.0 bar (%)	1.55	6.70	11.8
Microbial biomass (mg C/100g)			
Pre-experiment	7.8 (1.56 %OC)		48.3 (23 %OC)
Post-experiment	6.6 (1.32 %OC)		63.4 (30.2 %OC)

USDA: United States Department for Agriculture
[1] Calculated from organic matter according to OC = OM/1.724

B. STUDY DESIGN

1. Experimental conditions

'Static' test systems were used, consisting of Erlenmeyer flasks filled with soil. The tests were performed at different conditions as summarised in the table below.

Table 8.1.1.1-23: Incubation groups

Incubation group	Soil	Moisture content [% of MWHC]	Incubation Temperature [±2 °C]	Nominal Application rate 2 [mg/kg]
A	Speyer 2.1	40	20	4.0
B	Speyer 2.1	20	20	4.0
C	Speyer 2.1	40	8	4.0
D [1]	Speyer 2.1, sterile	40	20	4.0
E	Speyer 2.1	40	20	0.4
F [1]	Beedon Manor	40	20	4.0
1 Experiments conducted in the addendum to the study 2 Application rate expressed as mg glyphosate-trimesium/kg soil dry weight				

The flasks containing non-sterile soil were incubated at 8 or 20°C, and deionised water was added as appropriate to maintain the moisture content.

Two flasks with soil Speyer 2.1 were gamma irradiated for sterilisation. The sterilised soil samples were incubated at 20 °C in the dark. Moistened air was passed through as appropriate to maintain the moisture content.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel. The non-sterile test systems were acclimated for approximately two months for Speyer 2.1 soil and approximately one week for Beedon Manor soil at test conditions. The sterile test systems were acclimated for approximately 5-7 days at test conditions.

The study application rate was either 0.4 or 4 mg/kg soil (dry weight), corresponding to the anticipated use rate of 0.4 or 4 kg glyphosate-trimesium/ha. A test solution of [14C]glyphosate-trimesium, radiolabelled in the phosphonomethylglycine anion (PMG), with a concentration of 0.02 or 0.2 mg/mL was prepared in water. 1 mL of this solution was applied to each test system.

Test systems were incubated under aerobic conditions in the dark for 104 days for the non-sterile soils Speyer 2.1 and Beedon Manor or 70 days for the sterile soil Speyer 2.1.

2. Sampling

Duplicate samples of each system were processed and analysed at 0, 2, 4, 8, 16, 33, 64, and 104 days after treatment (DAT) for the non-sterile Speyer 2.1 and Beedon Manor soil. Duplicate samples from each system were processed and analysed at 0, 2, 4, 7, 16, 34, and 70 DAT for the sterile Speyer 2.1 soil.

3. Analytical procedures

At each sampling interval, samples of soil Speyer 2.1 were extracted three times successively with 50 mL of 0.5 M aqueous ammonia solution using a mechanical shaker for 30 minutes.

Samples of soil Beedon Manor were extracted once with 1 M aqueous ammonia solution and five times with 0.5 M aqueous ammonia solution for 15 minutes using a mechanical shaker.

Extracts and soil were separated each by centrifugation and decantation. Aliquots of the extracts were filtered, and the filtered extracts were neutralised with formic acid.

Extracts of Speyer 2.1 soil were freeze-dried, re-suspended in 1 M formic acid and basified with ammonia. The homogenised suspension was directly used for chromatography.

Extracts of Beedon Manor soil were freeze-dried, re-suspended in 1 M ammonia solution, and the suspension was used for chromatography. The original flasks were rinsed with 1 M formic acid and the rinsings were weighed and analysed for radioactivity by LSC. Procedural recoveries for the work-up steps filtration and freeze-drying are presented in the table below.

Table 8.1.1.1-24: Procedural recoveries for filtration and freeze drying

Incubation group	Procedural recoveries for filtration [%]	Procedural recoveries for freeze-drying [%]
A	71.29 - 100.09	99.70 - 122.01
B	87.39 - 94.78	84.44 - 111.48

C	82.03 - 95.13	100.50 - 121.21
D	89.65 - 99.42	81.87 - 103.38
E	91.39 - 97.83	88.33 - 134.28
F	85.30 - 101.46	77.96 - 94.34

For each dose group, portions of extracted soil at 0 DAT were combusted and analysed by LSC.

Residues in soil extracts were quantified by TLC on silica plates using two different solvent systems (Solvent system 1: methanol/ammonia/10% trichloroacetic acid solution/water, 12/3/1/6; Solvent system 5: methanol/ethanol/ammonia/water, 3/3/3/3).

Glyphosate and AMPA were identified by normal phase TLC co-chromatography with reference items using the two different solvent systems described above.

II. RESULTS AND DISCUSSION

A. DATA

Recovery of radioactivity in soil extracts and combusted soil for 0 DAT is presented in Table 8.1.1.1-25. Extractable radioactivity and results of TLC analysis of soil extracts are summarised below for the respective soils and test conditions.

Soil extracts were analysed by two TLC solvent systems but it is not reported which method was used as primary method. The results of analysis of extractable residues with the two TLC solvent systems were found to be very similar at each sampling interval. Therefore, further discussion and kinetic evaluation refer to average values of the two TLC solvent systems.

Table 8.1.1.1-25: Recovery of radioactivity for soils Speyer 2.1 and Beedon Manor at 0 DAT from extracts and extracted soil after combustion (% AR)

		Incubation group					
Fraction	Replicate	A, Speyer 2.1	B, Speyer 2.1	C, Speyer 2.1	D, Speyer 2.1	E, Speyer 2.1	F, Beedon Manor
Soil extract	A	95.29	96.20	95.51	94.21	96.15	65.84
	B	95.33	94.15	94.74	91.39	98.01	58.80
	Mean	95.31	95.18	95.13	92.80	97.08	62.32
Residual Combusted Soil	A	3.89	3.86	3.38	3.91	5.28	30.79
	B	3.73	3.49	3.54	3.91	5.08	30.98
	Mean	3.81	3.68	3.46	3.91	5.18	30.89
Total	A	99.18	100.06	98.89	98.12	101.43	96.63
	B	99.06	97.64	98.28	95.30	103.09	89.78
	Mean	99.12	98.86	98.59	96.71	102.26	93.21

DAT: days after treatment

Table 8.1.1.1-26: Soil Speyer 2.1, incubation group A: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	95.29	84.22	77.52	70.80	64.15	56.53	48.24	43.79
	B	95.33	84.74	79.31	70.51	63.63	56.50	50.74	45.02
	Mean	95.31	84.48	78.42	70.66	63.89	56.52	49.49	44.41
Total in TLC sample	A	86.02	65.15	74.29	69.38	65.00	55.17	50.05	39.67
	B	80.87	81.60	74.99	62.76	61.07	55.07	45.37	40.54
	Mean	83.45	73.38	74.64	66.07	63.04	55.12	47.71	40.11
Results for TLC solvent system 1									
Glyphosate	A	73.08	53.19	52.25	45.19	34.75	20.62	11.52	7.98
	B	75.48	63.76	51.86	40.38	31.72	20.63	13.22	8.63
	Mean	74.28	58.48	52.06	42.79	33.23	20.62	12.37	8.30
AMPA	A	11.02	7.29	17.30	20.10	25.60	29.13	35.46	29.57
	B	4.71	9.24	17.67	18.56	24.53	30.02	29.91	29.48
	Mean	7.87	8.26	17.48	19.33	25.06	29.57	32.68	29.53
Other	A	0.00	1.66	1.09	1.47	2.63	2.69	1.57	0.77

Table 8.1.1.1-26: Soil Speyer 2.1, incubation group A: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	B	0.00	2.27	0.61	1.29	1.29	1.79	0.83	0.99
	Mean	0.00	1.96	0.85	1.38	1.96	2.24	1.20	0.88
Origin	A	1.68	2.98	3.63	2.53	1.82	2.66	1.51	1.15
	B	0.49	6.07	4.25	2.41	3.31	2.52	1.20	1.43
	Mean	1.09	4.52	3.94	2.47	2.56	2.59	1.35	1.29
Unresolved background	A	0.24	0.04	0.02	0.09	0.20	0.07	0.00	0.19
	B	0.18	0.26	0.60	0.13	0.23	0.11	0.21	0.01
	Mean	0.21	0.15	0.31	0.11	0.21	0.09	0.11	0.10
Results for TLC solvent system 5									
Glyphosate	A	76.83	54.17	56.90	48.52	35.15	21.43	14.65	6.93
	B	74.11	66.20	58.04	43.29	32.34	21.77	13.39	7.46
	Mean	75.47	60.19	57.47	45.91	33.74	21.60	14.02	7.19
AMPA	A	4.77	6.89	13.31	17.14	25.30	29.33	32.37	30.28
	B	3.77	9.58	13.01	16.23	23.83	29.60	29.45	29.85
	Mean	4.27	8.24	13.16	16.69	24.57	29.47	30.91	30.06
Other	A	0.94	1.21	1.74	1.26	2.41	1.73	1.31	1.14
	B	0.67	0.92	0.98	0.85	1.66	1.06	1.05	1.29
	Mean	0.80	1.06	1.36	1.06	2.03	1.39	1.18	1.21
Origin	A	3.27	2.40	2.22	2.39	1.98	2.67	1.57	1.16
	B	2.16	4.63	2.66	2.09	3.15	2.38	1.33	1.84
	Mean	2.71	3.51	2.44	2.24	2.56	2.53	1.45	1.50
Unresolved background	A	0.21	0.49	0.13	0.06	0.16	0.01	0.15	0.15
	B	0.17	0.28	0.28	0.29	0.10	0.25	0.14	0.11
	Mean	0.19	0.38	0.21	0.18	0.13	0.13	0.15	0.13
Mean of solvent system 1 and 5[1]									
Glyphosate	A	74.96	53.68	54.58	46.86	34.95	21.03	13.09	7.46
	B	74.80	64.98	54.95	41.84	32.03	21.20	13.31	8.05
	Mean	74.88	59.33	54.76	44.35	33.49	21.11	13.20	7.75
AMPA	A	7.90	7.09	15.31	18.62	25.45	29.23	33.92	29.93
	B	4.24	9.41	15.34	17.40	24.18	29.81	29.68	29.67
	Mean	6.07	8.25	15.32	18.01	24.82	29.52	31.80	29.80
Other	A	0.47	1.44	1.42	1.37	2.52	2.21	1.44	0.96
	B	0.34	1.60	0.80	1.07	1.48	1.43	0.94	1.14
	Mean	0.40	1.52	1.11	1.22	2.00	1.82	1.19	1.05
Origin	A	2.48	2.69	2.93	2.46	1.90	2.67	1.54	1.16
	B	1.33	5.35	3.46	2.25	3.23	2.45	1.27	1.64
	Mean	1.90	4.02	3.19	2.36	2.57	2.56	1.40	1.40
Unresolved background	A	0.23	0.27	0.08	0.08	0.18	0.04	0.08	0.17
	B	0.18	0.27	0.44	0.21	0.17	0.18	0.18	0.06
	Mean	0.20	0.27	0.26	0.14	0.17	0.11	0.13	0.12

DAT: days after treatment

[1]Values calculated by the applicant while writing this summary.

Table 8.1.1.1-27: Soil Speyer 2.1, incubation group B: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 20 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	96.20	82.88	76.74	69.82	62.88	55.83	48.16	41.37
	B	94.15	82.72	77.43	NS	62.91	55.48	48.71	41.98
	Mean	95.18	82.80	77.09	69.82	62.90	55.66	48.44	41.68
Total in TLC sample	A	88.70	75.96	70.26	52.87	54.56	53.00	43.15	38.66
	B	86.50	76.66	67.29	NS	55.71	53.01	42.75	38.57
	Mean	87.60	76.31	68.77	52.87	55.14	53.01	42.95	38.62
Results for TLC solvent system 1									

Table 8.1.1.1-27: Soil Speyer 2.1, incubation group B: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 20 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Glyphosate	A	75.20	55.95	46.05	32.89	32.36	20.59	11.24	7.86
	B	75.50	55.79	40.81	NS	26.85	20.57	12.26	6.81
	Mean	75.35	55.87	43.43	32.89	29.60	20.58	11.75	7.33
AMPA	A	3.93	10.63	17.97	15.88	16.51	27.66	28.54	26.74
	B	4.33	10.41	18.20	NS	25.11	28.09	27.02	28.08
	Mean	4.13	10.52	18.08	15.88	20.81	27.88	27.78	27.41
Origin	A	6.03	7.31	5.22	2.06	2.36	2.65	1.09	1.74
	B	3.85	7.96	7.33	NS	2.67	2.10	0.96	1.71
	Mean	4.94	7.64	6.27	2.06	2.52	2.38	1.03	1.72
Other	A	2.98	1.82	0.95	1.54	3.01	1.86	2.18	2.04
	B	2.77	2.04	0.92	NS	1.01	2.21	2.25	1.75
	Mean	2.87	1.93	0.93	1.54	2.01	2.03	2.21	1.90
Unresolved background / 1	A	0.56	0.25	0.07	0.51	0.32	0.25	0.10	0.28
	B	0.06	0.45	0.04	NS	0.07	0.04	0.25	0.22
	Mean	0.31	0.35	0.06	0.51	0.19	0.14	0.18	0.25
Results for TLC solvent system 5									
Glyphosate	A	79.96	59.38	51.15	35.39	30.14	21.16	11.65	8.21
	B	77.35	61.46	49.24	NS	30.23	21.33	13.59	6.09
	Mean	78.66	60.42	50.20	35.39	30.18	21.25	12.62	7.15
AMPA	A	3.26	9.28	13.35	14.73	16.59	26.27	28.08	26.36
	B	4.06	8.04	13.10	NS	21.17	27.92	26.27	29.03
	Mean	3.66	8.66	13.22	14.73	18.88	27.10	27.17	27.70
Origin	A	3.59	4.20	3.25	1.51	3.65	2.60	1.51	1.90
	B	3.70	4.83	2.99	NS	2.21	1.92	0.87	1.26
	Mean	3.65	4.51	3.12	1.51	2.93	2.26	1.19	1.58
Other	A	1.50	1.88	1.97	1.19	2.19	2.36	1.86	1.79
	B	1.38	1.90	1.51	NS	1.96	1.59	1.97	1.58
	Mean	1.44	1.89	1.74	1.19	2.07	1.98	1.91	1.69
Unresolved background	A	0.38	1.20	0.53	0.06	2.00	0.62	0.05	0.40
	B	0.02	0.42	0.44	NS	0.14	0.25	0.06	0.61
	Mean	0.20	0.81	0.49	0.06	1.07	0.44	0.06	0.51
Mean of solvent system 1 and 5[1]									
Glyphosate	A	77.58	57.67	48.60	34.14	31.25	20.88	11.45	8.04
	B	76.43	58.63	45.03	0.00	28.54	20.95	12.93	6.45
	Mean	77.00	58.15	46.81	22.76	29.90	20.91	12.19	7.24
AMPA	A	3.60	9.96	15.66	15.31	16.55	26.97	28.31	26.55
	B	4.20	9.23	15.65	0.00	23.14	28.01	26.65	28.56
	Mean	3.90	9.59	15.66	10.20	19.85	27.49	27.48	27.55
Origin	A	4.81	5.76	4.24	1.79	3.01	2.63	1.30	1.82
	B	3.78	6.40	5.16	0.00	2.44	2.01	0.92	1.49
	Mean	4.29	6.08	4.70	1.19	2.72	2.32	1.11	1.65
Other	A	2.24	1.85	1.46	1.37	2.60	2.11	2.02	1.92
	B	2.08	1.97	1.22	0.00	1.49	1.90	2.11	1.67
	Mean	2.16	1.91	1.34	0.91	2.04	2.01	2.07	1.79
Unresolved background	A	0.47	0.73	0.30	0.29	1.16	0.44	0.08	0.34
	B	0.04	0.44	0.24	0.00	0.11	0.15	0.16	0.42
	Mean	0.26	0.58	0.27	0.19	0.63	0.29	0.12	0.38

DAT: days after treatment
NS: No sample taken
[1]Values calculated by the applicant while writing this summary

Table 8.1.1.1-28: Soil Speyer 2.1, incubation group C: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 8 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	95.51	92.04	90.96	85.43	83.08	74.10	67.93	60.01
	B	94.74	91.98	91.02	86.16	82.68	73.33	68.04	59.44
	Mean	95.13	92.01	90.99	85.80	82.88	73.72	67.99	59.73
Total in TLC sample	A	91.50	78.94	89.15	84.58	85.58	75.29	57.19	53.05
	B	88.85	89.85	80.34	80.54	80.55	82.64	60.09	53.78
	Mean	90.18	84.40	84.75	82.56	83.07	78.97	58.64	53.41
Results for TLC solvent system 1									
Glyphosate	A	81.61	63.88	74.91	65.24	63.60	53.68	34.90	22.81
	B	78.78	77.01	67.37	68.48	58.80	59.96	36.62	28.50
	Mean	80.20	70.44	71.14	66.86	61.20	56.82	35.76	25.66
AMPA	A	4.52	6.53	8.50	12.00	15.10	16.85	18.86	25.98
	B	4.27	5.17	7.50	8.64	16.30	18.30	20.45	22.37
	Mean	4.40	5.85	8.00	10.32	15.70	17.57	19.66	24.17
Origin	A	5.29	5.84	4.48	5.26	4.90	2.37	1.33	2.29
	B	5.49	4.72	3.42	1.80	3.84	2.30	1.54	1.41
	Mean	5.39	5.28	3.95	3.53	4.37	2.33	1.44	1.85
Other	A	0.00	2.54	1.25	2.01	1.33	2.21	1.92	1.21
	B	0.00	2.21	1.84	1.59	1.35	1.77	l.45	1.34
	Mean	0.00	2.38	1.54	1.80	1.34	1.99	1.68	1.28
Unresolved background	A	0.08	0.15	0.02	0.06	0.65	0.18	0.17	0.75
	B	0.30	0.75	0.22	0.03	0.26	0.32	0.04	0.15
	Mean	0.19	0.45	0.12	0.05	0.45	0.25	0.10	0.45
	Results for TLC solvent system 5								
Glyphosate	A	80.01	69.55	78.04	72.49	67.88	54.89	36.23	27.35
	B	77.65	77.60	70.31	70.10	63.84	59.83	38.20	28.43
	Mean	78.83	73.57	74.17	71.30	65.86	57.36	37.22	27.89
AMPA	A	3.99	4.61	6.86	7.70	12.26	16.12	16.79	21.66
	B	5.15	5.01	6.03	7.68	12.07	18.01	18.26	22.75
	Mean	4.57	4.81	6.44	7.69	12.17	17.06	17.53	22.20
Origin	A	6.37	2.39	2.70	2.87	3.71	2.57	2.26	1.63
	B	4.79	4.60	2.94	1.27	3.17	2.59	1.48	1.81
	Mean	5.58	3.50	2.82	2.07	3.44	2.58	1.87	1.72
Other	A	1.13	1.98	1.45	1.37	1.57	1.44	1.82	2.07
	B	0.95	2.02	1.03	1.26	1.45	1.45	1.92	0.67
	Mean	1.04	2.00	1.24	1.32	1.51	1.45	1.87	1.37
Unresolved background	A	0.02	0.42	0.11	0.16	0.16	0.28	0.07	0.33
	B	0.31	0.62	0.04	0.22	0.02	0.75	0.23	0.10
	Mean	0.16	0.52	0.07	0.19	0.09	0.52	0.15	0.22
	Mean of solvent system 1 and 5[1]								
Glyphosate	A	80.81	66.72	76.48	68.87	65.74	54.29	35.57	25.08
	B	78.22	77.31	68.84	69.29	61.32	59.90	37.41	28.47
	Mean	79.51	72.01	72.66	69.08	63.53	57.09	36.49	26.77
AMPA	A	4.26	5.57	7.68	9.85	13.68	16.49	17.83	23.82
	B	4.71	5.09	6.77	8.16	14.19	18.16	19.36	22.56
	Mean	4.48	5.33	7.22	9.01	13.93	17.32	18.59	23.19
Origin	A	5.83	4.12	3.59	4.07	4.31	2.47	1.80	1.96
	B	5.14	4.66	3.18	1.54	3.51	2.45	1.51	1.61
	Mean	5.49	4.39	3.39	2.80	3.91	2.46	1.65	1.79
Other	A	0.57	2.26	1.35	1.69	1.45	1.83	1.87	1.64
	B	0.48	2.12	1.44	1.43	1.40	1.61	1.92	1.01
	Mean	0.52	2.19	1.39	1.56	1.43	1.72	1.89	1.32
Unresolved background	A	0.05	0.29	0.07	0.11	0.41	0.23	0.12	0.54
	B	0.31	0.69	0.13	0.13	0.14	0.54	0.14	0.13
	Mean	0.18	0.49	0.10	0.12	0.27	0.38	0.13	0.33

DAT: days after treatment

Table 8.1.1.1-28: Soil Speyer 2.1, incubation group C: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 8 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104

[1]Values calculated by the applicant while writing this summary

Table 8.1.1.1-29: Soil Speyer 2.1, incubation group D: Distribution of radioactivity in soil extracts following TLC analysis for sterile soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT						
		0	2	4	7	16	34	70
Soil extract	A	94.21	84.99	85.62	78.41	77.58	74.63	55.43
	B	91.39	79.47	85.42	82.28	75.34	60.35	58.09
	Mean	92.80	82.23	85.52	80.35	76.46	67.49	56.76
Total in TLC sample	A	81.38	75.71	75.33	67.51	60.38	65.06	50.42
	B	84.39	72.28	72.18	70.41	63.40	53.29	50.23
	Mean	82.88	73.99	73.76	68.96	61.89	59.17	50.33
	Results for TLC solvent system 1							
Glyphosate	A	71.52	61.81	62.02	51.41	41.94	45.62	23.23
	B	72.97	60.38	59.46	56.93	41.36	29.46	23.87
	Mean	72.25	61.10	60.74	54.17	41.65	37.54	23.55
AMPA	A	4.42	5.61	7.50	10.82	11.65	12.98	21.84
	B	4.01	6.19	9.42	7.43	16.81	17.55	19.45
	Mean	4.21	5.90	8.46	9.12	14.23	15.26	20.65
Origin	A	3.64	5.76	3.03	2.58	4.17	4.22	2.61
	B	4.36	3.19	1.83	3.15	2.09	3.48	3.17
	Mean	4.00	4.47	2.43	2.87	3.13	3.85	2.89
Other	A	0.89	1.20	1.48	1.24	1.33	1.22	1.74
	B	1.78	1.07	1.39	1.36	1.53	1.63	2.26
	Mean	1.33	1.14	1.44	1.30	1.43	1.42	2.00
Unresolved background	A	0.91	1.32	1.30	1.47	1.29	1.01	1.00
	B	1.27	1.45	0.08	1.53	1.61	1.17	1.49
	Mean	1.09	1.39	0.69	1.50	1.45	1.09	1.25
	Results for TLC solvent system 5							
Glyphosate	A	70.56	64.28	61.53	53.64	45.01	49.13	24.64
	B	74.77	61.96	61.01	58.55	43.55	30.90	24.68
	Mean	72.66	63.12	61.27	56.09	44.28	40.01	24.66
AMPA	A	4.20	5.25	7.27	8.70	9.76	10.14	20.41
	B	4.19	5.28	7.32	6.89	14.67	17.18	19.68
	Mean	4.19	5.27	7.30	7.79	12.22	13.66	20.04
Origin	A	3.99	3.84	3.53	2.44	3.05	3.76	2.36
	B	3.23	2.55	1.47	2.92	2.02	3.39	3.19
	Mean	3.61	3.19	2.50	2.68	2.53	3.57	2.78
Other	A	1.52	1.50	1.73	1.59	1.64	1.52	2.17
	B	1.35	1.46	1.54	1.32	2.31	1.35	1.90
	Mean	1.44	1.48	1.64	1.45	1.98	1.44	2.04
Unresolved background	A	1.11	0.85	1.29	1.15	0.91	0.51	0.84
	B	0.85	1.02	0.84	0.74	0.85	0.46	0.78
	Mean	0.98	0.93	1.06	0.95	0.88	0.49	0.81
	Mean of solvent system 1 and 5[1]							
Glyphosate	A	71.04	63.05	61.78	52.53	43.48	47.38	23.94
	B	73.87	61.17	60.24	57.74	42.46	30.18	24.28
	Mean	72.46	62.11	61.01	55.13	42.97	38.78	24.11
AMPA	A	4.31	5.43	7.39	9.76	10.71	11.56	21.13
	B	4.10	5.74	8.37	7.16	15.74	17.37	19.57
	Mean	4.21	5.58	7.88	8.46	13.22	14.46	20.35
Origin	A	3.82	4.80	3.28	2.51	3.61	3.99	2.49
	B	3.80	2.87	1.65	3.04	2.06	3.44	3.18

Table 8.1.1.1-29: Soil Speyer 2.1, incubation group D: Distribution of radioactivity in soil extracts following TLC analysis for sterile soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT						
		0	2	4	7	16	34	70
	Mean	3.81	3.84	2.47	2.77	2.83	3.71	2.83
Other	A	1.21	1.35	1.61	1.42	1.49	1.37	1.96
	B	1.57	1.27	1.47	1.34	1.92	1.49	2.08
	Mean	1.39	1.31	1.54	1.38	1.70	1.43	2.02
Unresolved background	A	1.01	1.09	1.30	1.31	1.10	0.76	0.92
	B	1.06	1.24	0.46	1.14	1.23	0.82	1.14
	Mean	1.04	1.16	0.88	1.22	1.17	0.79	1.03

DAT: days after treatment

[1]Values calculated by the applicant while writing this summary

Table 8.1.1.1-30: Soil Speyer 2.1, incubation group E: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 0.4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	96.15	86.79	83.24	73.48	67.66	60.91	55.78	47.79
	B	98.01	86.70	81.46	NS	67.43	60.42	54.80	48.36
	Mean	97.08	86.75	82.35	73.48	67.55	60.67	55.29	48.08
Total in TLC sample	A	101.29	90.92	101.61	72.32	63.58	59.19	52.20	48.72
	B	79.67	95.27	88.49	NS	66.80	63.55	55.24	42.30
	Mean	90.48	93.09	95.05	72.32	65.19	61.37	53.72	45.51
	Results for TLC solvent system 1								
Glyphosate	A	84.18	71.36	74.71	44.62	33.06	22.47	12.68	7.99
	B	65.74	76.06	64.88	NS	31.51	23.13	12.61	6.90
	Mean	74.96	73.71	69.80	44.62	32.28	22.80	12.65	7.45
AMPA	A	6.13	10.37	17.32	17.43	23.78	27.22	30.58	32.79
	B	4.37	11.42	14.16	NS	25.13	29.79	31.77	26.64
	Mean	5.25	10.90	15.74	17.43	24.46	28.50	31.17	29.71
Origin	A	10.55	6.36	6.92	7.71	5.67	6.06	4.97	4.04
	B	9.00	6.07	6.42	NS	7.55	6.53	6.91	6.30
	Mean	9.78	6.22	6.67	7.71	6.61	6.30	5.94	5.17
Other	A	0.00	2.39	2.45	2.10	0.93	3.41	3.55	3.62
	B	0.00	1.54	2.64	NS	2.43	3.64	3.66	2.17
	Mean	0.00	1.97	2.54	2.10	1.68	3.52	3.61	2.90
Unresolved background	A	0.43	0.43	0.21	0.46	0.15	0.02	0.41	0.27
	B	0.56	0.18	0.39	NS	0.18	0.47	0.30	0.29
	Mean	0.49	0.30	0.30	0.46	0.16	0.25	0.35	0.28
	Results for TLC solvent system 5								
Glyphosate	A	86.68	72.26	75.43	48.82	32.96	23.52	12.71	7.25
	B	65.76	75.55	65.88	NS	35.27	25.03	13.12	6.88
	Mean	76.22	73.91	70.66	48.82	34.11	24.28	12.91	7.07
AMPA	A	5.26	10.81	19.02	16.12	24.31	27.62	30.97	34.58
	B	4.02	11.37	15.75	NS	24.83	32.21	32.34	27.94
	Mean	4.64	11.09	17.39	16.12	24.57	29.92	31.66	31.26
Origin	A	8.00	6.17	5.54	5.29	3.41	6.00	6.51	5.71
	B	8.27	6.33	4.62	NS	4.42	4.04	8.03	6.12
	Mean	8.14	6.25	5.08	5.29	3.92	5.02	7.27	5.92
Other	A	1.34	1.40	1.47	2.00	2.79	1.97	1.26	0.98
	B	1.59	1.91	1.28	NS	1.67	1.83	1.54	1.02
	Mean	1.47	1.65	1.38	2.00	2.23	1.90	1.40	1.00
Unresolved background	A	0.01	0.27	0.14	0.11	0.10	0.09	0.76	0.20
	B	0.03	0.10	0.94	NS	0.61	0.44	0.21	0.34
	Mean	0.02	0.18	0.54	0.11	0.35	0.26	0.48	0.27
	Mean of solvent system 1 and 5[1]								
Glyphosate	A	85.43	71.81	75.07	46.72	33.01	23.00	12.70	7.62

Table 8.1.1.1-30: Soil Speyer 2.1, incubation group E: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 0.4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	B	65.75	75.81	65.38	0.00	33.39	24.08	12.87	6.89
	Mean	75.59	73.81	70.23	31.15	33.20	23.54	12.78	7.26
AMPA	A	5.70	10.59	18.17	16.78	24.05	27.42	30.78	33.69
	B	4.20	11.40	14.96	0.00	24.98	31.00	32.06	27.29
	Mean	4.95	10.99	16.56	11.18	24.51	29.21	31.42	30.49
Origin	A	9.28	6.27	6.23	6.50	4.54	6.03	5.74	4.88
	B	8.64	6.20	5.52	0.00	5.99	5.29	7.47	6.21
	Mean	8.96	6.23	5.88	4.33	5.26	5.66	6.61	5.54
Other	A	0.67	1.90	1.96	2.05	1.86	2.69	2.41	2.30
	B	0.80	1.73	1.96	0.00	2.05	2.74	2.60	1.60
	Mean	0.73	1.81	1.96	1.37	1.96	2.71	2.50	1.95
Unresolved background	A	0.22	0.35	0.18	0.29	0.13	0.06	0.59	0.24
	B	0.30	0.14	0.67	0.00	0.40	0.46	0.26	0.32
	Mean	0.26	0.25	0.42	0.19	0.26	0.26	0.42	0.28

DAT: days after treatment
NS: No sample taken
[1]Values calculated by the applicant while writing this summary

Table 8.1.1.1-31: Soil Beedon Manor, incubation group F: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	**Replicate**	**DAT**							
		0	**2**	**4**	**8**	**16**	**33**	**64**	**104**
Soil extract	A	65.84	48.39	47.88	39.53	30.75	27.45	16.73	9.12
	B	58.80	47.96	48.14	39.60	32.03	22.37	16.49	9.43
	Mean	62.32	48.18	48.01	39.57	31.39	24.91	16.61	9.28
Total in TLC sample	A	47.59	37.16	37.13	28.51	23.47	20.02	11.22	6.03
	B	48.47	35.18	31.07	29.44	23.80	18.17	10.94	6.66
	Mean	48.03	36.17	34.10	28.98	23.63	19.10	11.08	6.34
Results for TLC solvent system 1									
Glyphosate	A	30.21	22.10	16.95	10.72	5.17	2.26	0.79	0.23
	B	35.53	19.67	13.49	10.47	5.45	2.10	0.56	0.45
	Mean	32.87	20.88	15.22	10.60	5.31	2.18	0.67	0.34
AMPA	A	3.92	7.34	10.79	13.76	12.23	12.54	6.67	3.55
	B	3.51	6.70	9.07	13.46	12.10	11.95	6.80	3.70
	Mean	3.72	7.02	9.93	13.61	12.17	12.25	6.73	3.62
Origin	A	11.56	6.15	8.22	2.91	4.51	3.17	2.46	1.37
	B	8.26	7.50	7.54	4.21	5.08	2.34	2.11	1.78
	Mean	9.91	6.82	7.88	3.56	4.80	2.76	2.29	1.58
Other	A	0.88	1.24	0.93	1.08	1.55	2.00	1.28	0.88
	B	0.95	1.01	0.92	1.28	1.12	1.75	1.45	0.69
	Mean	0.92	1.12	0.93	1.18	1.34	1.87	1.37	0.79
Unresolved background	A	1.01	0.35	0.25	0.04	0.00	0.05	0.02	0.00
	B	0.20	0.31	0.05	0.02	0.04	0.02	0.03	0.04
	Mean	0.61	0.33	0.15	0.03	0.02	0.03	0.02	0.02
Results for TLC solvent system 5									
Glyphosate	A	33.63	22.81	17.94	11.05	4.76	2.60	0.83	0.59
	B	35.60	19.23	14.28	10.39	4.94	2.37	0.76	0.99
	Mean	34.62	21.02	16.11	10.72	4.85	2.49	0.79	0.79
AMPA	A	3.84	7.42	11.02	13.39	12.52	12.80	7.08	3.32
	B	3.75	7.43	8.77	13.56	12.43	12.35	6.97	3.26
	Mean	3.79	7.43	9.90	13.48	12.47	12.58	7.03	3.29
Origin	A	8.46	5.53	6.89	2.49	4.84	3.28	2.20	1.21
	B	8.01	7.58	7.08	4.10	4.91	2.15	2.04	1.55
	Mean	8.23	6.55	6.98	3.29	4.87	2.71	2.12	1.38

Table 8.1.1.1-31: Soil Beedon Manor, incubation group F: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	**2**	**4**	**8**	**16**	**33**	**64**	**104**
Other	A	1.49	0.99	1.17	1.57	1.34	1.33	1.05	0.89
	B	0.99	0.76	0.92	1.36	1.52	1.30	1.15	0.85
	Mean	1.24	0.88	1.04	1.47	1.43	1.31	1.10	0.87
Unresolved background	A	0.17	0.42	0.10	0.01	0.01	0.00	0.06	0.02
	B	0.11	0.17	0.02	0.03	0.00	0.00	0.02	0.01
	Mean	0.14	0.29	0.06	0.02	0.00	0.00	0.04	0.01
Mean of solvent system 1 and 5[1]									
Glyphosate	A	31.92	22.46	17.45	10.89	4.97	2.43	0.81	0.41
	B	35.57	19.45	13.89	10.43	5.20	2.24	0.66	0.72
	Mean	33.74	20.95	15.67	10.66	5.08	2.33	0.74	0.57
AMPA	A	3.88	7.38	10.91	13.58	12.38	12.67	6.88	3.44
	B	3.63	7.07	8.92	13.51	12.27	12.15	6.89	3.48
	Mean	3.76	7.22	9.91	13.54	12.32	12.41	6.88	3.46
Origin	A	10.01	5.84	7.56	2.70	4.68	3.23	2.33	1.29
	B	8.14	7.54	7.31	4.16	5.00	2.25	2.08	1.67
	Mean	9.07	6.69	7.43	3.43	4.84	2.74	2.20	1.48
Other	A	1.19	1.12	1.05	1.33	1.45	1.67	1.17	0.89
	B	0.97	0.89	0.92	1.32	1.32	1.53	1.30	0.77
	Mean	1.08	1.00	0.99	1.32	1.38	1.60	1.23	0.83
Unresolved background	A	0.59	0.39	0.18	0.03	0.01	0.03	0.04	0.01
	B	0.16	0.24	0.04	0.03	0.02	0.01	0.03	0.03
	Mean	0.37	0.31	0.11	0.03	0.01	0.02	0.03	0.02

DAT: days after treatment

[1]Values calculated by the applicant while writing this summary

B. MATERIAL BALANCE

The material balance of radioactivity for all incubation groups at 0 DAT ranged from 93.21 to 102.26 % AR. No full material balance was determined for soil samples beyond DAT 0 of all incubation series, i.e. nor non-extractable radioactivity (NER) neither volatile radioactivity was determined.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Following application of 4 mg/kg to soil Speyer 2.1, the amount of radioactivity in the soil extract decreased from 0 to 104 DAT from 95.31 to 44.41 % AR at 20°C and 40% MWHC, from 95.18 to 41.68 % AR at 20°C and 20% MWHC, and from 95.13 to 59.73 % AR at 8°C and 40% MWHC.

Following application of 4 mg/kg to soil in Beedon Manor, the amount of radioactivity in the soil extract decreased from 0 to 104 DAT from 62.32 to 9.28 % AR at 20°C and 40% MWHC.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of radioactivity in the soil extract decreased from 0 to 104 DAT from 97.08 to 48.08 % AR at 20°C and 40% MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of radioactivity in the soil extract decreased from 0 to 70 DAT from 92.80 to 56.76 % AR at 20°C and 40% MWHC.

Non-extractable radioactivity (NER) in soils was not determined.

D. TRANSFORMATION OF THE TEST ITEM

All values provided are the mean values of the results of analysis by TLC with two different solvent systems.

Following application of 4 mg/kg to soil Speyer 2.1, the amount of glyphosate in the soil extract decreased from 0 to 104 DAT from 74.88 to 7.75 % AR at 20 °C and 40 % MWHC, from 77.00 to 7.24 % AR at 20 °C and 20 % MWHC, and from 79.51 to 26.77 % AR at 8 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Beedon Manor, the amount of glyphosate in the soil extract decreased from 0 to 104 DAT from 33.74 to 0.57 % AR in soil at 20 °C and 40 % MWHC.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of glyphosate in the soil extract decreased from 0 to 104 DAT from 75.59 to 7.26 % AR at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of glyphosate in the soil extract decreased from 0 to 70 DAT from 72.46 to 24.11 % AR at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Speyer 2.1, the amount of aminomethylphosphonic acid (AMPA) in the soil extract increased to a maximum of 31.80 % AR at 64 DAT at 20 °C and 40 % MWHC, 27.55 % AR at 104 DAT at 20 °C and 20 % MWHC, and 23.19 % AR at 104 DAT at 8 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Beedon Manor, the amount of AMPA in the soil extract increased to a maximum 13.54 % AR at 8 DAT at 20 °C and 40 % MWHC. The amount of AMPA subsequently decreased to 3.46 % AR at 104 DAT.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of AMPA in the soil extract increased to a maximum of 31.42 % AR at 64 DAT at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of AMPA in the soil extract increased to a maximum of 20.35 % AR at the end of the experiment (70 DAT) at 20 °C and 40 % MWHC.

E. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found under CA7.1.2.1.1/001.

III. CONCLUSIONS

Glyphosate was rapidly degraded in viable soil at an incubation temperature of 20 °C. AMPA was observed as the only significant metabolite of glyphosate to account for a 31.80 % AR in maximum (mean of two replicates).

Assessment and conclusion by applicant:

The study was conducted overall consistent with current guidelines, showing minor deviations. Determination of non-extractable radioactivity was performed for day 0, hence, a full material balance is available just for day 0. There was no determination of volatile radioactivity for the other sampling intervals. Furthermore, pesticide soil history was not reported for soil Beedon Manor. However, when putting the results of this study into context with overall information available on aerobic degradation in soil, the deviations are regarded of minor influence on the general outcome.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS

Some deviations from OECD 307 are identified.

There was no analysis for volatiles, and the determination of non-extractable radioactivity only at DAT0 prevents a complete mass balance for the whole study. However RMS highlights that this study was identified by the applicant as a rate of degradation study. Although mass balance is incomplete, it provides reliable information on occurrence of AMPA.

Pesticide history of the soil Beedon Manor is not reported. For this soil, the recovery at DAT0 is low, with only 34% AR as glyphosate. Results from Beedon Manor are therefore considered uncertain and are not relied on.

The procedural recoveries for filtration was sometimes low, which induces that at DAT0, the quantity of compounds analysed by TLC is already a little bit low. LOD/LOQ for analysis of glyphosate and AMPA is not clearly indicated.

It is noted that the radioactive quantity measured at "Origin" reaches up to 9% AR at DAT0. Loss of potential radioactivity as glyphosate or AMPA cannot be excluded, as no explanation was provided by the applicant. It is noted that this "origin" is however quite low in Speyer 2.1 soil with Group A.

The applicant proposed to average TLC results obtained with solvent system 1 and solvent system 5. This can be accepted in this case since results are quite similar.

The study is considered acceptable, except for soil Beedon Manor. RMS considers that only results from soil Speyer 2.1 conditions A (4 mg/kg, 20°C, 40% MWHC) should be taken forward for kinetic assessment since in case the same soil is incubated at various conditions, the recommended approach is to only use the incubation closest to reference conditions

[redacted], *1996*

Data point:	CA 7.1.1.1/002
Report author	[redacted]
Report year	1996
Report title	[P-Methylene-14C]glyphosate acid: aerobic soil metabolism
Report No	548W-1
Guidelines followed in study	US EPA 162-1 OECD guideline 307
Deviations from current test guideline	Major deviation from OECD 307: Oxygen was used instead of CO_2-free air for airing the headspace of the test vessels thus creating a best case regarding the supply with oxygen for the soil microbes and possibly accelerating glyphosate degradation.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: aerobic soil metabolism Test item: [14C] glyphosate, phosphonomethyl-label (97.4 % radiochemical purity) Test soil: Visalia (CA, USA) Soil type: sandy loam pH (matrix unknown): 8.3 Organic matter: 0.60 % Application rate: 4.74 mg/kg Test design: semi-static system with biometer flasks kept slightly pressurized with oxygen Volatiles trapping: CO2: 10 % KOH solution in trap Organic volatiles: foam plug Incubation: 25±1 °C (incubator, temperature controlled), soil moisture adjusted to 75 % of water holding capacity at 0.33 bar Sampling: 0, 1, 2, 3, 4, 8, 11, 14, 18, 24 and 31 days after treatment (DAT), duplicate samples Workup: threefold extraction with 1 M KH2PO4 (pH 2.0) at ambient temperature Analysis of radioactivity: Extracts: LSC (combined extracts) NER: combustion/LSC Volatiles: LSC; foam plug extracted with dichloromethane Identification of radioactive residues: HPLC/radiodetection and TLC/radiodetection co-chromatography with reference items.
Short description of results:	Recovery of radioactivity (mean values): 85.8 – 96.8 % AR Mineralization: 65.2 % AR at 24 DAT Other volatiles: not detected (< 2 x background)

Extractable radioactivity (mean values): 94.8 % AR at 0 DAT, 22.8 % AR at 31 DAT
Non-extractable radioactivity (mean values): 2.0 % AR at 0 DAT, 7.5 % AR at 8 DAT, 5.9 % AR at 31 DAT
Transformation of test item (HPLC analysis):
Glyphosate: 93.0 % AR at 0 DAT, 1.3 % AR at 31 DAT
AMPA: 1.6 % AR at 0 DAT, 20.2 % AR at 31 DAT, max. 24.4 % AR at 11 DAT
No unidentified metabolites >5 % AR.

Assessment and conclusion by RMS:

The applicant's summary is very brief. It was not completed by RMS since the study is not acceptable.

The use of oxygen instead of CO_2 free air may have a significant impact on the degradation behaviour of glyphosate and prevents the use of the study for addressing this data point. It is also noted that the organic matter content of the soil was 0.6% OM which corresponds to an organic carbon content <0.5%OC.

The study is not considered acceptable.

[redacted] *1996*

Data point:	CA 7.1.1.1/004
Report author	[redacted]
Report year	1996
Report title	[^{14}C]-Glyphosate: determination of soil degradation, bio-transformation and metabolism under aerobic conditions
Report No	96-120-1020
Guidelines followed in study	SETAC – Procedures for Assessing the Environmental Fate and Exotoxicity of Pesticides, 1995; Annex of FAO revised guidelines on environmental criteria for the registration of pesticides; BBA Guideline Part IV, 4-1 OECD Guideline 307
Deviations from current test guideline	From OECD 307: - No information on soil history - Application rate used does not cover the maximum intended application rate - Only one replicate was analysed per sampling point - LOD/LOQ not reported - inconsistencies between the tabulated results and the examples of chromatograms
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C- phosphonomethyl]-glyphosate
Lot No.: D1
Specific activity: 316 µCi/mg
Radiochemical purity: 99.6 %

2. Soil:

Soils were sampled from the fields and placed outside in the Springborn soil holding area. There, the soils were kept in wooden boxes underlying barley grass and seeded with Phacellia plants to provide natural conditions. The plots were irrigated if natural rainfall did not provide enough moisture. After four months

of storage, soil was collected from the Springborn soil holding area and sieved to ≤ 2 mm. The soil moisture content was determined and adjusted to the approximate incubation moisture. No pesticides or fertilizers were applied for at least four years. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-32: Characteristics of test soils

Parameter		**Results**		
Soil		**Speyer 2.1**	**Speyer 2.2**	**Speyer 2.3**
Country		Germany	Germany	Germany
Textural Class (DIN)		Sand	Loamy Sand	Loamy Sand
Sand (>63 µm) (%)		88.4	81.2	60.9
Silt (2 µm – 63 µm) (%)		9.8	13.4	29.6
Clay (< 2 µm) (%)		1.9	5.5	9.5
pH ($CaCl_2$)		5.9	5.6	6.4
pH (H_2O)[3]		6.4	6.1	6.9
Organic carbon (%)		0.62	2.32	1.22
Organic matter (%)[1]		1.07	3.99	2.10
Cation exchange capacity (meq/100 g)		5.0	10.9	10.2
Maximum Water Holding Capacity (%)		31	48	39
Microbial biomass (mg C/100g)	Before application[2]	90 (14.5 %OC)	71 (3.1%OC)	89 (20 °C) (7.3 %OC) 89 (10 °C) (7.3 %OC)
	Study end (90 DAT)	210 (33.9 %OC)	246 (10.6%OC)	173 (20 °C) (14.2%OC) 123 (10 °C) (10.1 %OC)

DAT = days after treatment

[1] Calculated from organic carbon according to OM = OC x 1.72

[2] acclimated for 2 d at 45% moisture at 0 bar

[3] calculated by RMS considering the formula pH_{H2O} = 0:982pH_{CaCl2} + 0.648 presented in the EFSA guidance for predicting environmental concentration in soil (2017)[3]

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used. Soil samples were incubated in 500 mL glass metabolism flasks. The flasks per experimental part were equipped with a trapping system: one washing bottle containing ethylenglycol was used to trap organic volatiles, three washing bottles containing 0.5 M NaOH solution were used to trap $^{14}CO_2$. The metabolism flasks were continuously ventilated with CO_2 free and moistened air at a flow rate of about 30 to 60 mL per minute.

100 g of sieved soil (dry weight equivalents) were weighed into each test vessel, soil moisture was adjusted to 45 % of the maximum water holding capacity (MWHC) and the test systems were acclimated for 3 days at test conditions.

A test solution of [^{14}C]glyphosate, mixed with unlabelled glyphosate was prepared in water. 0.2 mL of this solution were applied to each test system, resulting in a final concentration of 3.11 mg/kg dry soil.

Considering a 5cm depth and a default bulk density of the soil of 1.5 g/cm^3, it corresponds to a dose of 2332.5 g/ha of glyphosate.

Test systems were incubated under aerobic conditions in the dark for 90 days at 20 °C and 45 ± 2 % MWHC for soil Speyer 2.1 and 2.2 and for 60 days at 20 °C and at 10 °C at 45 ± 2 % MWHC for soil Speyer 2.3.

2. Sampling

One test system was processed and analysed 0, 1, 2, 4, 7, 15, 29 and 60 days after treatment (DAT), and additionally at 90 DAT for soil Speyer 2.1 and at 90 and 120 DAT for soil Speyer 2.2. All soil samples were processed on the designated sampling day. The ethylene glycol and NaOH traps were assayed at each sampling point.

3 EFSA Journal 2017;15(10):4982, source of the formula: Boesten et al. 2012

3. Analytical procedures

The analytical procedure was confirmed prior to the experimental start of the definitive test by extractability tests, which showed recoveries of 99 to 101%.

At each sampling interval, soil samples were extracted consecutively three times with 125 mL portions of 0.35 M aqueous H_3PO_4/0.09 M aqueous $CaCl_2$ per 50 g dry weight of soil by shaking the samples in an overhead shaker at about 60 rpm at ambient temperature. After centrifugation of each individual extract, extraction efficiency was determined by LSC. After exhaustive solvent extraction, extracts were pooled and the extraction efficiency was determined. Extracts were analysed qualitatively and quantitatively by HPLC via direct injection. The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Glyphosate and metabolites were identified by radio-HPLC and radio-TLC co-chromatography with reference items.

The identity of CO_2 in the sodium hydroxide traps was confirmed by the addition of barium hydroxide to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba1_4CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in the tables below for the respective soils.

Table 8.1.1.1-33: Degradation of [^{14}C]glyphosate in soil Speyer 2.1 under aerobic conditions (expressed as percent of applied radioactivity) at 20 °C

	DAT								
Radioactive Residues	0	1	2	4	7	15	29	60	90
Glyphosate	96.0	84.8	74.3	59.2	53.9	38.2	21.0	8.5	2.2
AMPA	1.3	12.1	12.9	25.1	27.3	27.5	37.9	42.3	**50.1**
Total extractable residues	97.2	97.0	87.2	84.4	81.2	65.7	58.9	50.8	52.3
Carbon dioxide	ND	5.1	7.4	12.8	17.9	23.2	32.4	39.4	43.0
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	0.5	0.7	1.0	1.1	1.7	1.7	2.0	1.9	2.5
Mass balance	97.7	102.7	95.6	98.2	100.8	90.7	93.3	92.1	97.8

DAT: days after treatment
ND: not determined

Table 8.1.1.1-34: Degradation of [^{14}C]glyphosate in soil Speyer 2.2 under aerobic conditions (expressed as percent of applied radioactivity) at 20 °C

	DAT									
Radioactive Residues	0	1	2	4	7	15	29	60	90	120
Glyphosate	99.2	96.1	84.2	77.1	71.8	60.3	41.7	26.7	25.9	19.0
AMPA	3.7	4.3	7.9	12.9	15.7	21.0	34.5	42.4	39.0	40.9
Total extractable residues	102.9	100.5	92.1	89.9	87.5	81.3	76.2	69.1	64.9	59.9
Carbon dioxide	ND	2.8	3.8	7.2	10.5	16.3	22.5	30.6	33.9	36.5
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	0.9	0.9	1.2	1.2	1.7	1.6	2.2	1.6	3.7	4.9
Mass balance	103.8	104.1	97.0	98.3	99.7	99.1	100.9	101.4	102.4	101.2

DAT: days after treatment
ND: not determined

Table 8.1.1.1-35: Degradation of [^{14}C]glyphosate in soil Speyer 2.3 under aerobic conditions (expressed as percent of applied radioactivity) at 20 °C

	DAT							
Radioactive Residues	0	1	2	4	7	15	29	60
Glyphosate	91.1	76.2	63.9	34.2	18.4	13.3	<0.1	3.0
AMPA	7.0	13.0	27.0	25.7	32.0	25.3	31.1	18.5

Total extractable residues	98.1	89.3	90.9	60.0	50.4	38.6	31.1	21.5
Carbon dioxide	ND	12.9	18.6	30.5	38.1	48.4	55.4	63.4
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	1.0	2.0	2.8	2.3	3.6	4.1	4.4	7.8
Mass balance	99.1	104.2	112.3	92.8	92.2	91.0	90.9	92.7

DAT: days after treatment
ND: not determined

Table 8.1.1.1-36: Degradation of [14C]glyphosate in soil Speyer 2.3 under aerobic conditions (expressed as percent of applied radioactivity) at 10 °C

	DAT							
Radioactive Residues	0	1	2	4	7	15	29	60
Glyphosate	93.6	87.3	80.0	62.2	54.9	35.9	21.7	7.5
AMPA	4.7	8.7	9.2	19.3	22.1	25.8	28.7	34.3
Total extractable residues	98.3	96.1	89.2	81.6	76.9	61.7	50.4	41.8
Carbon dioxide	ND	4.8	6.6	12.6	18.9	30.0	40.4	48.2
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	1.1	1.5	1.6	1.7	2.3	3.6	3.9	2.4
Mass balance	99.3	102.4	97.4	95.9	98.2	95.3	94.7	92.4

DAT: days after treatment
ND: not determined

B. MASS BALANCE

Material balances ranged from 90.7 to 102.7 % of applied radioactivity (% AR) for soil Speyer 2.1, from 97.0 to 104.1 % AR for soil Speyer 2.2, from 90.9 to 112.3 % AR for soil Speyer 2.3 at 20 °C and from 92.4 to 102.4 % AR for soil Speyer 2.3 at 10 °C.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 90 DAT from 97.2 to 52.3 % AR in soil Speyer 2.1, from 102.9 to 59.9 % AR at 120 DAT in soil Speyer 2.2 and from 98.1 to 21.5 % AR at 60 DAT. In soil Speyer 2.3 at 10 °C it decreased from 0 DAT to 60 DAT, from 98.3 to 41.8 % AR.

The amount of non-extractable residues (NER) increased from 0 DAT to the end of the study from 0.5 to 2.5 % AR in soil Speyer 2.1, from 0.9 to 4.9 % AR in soil Speyer 2.2 and from 1.0 to 7.8 % AR in Soil Speyer 2.3 at 20 °C. In soil Speyer 2.3 at 10 °C it increased from 1.1 to 2.4 % AR.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (120 DAT, 90 DAT or 60 DAT) were 43.0, 36.5 and 63.4 % AR in soils Speyer 2.1, Speyer 2.2 and Speyer 2.3 at 20 °C, respectively. In soil Speyer 2.3 at 10 °C the maximum amount was 48.2 % AR at the end of the study. Organic volatiles determined were <0.1 % AR for all soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

For all incubations at 20 °C, residues of glyphosate decreased quickly. In soil Speyer 2.1 it was detected with 96.0 % AR at 0 DAT and decreased to 2.2 % AR at 90 DAT. In soil Speyer 2.2 it was found with 99.2 % AR at 0 DAT and decreased to 19.0 % AR at 120 DAT. In soil Speyer 2.3, it was found with 91.1 % AR at 0 DAT and decreased to 3.0 % AR at 60 DAT.

In soil Speyer 2.3 at 10 °C, glyphosate was degraded slightly slower compared to the experiment at 20 °C with 93.6 % AR at 0 DAT and 7.5 % AR at 60 DAT.

Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected in all soils. In soil Speyer 2.1 it reached a maximum amount of 50.1 % AR at the end of the study (90 DAT). In soil Speyer 2.2 it was found with a maximum amount of 42.4 % AR at 60 DAT and showed a slight decrease to 40.9 % AR at the end of the study (120 DAT). In soil Speyer 2.3 at 20 °C AMPA was found with a maximum amount of 32.0 % AR at 7 DAT and decreased to 18.5 % AR at the end of the study (60 DAT).

In soil Speyer 2.3 incubated at 10 °C AMPA was found with a maximum amount of 34.3 % AR at the end of the experiment (60 DAT). No other metabolites were detected above 5 % AR at any time.

F. KINETICS

The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020a, CA 7.1.2.1.1/001).

III. CONCLUSIONS

[^{14}C]glyphosate showed a similar degradation behaviour in the three soils after treatment with 3.11 mg/kg. The main degradation product was carbon dioxide: Between 36.5 and 63.4 % of the applied radioactivity was mineralized, depending on the soil type and the incubation temperature. Glyphosate was degraded quickly in all incubation systems at 20 °C with amounts of 2.2 % AR at 90 DAT in the soil Speyer 2.1, 19.0 % AR at 120 DAT in the soil Speyer 2.2 and 3.0 % AR at 60 DAT in the soil Speyer 2.3 at the end of the study. At 10 °C, the decrease of glyphosate residues was slightly slower with 7.5 % AR in soil Speyer 2.3 at the end of the study at 60 DAT. AMPA was identified as the only major metabolite with a maximum amount of 50.1 % AR. Non-extractable residues amounted to maximum 7.8 % AR.

Assessment and conclusion by applicant:

The study conduct was consistent with the current guideline, showing minor deviations.

One replicate sample was processed and analysed per sampling point while the standard is work-up of duplicates. Instead, the number of sampling points was increased to i.e. eight being well beyond the minimum of five to six to allow for robust for kinetic analysis. The deviations are considered to not influence the overall outcome of the study.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS

The following deviations from OECD 307 are identified.

The treatment rate is below the maximum intended dose (worst GAP of 3600 g/ha; equivalent treatment dose in the study is 2307 g/ha). From all available data on degradation of glyphosate, there is no evidence of an impact of the application rate on the degradation of glyphosate, therefore no impact on the outcome of the study is expected.

No information on the soils history was provided. The percentage of sand in soils Speyer 2.1 and 2.2 (> 80%) seems quite high. As mentioned by the applicant, the OECD 307 guidance recommends ensuring the repeatability of the analysis, whereas only one replicate per sampling point was analysed. However RMS considers that these deviations have no impact on the overall outcome of the study.

LOD/LOQ for analysis of glyphosate and AMPA is not clearly indicated. However based on the presented results, it is expected that it is 0.1% AR.

Compared to other studies, RMS notes that few non-extractable residues are measured, which may be explained by the several extraction steps.

From the study report and tables presented above, it seems that all of the extractable radioactivity would either be glyphosate or AMPA. This is unexpected since in other studies, most of the time some additional radioactivity corresponding to other minor compounds is measured. RMS checked the HPLC analysis examples available in the study report. All chromatograms confirmed that HPLC analysis resulted in only 2 peaks, corresponding to glyphosate (peak 2) and AMPA (peak 1). However some inconsistencies were observed between values reported in the HPLC radiochromatograms and tabulated results in the study report. . RMS used the ROI% presented in the chromatograms and the amount of extracted radioactivity presented in the study report to recalculate the amount of glyphosate and AMPA as %AR, and to compare it with the values presented in the report.

RMS calculations from all chromatograms available in the study report are presented below:

		Results at DAT 0			
		Speyer 2.1	Speyer 2.2	Speyer 2. 3 (20°C)	Speyer 2.3 (10°C)
Results in % AR from study report	Glyphosate	96.0	99.2	91.1	93.6
	AMPA	1.3	3.7	7.0	4.7
	Total extractable residues	97.2	102.9	98.1	98.3
Results in % ROI from HPLC chromatograms	Peak 1 (AMPA)	3.65	1.30	4.83	7.08
	Peak 2 (glyphosate)	96.35	98.70	95.17	92.92
RMS calculation in % AR*	Peak 1 (AMPA)	3.5	1.3	4.7	7.0
	Peak 2 (glyphosate)	93.7	101.6	93.4	91.3

		Results at DAT 7			
		Speyer 2.1	Speyer 2.2	Speyer 2. 3 (20°C)	Speyer 2.3 (10°C)
Results in % AR from study report	Glyphosate	53.9	71.8	18.4	54.9
	AMPA	27.3	15.7	32.0	22.1
	Total extractable residues	81.2	87.5	50.4	76.9
Results in % ROI from HPLC chromatograms	Peak 1 (AMPA)	33.56	17.86	63.49	28.73
	Peak 2 (glyphosate)	66.44	82.14	36.51	71.27
RMS calculation in % AR*	Peak 1 (AMPA)	27.3	15.6	32.0	22.1
	Peak 2 (glyphosate)	53.9	71.9	18.4	54.8

		Speyer 2.1	Speyer 2.2	Speyer 2. 3 (20°C)	Speyer 2.3 (10°C)
at DAT		**90**	**120**	**30**	**60**
Results in % AR from study report	Glyphosate	2.2	19.0	<0.1	7.5
	AMPA	50.1	40.9	31.1	34.3
	Total extractable residues	52.3	59.9	31.1	41.8
Results in % ROI from HPLC chromatograms	Peak 1 (AMPA)	91.53	100	100	82.10
	Peak 2 (glyphosate)	8.47	0	0	17.90
RMS calculation in % AR*	Peak 1 (AMPA)	47.9	59.9	31.1	34.3
	Peak 2 (glyphosate)	4.4	0.0	0.0	7.5

* Calculated from Total extractable residues in % AR * HPLC results in % ROI
Results in grey come from the study report // Peak % from HPLC come from the figures presented // other results are calculated by RMS.

In 6 cases (day 7 for all soils, day 30 for Speyer 2.3 (20°C) and day 60 for Speyer 2.3 (10°C)), values calculated by RMS in % AR are exactly the same as the ones presented in the study report.

In 5 cases (day 0 for all soils and day 90 for Speyer 2.1), it can be seen that there are slight differences between concentrations of glyphosate and AMPA calculated by RMS and concentrations reported in the study report. Additionally, at day 0, it is noted that values calculated by RMS for Speyer 2.3 at 20°C correspond to values reported in the study report for Speyer 2.3 at 10°C, and vice versa. Therefore, RMS wonders whether there might be an inversion in the chromatograms or in the tabulated results presented in the report. Since the only identification of the chromatograms comes from the title of the figure that had been added during study reporting, it cannot be excluded that there was an error during the reporting.

Finally, for Speyer 2.2 at day 120, significant difference between RMS calculations and tabulated results from the study report is noted. According to the HPLC radiochromatograms, only peak 1 (AMPA) is found while tabulated results in the study report indicate that 40.9% AR correspond to AMPA and 19% AR to glyphosate.

The applicant was requested to provide further explanation on the differences observed and to provide all additional HPLC chromatograms available, to ensure that correct results are presented in the report. The answer from the applicant is reproduced below:

"GRG acknowledges the inconsistencies for the last sampling point (120 DAT) for soils Speyer 2.1 and Speyer 2.2 as indicated by AGG. Attempts for further clarification were made by inquiries to obtain access to the raw data of the study. However, confirmation was received that the raw data were discarded by the laboratory (Springborn Laboratories, Horn, Switzerland; now owned by Smithers Group Inc., Akron, OH, USA). Therefore, no further data can be provided.

An indication that the values in the tables are correct with erroneous chromatograms in the Appendix for the last sampling dates of soil 2.1 and soil 2.2 would be the conclusiveness of the sequence of data in the kinetic evaluation. The results compiled in the tables of the report (Table 1 and 2, p. 35) appear to be conclusive when following the individual time series of glyphosate and AMPA. This can also be shown by the good visual fits of the corresponding kinetic evaluation (see Tables 10 and 12 in [redacted], 2020a, KCA 7.1.2.1.1/001). If the tabulated values were erroneous, major deviations of the residues from the fitted curves would be expected. Therefore, it is supposed that the inconsistencies result from erroneously included chromatograms for soil 2.1 and 2.2 on the last sampling dates (DAT 120), but that tabulated values are most likely correct."

RMS disagrees that the kinetic results can be used as a confirmation that the results presented in the tables in the report are corrrect. Additionnally as presented above, the last sampling data are not the only samples for which differences were observed between the HPLC chromatograms and the data reported in the tables.

The main issue is that it is not possible to determine which between the tabulated results or the chromatograms are erroneous. The chromatograms for days between 7 and 90/120 (depending on the soil) are not available and it is therefore not possible to confirm the amounts reported at these dates. RMS considers that there is too much uncertainty and it is difficult to have confidence in the reported results.

As a consequence, the study is not considered acceptable.

[redacted] *1993*

Data point:	CA 7.1.1.1/007
Report author	[redacted]
Report year	1993
Report title	Rate of degradation and metabolism of [14C]-Glyphosate in soil under aerobic conditions
Report No	IWM-R93/047
Guidelines followed in study	Dutch Guideline for Biocides, section G.1.1 OECD guideline 307
Deviations from current test guideline	From OECD 307: - the work-up procedure was not suitable to result in adequate procedural recoveries. Soil extracts were concentrated by freeze-drying to result in high losses, i.e 15.3 to 57.6 % of extracted radioactivity could not be re-constituted for analysis, presumably due to high portions of test item bound to humic substances. In addition, high variability in results was observed between the duplicates per sampling interval investigated - soil history not reported - no limit of detection reported for TLC analytical method - for two soils Drovendaal & Lisse, no full material balance was established - for two soils representativeness as agricultural soil unknown - Microbial biomass < 1% OC in two soils (Drovendaal and Lisse)

GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C-phosphonomethyl]-glyphosate
Lot No.: CFA.745 batch 17
Specific activity: 12.3 MBq/mg
Radiochemical purity: ≥98.9 %

Identification: glyphosate (non-radiolabelled)
Lot No.: F92/-/086
Chemical purity: 99 %

2. Soil:

About one to two months prior to application, the soils were sampled from experimental stations (Droevendaal and Lisse) or an apple orchard (Maasdijk). Until the start of pre-incubation, soils were stored at 3 ± 2 °C for a maximum of 68 days. Soils were partly air-dried and sieved to ≤ 2 mm. The moisture adjusted to 0.32 bar and soil pre-incubated at 20 °C for seven days before application.

Table 8.1.1.1-37: Characteristics of test soils

Parameter		**Results**		
Soil name		**Droevendaal**	**Maasdijk**	**Lisse**
Soil		Humic sand	Sandy loam	Low humic-content (lhc) sand
Origin		Experimental farming station Droevendaal	Apple orchard located at the Maasdijk	Laboratory for Bulb-Research at Lisse
Country		Netherlands	Netherlands	Netherlands
Textural Class (USDA)		Sand	Sandy loam	Sand
Sand (>50 µm) (%)		88.6	64.0	96.7
Silt (2 µm – 50µm) (%)		8.1	24.1	0.5
Clay (< 2 µm) (%)		3.3	11.8	2.9
pH (KCl)		5.2	7.5	7.2
Organic carbon (%) [1]		2.32	1.10	0.64
Organic matter (%)		4.0	1.9	1.1
Moisture at pF 2.5 (g/100 g dry soil)		13.7	12.7	4.3
Microbial biomass (mg C/kg)	Start of the study (2 DAT)	10.2 (0.44 %OC)	19.6 (1.78 %OC)	2.2 (0.34 %OC)
	Study end (107 DAT)	7.0 (0.30 %OC)	13.6 (1.24 %OC)	1.3 (0.20 %OC)

DAT = days after treatment
[1] Calculated from organic carbon according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

The rate of degradation was determined in two soils (Droevendaal and Lisse) by measuring the extractable radioactivity in the soils and by characterisation of the glyphosate present in the extracts. The metabolism of glyphosate was determined in one soil (Maasdijk soil) by monitoring the evolution of 14C-carbon dioxide as a measure of mineralisation of the labelled carbon, by determining the extractable radioactivity in the soils and by characterisation of the radioactive compounds present. The amount of non-extractable residues was also determined.

Static test systems were used, consisting of glass flasks filled with 50 g of sieved soil (dry weight equivalents) and topped with a glass tube containing oil covered quartz wool for collection of organic volatiles and CO2-absorbing soda lime.

The study application rate was 3.8 mg/kg. The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate in 500 µL aqueous solution, resulting in 157 kBq [14C]-glyphosate and 0.18 mg unlabelled glyphosate per test system.

Test systems were incubated under aerobic conditions in the dark for 15 weeks at 20 ± 2 °C and a soil moisture of 0.32 bar. About every five weeks the loss of water was compensated.

2. Sampling

Duplicate test systems were processed and analysed 0, 1, 2, 4, 8 and 15 weeks after treatment. According to the tables in the report this corresponds to 0, 7, 14, 35, 70 and 100 days after treatment (DAT).

3. Analytical procedures

Before opening the Maasdijk soil test vessels, test systems were blown through with moist air to force volatiles into the traps.

At each sampling interval, soil samples of all soils were extracted with 0.5 N NH4OH solution for 5 minutes several times until the last extract contained < 5% of the applied radioactivity. Extracts and soil were separated by centrifugation for 5 minutes. All extracts were pooled and freeze-dried. The residue after freeze-drying was extracted with 18 % HCl solution. A considerable amount of radioactivity was not extractable from the residue after freeze-drying. This occurred already immediately after adding glyphosate to soil. It was assumed in the report that this fraction could partly be explained by glyphosate complexly bound to humic substances, which had been extracted from the soils at very alkaline conditions (NH_4OH).

The amounts of glyphosate and its metabolites were determined by thin layer chromatography TLC in concentrated extracts. Plates were developed in isobutyric acid:water:1-propanol:concentrated ammonium hydroxide:2-propanol:1-butanol (500:95:70:20:15:15) with 0.24 g of sodium-EDTA.

The test item and its metabolite aminomethyl phosphonic acid (AMPA) were identified by co-chromatography with reference items.

For the Maaskijk soil, the amount of volatiles (soda lime and and oil-covered glass wool) non-extractable residues was determined by LSC and combustion/LSC, respectively.

II. RESULTS AND DISCUSSION

Material balances ranged from 91.8 to 95.4 % AR (each mean of two replicates) for the Maasdijk soil. No material balances were determined for the two soils Droevendaal and Lisse.

The maximum amount of carbon dioxide reached at study end (100 DAT) was 79.6 % AR. Organic volatiles were found with a maximum amount of 0.3 % AR at 100 DAT (all values mean of two replicates). Volatiles were only determined for sandy loam soil.

The amount of radioactivity extractable with 0.5 M NH4OH decreased from 0 DAT to 100 DAT from 73.9 to 56.2 % AR with an intermediate minimum of 47.1 % AR at 14 DAT in the Droevendaal soil. In the Maasdijk soil, extractable radioactivity decreased from 90.4 at 0 DAT to 4.1 % AR at 100 DAT. In the Lisse soil, extractable radioactivity decreased from 98.1 at 0 DAT to 55.9 % AR at 100 DAT.

The amount of radioactivity extractable from freeze-dried residues, which is considered to be glyphosate, decreased from 23.9 % AR at 0 DAT to 28.2 % AR at 100 DAT in the Droevendaal soil. In the Maasdijk soil, it decreased from 41.1 % AR at 0 DAT to 4.3 % AR at 70 DAT and in the Lisse soil it decreased from 67.4% AR at 0 DAT to 30.4 % AR at 100 DAT.

A. DATA

Distribution of residues of [14C]-glyphosate in the tested soils are summarised in the tables below.

Table 8.1.1.1-38: Degradation of [14C]glyphosate in Droevendaal soil under aerobic conditions (values expressed as percent of applied radioactivity)

		DAT					
Compound	Replicate	0	7	14	35	70	100

NH_4OH extract [1]	A	73.7	62.7	46.4	56.6	58.5	56.7
	B	74.0	61.1	47.8	58.3	58.5	55.7
	Mean	73.9	61.9	47.1	57.5	58.5	56.2
Glyphosate [2]	A	16.1	26.0	31.1	25.1	20.7	25.4
	B	31.7	22.1	28.4	32.0	25.0	31.0
	Mean	23.9	24.1	29.8	28.6	22.9	28.2
Complexed Glyphosate [3]	A	57.6	36.7	15.3	31.5	37.8	31.3
	B	42.3	39.0	19.4	26.3	33.5	24.7
	Mean	50.0	37.9	17.4	28.9	35.7	28.0

[1] Radioactivity extractable with 0.5 M NH_4OH
[2] Radioactivity extracted with 18 % HCl after freeze-drying, considered to be glyphosate according to TLC ("free glyphosate")
[3] Radioactivity not extractable from freeze-dried residues, considered to be glyphosate complexly bound to humic substances
DAT: days after treatment

Table 8.1.1.1-39: Degradation of [14C]glyphosate in Maasdijk soil under aerobic conditions (values expressed as percent of applied radioactivity)

		DAT					
Compound	**Replicate**	**0**	**7**	**14**	**35**	**70**	**100**
NH_4OH extract [1]	A	89.4	37.2	24.9	16.1	8.7	4.2
	B	91.3	36.7	25.2	15.5	8.2	3.9
	Mean	90.4	37.0	25.1	15.8	8.5	4.1
Glyphosate [2]	A	36.8	13.0	9.6	8.4	4.6	ND
	B	45.4	15.5	10.5	7.5	3.9	ND
	Mean	41.1	14.3	10.1	8.0	4.3	ND
Complexed Glyphosate [3]	A	52.6	24.2	15.3	7.7	4.2	ND
	B	45.9	21.1	14.7	8	4.3	ND
	Mean	49.3	22.7	15.0	7.9	4.3	ND
Carbon Dioxide	A	ND	47	59.6	67.4	77.3	79.9
	B	ND	48.4	59.4	65.9	72.5	79.3
	Mean	ND	47.7	59.5	66.7	74.9	79.6
Volatile organic compounds	A	ND	0.1	0.4	0.1	0.1	0.3
	B	ND	0.1	0.4	0	0.3	0.2
	Mean	ND	0.1	0.4	0.1	0.2	0.3
Non-extractable Residues	A	3.3	7.2	7.8	8.1	9.1	7.5
	B	3.8	6.9	10.1	7.6	9.1	9.8
	Mean	3.6	7.1	9.0	7.9	9.1	8.7
Mass balance	A	92.7	91.4	92.7	91.7	95.2	91.9
	B	95.1	92.1	95.1	99	90.1	93.2
	Mean	93.9	91.8	93.9	95.4	92.7	92.6

[1] Radioactivity extractable with 0.5 M NH4OH, not used for material balance
[2] Radioactivity extracted with 18 % HCl after freeze-drying, considered to be glyphosate according to TLC
[3] Radioactivity not extractable from freeze-dried residues, considered to be glyphosate complexly bound to humic substances
DAT: days after treatment
ND: not determined

Table 8.1.1.1-40: Degradation of [14C]glyphosate in Lisse soil under aerobic conditions (values expressed as percent of applied radioactivity)

		DAT					
Compound	**Replicate**	**0**	**7**	**14**	**35**	**70**	**100**
NH_4OH extract [1]	A	98.2	83.3	80.3	68.5	55.7	58.2
	B	97.9	84.5	81.1	68.7	56.4	53.6
	Mean	98.1	83.9	80.7	68.6	56.1	55.9
Glyphosate [2]	A	61.7	54.6	36.4	40.6	40.2	30.3
	B	73.1	56.1	47.0	36.9	39.2	30.4
	Mean	67.4	55.4	41.7	38.8	39.7	30.4

Complexed Glyphosate [3]	A	27.7	28.7	43.8	28.7	15.5	27.9
	B	24.8	28.4	34.1	31.7	17.2	23.2
	Mean	26.3	28.6	39.0	30.2	16.4	25.6

[1] Radioactivity extractable with 0.5 M NH_4OH
[2] Radioactivity extracted with 18 % HCl after freeze-drying, considered to be glyphosate according to TLC ("free glyphosate")
[3] Radioactivity not extractable from freeze-dried residues, considered to be glyphosate complexly bound to humic substances
DAT: days after treatment

B. MASS BALANCE

Material balances ranged from 91.8 to 95.4 % AR (mean of two replicates) for the Maasdijk soil. Material balances were not established for the other two soils (Droevendaal and Lisse).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable with 0.5 M NH4OH decreased from 0 DAT to 100 DAT from 73.9 to 56.2 % AR with an intermediate minimum of 47.1 % AR at 14 DAT in the Droevendaal soil. In the Lisse soil, extractable radioactivity decreased from 98.1 at 0 DAT to 55.9 % AR at 100 DAT. In the Maasdijk soil, extractable radioactivity decreased from 90.4 at 0 DAT to 4.1 % AR at 100 DAT.

The amount of radioactivity extractable from freeze-dried residues, which is considered to be glyphosate, decreased from 23.9 % AR at 0 DAT to 28.2 % AR at 100 DAT in the Droevendaal soil. In the Lisse soil it decreased from 67.4% AR at 0 DAT to 30.4 % AR at 100 DAT and in the Maasdijk soil it decreased from 41.1 % AR at 0 DAT to 4.3 % AR at 70 DAT.

The amount of radioactivity not extractable from freeze-dried residues, which is considered to be glyphosate complexly bound to humic substances, decreased from 50.0 % AR at 0 DAT to 28.0 % AR at 100 DAT in the Droevendaal soil. In the Lisse soil it fluctuated between 16.4 and 39.0 % AR and in the Maasdijk soil it decreased from 49.3 % AR at 0 DAT to 4.3 % AR at 70 DAT.

The amount of non-extractable residues (NER), which was only determined for the Maasdijk soil, increased from 0 DAT to 70 DAT from 3.6 to 9.1 % AR and decreased to 8.7 % AR at 100 DAT (mean of two replicates).

D. VOLATILE RADIOACTIVITY

The maximum amount of carbon dioxide reached at study end (100 DAT) was 79.6 % AR. Organic volatiles were found with a maximum amount of 0.3 % AR at 100 DAT (all values mean of two replicates). Volatiles were only determined for sandy loam soil.

E. TRANSFORMATION OF THE TEST ITEM

All radioactivity extracted was considered to be glyphosate. According to TLC no known metabolites of glyphosate were found in the extracts after freeze-drying.

F. KINETICS

The DT_{50} value for the degradation of glyphosate was calculated by first order kinetics (not according to current guidance) to be <7 days in the Maasdijk soil, 180 days for Droevendaal soil and 110 days for Lisse soil. As the study is considered invalid, kinetic evaluation was not updated.

Assessment and conclusion by applicant:

The study shows major deficiencies. Problems probably resulting from inadequate work-up procedures caused high portions of radioactivity that remained sticking to the soil residues thus not allowing to re-constitute the formerly water soluble radioactive residues after freeze-drying into a solvent for analysis. Radioactivity lost during freeze-drying was assigned to glyphosate complexly bound to humic substances (co-)extracted. For two soils, no full material balance was established, i.e. non-extractable residues and volatiles were not determined.

Therefore, the study is considered invalid and was not used for endpoint derivation.

Assessment and conclusion by RMS:

Multiple deviations from the OECD 307 guidance were observed.

The history of the field is missing from the report. The microbial biomass is below 1% OC at all measured times for two out of the three soils. In these two soils (Drovendaal & Lisse), no mass balance was estimated. The applicant mentions that the representativeness of two of the soils (Drovendaal & Lisse) as agricultural soils is unknown, and RMS confirms that, at least for Lisse soil, the sole sand content (96.7% sand) seems to question its use. LOD/LOQ are not reported in the study report. In addition, the glyphosate recovery at DAT0 (extractable part) was very low in all three soils.

The study summary indicates that "a considerable amount of radioactivity was not extractable from the residue after freeze-drying", then "the test item and its metabolite aminomethyl phosphonic acid (AMPA) were identified by co-chromatography with reference items". Neither the amount of radioactivity not extracted nor the amount of AMPA analysed are reported.

The study is not acceptable.

[redacted], *1991* – [redacted], *1992*

Data point:	CA 7.1.1.1/008
Report author	[redacted]
Report year	1991
Report title	Aerobic metabolism of [14C]Glyphosate in sandy loam and silt loam soils with biometer flask
Report No	368
Guidelines followed in study	U.S. EPA 162-1 OECD guideline 307
Deviations from current test guideline	From OECD 307: - samples aerated with oxygen instead of air - procedural recoveries were rather variable (soil Kickapoo: 86.2 to 136.7 %, soil Dupo: 87.7 to 143.8 %); recoveries corrected for values below 90 % or above 110 % - mass balance <90 % for most of the sampling points in Kickapoo and some sampling points in Dupo, probably due to loss of carbon dioxide - high variation of recoveries between the two replicates of day 0 in Dupo soil - duration of study 12 months, no microbial biomass measurement during the study
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

Data point	CA 7.1.1.1/009
Report author	[redacted]
Report year	1992
Report title	Review of the Aerobic Metabolism of [^{14}C]Glyphosate in Soil - Addendum to Monsanto Report No. PTRL 368
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability	No

The degradation of [^{14}C]-glyphosate was investigated in two soils under aerobic conditions in the dark in the laboratory at 25 ± 0.1 °C and 75 ± 10 % of the field capacity for 12 months. The application rate of glyphosate was 4 mg/kg soil (dry weight).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]-phosphonomethyl-glyphosate
Lot No.: C-927.45
Specific activity: 3.98 mCi/mmole
Radiochemical purity: 98.8 %

2. Soil:

Soils were sieved to ≤ 2 mm and air-dried. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-41: Characteristics of test soils

Parameter	**Results**	
Soil	**Kickapoo**	**Dupo**
Country	Kentucky, USA	Missouri, USA
Pesticide use history	Soils had not been treated with pesticides during the past five years	
Textural Class	Sandy Loam	Silt Loam
Sand (%)	68	24
Silt (%)	22	68
Clay (%)	10	8
pH (Medium/method not reported)	7.3	7.5
Organic carbon (%) [1]	1.6	0.6
Organic matter (%)	2.8	1.0
Cation exchange capacity (meq/100 g)	9.0	10.7
Water Holding Capacity at 0.33 bar (%)	21.0	18.0
Microbiological characteristics (before study initiation) - (total colony forming units , CFU)		
Aerobic Bacteria	$6.2*10^5$	$4.0*10^6$
Actinomycetes	$5.8*10^5$	$3.2*10^6$
Fungi	$1.1*10^3$	$4.2*10^4$

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of biometer flasks filled with 50 g of sieved soil (dry weight equivalents). The biometer flasks had a side arm to which 50 mL of 1 N NaOH was added to trap CO_2. A pre-extracted (acetone) polyurethane foam plug was placed in the side arm connector to trap volatile organic compounds. An equilibrium was established in the biometer flasks by way of the humidified oxygen passed through the system which maintained a positive pressure on flasks to accommodate pressure differences realized by the adsorption of $^{14}CO_2$ into the NaOH upon its formation.

A test solution of [^{14}C]-glyphosate with a concentration of 2.4 µCi/mL was prepared in water. 2 mL of this solution were applied to each test system, resulting in a final concentration of 4 mg/kg. After application the soil moisture was adjusted to 75 % of the field capacity by addition of water.

Test systems were incubated under aerobic conditions in the dark for 12 months at 25 ± 0.1 °C and 75 ± 10 % of the field capacity.

Sterilised (autoclaved) samples were incubated in parallel and sampled 1, 3 and 6 month after treatment.

2. Sampling

Duplicate test systems were processed and analysed 0, 1, 3, 7, 14 days after treatment (DAT) and 1, 2, 3, 4, 6, 9 and 12 months after treatment. The foam plug and NaOH trap were assayed and changed at all sampling points up to month 4 and changed monthly afterwards.

3. Analytical procedures

At each sampling interval, soil samples were extracted three times with 0.5 N KOH. After centrifugation the pooled extracts were radioassayed by liquid scintillation counting (LSC). Selected samples were furthermore extracted with 1 N KOH and all extracts of a respective sample were pooled. Prior to HPLC analysis the pooled extracts were cleaned up by solid phase extraction and eluted with 0.1 N ammonium hydroxide. Recovery of radioactivity was investigated for the applied clean-up procedure. Recoveries were in the range from 86.2 to 136.7 % for soil Kickapoo and in the range from 87.7 to 143.8 % for soil Dupo. If recoveries were below 90 % or above 110 % calculations were corrected for the respective recovery.

The cleaned-up extracts were concentrated under reduced pressure using a rotary evaporator and then analysed by HPLC/radiodetection. The limit of detection (LOD) for the HPLC/radiodetection method was two times the background noise. The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Glyphosate and metabolites were identified by radio-HPLC co-chromatography with reference items using a different HPLC system as used for separation.

The identification of CO2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The formed precipitate was titrated with acid.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in the tables below for the respective soils.

Table 8.1.1.1-42: Degradation of (^{14}C)glyphosate in soil Kickapoo under aerobic conditions (% AR)

		Time after treatment											
		Days					**Months**						
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**1**	**2**	**3**	**4**	**6**	**9**	**12**
Glyphosate	A	50.6	32.8	18.9	10.6	5.4	2.8	1.6	2.0	2.1	1.9	0.9	0.5
	B	44.5	32.0	15.9	7.7	6.4	2.8	2.1	1.1	1.3	0.8	0.5	0.6
	Mean	**47.6**	32.4	17.4	9.2	5.9	2.8	1.9	1.6	1.7	1.4	0.7	0.5
AMPA	A	12.9	21.6	31.1	28.5	23.9	17.3	9.9	7.7	5.4	3.7	2.8	1.7
	B	19.0	25.0	20.0	18.0	28.6	17.0	12.1	5.5	4.1	3.5	0.1	2.1
	Mean	**16.0**	23.3	25.6	23.3	**26.3**	17.2	11.0	6.6	4.8	3.6	1.5	1.9
Unknown A	A	1.8	2.7	2.8	3.5	2.2	2.3	2.9	1.7	2.6	2.3	2.0	1.7
	B	2.5	2.5	2.1	2.4	1.8	2.7	3.0	2.0	2.4	1.9	1.4	1.3
	Mean	2.2	2.6	2.5	3.0	2.0	2.5	3.0	1.9	2.5	2.1	1.7	1.5
Unknown B	A	2.0	2.5	0.7	3.6	1.2	2.4	1.8	0.8	1.0	0.9	1.2	0.9
	B	2.6	2.3	2.4	2.4	1.3	2.2	2.0	1.3	1.4	1.0	0.7	0.5
	Mean	2.3	2.4	1.6	3.0	1.3	2.3	1.9	1.1	1.3	1.0	1.0	0.7
Unknown C	A	ND	ND	ND	0.5	ND	0.6	0.3	0.2	0.4	0.3	0.4	0.2
	B	ND	ND	ND	ND	ND	ND	ND	0.3	ND	0.3	0.1	0.2
	Mean	NA	NA	NA	NA	NA	NA	NA	0.3	NA	0.3	0.3	0.2
Others	A	ND	ND	ND	ND	0.4	1.3	ND	0.2	0.1	ND	0.2	ND
	B	0.8	0.7	0.4	ND	ND	ND	0.5	0.1	0.3	ND	0.1	0.2
	Mean	NA	NA	NA	NA	NA	NA	NA	0.2	0.2	NA	0.2	NA
Carbon Dioxide	A	NA	23.5	33.6	38.8	47.2	61.0	59.8	68.7	64.0	66.8	69.6	71.0
	B	NA	24.6	38.3	44.4	47.3	54.1	59.1	67.5	67.1	72.5	74.0	70.5
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable radioactivity	A	68.1	54.2	47.8	40.1	30.3	22.9	19.2	14.4	11.5	9.5	6.6	6.7
	B	70.6	62.5	45.7	39.0	38.6	28.2	17.9	12.8	11.6	9.1	6.6	6.3
Non-extractable radioactivity	A	3.2	4.9	7.8	7.1	9.5	7.5	8.5	7.8	8.1	11.9	6.1	7.8
	B	3.7	7.7	5.7	6.0	6.5	7.9	6.1	9.4	8.6	5.6	6.9	7.0
Mass balance	A	71.3	82.6	89.3	86.0	87.0	91.5	87.6	91.0	83.6	88.2	82.3	85.4
	B	74.3	94.8	89.6	89.4	92.4	90.2	83.1	89.6	87.4	87.3	87.5	83.9
	Mean	**72.8**	**88.7**	**89.5**	**87.7**	**89.7**	90.9	**85.4**	90.3	**85.5**	**87.8**	**84.9**	**84.7**

NA: not applicable; ND: not detected

Table 8.1.1.1-43: Degradation of (^{14}C)glyphosate in soil Dupo under aerobic conditions (% AR)

		Time after treatment											
		Days					Months						
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**1**	**2**	**3**	**4**	**6**	**9**	**12**
Glyphosate	A	64.3	49.7	27.3	13.9	7.0	2.7	1.5	1.7	1.1	0.7	0.8	0.4
	B	82.2	61.5	23.9	11.7	5.6	3.3	1.5	1.4	1.3	0.7	0.6	0.7
	Mean	73.3	55.6	25.6	12.8	6.3	3.0	1.5	1.6	1.2	0.7	0.7	0.6
AMPA	A	14.3	22.0	26.3	28.0	34.6	22.3	14.7	10.0	5.1	2.8	2.1	1.6
	B	18.7	19.1	21.7	23.4	22.7	26.3	14.9	11.4	8.8	4.3	2.2	1.5
	Mean	16.5	20.6	24.0	25.7	**28.7**	24.3	14.8	10.7	7.0	3.6	2.2	1.6
Unknown A	A	1.4	2.0	1.7	1.7	1.4	1.8	1.6	1.7	1.6	0.8	1.0	1.7
	B	0.9	2.4	1.3	1.6	2.8	2.2	1.4	1.7	1.8	1.3	1.1	0.9
	Mean	1.2	2.2	1.5	1.7	2.1	2.0	1.5	1.7	1.7	1.1	1.1	1.3
Unknown B	A	0.9	0.9	0.9	1.3	0.6	0.8	0.9	1.0	1.0	0.4	0.4	0.8
	B	0.9	1.0	1.2	1.3	3.1	1.3	0.7	0.9	0.9	0.7	0.6	0.3
	Mean	0.9	1.0	1.1	1.3	1.9	1.1	0.8	1.0	1.0	0.6	0.5	0.6
Unknown C	A	ND	ND	ND	ND	ND	ND	ND	ND	0.1	0.2	0.1	0.2
	B	ND	ND	ND	ND	ND	ND	ND	ND	0.2	0.1	0.1	0.2
	Mean	NA	NA	NA	NA	NA	NA	NA	NA	0.2	0.2	0.1	0.2
Others	A	ND	ND	1.0	ND	ND	ND	ND	ND	ND	0.1	ND	0.4
	B	4.6	ND	ND	ND	ND	ND	ND	ND	0.6	0.1	ND	ND
	Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.1	NA	NA
Carbon Dioxide	A	NA	16.9	32.2	36.9	46.5	58.7	65.2	71.7	76.4	79.7	81.9	78.0
	B	NA	16.3	32.8	38.4	47.2	57.4	63.8	72.1	75.4	80.4	83.8	78.6
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable radioactivity	A	81.3	75.5	65.2	45.5	39.9	28.2	21.4	11.4	8.8	6.9	5.4	4.4
	B	97.9	67.9	48.1	38.6	39.6	25.8	18.7	15.7	11.9	7.2	5.4	4.9
Non-extractable Radioactivity	A	2.7	7.0	7.0	5.0	7.8	5.7	7.0	6.2	4.7	5.0	5.3	3.7
	B	5.4	3.0	3.9	4.4	6.8	5.2	4.7	5.9	5.4	5.2	4.9	4.7
Mass balance	A	84.0	99.5	104.4	87.4	94.1	92.6	93.5	89.3	89.8	91.7	92.6	86.1
	B	103.2	87.1	84.8	81.4	93.6	88.4	87.2	93.6	92.8	92.9	94.1	88.3
	Mean	93.6	93.3	94.6	**84.4**	93.9	90.5	90.4	91.5	91.3	92.3	93.4	**87.2**

NA: not applicable; ND: not detected

B. MASS BALANCE

Material balances ranged from 71.3 to 94.8 % of applied radioactivity (% AR) for soil Kickapoo, from 84.0 to 103.2 % AR for soil Dupo.

The total recoveries of applied in sterilized samples was radiocarbon were 100.0 ± 1.5 and 102.0 ± 5.3% for the Kickapoo and Dupo soils, respectively.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in soil decreased from 0 DAT to 12 months after treatment from 69.4 to 6.5 % AR in soil Kickapoo and from 89.5 to 4.7 % AR in soil Dupo.

In soil Kickapoo, NER increased from 3.5 % AR at 0 DAT to 8.8 % AR 6 months after treatment and decreased to 7.4 % AR 12 months after treatment. In soil Dupo, NER increased from 4.1 % AR at 0 DAT to 6.1 % AR 3 months after treatment and decreased to 4.2 % AR 12 months after treatment (each value as mean of two replicates).

D. VOLATILE RADIOACTIVITY

In both test soils formation of 14CO2 increased steadily during the experimental period. Maximum amounts of carbon dioxide reached at 9 months of incubation were 71.8 and 82.9 % AR in soils Kickapoo and Dupo, respectively. At the end of the study a slight decrease to 70.8 and 78.3 % AR, for soils Kickapoo and Dupo,

respectively (each value as mean of two replicates). No organic volatiles were determined for both soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

In the sterilized samples, radiolabelled CO2 accounted for 42.5 and 38.2% AR after 6 months for Kickapoo and Dupo soils, respectively.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in soil extracts decreased from 0 DAT to 12 months of incubation from 47.6 to 0.5 % of the applied radioactivity in soil Kickapoo and from 73.3 to 0.6 % of the analysed radioactivity in soil Dupo (mean of two replicates).

Besides carbon dioxide, one major metabolite was detected. AMPA was detected with a maximum amount of 26.3 % AR at 14 DAT in soil Kickapoo and decreased to below 2 % of the applied radioactivity after 12 months of incubation. In soil Dupo, AMPA was detected with a maximum amount of 28.7 % AR at 14 DAT and decreased to below 2 % of the applied radioactivity after 12 months of incubation (all values mean of two replicates). No other metabolites were detected above 5 % AR at any time.

In the sterilized flasks, degradation of glyphosate was significantly less rapid. In soil Kickapoo, glyphosate accounted to 46.3% AR and AMPA to 12.9% AR after 6 months. In soil Dupo, glyphosate accounted to 44.6% AR and AMPA to 17.3% AR after 6 months.

F. KINETICS

The DT_{50} values for glyphosate were calculated using a non-linear, first-order kinetic model, not according to current guidance. Degradation of glyphosate was very fast with are DT_{50} values of 1.85 and 2.06 days for Kickapoo sandy loam and Dupo silt loam soils, respectively. As the study is considered invalid, kinetic evaluation was not updated.

III. CONCLUSIONS

Glyphosate is rapidly degraded in soil under aerobic conditions. The primary degradation products in both soils are CO_2 and aminomethyl phosphonic acid (AMPA). Several low-level unidentified metabolites are also produced. However, none of these unidentified products constitute greater than 3.6 % of the initial glyphosate concentration and, therefore, are considered insignificant. The proposed metabolic pathway involved the initial conversion of glyphosate to AMPA, followed by further metabolism of AMPA to CO_2.

Assessment and conclusion by applicant:

The study conduct had a major deficiency compared to current guidelines by pressing pure oxygen for aeration through the samples rather than to allow a gentle stream of air to pass through.

In addition, a number of minor deviations occurred including recoveries to be below 90 %, often for soil Kickapoo and occasionally for soil Dupo, presumably due to a loss of volatiles/CO_2.

Due to the major deficiency in conduct the study is considered invalid and was not used for endpoint derivation.

Assessment and conclusion by RMS:

Multiple deviations from OECD 307 were observed. The use of oxygen instead of CO_2 free air may have a significant impact on the degradation behaviour of glyphosate and prevents the use of the study for addressing this data point. Procedural recoveries were very variable. The mass balance for soil Kickapoo was below 90% AR at all except one sampling point and was occasionally below 90%ARin Dupo soil. In addition, the glyphosate recovery at DAT0 was quite low in both soils. Duration of the study was long (12 months) and no check of microbial biomass was done.

The study is not acceptable.

[redacted], *1985*

Data point:	CA 7.1.1.1/010
Report author	[redacted]
Report year	1985
Report title	Metabolism of SC-0224 in soil: Fate of the anion moiety
Report No	PMS-186
Guidelines followed in study	US EPA 162-1 OECD guideline 307
Deviations from current test guideline	Major deviations from OECD 307: - Test systems were aerated with pure oxygen instead of air. - For the main ('large-scale') degradation experiment, glyphosate and its metabolites were not identified. - For the 'small-scale' experiment, incubation temperature was not controlled or reported
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations	Study type: aerobic soil metabolism Test item: [14C] glyphosate, phosphonomethyl-label (97.4 % radiochemical purity) Test soil: Visalia (CA, USA) Soil type: sandy loam pH (water?): 8.3 Organic matter: 0.60 % Application rate: 4.74 mg/kg Test design: semi-static system with biometer flasks kept slightly pressurized with oxygen Volatiles trapping: CO2: 10 % KOH solution in trap Organic volatiles: foam plug Incubation: 25±1 °C (incubator, temperature controlled), soil moisture adjusted to 75 % of water holding capacity at 0.33 bar Sampling: 0, 1, 2, 3, 4, 8, 11, 14, 18, 24 and 31 days after treatment (DAT), duplicate samples Workup: threefold extraction with 1 M KH2PO4 (pH 2.0) at ambient temperature Analysis of radioactivity: Extracts: LSC (combined extracts) NER: combustion/LSC Volatiles: LSC; foam plug extracted with dichloromethane Identification of radioactive residues: HPLC/radiodetection and TLC/radiodetection co-chromatography with reference items.
Short description of results:	<u>Large-scale experiment</u>: Recovery of radioactivity: 90.5 – 103.1 % AR Mineralization: 83.1 % AR after 376 days Other volatiles: none Extractable radioactivity (mean values): 57.6 % AR at 0 DAT, 6.9 % AR at 150 DAT, for later samplings, no extraction performed. Non-extractable radioactivity (mean values): 40.3 % AR at 0 DAT, 16.6 % AR at 376 DAT Transformation of test item: not analysed <u>Small-scale experiment</u>: Recovery of radioactivity (mean values): 91.6 – 99.9 % AR Mineralization: 37.2 % AR after 21 days Other volatiles: not analysed

	Extractable radioactivity (mean values): 37.2 % AR at 0 DAT, 27.3 % AR at 21 DAT Non-extractable radioactivity (mean values): 17.3 % AR at 0 DAT, 31.5 % AR at 21 DAT Transformation of test item (TLC analysis): Glyphosate: 78.0 % AR at 0 DAT, 8.2 % AR at 21 DAT AMPA: 0.4 % AR at 0 DAT, 15.4 % AR at 21 DAT The half-life of glyphosate was estimated from the small-scale experiment to about 3 days.

Assessment and conclusion by RMS:

Multiple deviations from the OECD 307 guidance were observed. The use of oxygen instead of CO_2 free air may have a significant impact on the degradation behaviour of glyphosate and prevents the use of the study for addressing this data point. Additionally, for the main degradation experiment, glyphosate and its metabolites were not identified while for the small scale experiment the temperature was not checked.

The study is not acceptable.

[redacted], *1972*

Data point:	CA 7.1.1.1/011
Report author	[redacted]
Report year	1972
Report title	The degradation and metabolism of MON-0573 in soil
Report No	269
Guidelines followed in study	U.S. Department of Agriculture (ARS, Pesticides Regulation Division): Pesticide Registration (PR) Notice 70-15 "Guidelines For Studies to Determine the Impact of Pesticides on the Environment." June 23, 1970
Deviations from current test guideline	Major deviations from OECD 307: - soil history, sampling and storage not reported - mixed aerobic/anaerobic design in conduct strongly beyond actual standards and guidelines in soil degradation testing, i.e. soil suspended in aqueous solution during incubation followed by application of the test substance - work-up of aliquots only instead of complete soil sample - closed system without aeration during incubation - incubation at 30 °C
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: aerobic/anaerobic soil metabolism, degradation in water Test item: [^{14}C] glyphosate, phosphonomethyl-label (97 % radiochemical purity), 1-glycine label (96 % radiochemical purity), 2-glycine label (99 % radiochemical purity) Test soils (soil type): Ray (silt loam), Drummer (silty clay loam), Lintonia (sandy loam), Norfolk (sandy loam) pH: 6.5, 7.0, 6.0, 5.7 (method not stated) Organic matter: 1.0 %, 6 %, 1 %, 1 % The total study included various tests including aerobic and anaerobic degradation (samples water-logged) in non-sterile and sterilized soil (soil

	Ray only). Tests with exaggerated application rates performed for identification of metabolites (soil Ray). This summary focuses on the results of aerobic degradation tests. Application rate: 109 to 126 mg/kg for the different labels, 1000 mg/kg for metabolite identification with test substance applied to water phase, i.e. not applied directly to soil Test design: 5 g soil suspended in 100 mL water, continuously agitated by shaking; 100 g soil and 1000 mL for large scale tests Volatiles trapping: CO_2: ascarite trap Organic volatiles: no trapping Incubation: 30 °C, continuous shaking, soil flooded/suspended Sampling: 0, 1, 3, 7, 14, 21, 28 days after treatment (DAT) for soil Ray, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77, 84, 91, 105 and 112 DAT for soil Norfolk, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77 and 84 DAT for soil Drummer, 0, 1, 3, 7, 14, 21, 28 and 35 DAT for soil Lintonia, single samples collected per soil and sampling interval Workup: taking of an aliquot of the soil-water suspension, centrifugation, washing of soil with water, lyophilisation of soil, threefold extraction with 0.5 N aqueous NH_4OH solution at ambient temperature Determination of radioactivity: Extracts: LSC NER: combustion/LSC Volatiles: ascarite treated with HCl, trapping in 0.25 N NaOH, LSC Identification of radioactive residues: TLC/radiodetection co-chromatography with reference items, ^{1}H and ^{31}P-NMR
Short description of results:	Recovery of radioactivity: 68.7 – 109.8 % AR for all glyphosate labels and soils at the day of experiment termination Mineralization: 46.8 to 55.3 % AR for soil Ray, 5.8 to 9.3 % AR for soil Norfolk, 34.7 to 41.4 % AR for soil Drummer, 14.3 % AR for soil Lintonia (for all soils at termination) Other volatiles: not measured Extractable radioactivity: 2.7 to 22.9 % AR at 28 DAT for soil Ray, 65.4 to 81.8 % AR at 112 DAT for soil Norfolk, 12.0 to 19.6 % AR at 84 DAT for soil Drummer, 18.3 % AR at 35 DAT for soil Lintonia Non-extractable radioactivity: 8.5 to 40.3 % AR at 28 DAT for soil Ray, 4.6 to 13.5 % AR at 112 DAT for soil Norfolk, 16.7 to 33.9 % AR at 84 DAT for soil Drummer, 2.6 % AR at 35 DAT for soil Lintonia Transformation of test item (TLC analysis):

	Glyphosate: 0.2 to 7.4 % AR at 14 DAT and not detected at 28 DAT for soil Ray, 45.6 to 80.1 % AR at 14 DAT and 0.8 to 16.3 % AR at 112 DAT for soil Norfolk, 12.5 to 25.5 % AR at 14 DAT and 7.6 to 15.7 % AR at 84 DAT for soil Drummer, 69.5 % AR at 14 DAT and 59.5 % AR at 35 DAT for soil Lintonia AMPA: 8.5 % AR at 14 DAT and 4.4 % AR at 28 DAT for soil Ray; 0.5 % AR at 14 DAT and 1.7 % AR at 28 DAT for soil Norfolk, 1.8 % AR at 14 DAT, 8.4 % R at 56 DAT and 8.3 % AR at 84 DAT for soil Drummer, 6.9 % AR at 14 DAT and 6.6 % AR at 35 DAT for soil Lintonia (phosphonomethyl-label only for all soils) No unknown metabolites were observed at >5 % AR.

Assessment and conclusion by RMS:

As indicated by the applicant, multiple deviations from OECD 307 were observed. The history of the field is missing from the report. No indication on whether the soil was freshly collected was reported, nor the storage conditions. The study was performed with soil suspended in aqueous solution during incubation followed by application of the test substance and the incubation temperature was 30°C. During incubation, the system was closed, without aeration.

The study is not acceptable.

[redacted], *1972*

Data point:	CA 7.1.2.1.1/009
Report author	[redacted]
Report year	1972
Report title	The rate of dissipation of MON-0573 in soil
Report No	271
Guidelines followed in study	U.S. Department of Agriculture (ARS, Pesticides Regulation Division): Pesticide Registration (PR) Notice 70-15 "Guidelines For Studies to Determine the Impact of Pesticides on the Environment." June 23, 1970
Deviations from current test guideline	-'mixed design' as soil degradation and plant test - planting pots as test vessels incubated in greenhouse under unspecified conditions of light and moisture - incubation temperature (32 °C) out of standard range of testing (20 to 25°C) - influence of corn plants on degradation in soil - addition of media like Hoagland solution during the study with unknown effects on outcome of test - no full material balance established due to open test systems - microbial activity of soils not reported
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: aerobic soil metabolism Test item: [^{14}C] glyphosate, phosphonomethyl-label (97 % radiochemical purity) and non-labelled glyphosate Test soils (soil type): Ray (silt loam), Drummer (silty clay loam), Norfolk (sandy loam) pH: 6.5, 7.0, 5.7 (medium not stated) Organic matter: 1.0 %, 6 %, 1 % Test containers were planted with corn seeds immediately after application. Plants were also analysed. Large scale-experiments for characterization of metabolites conducted with each soil type.

	Application rate: single application of 4 mg/kg or 8 mg/kg, corresponding to 4.48 kg/ha and 8.96 kg/ha; 202.32 mg/kg for large scale experiment Test design: blackened pyrex planters, planted with four corn seeds per test vessel, soil moisture set to 11 % water content prior to application, addition of Hoagland solution 10, 11 and 12 weeks after application Volatiles trapping: no trapping of volatiles Incubation: 32 °C, greenhouse Sampling: 0, 7, 14, 28, 41/42, 55/56, 83/84, 111/112 days after treatment (DAT), single samples per application rate Workup: threefold extraction with 0.5 N aqueous NH_4OH solution at ambient temperature (five extractions for soil Drummer); extraction efficiency tested 76.3 to 100.1 %; clean-up by diethylaminoethylcellulose (DEAE) and ion exchange chromatography Determination of radioactivity: Extracts: LSC NER: combustion/LSC Volatiles: not collected Identification of radioactive residues: TLC co-chromatography with reference items; additional characterization by NMR
Short description of results:	Recovery of radioactivity: not applicable due to open test systems Mineralization: not applicable due to open test systems Extractable radioactivity: For the 4 mg/kg application rate: 67.8 % AR at 0 DAT to 10.9 % AR at 112 DAT for soil Ray, 88.8 % AR at 0 DAT to 74.2 % AR at 112 DAT for soil Norfolk, 60.3 % AR at 0 DAT to 13.5 % AR at 112 DAT for soil Drummer. For the 8 mg/kg application rate: 78.5 % AR at 0 DAT to 9.6 % AR at 111 DAT for soil Ray, 89.8 % AR at 0 DAT to 77.6 % AR at 111 DAT for soil Norfolk, 60.3 % AR at 0 DAT to 12.8 % AR at 111 DAT for soil Drummer. Non-extractable radioactivity: For the 4 mg/kg application rate: 29.9 % AR at 0 DAT to 12.5 % AR at 112 DAT for soil Ray, 8.8 % AR at 0 DAT to 18.6 % AR at 112 DAT for soil Norfolk, 33.8 % AR at 0 DAT to 22.3 % AR at 112 DAT for soil Drummer. For the 8 mg/kg application rate: 20.6 % AR at 0 DAT to 9.6 % AR at 111 DAT for soil Ray, 8.6 % AR at 0 DAT to 12.4 % AR at 111 DAT for soil Norfolk, 35.4 % AR at 0 DAT to 20.0 % AR at 111 DAT for soil Drummer. Transformation of test item (TLC analysis): 4 mg/kg application rate Glyphosate: 62.5 % AR at 0 DAT, 3.1 % AR at 112 DAT for soil Ray; 86.0 % AR at 0 DAT, 57.5 % AR at 112 DAT for soil Norfolk, 56.6 % AR at 0 DAT, 1.1 % AR at 112 DAT for soil Drummer. AMPA: 5.1 % AR at 0 DAT, 25.6 % AR at 14 DAT, 7.8 % AR at 112 DAT for soil Ray; 2.8 % AR at 0 DAT, 16.7 % AR at 112 DAT for soil Norfolk, 3.7 % AR at 0 DAT, 23.1 % AR at 84 DAT, 12.4 % AR at 112 DAT for soil Drummer 8 mg/kg application rate Glyphosate: 76.1 % AR at 0 DAT, 1.8 % AR at 55 DAT, 2.9 % AR at 111 DAT for soil Ray; 87.4 % AR at 0 DAT, 67.0 % AR at 111 DAT for soil Norfolk, 58.4 % AR at 0 DAT, not detected at 111 DAT for soil Drummer. AMPA: 2.4 % AR at 0 DAT, 26.7 % AR at 14 DAT, 6.7 % AR at 111 DAT for soil Ray; 2.4 % AR at 0 DAT, 10.5 % AR at 111 DAT for soil Norfolk, 1.9 % AR at 0 DAT, 17.5 % AR at 83 DAT, 12.7 % AR at 111 DAT for soil Drummer No unknown metabolites observed at >5 % AR.

Assessment and conclusion by RMS:

Multiple deviations from the OECD 307 were observed. The history of the field is missing from the report. The microbial activity of the soils was not reported. The study is a mixed design for soil

degradation and plant test. No full material balance was established. The conditions of incubation are quite far from the recommendations and incubation temperature was 32°C.

The study is not acceptable.

[redacted], *1991*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.1.1/006
Report author	[redacted]
Report year	1991
Report title	Glyphosate-Trimesium: Laboratory degradation in four soils
Report No	RJ1064B
Guidelines followed in study	BBA guidelines Part IV, 4-1
Deviations from current test guideline	From OECD 307: - no information on test item ("technical material") - organic carbon content of East Jubilee and 18 Acres soils above the recommended 2.5% - residues were corrected for external procedural recoveries based of fortified results, (55 – 82 %) depending on soil type; the correction performed is not reproducible (raw data not available)- only average recoveries of fortified controls reported, mean recovery <70% for soils Jubilee and 18-Acres - storage times and conditions of soils after sampling prior to use is not reported - no information on storage of samples
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: glyphosate-trimesium

No details on purity or lot no. of the test item are reported. It is stated that a solution of technical glyphosate-trimesium was used. Methods and results for glyphosate (PMG) are presented in this summary and reported information for the trimesium cation (TMS+) are not considered here.

2. Soil:

Soils were obtained from stocks. No herbicide treatment was applied to the soils for 5 years. Soils were air-dried and sieved to ≤ 2 mm. The soils were each freshly sampled to a depth of 15 cm (after removal of the turf). Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-44: Characteristics of test soils

Parameter	Results			
Soil	**Speyer 2.1**	**Speyer 2.2**	**East Jubilee**	**18 Acres**
Country	Germany	Germany	England	England
Textural Class (USDA)	Loamy sand	Loamy sand	Sandy loam	Sandy clay loam
Sand (%)	87	85	66	52
Silt (%)	7	8	17	22
Clay (%)	6	7	17	26
pH (water)	6.6	6.0	5.7	6.2
Organic carbon (%) [1]	0.5	2.4	3.0	5.2
Organic matter (%)	0.8	4.1	5.1	9.0
Moisture at 0.33 bar	4.91	11.85	12.76	23.68

Moisture at 15 bar		2.55	5.72	8.67	16.52
Cation exchange capacity (meq/100 g)		2.9	7.8	9.6	17.6
Microbial biomass (mg C/100 g soil)	Begin of study	13.9 (2.78 %C)	39.6 (1.65 %C)	59.0 (1.97 %C)	>112.5 (2.15 %C)
	Study end	7.9 (1.58 %C)	24.8 (1.03 %C)	25.0 (0.83 %C)	69.1 (1.33 %C)

USDA: United States Department for Agriculture
[1] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

Static test systems were used, consisting of Erlenmeyer flasks filled with soil and sealed with a plastic foam plug. As a non-radiolabelled test item was used, volatiles were not collected.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel and the moisture was adjusted to 40 % of the determined moisture holding capacity. The soils were pre-incubated at 20 °C for 14 days before application.

The study application rate was 5.0 mg/kg, corresponding to an application rate of 5 kg glyphosate-trimesium/ha. The test item glyphosate-trimesium was applied to each test vessel in 0.25 mL of an aqueous test solution.

Test systems were incubated under aerobic conditions in the dark for 108 days at 20 °C. The moisture was maintained at 40 % moisture holding capacity during the study by addition of de-ionised water.

2. Sampling

Duplicate test systems were processed and analysed 0, 2, 4, 8, 16 or 18, 32 or 46, 64 or 72 and 108 days after treatment (DAT).

3. Analytical procedures

At each sampling interval, soil samples were extracted with 0.5 M NH_4OH solution. After centrifugation an aliquot of the extract was evaporated to dryness and re-suspended in acidic solution. The pH was adjusted to 9-10 by addition of 2 M NaOH and the samples were derivatised with 9-fluorenylmethylchloroformate.

Glyphosate in the derivatised samples was quantified by HPLC using anion exchange chromatography and fluorescence detection. Residue concentrations were quantified by external standardisation and corrected for recoveries of fortified control samples (for values <100 %). The mean recoveries were 82 % (CV 12%) for soil Speyer 2.1, 80 % (CV 12%) for soil Speyer 2.2, 60 % (CV 13%) for soil Jubilee and 55 % (CV 14%) for soil 18-Acres. The limit of determination (LOD) of the method was 0.05 mg/kg.

Control samples were incubated alongside treated samples and analysed at the 0, 64/72 and 108 day intervals. No residues of glyphosate above the limit of determination (LOD) were determined in any of the control samples for any of the four soils.

II. RESULTS AND DISCUSSION

A. DATA

Degradation of glyphosate in the tested soils is summarised in the tables below. Values were corrected for recoveries of fortified control samples.

Table 8.1.1.1-45: Residues of glyphosate in Speyer 2.1 soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	Replicate	0	2	4	8	16	32	64	108
Glyphosate	A	3.1	3.3	2.4	1.9	1.7	1.6	0.29	0.17
	B	3.5	3.6	2.2	1.9	1.8	1.5	0.29	0.16
	Mean	3.3	3.4	2.3	1.9	1.8	1.6	0.29	0.17

DAT: days after treatment

Residues have been corrected from external recovery values. For PMG, analysis mean recovery for this soil = 82% (CV = 12%)
Limit of determination: 0.05 mg/kg for PMG
Sample residues have not been corrected for control values or for recovery values greater than 100%

Table 8.1.1.1-46: Residues of glyphosate in Speyer 2.2 soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	Replicate	0	2	4	8	16	32	64	108
Glyphosate	A	3.0	2.5	2.5	2.1	1.9	1.8	0.97	0.64
	B	3.0	2.7	2.5	2.3	1.9	1.9	0.90	0.44
	Mean	3.0	2.6	2.5	2.2	1.9	1.9	0.94	0.54

DAT: days after treatment
Residues have been corrected from external recovery values. For PMG, analysis mean recovery for this soil = 80% (CV = 12%)
Limit of determination: 0.05 mg/kg for PMG
Sample residues have not been corrected for control values or for recovery values greater than 100%

Table 8.1.1.1-47: Residues of glyphosate in East Jubilee soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	Replicate	0	2	4	8	18	46	72	108
Glyphosate	A	2.1	2.1	2.2	2.0	1.7	1.6	0.81	0.63
	B	2.6	2.2	1.8	1.8	1.6	1.3	0.76	0.60
	Mean	2.3	2.1	2.0	1.9	1.7	1.5	0.79	0.62

DAT: days after treatment
Residues have been corrected from external recovery values. For PMG, analysis mean recovery for this soil = 60% (CV = 13%)
Limit of determination: 0.05 mg/kg for PMG
Sample residues have not been corrected for control values or for recovery values greater than 100%

Table 8.1.1.1-48: Residues of glyphosate in 18 Acres soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	Replicate	0	2	4	8	18	46	72	108
Glyphosate	A	2.2	2.1	1.8	2.1	1.9	1.6	0.99	0.67
	B	2.4	2.1	2.4	1.9	1.6	1.6	0.95	0.61
	Mean	2.3	2.1	2.1	2.0	1.7	1.6	0.97	0.64

DAT: days after treatment
Residues have been corrected from external recovery values. For PMG, analysis mean recovery for this soil = 55% (CV = 14%)
Limit of determination: 0.05 mg/kg for PMG
Sample residues have not been corrected for control values or for recovery values greater than 100%

B. MASS BALANCE

No material balances were established.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of extractable and non-extractable residues was not determined.

D. VOLATILE RADIOACTIVITY

The amount of volatiles was not determined.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate decreased from 3.3 mg/kg at 0 DAT to 0.17 mg/kg at 108 DAT in soil Speyer 2.1, from 3.0 mg/kg at 0 DAT to 0.54 mg/kg at 108 DAT in soil Speyer 2.2, from 2.3 mg/kg at

0 DAT to 0.62 mg/kg at 108 DAT in soil East Jubilee and from 2.3 mg/kg at 0 DAT to 0.64 mg/kg at 108 DAT in soil 18 Acres (mean of duplicates).

F. KINETICS

The DT_{50} value for the degradation of glyphosate was calculated by a second order model to be 24 days in Speyer 2.1 soil, 46 days in Speyer 2.2 soil, 58 days in East Jubilee soil and 62 days in 18 Acres soil.

Assessment and conclusion by applicant:

The study shows major deficiencies. A major deficiency is that residues of glyphosate were quantified by external standardisation and corrected for external recoveries of fortified control samples in case the measured recovery of glyphosate was below 100% - While residues were not corrected for recoveries higher than 100 %. The report just gives tables with values corrected, no tables are included with initial, uncorrected values. For the external recoveries mean values are reported only. Consequently, the original uncorrected amount of glyphosate in the sample cannot be assessed. The mean external recoveries were below 70 % for two soils.

Therefore, the study is considered invalid and therefore not used in risk assessment.

Assessment and conclusion by RMS:

Multiple deviations from the OECD 307 were observed.

No information on the test item nor on the conditions of storage of samples was reported in the study. Organic carbon content of East Jubilee and 18 Acres are above the recommended 2.5%. Only glyphosate was analysed (whereas other peaks are observed on the chromatograms).

Regarding the correction for external recovery, the procedure reported in the study report is very unclear. From RMS understanding, the following issues are noted:

- As explained above by the applicant, the measured residues were corrected for external recovery values when measured recovery of glyphosate was below 100%, while no correction was done in other cases. Therefore some of the data could have been handled differently.
- Uncorrected raw data are not available. The procedure is not reproducible, as it is not possible to back calculate the raw data and to identify which data were corrected and which were not.
- For Jubilee and 18-Acres soils, the mean recovery were 60% and 55%, *i.e.* outside the recommended range of 70-110%.
- For Speyer 2.1 and Speyer 2.2, the mean recoveries and coefficient of variation are in the recommended range (70-110%, with CV < 20%). However, since individual recoveries are not presented, it is not possible to check for possible outliers.

As a consequence, this prevents validating the correction procedure and the data are considered quite uncertain. In RMS opinion, results cannot be used for route and rate of degradation.

The study is not acceptable.

[redacted], *1991*

Data point:	CA 7.1.2.1.1/007
Report author	[redacted]
Report year	1991
Report title	Behaviour of Glyphosate in water and soil, Part 5 Degradation in soil
Report No	PR90/002
Guidelines followed in study	BBA-guideline for testing of pesticides, Part IV 4-1
Deviations from current test guideline	From OECD 307: - variable incubation temperature (22-26°C)

	- limited information on study conduct and results (e.g. no information on application solution, application technique) - No numeric results reported for metabolite AMPA (only graphs) - Tabulated results for glyphosate only shown in DT_{50} evaluation tables, only mean values reported (no individual replicates, no standard deviation) - Recovery at day 0 rather variable from 36 – 84 % - Recoveries of fortified samples outside the range of 70-110% (89-142% for glyphosate and 117-181% for AMPA) - No soil history data provided - No pre-equilibration of soil prior to application - No information on soil storage condition and length prior to use No information on storage of soil extracts prior to and after analysis
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: aerobic rate of degradation Test item: glyphosate, non-labelled (99 % purity) Test soil (origin/soil pH/organic carbon content): LUFA F1 (Speyer, Germany/ 5.7/ 0.70), LUFA F2 (Speyer, Germany/ 6.4/ 1.34), LUFA 2.2 (Speyer, Germany/ 5.6/ 2.29), Eigenboden (Goch, Germany/ 6.2/ not reported) Test concentration: 1 mg/kg soil Test design: static system with flasks loosely closed with cotton wool Volatile trapping: None Incubation: 22-26 °C (ambient temperature) in the dark, soil moisture adjusted to 40 % of maximum water holding capacity Sampling: 0, 2, 7, 15, 30, 60 and 100 days after treatment (DAT), duplicate samples Workup: soil extracted with water/phosphoric acid at ambient temperature, derivatization to trifluoroacetyl ester, clean-up by HPLC, quantification by GC-ECD; recoveries of analytical method for glyphosate were from 89 to 142 % (mean values). Limit of detection: 20 µg a.s./kg soil Identification of glyphosate and AMPA residues: calibration of GC-system with reference substances
Short description of results:	No full mass balances and information about non-extractable residues owing to the non-labelled character of the test substance. Derivatisation of test item (GC-ECD analysis of TFA derivate, values for AMPA estimated from figures): Soil LUFA F1: Glyphosate: 0.84 mg/kg at 0 DAT, 0.02 mg/kg at 100 DAT AMPA: 0.09 mg/kg at 0 DAT, 0.25 mg/kg at 30 DAT, 0.2 mg/kg at 100 DAT Soil LUFA F2: Glyphosate: 0.36 mg/kg at 0 DAT, 0.001 mg/kg at 100 DAT AMPA: not detected at 0 DAT, 0.15 mg/kg at 7 DAT, 0.12 mg/kg at 100 DAT Soil LUFA 2.2: Glyphosate: 0.63 mg/kg at 0 DAT, 0.05 mg/kg at 100 DAT AMPA: not detected at 0 DAT, 0.15 mg/kg at 15 DAT, 0.15 mg/kg at 100 DAT Soil Eigenboden: Glyphosate: 0.56 mg/kg at 0 DAT, 0.02 mg/kg at 100 DAT AMPA: not detected at 0 DAT, 0.23 mg/kg at 30 DAT and 100 DAT The half-life for glyphosate (square root first order) was estimated to 3.78 days for LUFA F1, 1.57 days for LUFA F2, 8.04 days for LUFA 2.2 and 10.36 days for soil Eigenboden.

Assessment and conclusion by RMS:

Multiple deviations from the OECD 307 were observed. Among them, the variable incubation temperature, no history of the soil provided, no information on storage conditions of the soil before the study or of the soil extracts. The recoveries at DAT0 were low in some cases and only mean values of the duplicates were reported. No information on the application solution and technique were provided either. Recoveries are variable. Tabulated results for AMPA are not reported.

The study is not acceptable.

[redacted], *1980*

Data point:	CA 7.1.2.1.1/008
Report author	[redacted]
Report year	1980
Report title	Soil dissipation of Glyphosate following multiple applications under laboratory conditions
Report No	MSL-1173
Guidelines followed in study	None
Deviations from current test guideline	From OECD 307: - The incubation included phases of light - Temperature varied significantly during incubation - Recoveries of the analytical procedures below actual standards - Soil origin, collection, pesticide history, handling, storage till incubation not reported - Soil moisture not reported and not controlled during incubation
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: aerobic soil metabolism Test item: glyphosate, non-labelled Test soil: Two, i.e. Drummer and Spinks Soil type: silty clay loam (Drummer), sandy loam (Spinks) pH: 6.2, 4.7 (method not reported) Organic matter: 5.6 %, 2.3 % (combustion) and 3.4 %, 1.8 % (Walkeley-Blick) Application rate: 12.5 mg/kg, reflecting three seasonal applications Test design: pans in a plant growth chamber Volatiles trapping: no trapping of volatiles Incubation: light/dark cycles each of 12 hours including temperature change (30 °C during day, 25 °C at night), soils kept moist with no control of moisture Sampling: 0, 1.5, 3, 6, 12 and 24 weeks after treatment, single samples Workup/Analysis: three successive extraction steps each with 0.5 N aqueous NH_4OH at ambient temperature, purification of soil extracts by ion exchange chromatography, i.e. elution from anion exchange resin followed by cation exchange resin, conversion to N-trifluoroacetyl methyl ester derivative and its quantification by GC-FPD; recovery of analytical method 43.8-98.0 % for glyphosate, 49.7-98.5 % for AMPA, overall average 63.8 to 78.7 %
Short description of results:	Results based on GC-FPD analysis for Drummer soil: Glyphosate: 11.3 mg/kg at 0 DAT, 0.4 mg/kg at 24 weeks AMPA: 0.4 mg/kg at 0 DAT, 2.4 mg/kg at 6 weeks, 1.0 mg/kg at 24 weeks Spinks soil: Glyphosate: 14.1 mg/kg at 0 DAT, 0.2 mg/kg at 24 weeks AMPA: 1.0 mg/kg at 0 DAT, 4.3 mg/kg at 6 weeks, 2.3 mg/kg at 24 weeks

No analysis for other metabolites.

The half-life for glyphosate was reported as 2.2 weeks (*ca.* 15 days) for Drummer soil and 1.6 weeks (*ca.* 11 days) for Spinks soil.

Assessment and conclusion by RMS:

Multiple deviations from the OECD 307 were observed. Among them, no history of the soil provided, variable incubation temperature, no information on soil moisture, moisture not controlled during incubation, dark conditions not respected, recoveries of analytical procedure below actual standards.

The study is not acceptable.

Relevant articles from literature search

Within the Literature Review Report performed for glyphosate on peer reviewed publications (2010-2020), two publications were identified that could provide information potentially relevant to this data point.

Table 8.1.1.1-49: Aerobic degradation – relevant articles from literature search

Study	Study type	Substance(s)	Status
Sun *et al.*, 2019	Degradation in laboratory	Glyphosate	Reliable with restrictions
Muskus *et al.*, 2019	Degradation in laboratory	Glyphosate	Reliable with restrictions

Sun et al. 2019

Data point:	CA 7.1.1.1/012
Report author	Sun et al.
Report year	2019
Report title	Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil water system
Document No	Water Research 163 (2019) 114840
Guidelines followed in study	None
Deviations from current test guideline	Many details are missing in the report to evaluate against OECD 307: e.g. soil properties, exact soil water content during incubation, the size/mass of soil samples, procedures of work-up including procedural recoveries for glyphosate and AMPA (except for figure)
GLP	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

In this study, liquid chromatography mass spectrometry (LC-MS) and electrospray ionization (ESI) source Q Extractive Orbitrap mass spectrometry (ESI-Orbitrap MS) was used to identify non-labelled/stable-labelled glyphosate degradation products and combined with sequential extraction and stable isotopes to investigate the degradation of glyphosate and transformation of phosphorous (P) product in a soil-water system. The LC-MS and ESI-Orbitrap MS results showed that glycine formed during the early stage but was rapidly utilized by soil microorganisms. AMPA started to accumulate at the late stage and was found to be 3-6 times more resistant than glyphosate against degradation; while no sarcosine was formed. The ^{18}O labeling and phosphate oxygen isotope results allowed a clear distinction of the fraction of inorganic P (P_i) derived from glyphosate, about half of which was then rapidly taken up and recycled by soil microorganisms. Our results provide the first evidence of the preferential utilization of glyphosate-derived P_i by microorganisms in the soil-water system. The rapid cycling of P_i derived from this disregarded source has important implications on nutrient management as well as water quality.

Materials and methods

Reagents and chemicals

Glyphosate (≥96 %), (Aminomethyl) phosphonic acid (≥98 %) and 9-Fluorenyl-methoxycarbonyl chloride (FMOC-Cl) (≥97 %) were obtained from Sigma-Aldrich. Isotope labeled compounds including glyphosate-2-^{13}C, ^{15}N, glycine-d_5 and sarcosine-d_3 (methyl-d_3) were purchased from Sigma-Aldrich. Other chemicals including glycine (≥99 %) and sarcosine (≥98 %) were purchased either from Acros Organics or Fisher Scientific. All the reagents were of analytical grade and stock solution were prepared with DI water.

Soil collection and incubation

A typical silt loam soil (0-15 cm depth) from the Agricultural Experiment Station research farm at the University of Delaware was used in this study. The detailed information about the soil characterization has been reported in a previous publication. After removing any plant residues and granular rock particles, the soil samples were air-dried, homogenized, passed through a 2 mm sieve, and stored until analyses.

Table 8.1.1.1-50: Physical and chemical properties of soils collected from four depths

Depth	pH†	Density	Total P‡	Mehlich-3 P	OM§	Sand	Silt	Clay
cm		g cm^{-3}	mg kg^{-1}		%	%		
0–7.5	6.3	1.03	914.35 ± 0.03¶	212.71 ± 0.30	2.5	15	61	24
22.5–30	6.3	1.17	692.62 ± 0.03	142.73 ± 0.02	1.7	13	59	28
37.5–45	5.7	1.08	469.10 ± 0.01	48.96 ± 0.01	1.4	9	61	30
75–82.5	5.3	1.09	488.27 ± 0.05	74.36 ± 0.05	1.4	11	57	32

† Soil pH values were measured at a 1:1 soil/water ratio.
‡ Total P was measured after microwave digestion of soil by using inductively coupled plasma optical emission spectrometry.
§ Organic matter.
¶ Mean ± standard deviation

A flowchart of the experimental and analytical approach used is shown in Figure 8.1.1.1-1. The first degradation experiment was run to identify glyphosate and its degradation products in soil as well as to determine the degradation kinetics and half-lives of major products. The soil was incubated with 1 µmol/g unlabeled glyphosate at 20ºC in the dark with 60 % water content for 175 d. A separate experiment with dual isotope (13C and 15N) labeled glyphosate (1 µmol/g) spiked in soil was performed for 35 d to accurately identify degradation products. The control experiment was performed under the same condition but without glyphosate. The natural soil incubation included both biotic and abiotic degradations. Identical experiment run with autoclaved water and soil served as abiotic degradation. At selected time points, 5 g subsamples were collected into 50 mL centrifuge tubes and stored at -20ºC until further analysis. All experiments were run in duplicate under the same condition.

In order to identify P distribution and bioavailability during glyphosate degradation, the second set of experiments was performed in two 18O-labeled waters (δ18OH2O = -6.51 and + 18.27 ‰). To collect sufficient P for isotope analyses, 5 µmol/g unlabeled glyphosate was spiked into 300 g soil, and incubated with 600 mL 18O-labeled water at 20ºC in the dark for 161 d. The spiked glyphosate concentration is much higher than application dose in agriculture (about 1 kg/ha), but is required to obtain reliable phosphate isotopic analyses. The experimental containers were tightly capped to avoid any water evaporation that compromises the water oxygen isotopes. The containers were shaken every day for ~15 min to homogenize the system and then briefly ventilated to replenish ambient oxygen and to preserve the oxic condition. The control experiments were run under the same condition but in the absence of glyphosate. Subsampling and processing followed a similar procedure as described above.

Extraction and analyses of glyphosate, AMPA, glycine, and sarcosine

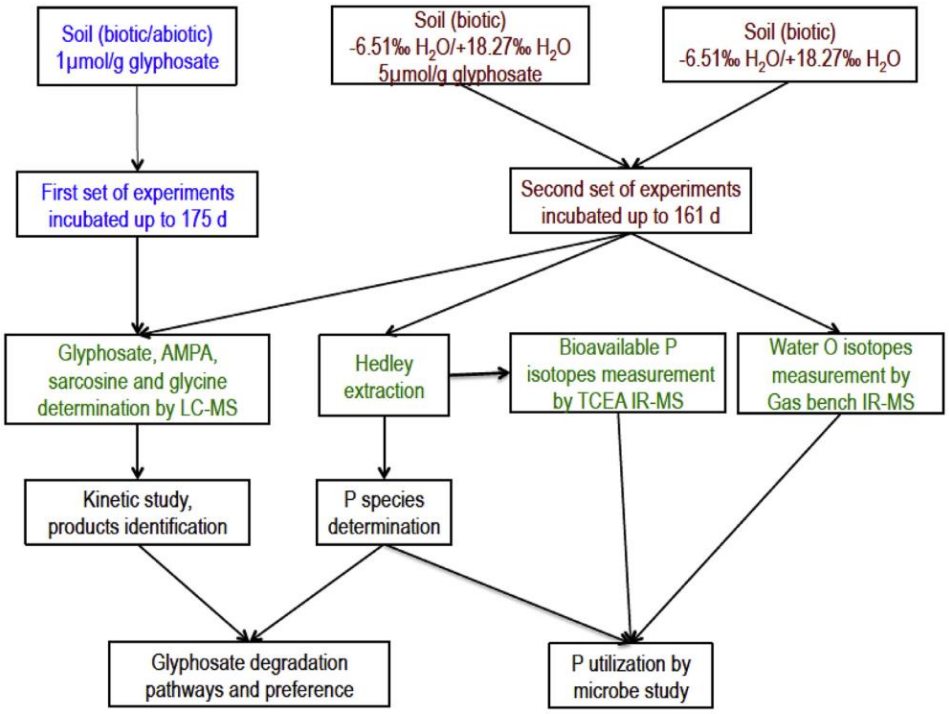
The extraction of glyphosate, AMPA, glycine, and sarcosine was based on the published method. Briefly, 1 g lyophilized soil samples degradation experiments were extracted with 5 mL 0.6 M KOH for 1 h by shaking at 140 rpm, then centrifuged at 2755 x g for 30 min. One mL of supernatant was removed and neutralized by HCl and then 0.12 mL of borate buffer (pH=9) and 0.12 mL FMOC-Cl (12 g/L) were added and shaken for 1 min on a vortex mixer. After an overnight reaction at room temperature, the mixture was filtered with a 0.45 µm syringe filter for LC-MS analysis.

Glyphosate, AMPA, glycine, and sarcosine standards were prepared to develop the separation method by using an Acclaim 120, C18 column (2.1 x 250 mm) under a gradient eluent program. After testing and

running several programs, the optimized gradient was identified to be effective with a mixture of two mobile phases with a flow rate of 0.35 mL/min with (A) acetonitrile and (B) 5 mmol/L HAc/NH4Ac: 0-6 min, 20-40 % A, 80-60 % B; 6-9 min, 40-75 % A, 60-25 % B; 9-10.2 min, 75-100 % A, 65-0 % B; 10.2-12 min, 100 % A, 0 % B; 12-12.1 min, 100-20 % A, 0-80 % B; 12.1-14 min, 20 % A, 80 % B.

The chromatographic separation for each sample required 14 min.

Figure 8.1.1.1-1: Flowchart outlining glyphosate degradation experiments in the water-soil system



Glyphosate and its degradation products were identified and quantified by a Waters single quadrupole LC-MS equipped with PDA and SQ detector. The optimized MS parameters are as follows: ESI positive mode, capillary voltage 3 kV, cone voltage 40 V, desolvation temperature 200ºC, desolvation gas flow 650 L/hr, and full mass scan from 100 to 500 m/z. The unlabeled glyphosate and labeled sarcosine were quantified with labeled glyphosate and unlabeled sarcosine as internal standards. Similarly, labeled glycine was quantified by labeled glycine as an external standard to avoid any interference from glycine already present in soil. AMPA was determined by the soil spiked external standards. Labeled glyphosate degradation samples were analyzed with a high resolution mass spectrometry-Q Extractive Orbitrap Mass Spectrometry (Thermo, Germany) at the University of Delaware. Orbitrap MS data were acquired under the positive mode with scan range from 100 to 1000 m/z. Glycine formation during labeled glyphosate degradation were determined by external standard prepared by spiking labeled glycine in soil to avoid the interference of soil original glycine.

The extraction and derivatization methods for glyphosate, AMPA, glycine, and sarcosine were validated by spiking known amounts of these compounds in soil. The recovery ranged from 85 to 107 % for glyphosate, 79-93 % for AMPA, 74-88 % for glycine, and 80-97 % for sarcosine with RSD below 20 %, which is considered satisfactory. The limit of quantification (LOQ) for glyphosate and AMPA is 10 nmol/g soil and for glycine and sarcosine is 50 nmol/g in single quadrupole LC-MS, while it was largely improved by using Orbitrap (0.5 nmol/g).

Distribution of P derived from glyphosate into soil P pools

To differentiate and quantify the distribution of glyphosate-derived P in soil, samples from both control and glyphosate spiked soils (from the second set of experiments) were analyzed. A 0.3 g lyophilized soil was

weighed and extracted with 30 mL DI water for 2 h using the modified Hedley et al. (1982) sequential extraction method (Tiessen et al., 1984). The supernatant was collected as H2O extractable Pi (most labile Pi), and residual soil was extracted with 30 mL of 0.5 M NaHCO3 for 16 h to collect labile and weakly adsorbed Pi. Inorganic P from those two pools represents microbially available Pi. The soil was further extracted for 16 h first with 30 mL of 0.1 M NaOH and then with 1 M HCl to obtain the NaOH extractable Pi (strongly sorbed P, fixed by Fe and Al oxides) and HCl extractable Pi (strongly fixed Ca-P), respectively. The concentration of Pi in each pool was measured by using the phosphomolybdate blue method. The residual P in the soils after the completion of sequential extraction was quantified using ICP-MS.

Measurement of oxygen isotope ratios

Soil samples from control and glyphosate spiked (5 µmol/g) experiments with two 18O-labeled waters were centrifuged first to extract waters to measure water oxygen isotopes (δ 18OW) by CO2 equilibration method. The measurement was done in a Finnigan GasBench II coupled with an isotope ratio mass spectrometer (IRMS; Thermo, Darmstadt, Germany) in the Environmental Biogeochemistry Laboratory at the University of Delaware.

To understand the P bioavailability, the H2O- and NaHCO3- extracted Pi pools were combined and processed for the measurement of phosphate oxygen isotope ratios (δ 18OP). Five grams of lyophilized soil samples from the second set of degradation experiments were processed following the Joshi et al (2018) method to purify and finally convert Pi into silver phosphate. The O-isotope ratios were measured by a thermochemolysis/elemental analyzer (TC/EA) couples with IRMS. All isotopes from samples and standards were run at least in triplicate.

The measured δ 18OP values of Pi were calibrated against two silver phosphate standards (YR 1aR-2 and YR 3-2, with the δ 18OP values of -5.49 and +33.63 ‰, respectively). Similarly, the δ 18OW values of porewater were calibrated with two USGS water standards (δ 18OW values of -1.97 and -9.25 ‰, respectively). All isotope values are reported in per mil (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW).

Results and Discussion

Degradation kinetics of glyphosate and its metabolites

The typical chromatography spectra of glyphosate, AMPA, sarcosine, and glycine are shown in Figure 8.1.1.1-2. Based on the LC-MS results, the concentrations of the compounds were calculated and are shown in Figure 8.1.1.1-3. Glyphosate gradually degraded over time and the extent of degradation reached >80 % by 35 d of incubation but traces of residual glyphosate were still detected until 175 d.

AMPA, the major metabolite of glyphosate, appeared after several days, accumulated during incubation, and reached its maximum concentration at 35 and 56 d in the experiment with 1 µmol/g and 5 µmol/g glyphosate, respectively. Afterwards, its degradation dominated over accumulation. Neither the degradation of glyphosate nor the formation of AMPA was observed in the sterilized soil incubation (abiotic only experiment), indicating microorganisms play a crucial role in degrading glyphosate in soils.

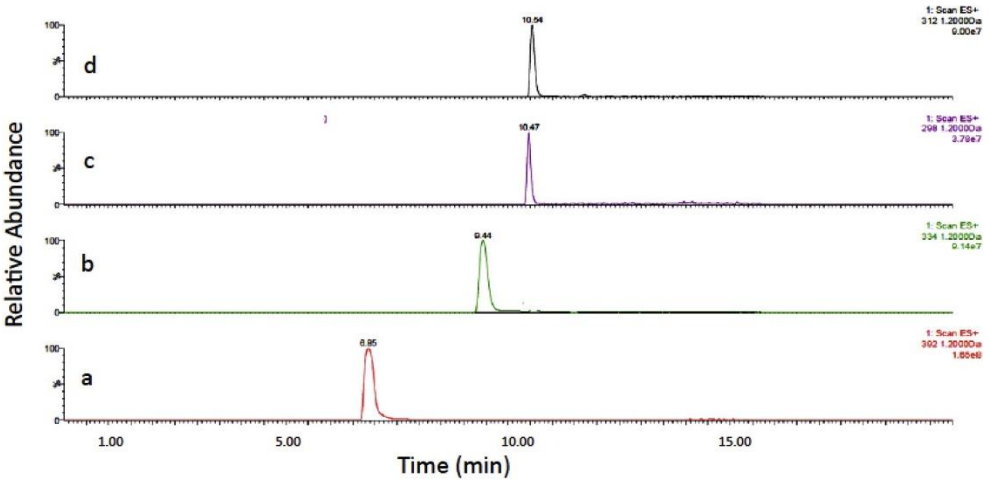
The degradation of glyphosate with time is often described according to first-order kinetics:

$$\ln(C/C0) = -kt \quad (1)$$

$$t1/2 = \ln2/k \quad (2)$$

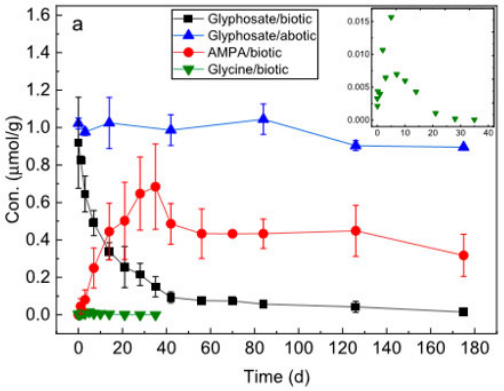
where C_0 is the initial concentration, C is the concentration at time t, and k is the degradation rate constant. The maximum accumulated concentration of AMPA is used as its initial concentration since more than 80 % of glyphosate was degraded at the time. The results show that both glyphosate and AMPA degradation follow first order kinetics with a strong correlation coefficient (R^2 >0.85). The calculated half-lives of glyphosate under two sets of experiments are 28.9 and 31.5 d, respectively, consistent with the published results. A calculation based on the maximum amount of AMPA accumulated in the soil shows that the AMPA accounts for 48-68 % of the products from glyphosate degradation. It shows much longer half-lives (138.6 and 173.3 d), which highlights the high risk because of its toxicity and persistence in the environment.

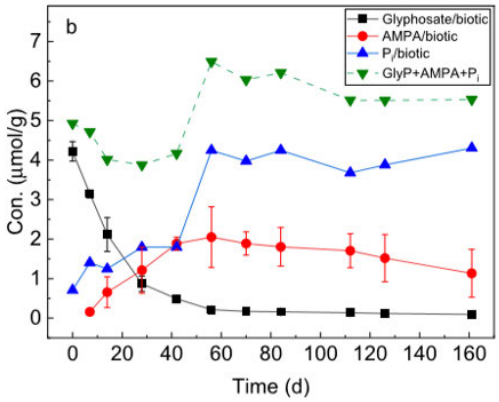
Figure 8.1.1.1-2: Typical spectrum of glyphosate, AMPA, glycine and sarcosine analyzed by LC-MS (soil spiked with 1 µmol/g standards). a) glyphosate, b) AMPA, c) glycine, and d) sarcosine



Glycine is a common amino acid and commonly present in soil and other environment. The isotope labeled glyphosate provides the reliability of detection because the labeled element is present in glycine as well. Labeled glycine appeared only after few days, accumulated, and reached the highest concentration after 5 d and then decreased but was still detectable after 35 d incubation (Figure 8.1.1.1-3**a).**

Figure 8.1.1.1-3: Kinetics of glyphosate biotic (natural soil) and abiotic (sterilized soil) degradation and its products. a) incubated with 1 µmol/g glyphosate, and b) incubated with 5 µmol/g glyphosate. Please note that the natural soil incubation includes both biotic and abiotic components of degradation





The concentration of labeled glycine is low, probably due to glycine derived from glyphosate was readily incorporated into microbial biomass soon after it formed. Results from a separate labeled glycine incubation experiment showed a rapid decline of soil-spiked glycine (1 µmol/g) with half-life of 0.89 d. Abiotic experiment showed no significant decline in glycine concentration in sterilized soil, validating methodology as well as indicating that soil microorganisms play a major role in glycine transformation. A recent study

of labeled glyphosate reported the distribution of ^{13}C and ^{15}N into several amino acids including glycine, which our results corroborate. These findings, together, confirm that glyphosate derived glycine in the experiments should have rapidly utilized and metabolized by soil microorganisms.

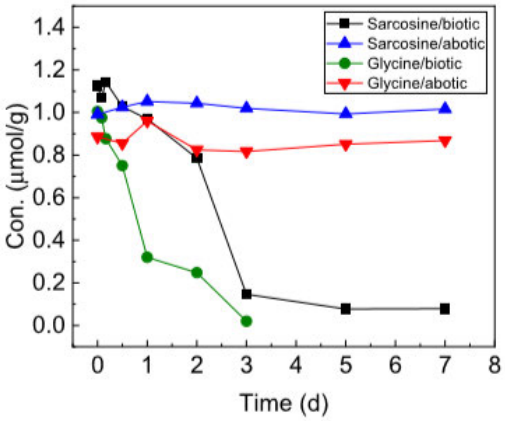


Figure 8.1.1.1-4: Biotic (natural soil) and abiotic (sterilized soil) degradation of glycine and sarcosine in soil with spiked concentration of 1 µmol/g of each. Please note that the natural soil incubation includes both biotic and abiotic components of degradation

Sarcosine is a commonly recognized precursor to glycine during glyphosate degradation primarily on pure cultures that include bacteria isolated from soils, but rarely from the natural or simulated environments. In this study, sarcosine was not detected in any soil treatments including labeled glyphosate and high glyphosate (5 µmol/g) incubations. There might be three possibilities for the observed results: inefficient extraction from soil, fast oxidation of sarcosine, or presence below the detection limits of the analytical method. However, the recovery test performed by artificially spiking sarcosine in the same soil revealed that the method used could efficiently extract and accurately quantify sarcosine (yield 80-97 %). The individual incubation experiment showed that sarcosine could be degraded fast in the biotic experiment (with half-life of 0.99 d) but no significant decline in sterilized soil, which indicates degradation possible only by soil microorganisms. These lines of evidences suggest analytical method is not the reason, particularly since the high resolution Obitrap MS (detection limit of 0.5 nmol/g of sarcosine and glycine) was used. In the labeled glyphosate degradation experiments, soil samples were collected in several time points (0, 1, 2, 4 h, until 35 d). The analytical method used successfully monitored the glycine formation and accumulation under extremely low concentration. If sarcosine was actually formed as a precursor to glycine, it should have detected by Obitrap MS since both sarcosine and glycine have similar half-lives. In a recent study, sarcosine was not detected in the abiotic degradation of glyphosate catalyzed by Mn minerals. These authors used advanced analytical methods including NMR, HPLC, and density functional theory (DFT) based electronic structure calculations and concluded that sarcosine was not a necessary intermediate product. Overall, the reliable extraction and analytical methods and intensive time point sampling verified that sarcosine was not formed during glyphosate degradation by soil microorganisms in this study.

Distribution of glyphosate-derived phosphorous in soil

Concentrations of four soil Pi pools in the control and glyphosate-spiked soils during the second set of incubations are shown below. The experiments performed in two 18O-labeled waters are considered duplicates because the difference in water oxygen isotopes does not impact the kinetics and extent of glyphosate degradation. Clearly, the control soil without glyphosate already contains high Pi and concentrations of Pi in different pools vary. It is noticeable, however, that the concentrations of Pi in these four pools remained essentially constant during the long-term incubation, with H2O-Pi (1.01 ± 0.08 µmol/g), NaHCO3-Pi (4.21 ± 0.23 µmol/g), NaOH-Pi (10.08 ± 0.91 µmol/g), and HCl-Pi (0.52 ± 0.06 µmol/g). This means that no significant transfer of P pools and organic-inorganic transformation occurred during the long-term incubation. The NaOH-Pi pool was the largest, indicating that Fe and Al minerals associated P is the major P sink in this soil, which is consistent with several other soils.

The results from the experiment in which glyphosate was spiked show that Pi derived from glyphosate transferred into different pools, resulting in an increase of corresponding pool size. The maximum concentration of H2O-Pi was 2.11 µmol/g at 70 d of incubation. The difference between control (soil without glyphosate) and glyphosate spiked soil shows that there was 1.06 µmol/g glyphosate-derived Pi transferred into this pool. Similarly, a significant net increase of Pi was observed in NaHCO3-Pi (1.40 µmol/g), NaOH-Pi (1.93 µmol/g), and HCl-Pi (0.23 µmol/g) pools, with the highest Pi concentrations measured around 56-126 d of incubation. It is interesting that the order of P pool was the same as that in the original (control) soil: NaOH-Pi >NaHCO3-Pi >H2O-Pi >HCl-Pi. Calculated P mass balance shows that the total increase in Pi among 4 pools was 4.30 µmol/g at the end of incubation, which accounts for ~86 % of spiked glyphosate (5 µmol/g). The residual P in the control and glyphosate spiked soils were similar (7.99 ± 0.69 and 7.67 ± 0.69 µmol/g, respectively), indicating that there was no significant incorporation of glyphosate-derived P in the residual P pool. It also means that the Hedley extraction could efficiently extract almost all P and account P derived from biodegradation of glyphosate.

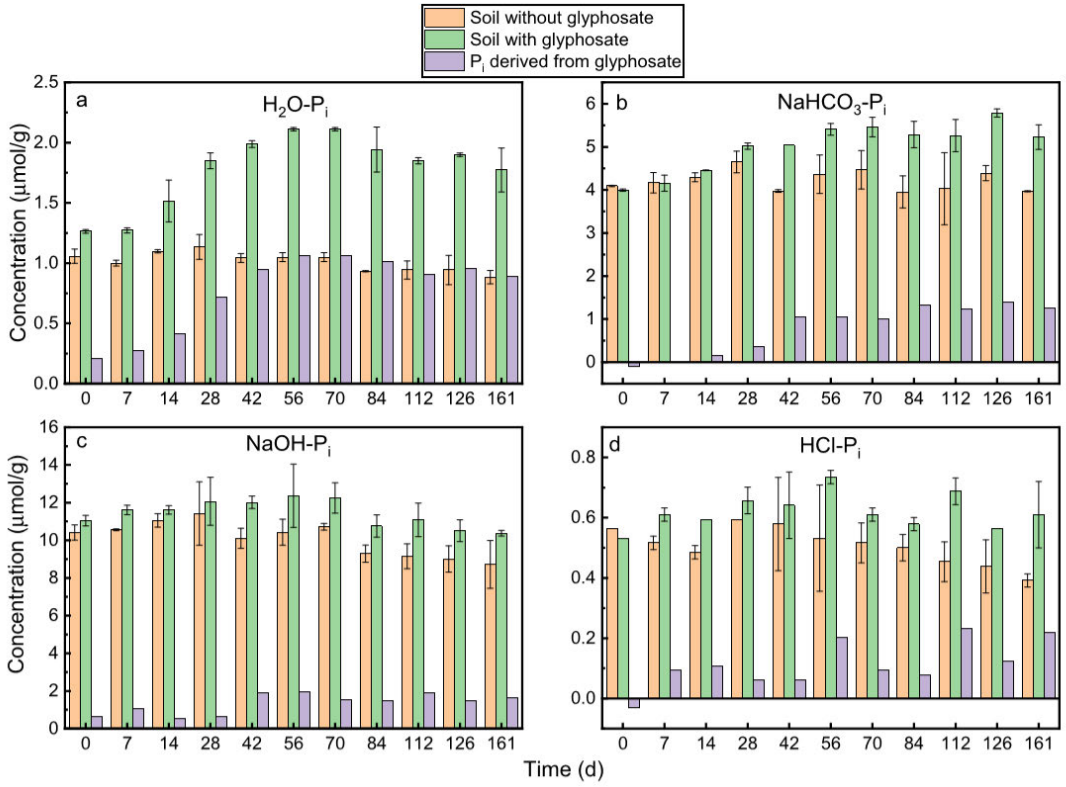


Figure 8.1.1.1-5: Concentrations of P in different pools in original soil and glyphosate incubated soil during biotic degradation. H_2O and $NaHCO_3$ extracted P pools are considered bioavailable P in soil. Soil was spiked with 5 µmol/g glyphosate. Glyphosate derived P was calculated as the different between soil with and without glyphosate

n terms of distribution P_i derived from glyphosate, the H_2O- and $NaHCO_3$-P_i pools, which are considered readily available P_i for uptake by microorganisms and plant roots, received almost half (44 %) of it. Meanwhile, around 33-38 % of glyphosate P transformed into the NaOH-P_i pool, an unavailable or moderately bioavailable P pool depending on the soil P conditions and plant efficiency and time. This means that this conditionally unavailable P pool might be further transported into open water systems by leaching or soil erosion and could increase the risk of polluting waters. The HCl-P_i, which is not directly utilized by plants and microorganisms and normally remains as an unavailable P pool in agricultural soil, only received 3-5 % of P derived from glyphosate. These results highlight the fact that P load derived from a large amount of glyphosate application (with estimated 130 million kg used in the U.S.) cannot be ignored.

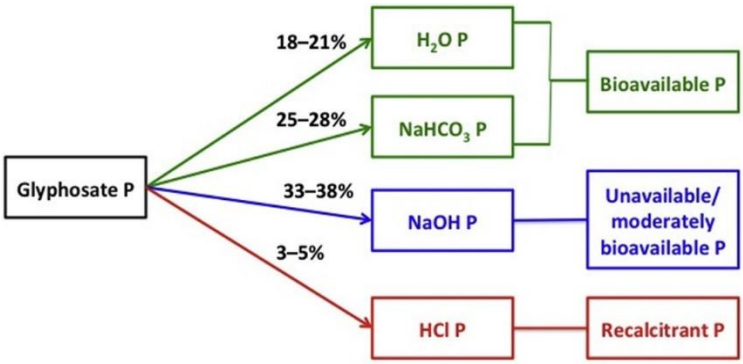


Figure 8.1.1.1-6: Distribution of glyphosate-derived P to different P pools during its biodegradation in the soil-water system. Soil incubated with 5 µmol/g glyphosate

Given that the Pi derived from glyphosate is steady means that it was gradually released as the degradation continues and distributed more into the bioavailable pool, and it may be a better P source for plants. Phosphorus fertilizer is the major P supply for plants with estimated 4 billion kg used in the U.S in 2014 with 50-70 % use efficiency. However, its fast P release kinetics do not match the dynamic needs of different crop growth stages well and this offset causes nutrient loss from soil to aquatic systems. Given the slow but steady P release from glyphosate degradation, it might be slightly more synchronous than commercial fertilizers, but still too fast than plant needs. Furthermore, multiple sprays of glyphosate during the crop lifetime (average of 1.6 times per crop year) support the possibility of fractionating more into bioavailable P that plants can readily take up. This demands reconsidering glyphosate not only use as a herbicide but a bonus P source to crops and should be included in estimations of crop P needs to improve the P efficiency of plant uptake as well reducing the P loss from agricultural soils.

Bioavailability of glyphosate-derived phosphorus

Once inside the cell, Pi is involved in several metabolic reactions catalyzed by enzymes including incorporation into cell biomass and ATP-ADP conversion. One of the unique enzymes is pyrophosphatase (PPase), which is highly conserved across all three domains of life, catalyzes the hydrolysis of pyrophosphate into Pi. This is a reversible reaction and leads to exchange of all four O atoms in Pi with O in ambient water and thus achieves O-isotopic equilibrium between phosphate and water. The equilibrium isotope depends on the temperature and water oxygen isotope value.

To further test the bioavailability and rate of microbial utilization of glyphosate-derived Pi, phosphate oxygen isotopes (δ18OP) of Pi in the soil incubated with and without glyphosate were measured and compared with the equilibrium isotope values calculated from the temperature and oxygen isotopes of water (δ18Ow) in the experiments. The δ18Ow values remained constant at -6.51 ± 0.30 ‰ and +18.27 ± 0.12 ‰ for two 18O-labeled water experiments in the long-term incubation except at the end of the experiment (161 d), when an inadvertent evaporation resulted in slight enrichment of isotopes (-4.90 ‰ and +20.71 ‰, respectively). The expected isotopic equilibrium value (δ18OP-eq) was calculated based on the (Chang and Blake, 2015) equation as:

$$\delta^{18}O_{p-eq} = e^{\left(\frac{14.43}{T} - 0.0265\right)} \times (\delta^{18}O_w + 1000) - 1000 \qquad (3)$$

The δ18OP-eq values in the experiments incubated with -6.51 ‰ and +18.27 ‰ water are +15.83 ± 0.31 ‰ and +41.16 ± 0.12 ‰, respectively, and remained constant during the incubation period (except at 161 d, in which water mass was not conserved). The starting isotope values of extracted Pi were consistent in all treatments: 20.77 ± 0.26 ‰, 21.02 ± 0.10 ‰, 21.38 ± 0.42 ‰ and 21.21 ± 0.16 ‰ in two controls (soil without glyphosate) and two glyphosate spiked experiments with -6.51 ‰ and +18.27 ‰ 18O-labeled waters, respectively. It means that there are no different O sources or contaminants that might have impacted isotope values during the incubation period, besides the degradation of glyphosate.

The measured δ18OP values in the bioavailable P in 18O spiked (+18.27 ‰) water became gradually heavier, shifting towards the equilibrium values (+41.16 ‰) and reached 32.04 ‰ at the end of incubation.

This result reveals the rapid uptake of the available P by soil microorganisms and the release of cycled P back to the soil. At the early stage, δ18OP values of Pi in the soil spiked with glyphosate were consistent with those in original control experiments. However, they became lighter after 14 d and remained 1.2-2.5 ‰ lighter for a long period. This is due to the contribution from a much lighter isotope value of Pi (~4-9 ‰) derived from glyphosate. The newly derived Pi from glyphosate degradation mixed with soil Pi pool and turned them into isotopic lighter and away from the equilibrium value (around +41.2 ‰). This result is consistent with Pi distribution that Pi was heavily released from glyphosate from 14 d to 84 d and preserving isotope record of the lighter glyphosate derived Pi in the system. However, the difference in isotope values between those two treatments gradually narrowed and eventually erased at 161 d, indicating that the soil microorganisms were efficient to uptake and cycle almost all of bioavailable P in the soil both from originally present soil and from glyphosate derived Pi.

The isotope trend in the experiments performed in -6.51 ‰ water is comparable to heavy water, but with a minor difference. For example, the δ18OP values in glyphosate spiked soil became much lighter and reached the equilibrium value sooner than those from control soil (without glyphosate). The reason is that the Pi derived from glyphosate carries much lighter δ18OP values (as explained above), which brings the isotope values close to equilibrium (which is lighter: +15.83 ± 0.31 ‰, due to the lighter water oxygen isotopes). The gap between the two treatments was 0.8 ‰, and then increased to 2.3 ‰ due to the large contribution of lighter isotopes of glyphosate derived Pi, but with the enhancement of microbe turnover, it decreased again but still 1.6 ‰ off at the end of the incubation.

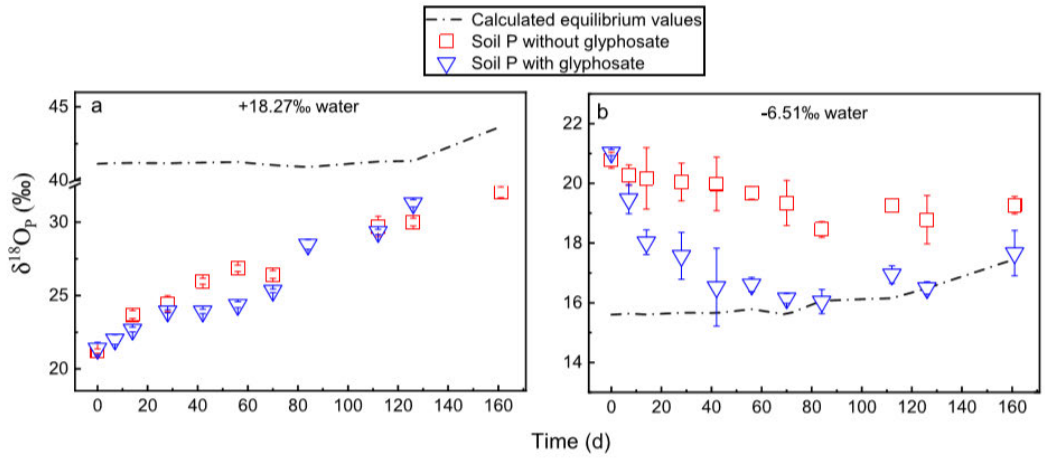


Figure 8.1.1.1-7: Changes in phosphate oxygen isotopes during glyphosate biodegradation in the water-soil system. The calculated equilibrium values assumes all P is completely recycled by microorganisms. The closer the isotope values toward the equilibrium values, the higher the extent of P cycling

The observed results explained above provide several new insights on degradation of glyphosate and its metabolites and recycling of glyphosate derived-P and together have several implications on the fate and impact of glyphosate in soils.

First, it proves that the isotope signature of glyphosate degradation can be detected in the experiments mimicking environmental systems. Second, it indicates that the degradation of glyphosate is faster than the microbial uptake and turn-over of P, so that the unique signature could be measured at the early to middle stage of the reaction.

Third, if the δ18OP values of the P derived from organic compounds are farther/closer to the equilibrium range compared to those present in-situ, they could easily shift/overprint bulk isotope value (due to mixing), leading to the inaccurate estimation of the biological activities.

Microbial turnover of P in the soil-water system

To evaluate the extent of P taken up and recycled by soil microorganisms, the P turnover was calculated from the starting δ18OP values (δ18OP-t0) at 0 h, measured values at time t (δ18OP-t) and the equilibrium values (δ18OP-eq):

$$\%P\ turnover = \frac{(\delta^{18}O_{p-t} - \delta^{18}O_{p-to})}{(\delta^{18}O_{p-eq} - \delta^{18}O_{p-t})} \times 100 \qquad (4)$$

As the equation shows, the closer the values of δ18OP-t0 to δ18OP-eq, the higher the microbial turnover efficiency. The results show that Pi in the control experiment was rapidly exchanged by soil microorganisms and driven closer to the equilibrium values, with the turnover efficiency of 22-28 % at 56 d and 45-48 ‰ at 161 d in two 18O-labeled waters. As expected, the efficiency of P turnover was similar irrespective of the starting isotopic values of 18O-labeled water (-6.51 ‰ or +18.27 ‰).

In the glyphosate spiked experiments, the $\delta^{18}O_P$ value at time t ($\delta^{18}O_{P\text{-}t/spike}$) is the sum of glyphosate derived P_i ($\delta^{18}O_{P\text{-}t/gly}$) and the original P_i from control soil ($\delta^{18}O_{P\text{-}t/con}$), which can be calculated from a simple mass balance equation as follows:

$$\delta^{18}O_{p-t/spike} = x\delta^{18}O_{p-t/gly} + (1-X)\delta^{18}O_{p-t/con} \qquad (5)$$

where x is the fraction of Pi derived from glyphosate in the spiked samples. We calculated the starting isotope values of glyphosate derived Pi in two 18O-labeled water systems at 0 h using previous results, which are +6.92 ‰ and ±12.14 ‰ in -6.51 ‰ and +18.27 ‰ waters, respectively. Based on the starting values of glyphosate-derived Pi, its microbial turnover was calculated using equation (4). The trend of P turnover in the soils receiving glyphosate-derived Pi was similar to that of control soil (without glyphosate), but the recycling efficiency was higher (67-75 ‰). Overall, phosphate oxygen isotopes allowed discrimination of sources and variable recycling efficiency of soil P vs glyphosate-derived Pi.

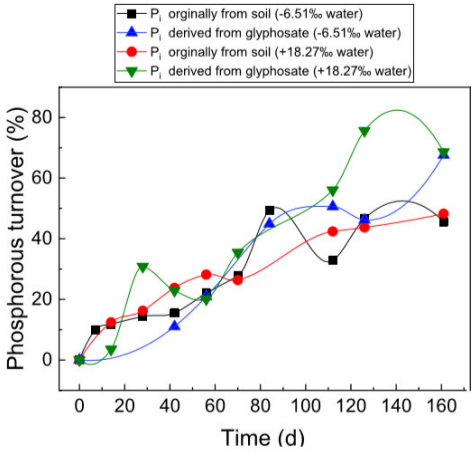


Figure 8.1.1.1-8: Microbial turnover efficiency of soil P and glyphosate-derived P

Glyphosate degradation pathways in soil

To understand the degradation pathways and specific preferences in the soil system studied, the released P_i extractable from four pools were combined together. The total P mass from glyphosate source was also calculated by adding glyphosate, AMPA, and released P_i and are shown in Figure 8.1.1.1-3b. The released P_i steadily increased and reached the peak concentration around 56 days. AMPA remained at the accumulation stage and started to degraded at that time only when more than 80 % of glyphosate was already degraded. There was slight decrease in total P (from original concentration of 4.92 µmol/g) at the early stage of degradation, and then remained almost constant during the incubation period. Consider the efficient extraction of the glyphosate-derived P_i, it implies that there might be some other non-detected P speciation during the early stage of glyphosate degradation besides glyphosate, AMPA, and inorganic P. A potential P compound could be methylphosphonic acid, which can be generated synchronously if glycine forms directly from glyphosate. Based on the data generated in this study and foregoing assumptions and published results, revised pathways and temporal preference of glyphosate degradation in the soil-water system is proposed. Under the action of soil microorganisms, at the early stage of degradation, glyphosate is cleaved at C(3)-N position to form glycine and methyl-phosphonic acid, the latter one is further degraded to form P_i, which accumulates in the system. Another bond cleavage occurs at C(2)-N position and form

AMPA and glyoxylic acid. AMPA accumulates at the late stage of degradation. No sarcosine was generated in the soil-water system in this study, so it is not the required intermediate metabolite to form glycine.

Conclusion

In this study, we studied degradation glyphosate and its metabolites and successfully utilized phosphate oxygen isotopes to confirm the biological availability of glyphosate-derived P in the simulated soil-water system. The broader conclusions derived from this study and the implications thereof are as follows:

1) A satisfactory method of extraction and separation of glyphosate and its major metabolites in soil was developed, which could be used to identify the fate of glyphosate in a variety of environments. The absence of degradation in sterilized soil showed the soil microorganisms play the essential role on the degradation of glyphosate. Temporal presence of glycine and AMPA varied as well as their microbial uptake and degradation. AMPA was found to be 3-6 times resistant than glyphosate against degradation, which brings a higher concern to the safety of environment.

2) The distribution of glyphosate-derived Pi in a soil was investigated. About half of the glyphosate-derived Pi transferred into the readily bioavailable P pool. A slow but steady release of Pi from the degradation of glyphosate could mean that its supply could be slightly more synchronous with plant P demand during plant growth especially because it is applied more than one time during a crop cycle. This means that a higher proportion of glyphosate-derived P, than P from commercial fertilizers which release P all at once, could be taken up by plants.

3) Glyphosate-derived Pi has a distinct isotopic signature and can aid in identification of its source. The natural environment, however, is complex and could pose additional challenges, most likely due to the low content of glyphosate and inappropriate sampling time could miss to detect significant offset of isotope values. This is because isotope signature could be erased or overprinted due to biological cycling of glyphosate-derived P.

4) 18O-labeling in water and application of phosphate oxygen isotope method allowed explicit understanding of microbial uptake and extent of biological turnover of glyphosate derived-P. The microbial turnover of original P in soil and glyphosate-derived P was comparable, but it was found that the microorganisms were more efficient to utilize and recycle glyphosate-derived P. The research tool developed could be further used to investigate the extent of microbial activities in soils and other natural environments.

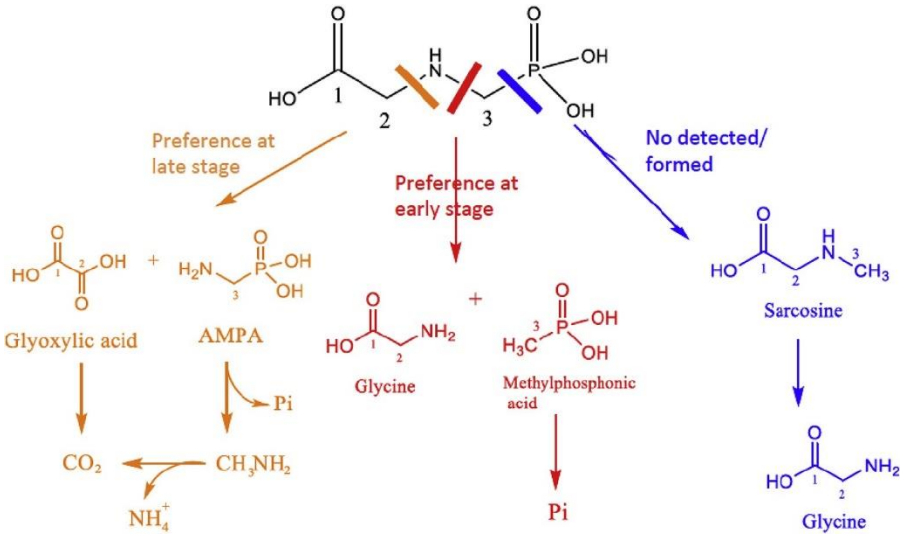


Figure 8.1.1.1-9: (Bio)degradation pathways of glyphosate and preference of degradation in the water-soil system used in this study

Assessment and conclusion by applicant:

The article deals with aerobic soil degradation with non-labelled and stable-labelled glyphosate. AMPA formation was confirmed to occur as metabolite. The article focuses mainly on the fate of the phosphorous moiety to investigate for potential transformation products of glyphosate origin.

In general, the methods and results are described, but there is a lack of details reported to allow for the evaluation of all potential deviations from OECD 307. For example, soil properties are not reported, exact soil water content during incubation is unclear, the size/mass of soil samples incubated is not clearly stated (1 g soil was used for extraction). Further, procedures of work-up including procedural recoveries for glyphosate and AMPA are presented in figures, but not in detail (tabulated values). DT_{50} values according to SFO were calculated for glyphosate and AMPA (based on max. concentration) but no details on quality of fits and statistics are provided. No new transformation products were reported.

The article is therefore considered as reliable with restrictions.

Assessment and conclusion by RMS:

As indicated by the applicant, the study focuses mainly on the formation and bioavailability of phosphorous from glyphosate in soil. The study confirms the results of the available studies on route of degradation of glyphosate and no additional significant degradates are put forward.

The article provides supportive information on the route of degradation of glyphosate in soil.

Muskus et al. 2019

Data point:	CA 7.1.1.1/013, CA 7.1.2.1.1
Report author	Muskus A. M. *et al.*
Report year	2019
Report title	Effect of temperature, pH and total organic carbon variations on microbial turnover of $^{13}C_3{}^{15}N$-glyphosate in agricultural soil
Document No	Science of the Total Environment 658 (2019) 697-707
Guidelines followed in study	none
Deviations from current test guideline	not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

Full summary

The pH or TOC of an agricultural original soil (pH 6.6, TOC 2.1 %) was modified using sulfuric acid or farmyard manure (FYM), respectively. Each treatment: original (I), 3 % TOC (II), 4 % TOC (III), pH 6.0 (IV) and pH 5.5 (V) was amended with $^{13}C_3{}^{15}N$-glyphosate and incubated at 10°C, 20°C and 30°C for 39 days. The temperature was the main factor controlling the mineralization and the extractable $^{13}C_3{}^{15}N$-glyphosate, whereas higher TOC content and lower pH resulted in enhanced formation of ^{13}C-bioNER. After 39 days the cumulative mineralization of ^{13}C-glyphosate was in the range of 12-22 % (10°C), 37-47 % (20°C) and 43-54 % (30°C). Extractable residues of ^{13}C-glyphosate were in the range of 10-21 % (10°C) and 4-10 % (20°C and 30°C); whereas those of ^{15}N-glyphosate were as follows 20-32 % (10°C) and 12-25 % (20°C and 30°C). The ^{13}C-NER comprised about 53-69 % of ^{13}C-mass balance in soils incubated at 10°C, but 40-50 % in soils incubated at 20°C and 30°C. The ^{15}N-NERwere higher than the ^{13}C-NER and varied between 62 % and 74 % at 10°C, between 53 % and 81 % at 20°C and 30°C. A major formation of ^{13}C-bioNER (72-88 % of ^{13}C-NER) at 20°C and 30°C was noted in soil amended with FYM. An increased formation of ^{15}N-bioNER (14-17 % of ^{15}N-NER) was also observed in FYM-amended soil. The xenobiotic ^{15}N-NER had a major share within the ^{15}N-NER and thus need to be considered when assessing the environmental risk of glyphosate-NER.

Materials and methods

Soil and farmyard manure
The soil used for the incubation experiment was the Ap horizon of a haplic Chernozem collected from the long-term experimental area "Static Fertilization Experiment" located in Bad Lauchstädt, Germany. The silt loam soil contained 21 % of clay, 68 % of silt and 11 % of sand, and it was amended with 30 t/ha farmyard manure every second year for >100 years. In addition, the soil received 12 kg P/ha and 50 kg K/ha each year. The soil contained 2.1 % (w/w) TOC and 0.17 % (w/w) total N (based on our measurements in the lab). The maximum water holding capacity (WHC) was 37.5 % and the pH (in water) was 6.6. This experimental site has also a long-term history of pesticides application (>30 years including glyphosate). The soil prior to incubation was stored at 4°C for three months.

The cow farmyard manure (FYM) which has been used for fertilization of soil in "Static Fertilization Experiment" was also used to modify the TOC level of the reference soil in our simulation experiments. FYM contained 34 % TOC (w/w), 2.5 % (w/w) total N and had pH (in water) 8.72. Due to the addition of FYM, the pH of soil with 3 % TOC and 4 % TOC increased to 7.0 and 7.3, respectively.

Chemicals
Co-labeled $^{13}C_3{}^{15}N$-glyphosate was purchased from Iso-Sciences Company (Trevose, PA, USA). The isotopic enrichment of the labeled glyphosate was 99 % for ^{13}C and 98 % for ^{15}N, the chemical purity was 98 %.

Incubation experiment
The fresh soil was first sieved through a 2 mm screen. Thereafter, the soil was modified to obtain three levels of TOC (the original TOC content of 2.1 %, 3 % TOC and 4 % TOC) and three levels of pH (the original soil pH of 6.6, pH 6.0 and pH 5.5). The original soil without any modification (I) had 2.1 % TOC and pH 6.6. To study the effect of increased TOC content on glyphosate turnover, the original soil was amended with FYM to obtain a final concentration of 3 % TOC (II) or 4 % TOC (III). The influence of lower soil pH (compared to that of the original soil) on glyphosate transformation was investigated after the prior adjustment of pH of original soil to pH 6.0 (IV) with 0.1 M H_2SO_4 and to pH of 5.5 (V) using 1 M H_2SO_4. The targeted levels of TOC (2.1 %-4 %) and of pH (5.5-6.6) are characteristic for soils in the temperate climate; therefore, they were chosen for this study.

Prior to the addition to the soil, FYM was dried, homogenized and sieved through 2 mm screen. To set up the defined 3 % and 4 % TOC level, soil preliminary tests with FYM were conducted. After addition of calculated amounts of FYM, the soil was kept at 20°C to equilibrate. The content of TOC of each tested soil was measured at different times after mixing using an elemental analyzer-combustion-isotope ratio mass spectrometry (EA-C-IRMS; Finnigan MAT 253, Thermo Electron, Bremen, Germany). Based on the results from preliminary tests, an addition of 3.5 % (w/w) of FYM was needed to increase the original TOC level of soil 2.1 % to 3 %. To obtain the 4 % TOC level, 7 % of (w/w) of FYM was added.

To lower the pH of the soil sulfuric acid (H_2SO_4) was added. In addition, the concentration and the amount of sulfuric acid was chosen carefully to cause minimum harm to the soil bacteria and minimum water loss of soil. After addition of the calculated amounts of H_2SO_4, the soil was stored at 20°C to equilibrate. The pH of the soil was measured on different time intervals in order to check the stability of pH. Based on the preliminary results, 1 mL of 0.1 M H_2SO4 was added to 20 g of soil to adjust the original pH of soil 6.6 to pH 6.0. To lower the original pH of soil 6.6 to pH 5.5, 1 mL of 1 M H_2SO_4 was added to 20 g of soil.

Each treatment was incubated for 39 days after adding either $^{13}C_3{}^{15}N$-labeled glyphosate or, unlabeled glyphosate (unlabeled control). In addition, soil without glyphosate was incubated (non-amended control). Both unlabeled and non-amended controls were used to correct the natural abundance of ^{13}C and ^{15}N. The soil in the glyphosate amended treatments was spiked with either labeled or unlabeled glyphosate dissolved in methanol to yield a final concentration of 50 mg glyphosate/kg soil. The high concentration of labeled glyphosate was needed to obtain a reliable isotopic enrichment against the ^{13}C and ^{15}N backgrounds. Thereafter, the water content of all soil treatments was adjusted to a 60 % of maximum WHC with distilled water and the soil was placed into Duran glass bottles. All five treatments were incubated in triplicate at 10°C, 20°C and at 30°C for a period of 39 days in darkness as recommended in the OECD guideline 307

(OECD, 2002). The CO_2 evolved by soil respiration (total CO_2) and from glyphosate mineralization ($^{13}CO_2$) was measured after 3, 5, 10, 21 and 39 days. At the end of incubation all soil samples were used to determine the mass balance of ^{13}C- and ^{15}N-glyphosate.

Mass balance of ^{13}C- and ^{15}N-glyphosate in soil
In all five soil treatments, a turnover mass balance for $^{13}C_3{}^{15}N$-glyphosate was determined, comprising ^{13}C-CO_2 (mineralization), borate buffer extractable $^{13}C_3{}^{15}N$-glyphosate and $^{13}C^{15}N$-AMPA, and total ^{13}C- and ^{15}N-NER. To assess the extent of ^{13}C and ^{15}N incorporation from $^{13}C_3{}^{15}N$-glyphosate into the biogenic NER, the amounts of ^{13}C and ^{15}N- total amino acids (tAAs) were determined.

Mineralization
The measurement of $^{13}CO_2$ trapped in 2 M NaOH was based on the determination of the total CO_2 (soil respiration) and its isotopic composition. The total content of CO_2 was quantified using a total organic carbon analyzer (Multi N/C 21005, Jena, Germany). The isotopic composition of the released $^{13}CO_2$ from each sample was measured using a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS; Finnigan MAT 252, Thermo Electron, Bremen, Germany, coupled to Hewlett Packard 6890 GC; Agilent Technologies, Germany), equipped with a Porabondt Q-HT Plot FS column (50 m × 0.32 m × 5 µm; Chrompack, Middburg, Netherlands).

Extractable glyphosate and AMPA
The concentrations of labeled and unlabeled glyphosate and AMPA in soil samples were determined using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) after extraction, clean-up and derivatization. First, glyphosate and AMPA were extracted from 1g of soil with 20 mL of a 40 mM sodium borate buffer solution (pH 8) using an overhead shaker for 1 h. Thereafter, the extracts were separated from the soil particles by centrifugation of the soil-extract mixture at 2362 *g* for 10 min. The soil extracts were then transferred to conical 50 mL tubes and 2 mL of each sample was taken for purification over OASIS HLB 6 mL (200 mg) SPE cartridges. After the sample passed through the column, the internal standard glufosinate was added to each sample. In the purified extract, glyphosate, AMPA and glufosinate were derivatized with 200 µL of a FMOC-Cl (60 mg/mL in acetonitrile) for 60 min at room temperature. The derivatization reaction was stopped by addition of 30 µL concentrated formic acid (98-100 %), and the samples were stabilized with 150 µL 1 M EDTA prior to LC-MS/MS analyses.

The analysis of glyphosate, AMPA and glufosinate was performed using an Agilent 1260 LC system coupled to an ABSciex QTrap 6500 MS/MS operated with negative mode electrospray ionization. A ZORBAX Extend-C18 analytical column (100 × 2.1 mm, 3.5 µm particle size, Agilent) was used for separation. 5 mM ammonium acetate and acetonitrile were used as a mobile phase A and B, respectively. The LC gradient for the separation was as follows: an isocratic phase (95 % A: 5 % B) from 0 to 4 min, followed by a 2-stepwise increase of B from 5 to 95 % from 4 to 16 min; then an isocratic state from 16 to 21 min and finally a re-equilibration period of 7 min with the initial eluent composition. The total run time was 28 min. The flow rate was 0.4 mL/min and 5 µL of each sample were injected.

The quality of the measured concentration of unlabeled and labeled glyphosate and AMPA in soil extracts was controlled by the measurement of (I) blank samples every three injections, and (II) purified soil extracts every six injections. Blank samples contained 20 % of acetonitrile in water, whereas purified soil extracts were spiked with 100 mg/L of unlabeled glyphosate, $^{13}C_3{}^{15}N$-glyphosate and AMPA and with 40 mg/L of internal standard glufosinate. The calculated relative standard deviation (RSD) of the measured soil extracts daily was 10 % for unlabeled glyphosate, 5-10 % for AMPA, and 4-5 % for $^{13}C_3{}^{15}N$-glyphosate. The RSD for $^{13}C^{15}N$-AMPA was not possible to calculate because this reagent was not available. The sample batch quantification was calculated through a calibration curve measured at the beginning and at the end of each batch.

Neither the limit of detection (LOD) nor the limit of quantification (LOQ) depended on the isotopic composition of the analyte. For glyphosate LOD was 1 µg/L and LOQ was 10 µg/L and for AMPA LOD was 10 µg/L and LOQ was 20 µg/L. The recovery of glyphosate and AMPA including the extraction, clean-up and derivatization steps was tested using soil spiked with either a high (500 µg/L) or a low (10 µg/L) glyphosate and AMPA concentrations.

The results from this experiment showed that the soil matrix did not interfere with the ionization process, as recoveries were 100 % ± 7 % for low and 97 % ± 3 % for high concentrations of glyphosate-FMOC and AMPA-FMOC. Additionally, no interferences during derivatization were found as indicated by similar recoveries of the analytes from the soil extract to those in the solvent (121 % ± 8 % for low and 100 % ± 13 % for high concentrations). The overall recovery of glyphosate and AMPA reached 93 % ± 3 %.

Total NER
After the extraction of glyphosate and AMPA, the pre-extracted soil samples were air-dried. An aliquot of 2-4 mg of the dry soil was taken for analyses of the total NER using EA-C-IRMS (Finnigan MAT 253, Thermo Electron, Bremen, Germany) coupled to Euro EA 3000 (Eurovector, Milano, Italy). The temperature of the oxidation oven was 1020°C and the one of the reduction oven was 650°C. The amount of NER was calculated based on comparison of the ^{13}C and ^{15}N excess in labeled samples over the corresponding unlabeled samples.

Total bioNER
Proteins comprise approximately 50 % of the dry weight of microbial biomass and show a relatively high stability in soils. Therefore, the measured amount of tAAs hydrolyzed from proteins was used to estimate biogenic NER formation from $^{13}C_3{}^{15}N$-glyphosate.

Xenobiotic NER
The $^{13}C(^{15}N)$-xenobiotic NER represent the difference between the total ^{13}C (^{15}N)-NER measured by EA-irMS and the total $^{13}C(^{15}N)$-bioNER.

Data analysis and statistics
All incubation experiments and analyses were performed in triplicate and results are presented as averages with standard deviation.

The contents of ^{13}C in $^{13}CO_2$, and $^{13}C/^{15}N$ in $^{13}C/^{15}N$-NER or in $^{13}C/^{15}N$-tAA, were based on the quantification of their total carbon (^{12}C + ^{13}C) or total nitrogen (^{14}N + ^{15}N) pools and the $^{13}C/^{15}N$ excess over the unlabeled and non-amended controls. Total recovery in the mass balances for ^{13}C ranged from 68 % to 102 % and for ^{15}N between 68 % and 106 %. The results are shown as percentages of the ^{13}C and ^{15}N in the respective fraction in relation to the initially applied $^{13}C_3{}^{15}N$-glyphosate. The analytical uncertainty of ^{13}C and ^{15}N isotope signatures based on Gaussian error propagation in each fraction was <1 % and <5 % (of atom percent [at.%] ^{13}C or at.% ^{15}N) for unlabeled and labeled samples, respectively.

The gaseous $^{15}N_2$ from ^{15}N-glyphosate was not measured; but it is expected to be rather low. The $^{15}NO_3^-$ and $^{15}NH_4^+$ which might a result of the mineralization of ^{15}N-glyphosate were not analyzed. $^{15}NO_3^-$ and $^{15}NH_4^+$ might have been overlooked in the ^{15}N-extract (extractable glyphosate and AMPA) or in the bulk soil as ^{15}N-NER. The total recovery of ^{15}N for soils was >80 % (except from 68 % for the original soil at 20°C and 74 % for 3 % TOC at 30°C). Therefore, the conversion of ^{15}N-glyphosate to $^{15}NO_3^-$, $^{15}NH_4^+$ or $^{15}N_2$ does not seem to be a major route for dissipation of this herbicide from soil.

Dissipation half-life (DT_{50}) and degradation rate constant (k) of glyphosate
The dissipation half-life (DT_{50}) of glyphosate and the degradation rate constant (k) of glyphosate were calculated assuming a single first order (SFO) degradation kinetics frequently used for determination of DT_{50} of glyphosate in soils.

Statistics
A two-way ANOVA without replication was performed to test the combined effect of soil properties (pH or TOC) and temperature variations on mineralization kinetics of $^{13}C_3{}^{15}N$-glyphosate, $^{13}C(^{15}N)$-tAAs, the extractable $^{13}C_3{}^{15}N$-glyphosate or $^{13}C^{15}N$-AMPA, and $^{13}C(^{15}N)$-NER. The parameters were estimated from triplicates and the statistical analysis was conducted with Microsoft Excel 2010 software. The differences between the results were considered as significant at $p < 0.05$.

Results

Mineralization kinetics of $^{13}C_3$-glyphosate

The cumulative mineralization of 50 mg/kg of $^{13}C_3{}^{15}N$-glyphosate over 39 days in dependence of TOC and pH at 10, 20 and 30°C is shown below. The mineralization of $^{13}C_3$-glyphosate in all set-ups and at all temperatures followed a similar pattern. The mineralization kinetics can be described as bi-phasic (Registration EU, 2011) with no lag phase and an initially high rate (up to day 10) followed by a slower increase until the end of the incubation period.

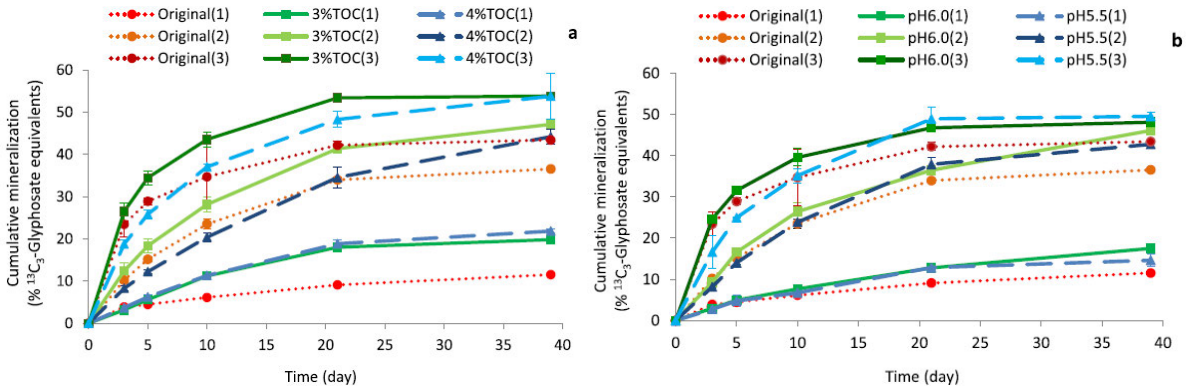


Figure 8.1.1.1-10: Cumulative mineralization of initial $^{13}C_3$-glyphosate equivalents applied to soil (n = 3, bars represent standard deviation). 1: 10°C, 2: 20°C, 3: 30°C. a: Original soil (2.1 % TOC. pH 6.6) and TOC treatment at 10°C (1), at 20°C (2) and at 30°C (3). b: Original soil (2.1 % TOC. pH 6.6) and pH treatment at 10°C (1), at 20°C (2) and at 30°C (3).

The absence of a lag phase in all settings indicates that the endogenous soil microflora was capable to use glyphosate as a source of energy without prior acclimation.

The decrease of the mineralization rate of glyphosate over time can be related to the time-dependent glyphosate availability to microbial degraders. The bioavailability of glyphosate can be limited due to its sorption or degradation.

The temperature was the main factor controlling the cumulative mineralization kinetics of the applied $^{13}C_3$-glyphosate in all treatments (ANOVA, $p < 0.05$). The mineralization was as follows 30°C >20°C >10°C. In addition to the temperature, pH and FYM influenced significantly the mineralization of $^{13}C_3$-glyphosate.

The mineralization of the initial $^{13}C_3$-glyphosate equivalents was lowest in the original soil and it reached at the end 12 % (10°C), 37 % (20°C) and 43 % (30°C). In pH 5.5 and pH 6.0 treatment and at 10°C it increased to 15 % and to 18 % of the $^{13}C_3$-glyphosate equivalents, respectively. An increased mineralization of $^{13}C_3$-glyphosate was also noted for both pH treatments at higher temperatures and it ranged between 43 % and 46 % (20°C) and 48-50 % (30°C). The highest ultimate mineralization was observed in FYM-amended soils and at all temperatures. At 10°C it amounted to 20-22 %, at 20°C to 44-47 % and at 30°C to 54 % of the $^{13}C_3$-glyphosate equivalents.

The temperature affected the microbial activity and thus controlled the extent of $^{13}C_3$-glyphosate mineralization. However, shifts in the soil properties, in particular increasing TOC content due to FYM amendment, also resulted in small increases of mineralization of this herbicide. The decrease of pH of the original soil to pH 6.0 and 5.5 through the addition of H_2SO_4 could change the net surface charge of the original soil. As a result, more glyphosate could have been desorbed from the solid matrix and thereby its availability to microbial degraders increased. An addition of H_2SO_4 to soil, to lower pH, could also result in remobilization of nutrients supporting the growth of glyphosate degraders. Therefore, an increased availability of glyphosate and nutrients to microorganisms could explain slightly glyphosate mineralization at lower pH.

Extractable $^{13}C_3{}^{15}N$-glyphosate and $^{13}C{}^{15}N$-AMPA

In line with mineralization data, temperature also was the main factor controlling the content of the extractable $^{13}C_3{}^{15}N$-glyphosate in all treatments (ANOVA, p <0.05). However, the extractable $^{13}C_3{}^{15}N$-glyphosate decreased with increased temperature. Regardless of pH or TOC, about 4.2-16 % of initial $^{13}C_3{}^{15}N$-glyphosate equivalents were recovered as $^{13}C_3{}^{15}N$-glyphosate at 10°C. At higher temperatures, these values ranged between 0.8 % and 4.5 % (20°C) and between 0.1 % and 4.6 % (30°C).

The extractable $^{13}C_3{}^{15}N$-glyphosate in 3 % TOC treatment amounted to 16 % (10°C), 1.8 % (20°C) and to 3.9 % (30°C) of the initial $^{13}C_3{}^{15}N$-glyphosate equivalents. In soil with 4 % of TOC, these values were as follows: 15 % (10°C), 4.5 % (20°C) and 4.6 % (30°C). Similar to what observed for mineralization, temperature + TOC variation also significantly influenced the extractable $^{13}C_3{}^{15}N$-glyphosate in soil with 3 % and 4 % TOC (ANOVA, p <0.05). The extractable $^{13}C_3{}^{15}N$-glyphosate in the original soil and pH treatment (pH 5.5 and 6.0) was lower than in the TOC treatment and ranged between 0.07 % and 8.8 %.

No straightforward and significant correlation between the extractable $^{13}C^{15}N$-AMPA and temperature, TOC or pH could be found. About 4.1-7.2 % of initial $^{13}C_3$-glyphosate equivalents were recovered as ^{13}C-AMPA at 10°C, whereas at 20°C and 30°C were accordingly 3.2-7.4 % and 3.9-5.4 %. Additionally, the values of ^{15}N-AMPA were 3-fold higher than the ^{13}C-AMPA. The percentages of measured ^{13}C- or ^{15}N-AMPA are referred to the initial amount of $^{13}C_3$- or ^{15}N-glyphosate equivalents. $^{13}C_3$-glyphosate contains three labeled ^{13}C atoms versus only one ^{15}N labeled atom of ^{15}N-glyphosate. Due to the fact that soil samples were only available from the final sampling, it is not possible to conclude on the kinetics (assuming a SFO kinetic) and thus on the influence of formation, sorption, desorption or degradation process of this transformation product.

Table 8.1.1.1-51: The extractable $^{13}C_3$-glyphosate and ^{13}C-AMPA as percentages of the initially added $^{13}C_3$-glyphosate to soil (n = 3, mean values ± standard deviation). a: Original soil and TOC treatment at 10°C, 20°C and 30°C. b: Original soil and pH treatments at 10°C, 20°C and 30°C

a

Temperature	Compound	% of initial $^{13}C_3$-glyphosate equivalents		
		Original	3% TOC	4% TOC
10 °C	Glyphosate	8.8 ± 0.7	15.6 ± 1.8	14.8 ± 0.7
	AMPA	7.2 ± 0.9	5.4 ± 1.9	4.1 ± 1.5
20 °C	Glyphosate	0.8 ± 0.2	1.8 ± 0.5	4.5 ± 0.4
	AMPA	4.6 ± 0.3	3.2 ± 0.8	3.6 ± 0.1
30 °C	Glyphosate	0.1 ± 0.1	1.4 ± 1.3	4.6 ± 4.7
	AMPA	3.9 ± 0.7	3.9 ± 0.9	5.4 ± 2.7

b

Temperature	Compound	% of initial $^{13}C_3$-glyphosate equivalents		
		Original	pH 6.0	pH 5.5
10 °C	Glyphosate	8.8 ± 0.7	6.7 ± 2.0	4.2 ± 2.6
	AMPA	7.2 ± 0.9	5.3 ± 0.8	5.4 ± 2.4
20 °C	Glyphosate	0.8 ± 0.2	1.2 ± 0.2	3.0 ± 0.8
	AMPA	4.7 ± 0.3	5.8 ± 0.2	7.4 ± 1.5
30 °C	Glyphosate	0.1 ± 0.1	0.1 ± 0.0	0.6 ± 0.0
	AMPA	3.9 ± 0.7	3.9 ± 0.0	5.1 ± 1.0

The DT_{50} values of $^{13}C_3{}^{15}N$-glyphosate for all treatments were lower at 20°C and 30°C than that at 10°C. The DT_{50} values for the original soil were as follows: 11 days (10°C), 5 days (20°C) and 3 days (30°C). The reported DT_{50} values were lowest for the pH 5.5 and 6.0 treatments (8-10 days at 10°C, 6-7 days at 20°C and 4-5 days at 30°C) and highest for the 3 % and 4 % TOC treatments (14 days at 10°C, 6-9 days at 20°C and 6-8 days at 30°C).

Mass balance of $^{13}C_3{}^{15}N$-glyphosate and glyphosate-NER in soils

The temperature and soil parameter variation had an effect on the distribution of ^{13}C and ^{15}N in the mass balance of $^{13}C_3{}^{15}N$-glyphosate including ^{13}C and ^{15}N-NER formation. Extractable $^{13}C_3$-glyphosate ($^{13}C_3$-glyphosate + ^{13}C-AMPA) were in the range of 10-22 % (10°C) and 4-10 % (20°C and 30°C). The total ^{13}C-NER (sum of xenobiotic NER, proteinaceous bioNER and other bioNER) comprised about

58-75 % of ^{13}C-mass balance in soils incubated at 10°C, but only 40-55 % in soils at 20°C and 30°C. Higher formation of ^{13}C-NER (particularly the xenobiotic ^{13}C-NER) in soils at 10°C than that at 20°C and 30°C is associated with the slower mineralization of $^{13}C_3$-glyphosate at lower temperature.

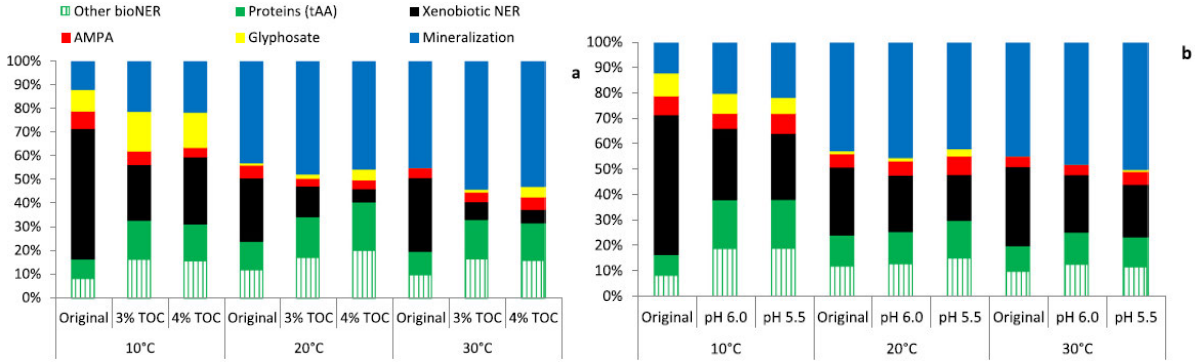


Figure 8.1.1.1-11 Mass balance (normalized to 100 % recovery) of $^{13}C_3$-glyphosate in soil treatments incubated at 10°C, 20°C and 30°C for 39 days (means of three replicates). Proteinaceous bioNER are ^{13}C-tAA. Other bioNER were calculated based on known 50 % content of proteins (^{13}C-tAAs). a: Original soil (2.1 % TOC, pH 6.6) compared to the TOC treatments. b: Original soil (2.1 % TOC, pH 6.6) compared to the pH treatments.

The total recovery of the ^{13}C label from $^{13}C_3$-glyphosate was in the range of 67.6-101.1 % at 10°C, 85.6-102.4 % at 20°C and 97.1-102 % at 30°C. Approximately 72 % of the ^{13}C-NER in the original soil at 10°C was represented by potentially toxic xenobiotic NER and only 38 % by the non-toxic bioNER. Similar observations were made for the ^{13}C-bioNER in the original soil at 20°C and 30°C (38-47 %). Mineralization of $^{13}C_3$-glyphosate in the original soil at each temperature was lower than in the other soil treatments. In addition, the sorption potential of $^{13}C_3$-glyphosate or ^{13}C-AMPA in the original soil (pH 6.6) could be higher than in the other soils resulting in an increased formation of xenobiotic ^{13}C-NER. A decrease of soil pH to pH 6.0 or 5.5 promoted the formation of ^{13}C-bioNER at each temperature and it comprised about 52-62 % of the total ^{13}C-NER. High formation of xenobiotic NER, in particular in the soils with lower pH (6.0 and 5.5) and original soil may be a result of sorption processes of $^{13}C_3$-glyphosate or its transformation product ^{13}C-AMPA.

Amendment of soil with FYM resulted in a major formation of ^{13}C-bioNER which amounted to 72 % (3 % TOC) and 87 % (4 % TOC) of the total NER at 20°C, and to 81 % (3 % TOC) and 85 % (4 % TOC) at 30°C. This agrees well with the highest mineralization and highest amount of extractable $^{13}C_3$-glyphosate in the FYM-amended soils.

The recovery of the ^{15}N label from ^{15}N-glyphosate was in the range of 84-98 % at 10°C, 68-106 % at 20°C and 74-94 % at 30°C. Extractable residues of ^{15}N-glyphosate were slightly higher than those of ^{13}C-glyphosate and ranged from 21 to 32 % (10°C) and 12 to 25 % (20°C and 30°C). The amounts of total ^{15}N-NER were much higher than the ^{13}C-NER and varied from 65 % to 80 % at 10°C, and from 75 % to 85 % at 20°C and 30°C. In contrast with the ^{13}C-bioNER, the ^{15}N-bioNER comprised only a small proportion of the ^{15}N-NER, ranging from 6.2 % to 17 % at 10°C, from 11 % to 16 % at 20°C and from 10 % to 18 % at 30°C.

Similar to what was noticed for the ^{13}C-mass balance, FYM-amendment promoted the formation of ^{15}N-bioNER in soils (14-18 % of ^{15}N-NER). However, xenobiotic ^{15}N-NER had a major share within the ^{15}N-NER even in the FYM amended soil.

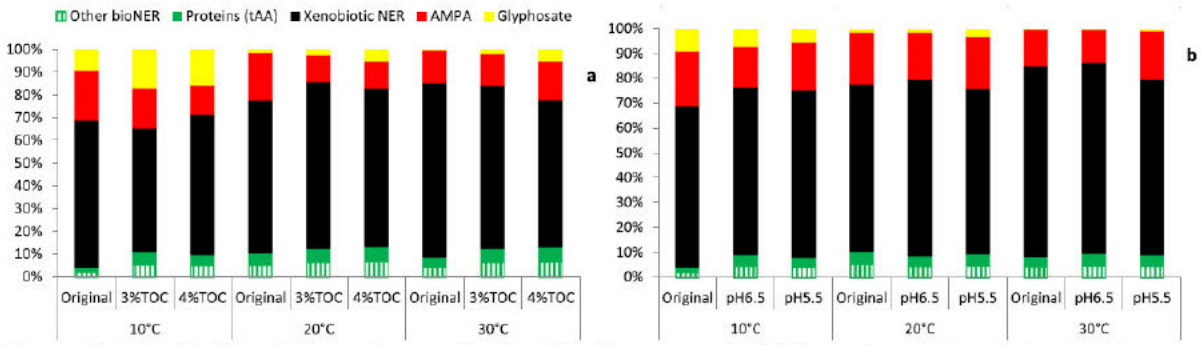


Figure 8.1.1.1-12: Mass balance (normalized to 100 % recovery) of ^{15}N-glyphosate in soil treatments incubated at 10°C, 20°C and 30°C for 39 days (means of three replicates). Proteinaceous bioNER are ^{13}C-tAA. Other bioNER were calculated based on known 50 % content of proteins (^{13}C-tAAs). a: Original soil (2.1 % TOC. pH 6.6) and TOC treatment. b: Original soil (2.1 % TOC. pH 6.6) and pH treatment

The difference in the $^{13}C/^{15}N$ label distribution within the mineralization (^{13}C), extractable, NER and bioNER 10°C between the pH and FYM treatments versus the original soil was more pronounced than at 20 or 30°C. This indicates that both pH and TOC factor play an important role in microbial conversion of $^{13}C_3{}^{15}N$-glyphosate. The microbial metabolism of $^{13}C_3{}^{15}N$-glyphosate was slower at lower temperature; thus, the effect of pH or TOC was more noticeable than that at higher temperatures, where glyphosate/AMPA degradation was already more or less complete according to the results of the SFO kinetic.

Conclusion

Higher temperature and TOC content resulted in enhanced microbial conversion of glyphosate to CO_2 and bioNER. The influence of the pH or TOC treatment on the extent of glyphosate mineralization and bioNER formation was more discernible at lowest temperature highlighting the importance of the soil pH and TOC variations on glyphosate degradation. An amendment of soils with 3 % TOC could be a promising strategy to stimulate the activity of endogenous microorganisms and to accelerate the microbial degradation of glyphosate. However, even in the treatments with highest mineralization and bioNER formation, a small percentage of the ^{13}C was still recovered as xenobiotic NER.

Assessment and conclusion by applicant:

Study of effect of temperature, soil pH, total organic carbon on degradation of ^{13}C and ^{15}N glyphosate to nonaextractable residues. Study conducted in Germany. Provides supplemental information as non-extractable residues are not directly considered in the risk assessment.

Assessment and conclusion by RMS:

The degradation of glyphosate was studied in this article. The extractability of glyphosate in the experiment is very low (0.8 % and 4.5 % (20°C) $^{13}C_3{}^{15}N$-glyphosate equivalents were recovered as $^{13}C_3{}^{15}N$-glyphosate and only 3.9% extractable.

The article provides supportive information on the degradation of glyphosate but no reliable endpoints can be derived for use in risk assessment.

B.8.1.1.1.2. Anaerobic degradation (laboratory studies)

The route of degradation of glyphosate under laboratory anaerobic conditions was investigated in 6 existing studies. No new study was provided in this renewal dossier.

Table 8.1.1.1-52: List of existing studies on anaerobic soil degradation with glyphosate (route)

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021
CA 7.1.1.2/003	[redacted], 2003	Yes, accepted in RAR (2015)	Acceptable

CA 7.1.1.2/002	[redacted], 2004	Yes, considered as supplementary information in RAR (2015)	Supportive
CA 7.1.1.2/004	[redacted], 2000	Yes, accepted in RAR (2015)	Supportive
CA 7.1.1.2/001	[redacted], 2004	Yes, considered as supplementary information in RAR (2015)	Not acceptable
CA 7.1.1.2/005	[redacted], 1987	Yes, accepted in RAR (2015)	Not acceptable
CA 7.1.1.2/006	[redacted], 1972	Yes, not mentioned in RAR (2015) but not accepted in DAR (2001)	Not acceptable

Within the search for peer reviewed scientific literature (2010-2020), no article was identified that would provide information relevant to this data point.

[redacted] *2003*

Data point:	CA 7.1.1.2/003
Report author	[redacted]
Report year	2003
Report title	The degradation of [14C]-Glyphosate in soil under anaerobic conditions
Report No	22581
Guidelines followed in study	OECD 307
Deviations from current test guideline	From OECD 307: none
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate (N-(phosphonomethyl) glycine)

Lot No.: 2213-04.3

Specific activity: 38.79 µCi/mg or 6.56 mCi/mmol

Radiochemical purity: Radiochemical purity 97.28% (HPLC)

2. Soil:

The soil was collected from the upper 20 cm layer of a grassland site by removing surface vegetation and collecting the top soil. Soil was sieved (2 mm) prior to use on the study. The sandy loam soil was supplied by Landlook, Midlands, UK from a site with no pesticide or organic fertiliser treatments for at least five years prior to collection. Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-53: Characteristics of test soil

Parameter	
Soil	Landlook,
Country	UK
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	69.43
Silt (2 µm – 50µmm) (%)	18.85
Clay (< 2 µm) (%)	11.72
pH (KCl)	5.9
Organic carbon (%)	1.8

Cation exchange capacity (meq/100 g)	15.7
Water Holding Capacity at 1/3 bar (%)	16.0
Microbial biomass (mg C/100g)	32.4 (1.8% OC)

B. STUDY DESIGN

1. Experimental conditions

A total of 22 soil samples (ca. 50 g oven dry equivalent) were prepared. The moisture content of each soil sample was adjusted to ca 50 % maximum water holding capacity and maintained at this level throughout the aerobic phase of the study. Soil samples were pre-incubated under aerobic conditions at 20 ± 2 C for 8 days prior to application for an acclimation period.

The dosing solution was prepared by combining an aliquot of non-labelled glyphosate standard with an aliquot of [^{14}C]-glyphosate test substance dissolved in water. The resulting [^{14}C]-glyphosate treatment solution which had a specific activity of 29.96 µCi/mg was used for dosing. Treatment solution was applied drop-wise to the surface of the soil with the radioactive application of 7.82 µCi, equivalent to an application rate of 5.22 mg/kg (dry weight equivalent).

Following test material application, the samples were re-connected to the continuous air flow system and incubated under aerobic conditions at 20 ± 2 C in the dark for a period of 10 days after application of glyphosate (pre-determined aerobic half-life during a preliminary test). The gas mixture leaving each flask was passed through four traps, the first one acting as a safety trap, the second one contained ethanediol to trap non-specific organic volatiles and the final 2 traps contained 2 M sodium hydroxide to trap liberated $^{14}CO_2$.

Following removal of day 10 aerobic incubates, all remaining soil samples were then flooded by the addition of approximately 100 mL milli-Q water to give a depth of 1-3 cm. A stream of moist nitrogen was then introduced to the test system. The samples were maintained under anaerobic conditions at 20 ± 2 C in the dark for a period of 120 days post-flooding. Two additional samples were prepared for the in situ redox measurements during the anaerobic phase. The measured redox potential indicates that the test system achieved anaerobic conditions 14 days post flooding.

2. Sampling

During the aerobic phase (10 days), duplicate soil samples were taken for analysis at zero time (immediately post-application) and at the pre-determined aerobic soil half-life (10 days after application). Duplicate soil samples were removed and analysed at 1 h, and 3, 7, 14, 28, 56, 84 and 120 days post-flooding. Trapping solutions were removed and analysed at the time of removal of the respective incubation flasks.

3. Analytical procedures

For the aerobic phase of the study, soil samples were transferred into centrifuge bottles and extracted three times with 0.5 M ammonium hydroxide (100 mL) using an end-over-end shaker for a period of approximately one hour. After shaking, the extract was separated from the residue by centrifugation (2200 g, 30 min) and the radioactivity in the supernatant was determined by liquid scintillation counting. The quantitative distribution of radiolabelled components in the combined soil extracts was determined using ion exchange HPLC.

For the anaerobic phase of the study, the surface water was separated from soils by decanting. The remaining soils were processed in the same way as aerobic soil sample. Surface waters containing > 5 % of applied radioactivity were also subjected to chromatographic analysis (HPLC and TLC). Following extraction, the radioactivity remaining in the post-extracted soil was determined by combustion analysis.

The distribution of radioactivity in organic matter in selected post-extracted soil samples was determined. Each sample was extracted by shaking in 0.5 M sodium hydroxide (ca 100 mL) for about one hour. The extracts were separated by centrifugation from soil residues and the radioactive content of the soil (humin) was determined by combustion analysis. The extract was adjusted to ca pH 1 using concentrated hydrochloric acid, to precipitate the humic acid fraction. The extract was centrifuged and the supernatant, containing the fulvic acid fraction, was removed and aliquots were submitted for liquid scintillation

counting. Radioactivity associated with the humic acid fraction was quantified by dissolving directly in scintillation fluid.

Prior to chromatographic analysis for each individual soil sample, an aliquot of each extract was combined. All combined soil extracts and surface water samples containing > 5% of the applied radioactivity were analysed using HPLC.

For TLC analyses, aliquots of selected sample extracts and surface waters were applied to Polygram Ionex-25 SA-Na TLC plates which were subsequently developed in 0.01 M potassium dihydrogen phosphate (adjusted to ca pH 2 with concentrated phosphoric acid): methanol (9:1, v/v). Non-radiolabelled glyphosate and AMPA prepared in Milli-Q grade water were chromatographed at each sample. Following chromatography, the areas of radioactivity present on TLC plates were quantified using a Molecular Dynamics phosphor imager or a Fuji FLA5000 phosphor imager. Standards were visualised using Ninhydrin spray reagent.

For combustion analyses, cellulose powder and Combustaid® (ca 100 µL) were added to triplicate portions of air-dried soil residues (ca 0.3 g) prior to combustion in oxygen using a Packard Sample Oxidiser, Model 307. The combusted products were absorbed in Carbo-Sorb®, mixed with Permafluor®E+ and the radioactivity determined by liquid scintillation counting.

All extract aliquots, surface water aliquots, trap solution aliquots and apparatus wash aliquots were added directly to scintillates and submitted for liquid scintillation counting. All radioassays were performed in duplicate. Radioactivity was quantified using a liquid scintillation analyser (Packard 1600TR or Packard 2100TR), with automatic quench correction by external standard-channels ratio. A limit of reliable determination of 30 dpm above background has been instituted in these laboratories.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised below.

Table 8.1.1.1-54: Recovery radioactivity from water and soil under aerobic followed by anaerobic conditions following application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of Applied Radioactivity						
			Water	Soil extract	$^{14}CO_2$[1]	Volatiles[2]	Non-extractable residue	Apparatus wash	Mass Balance
Zero time	Aerobic phase	Rep A	NS	107.42	NS	NS	2.33	NS	109.75
		Rep B	NS	106.08	NS	NS	2.45	NS	108.53
		Mean	-	106.75	-	-	2.39	-	109.14
Day 10		Rep A	NS	75.59	12.37	0.01	15.11	0.01[3]	103.09
		Rep B	NS	76.66	12.50	ND	14.78	0.01[3]	103.95
		Mean	-	76.13	12.44	0.01	14.95	0.01[3]	103.52
1 h	Anaerobic phase	Rep A	0.49	76.96	12.49	ND	12.65	0.05	102.64
		Rep B	0.49	78.18	12.46	ND	13.21	0.02[3]	104.36
		Mean	0.49	77.57	12.48	-	12.93	0.04[3]	103.50
Day 3		Rep A	1.20	72.76	13.13	0.01	16.38	0.01[3]	103.49
		Rep B	1.47	74.61	12.97	0.01	16.73	0.01[3]	105.80
		Mean	1.34	73.69	13.05	0.01	16.56	0.01[3]	104.65
Day 7		Rep A	0.97	71.36	12.14	ND	17.68	0.02[3]	102.17
		Rep B	1.17	70.46	1.64[4]	ND	17.88	0.01[3]	91.16
		Mean	1.07	70.91	6.89	-	17.78	0.02[3]	96.67
Day 14		Rep A	1.82	67.30	13.08	ND	20.22	0.01[3]	102.43
		Rep B	2.06	66.94	13.32	0.01	21.05	0.01[3]	103.39
		Mean	1.94	67.12	13.20	0.01	20.64	0.01[3]	102.91
Day 28		Rep A	5.90	65.25	13.40	0.01	20.57	0.01[3]	105.14
		Rep B	4.36	67.61	13.44	0.01	20.41	ND	105.83
		Mean	5.13	66.43	13.42	0.01	20.49	0.01[3]	105.49
Day 56		Rep A	5.41	62.15	12.18	0.01	24.74	0.01[3]	104.50
		Rep B	5.48	63.06	1.73[4]	0.01	24.52	0.01[3]	94.81

		Mean	5.45	62.61	6.96	0.01	24.63	0.01[3]	99.66
Day 84		Rep A	5.72	62.19	12.52	0.52	22.02	0.03[3]	103.00
		Rep B	6.37	61.73	13.01	0.27	22.53	0.01[3]	103.92
		Mean	6.05	61.96	12.77	0.40	22.28	0.02[3]	103.46
Day 120		Rep A	6.31	61.55	11.89	0.28	22.54	ND	102.57
		Rep B	6.78	60.36	13.13	0.29	22.49	0.01[3]	103.06
		Mean	6.55	60.96	12.51	0.29	22.52	0.01[3]	102.82

[1] trapped with 2M sodium hydroxide
[2] non-specific organic volatiles: trapped with ethanediol
[3] results calculated from data less than 30 dpm above background
[4] low recoveries of $^{14}CO_2$ are probably caused by a leak in the flow-through apparatus
NS = no sample, ND = not detected

Table 8.1.1.1-55: Characterization of radioactivity in soil extracts under aerobic followed by anaerobic conditions following application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of applied radioactivity			
			Soil extracts			
			Glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aerobic phase	Rep A	104.41	3.01	ND	ND
		Rep B	101.20	4.88	ND	ND
Day 10		Rep A	55.85	18.92	0.82	ND
		Rep B	54.05	19.38	ND	3.23
1 h	Anaerobic phase	Rep A	57.50	19.46	ND	ND
		Rep B	56.99	21.19	ND	ND
Day 3		Rep A	53.35	19.41	ND	ND
		Rep B	54.85	19.76	ND	ND
Day 7		Rep A	53.08	18.28	ND	ND
		Rep B	51.84	18.62	ND	ND
Day 14		Rep A	46.38	20.92	ND	ND
		Rep B	46.44	20.49	ND	ND
Day 28		Rep A	39.06	26.19	ND	ND
		Rep B	42.37	25.24	ND	ND
Day 56		Rep A	31.59	30.56	ND	ND
		Rep B	40.52	22.54	ND	ND
Day 84		Rep A	32.85	29.34	ND	ND
		Rep B	31.74	29.99	ND	ND
Day 120		Rep A	31.62	29.93	ND	ND
		Rep B	33.40	26.96	ND	ND

NS = no sample, ND = not detected, NP = not profiled as < 5% applied radioactivity in sample

Table 8.1.1.1-56: Characterization of radioactivity in water under aerobic followed by anaerobic conditions after application of [14C]-glyphosate

Time point	Study phase	Sample	% of applied radioactivity			
			Water			
			Glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aerobic phase	Rep A	NS	NS	NS	NS
		Rep B	NS	NS	NS	NS
Day 10		Rep A	NS	NS	NS	NS
		Rep B	NS	NS	NS	NS
1 h	Anaerobic phase	Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 3		Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 7		Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 14		Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 28		Rep A	5.90	ND	ND	ND
		Rep B	NP	NP	NP	NP

Day 56		Rep A	5.41	ND	ND	ND
		Rep B	5.48	ND	ND	ND
Day 84		Rep A	5.46	0.26	ND	ND
		Rep B	5.47	0.90	ND	ND
Day 120		Rep A	6.31	ND	ND	ND
		Rep B	6.78	ND	ND	ND

NS = no sample, ND = not detected, NP = not profiled as < 5% applied radioactivity in sample

Table 8.1.1.1-57: Characterization of radioactivity in water/soil system under aerobic followed by anaerobic conditions following application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of applied radioactivity			
			Total			
			glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aerobic phase	Rep A	104.41	3.01	ND	ND
		Rep B	101.20	4.88	ND	ND
Day 10		Rep A	55.85	18.92	0.82	ND
		Rep B	54.05	19.38	ND	3.23
1 h	Anaerobic phase	Rep A	57.99[1]	19.46	ND	ND
		Rep B	57.48[1]	21.19	ND	ND
Day 3		Rep A	54.55[1]	19.41	ND	ND
		Rep B	56.32[1]	19.76	ND	ND
Day 7		Rep A	54.05[1]	18.28	ND	ND
		Rep B	53.01[1]	18.62	ND	ND
Day 14		Rep A	48.20[1]	20.92	ND	ND
		Rep B	48.50[1]	20.49	ND	ND
Day 28		Rep A	44.96	26.19	ND	ND
		Rep B	46.73[1]	25.24	ND	ND
Day 56		Rep A	37.00	30.56	ND	ND
		Rep B	46.00	22.54	ND	ND
Day 84		Rep A	38.31	29.60	ND	ND
		Rep B	37.21	30.89	ND	ND
Day 120		Rep A	37.93	29.93	ND	ND
		Rep B	40.18	26.96	ND	ND

NS = no sample, ND = not detected, NP = not profiled as < 5% applied radioactivity in sample
[1] Radioactivity in surface water for these samples accounted for <5% applied activity. It was assumed to be glyphosate and was included in total.

B. MASS BALANCE

The mean total recoveries from the sand loam soil incubated for 10 days, during the aerobic phase of the study were in a range of ca. 104 % to 109 % AR (mean of replicates).

The mean recoveries under anaerobic conditions incubated subsequently for up to 120 days were in the range of ca. 97 to ca. 105 % AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Extractable radioactivity accounted for 107 % AR at zero time (aerobic phase), declining to 63 % AR after 56 days post flooding (anaerobic phase), remaining relatively constant.

Radioactivity associated with surface water following flooding was observed to increase slowly from < 1 % applied after 1 hour to 5 % after 28 days. At day 120 levels of radioactivity associated with the surface water accounted for 7 % AR.

Non-extractable residue increased from a minimum of 2 % AR at zero time to a maximum of 25 % after 56 days. At day 120, levels recovered as non-extractable residues accounted for 23 % AR (mean of two replicates).

D. VOLATILE RADIOACTIVITY

$^{14}CO_2$ was observed during the aerobic phase of the study and accounted for 12 % of applied radioactivity in the majority of study samples prior to flooding. Production of $^{14}CO_2$ after initiation of anaerobic conditions decreased to levels of less than 2 % of applied radioactivity for the remainder of incubation. Radioactivity associated to ethanediol trap (non-specific volatiles) and apparatus washings accounted for < 0.1 % of applied radioactivity.

E. TRANSFORMATION OF THE TEST ITEM

At zero time (aerobic), levels of glyphosate in the water/soil system were quantified and subsequently declined to 55.0 % (mean of two replicates) after 10 days of aerobic incubation. Upon initiation of anaerobic conditions (1-hour post-flooding), levels of glyphosate accounted for 57.7 % applied radioactivity and decreased to 39.1 % after 120 days of incubation under anaerobic conditions.

The only significant degradation product detected was AMPA. At zero time AMPA accounted for 3.9 % applied radioactivity, increasing to a maximum of 30.2 % after 84 days and subsequently declining to 28.4 % after 120 days of incubation (all values representing mean of two replicates).

No other compounds were detected above 5 % AR at any time.

F. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.1.3/001).

III. CONCLUSIONS

Glyphosate was degraded rapidly during the aerobic period of the study. The only significant metabolites detected were AMPA and $^{14}CO_2$. Following initiation of anaerobic conditions, the rate of degradation was observed to slow down significantly. During the anaerobic phase, liberation of $^{14}CO_2$ was significantly reduced when compared to the aerobic ageing period, and AMPA was the only significant metabolite.

Assessment and conclusion by applicant:

The study adequately describes the degradation behavior of glyphosate in soil under anaerobic conditions of the laboratory. No deficiencies or deviations occurred. The study was used for subsequent kinetic evaluation following latest EU guidance.

The study is considered valid to address the data point.

Assessment and conclusion by RMS:

The study is well conducted. No deviation from OECD 307 is identified.

The study is acceptable.

[redacted] *2003*

Data point:	CA 7.1.1.2/002
Report author	[redacted]
Report year	2003
Report title	Route and Rate of Anaerobic Soil Degradation of Glyphosate According to SETAC, Part 1, 1.2 (March 1995)
Report No	IF-02/00005224
Guidelines followed in study	SETAC, Part 1, 1.2 (March 1995)
Deviations from current test guideline	From OECD 307: - Application of test substance to water layer after establishment of anaerobic conditions (no aerobic incubation phase prior flooding)
GLP/Officially recognised testing facilities	Yes

Previous evaluation	Yes, considered as supplementary information in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled test material

Identification: [^{14}C]-glyphosate

Lot No.: Amersham Pharmacia CFQ 12960/BE9180

Specific activity: 57 mCi/mg

Radiochemical purity: 98.1 %

Non-radiolabelled test compound

Identification: Glyphosate

Lot No.: sigma Aldrich 1025x

Chemical purity: 99.1 %

2. Soil:

Soil was sieved to ≤ 2 mm. The soil was received and stored in a sealed transport container at ambient temperature. It had no pesticide application for several years. Characteristics of the test soil are presented in the table below. The sieved soil was stored for 1 week under aerobic conditions in the dark at approximately 8°C.

Table 8.1.1.1-58: Characteristics of test soil

Parameter	Results
Soil	Hofheim
Country	Germany
Textural Class (USDA)	Silt loam
Sand (50 µm – 2 mm) (%)	29.9
Silt (2 µm – 50µmm) (%)	52.3
Clay (< 2 µm) (%)	17.8
pH (water)	6.06
pH ($CaCl_2$)	5.10
Organic carbon (%)	1.24
Organic matter (%)	2.14
Cation exchange capacity (meq/100 g)	13.5 meq/100g dry soil
Maximum Water Holding Capacity (%)	43.0
Bulk Density (disturbed) (g/cm^3)	1119 g/L
Microbial biomass after sampling (initial value) (mg C/100g dry soil)	24 (1.9% OC)
Microbial biomass at study end (day 120) (mg C/100g dry soil)	3

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Soil equivalent to 100 g dry weight (moistened to approximately 40 % maximum water holding capacity) were bottled into 1000 mL glass vessels. Bottled soil was flooded by addition of reagent water (water column height ca. 2 cm) and maintained under a dynamic atmosphere of nitrogen gas, in the dark, at 20 C°. Nitrogen gas leaving the system was passed sequentially through a series of traps to collect any carbon dioxide and organic volatiles produced. The study was carried out with duplicate specimens at each sampling point. Metabolism vessels and trapping system were connected via tubing. On a weekly basis the nitrogen gas stream of approximately 10-15 rnL/min was measured. Flooded soil was acclimatized under a dynamic atmosphere of nitrogen gas. A pre-incubation phase of approximately 2 months was needed to reach an anaerobic equilibrium of the test matrix based on measurable variables (redox potential of water and sediment oxygen concentration of water, and pH-value of water and soil).

The stability of [^{14}C]-glyphosate in the application solution was confirmed by LSC and radio-chromatography before and after application. Reserved test matrix for the determination of aged microbial activity of anaerobic soils was treated at 5.8 mg/kg dry soil using unlabelled glyphosate. .

Each individual test vessel was treated with the application solution to give glyphosate concentrations of 5.8 mg/kg. Aliquots of glyphosate application solution were added using a 250 µl syringe. The test item was applied in reagent water onto the surface of the water phase.

If the water level dropped more than 10 % below 2 cm equivalent to 180 mL (in weight equivalents), oxygen free reagent water was added until the desired water level was obtained. The redox potential of water and soil, oxygen concentration of water and pH value of water and soils were determined at each sampling interval or at about 14 day intervals using specimens for the determination of aged microbial activity of soils, and specimens taken for analysis.

2. Sampling

Duplicated specimen were taken at the following sampling times: zero time, 6 hours, and 1, 7, 14, 32, 60, 90 and 120 days. Aliquots from the volatile traps were radio-assayed at each sampling time (excluding zero time) or at about 14 day intervals, whichever came first.

3. Analytical procedures

After removal of the water phase from the test system by decantation, water and soil were assayed separately for their radiocarbon concentration and their radiocarbon composition.

The soils were transferred quantitatively into 750 mL vessels and extracted several times by shaking with 100 mL of 1 M Nik-solution. The extraction solvent was separated from the soil by centrifugation (10 minutes at 4500 rpm). The sequential extractability of radioactivity of each individual extract as well as the combined extraction solutions on a per specimen basis was radio-assayed by liquid scintillation counting (LSC). The final extraction step resulted in < 5 % of the applied radioactivity (% AR). The combined extraction solutions were adjusted to pH 2 by the addition of HCL and again centrifuged. There was no loss radioactivity during acidification of specimens.

Specimen extracts were subjected to further concentration using freeze drying if necessary, and specimen residues were reconstituted with reagent water. The volume of the specimen, extract concentrate was measured and subjected to LSC for confirmation that there was no loss of radioactivity during specimen concentration. Processed specimen extracts were subjected to radio-chromatography (HPLC and TLC). Each chromatographic analysis was performed in duplicate.

After exhaustive soil extraction the residual radioactivity in soils was assayed by combustion. Remaining soil was stored at room temperature in tightly closed storage containers.

The extracted soils of the 120 day samplings (air dried-and ground) were subjected to further characterisation of soil radioactivity which remained bound to the humic and fulvic acids and the humin fraction.

After separation from the soil phase, the volume of the water phase was measured and aliquots analysed by LSC. A concentration of the water phases was performed by freeze drying, if needed. Specimen residues were reconstituted with reagent water. The volume of the specimen extract concentrate was measured and subjected to LSC for confirmation that there was no loss of radioactivity during specimen concentration. Processed specimen extracts were subjected to radio-chromatography (HPLC and TLC) in duplicate.

Radioactivity in solution was determined by liquid scintillation counting (LSC) in triplicate per specimen.

Thin Layer Chromatography (TLC) was performed on pre-coated plates of lonex-25 SA-Na. One dimensional thin layer chromatography was used for the separation of specimen extract aliquots. The TLC plates were developed under chamber saturation conditions with a general target development distance of approximately 16 cm.

HPLC was based on a glyphosate cation-exchange column by Pickering. After HPLC separation of specimen aliquots, radioactive signals were detected by means of a radioactivity monitor/UV-photometer. The resulting peaks observed by the radioactive monitor were taken and quantified in relation to the summed radiochemical signals of the run time of interest (% area). Radioactive signals were quantified and characterised by comparing their retention time with the retention times of the pure reference items.

The identification of CO_2 was performed by precipitation $BaCO_3$ using barium chloride.

II. RESULTS AND DISCUSSION

A. DATA

pH values in water layer and soil remained relatively constant during the study. The water and soil phases remained anaerobic during the test period, reflected by negative redox potential and an oxygen concentration of zero in the water phases during all the study period.

Table 8.1.1.1-59: Recovery of radioactivity in water and soil under anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

Fraction	Replicate	DAT								
		0	0.25	1	7	14	32	60	90	120
Carbon dioxide	A	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
	B	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
	Mean	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
Volatile organic compounds	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total water	A	96.5	65.3	42.7	36.5	24.0	8.5	5.5	3.5	5.8
	B	96.4	62.2	44.5	38.1	17.7	7.0	6.9	4.7	5.7
	Mean	96.5	63.8	43.6	37.3	20.9	7.8	6.2	4.1	5.8
Total extractables soil	A	1.9	25.4	41.1	40.4	51.6	51.8	50.2	45.1	35.8
	B	1.8	28.2	40.7	37.5	54.7	53.4	49.5	42.8	32.7
	Mean	1.9	26.8	40.9	39.0	53.2	52.6	49.9	44.0	34.3
Non-extractable Residues	A	0.5	3.4	9.7	19.8	20.1	32.2	24.3	31.7	34.4
	B	0.6	7.4	8.7	21.8	27.0	29.9	29.3	31.5	37.8
	Mean	0.6	5.4	9.2	20.8	23.6	31.1	26.8	31.6	36.1
Mass balance	A	98.9	94.2	93.7	97.3	96.8	98.5	95.3	99.4	96.1
	B	98.8	97.9	94.1	98.0	100.5	96.3	101.0	98.1	96.3
	Mean	98.9	96.1	93.9	97.7	98.7	97.4	98.2	98.8	96.2

DAT: days after treatment
nd: not detected
np: not performed

Table 8.1.1.1-60: Characterisation of radioactivity in water and soil extracts under anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

Compound		Replicate	DAT								
			0	0.25	1	7	14	32	60	90	120
Glyphosate	Water	A	92.9	59.4	41.3	34.0	20.9	7.5	4.8	1.5	2.1
		B	93.0	58.3	42.2	36.0	15.8	5.8	6.2	2.4	2.3
		Mean	93.0	58.9	41.8	35.0	18.4	6.7	5.5	2.0	2.2
	Soil	A	n.p.	4.7	7.4	8.1	8.2	5.8	20.6	19.5	19.5
		B	n.p.	6.2	7.5	8.5	8.3	9.5	17.4	19.1	17.1
		Mean	n.p.	5.5	7.5	8.3	8.3	7.7	19.0	19.3	18.3
AMPA	Water	A	2.7	3.0	0.8	1.5	1.4	0.4	0.8	1.2	3.2
		B	2.2	2.3	1.1	1.2	1.3	0.6	0.8	1.4	3.0

Table 8.1.1.1-60: Characterisation of radioactivity in water and soil extracts under anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

Compound		Replicate	DAT								
			0	0.25	1	7	14	32	60	90	120
		Mean	2.5	2.7	1.0	1.4	1.4	0.5	0.8	1.3	3.1
	Soil	A	n.p.	4.7	7.4	8.1	8.2	5.8	20.6	19.5	19.5
		B	n.p.	6.2	7.5	8.5	8.3	9.5	17.4	19.1	17.1
		Mean	n.p.	5.5	7.5	8.3	8.3	7.7	19.0	19.3	18.3
Largest unknown	Water	A	1.0	1.5	0.6	1.0	0.7	0.5	nd	0.7	0.6
		B	1.3	1.1	0.7	1.0	0.4	0.6	nd	0.9	0.5
		Mean	1.2	1.3	0.7	1.0	0.6	0.6	nd	0.8	0.6
	Soil	A	n.p.	nd	nd	nd	1.1	1.6	nd	nd	2.4
		B	n.p.	nd	nd	nd	1.3	1.1	nd	nd	2.0
		Mean	n.p	nd	nd	nd	1.2	1.4	nd	nd	2.2
All unknowns	Water	A	1.0	3.1	0.8	1.0	1.8	0.8	nd	0.9	0.6
		B	1.3	1.6	1.3	1.0	0.9	0.6	nd	1.1	0.5
		Mean	1.2	2.4	1.1	1.0	1.4	0.7	nd	1.0	0.6
	Soil	A	n.p.	nd	nd	nd	1.8	2.6	nd	nd	3.1
		B	n.p.	nd	nd	nd	2.1	1.7	nd	nd	2.8
		Mean	n.p	nd	nd	nd	2.0	2.2	nd	nd	3.0

Table 8.1.1.1-61: Characterisation of radioactivity in flooded soil (sum of water and soil extracts) under anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	7	14	32	60	90	120
Glyphosate	A	92.9	80.2	75.0	66.4	62.5	51.0	34.4	27.1	15.5
	B	93.0	80.3	75.4	65.0	60.2	48.1	38.3	26.2	15.3
	Mean	93.0	80.3	75.2	65.7	61.4	50.6	36.4	26.7	15.4
AMPA	A	2.7	7.7	8.2	9.6	9.6	6.2	21.4	20.7	22.7
	B	2.2	8.5	8.6	9.7	9.6	10.1	18.2	20.5	20.1
	Mean	2.5	8.1	8.4	9.7	9.6	8.2	19.8	20.6	21.4
Largest unknown	A	1.0	1.5	0.6	1.0	1.8	2.1	nd	0.7	3.0
	B	1.3	1.1	0.7	1.0	1.7	1.7	nd	0.9	2.5
	Mean	1.2	1.3	0.7	1.0	1.8	1.9	nd	0.8	2.8
All unknowns	A	1.0	3.1	0.8	1.0	3.6	3.4	nd	0.9	3.7
	B	1.3	1.6	1.3	1.0	3.0	2.3	nd	1.1	3.3
	Mean	1.2	2.4	1.1	1.0	3.3	2.9	nd	1.0	3.5

Table 8.1.1.1-62: Fractionation of day 120 post extracted soil (in percent of applied radioactivity)

Experiment	Fulvic acid	Humic acid	Humin
120 DAT A 20°C	6.3	3.8	24.4
120 DAT B 20°C	5.2	2.6	29.9

B. MASS BALANCE

The mass balance range for the individual sampling times (0, 6 hrs, 1, 7, 14, 32, 60, 90, and 120 days) was 93.7 to 101.0 % AR.

Radioactivity disappeared very fast from the treated water phases. At zero time 96.4 and 96.5 % AR were found in the soil surface water. After 1 day of incubation approximately 40 % AR were detected in the water phases. At experimental end (120 day's) the remaining radioactivity in the water phases was 5.8 % AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The extractable radioactivity in the soil increased over time, reaching a plateau value of approximately 50 % AR by day 14 to 60. At experimental end the soil extractable radioactivity was reduced to 32.7 and 35.8 % AR. Respectively.

The non-extractable radioactivity in the soils increased over time. The residual radioactivity in the soil by day 120 was 34.4 and 37.8 % AR.

D. VOLATILE RADIOACTIVITY

After 120 days of anaerobic incubation the amounts of CO_2 evolved accounted for 20.1 % AR maximum. Liberated volatile organics were below 0.1 % of the applied radioactivity at experimental end (120 days).

E. TRANSFORMATION OF THE TEST ITEM

Glyphosate was present at zero time in the assayed water phases with an average value of approximately 93 % AR. It is level decreased rapidly to approximately 40 % and 5 % AR (equivalent to 2.32 and 0.29 mg/kg) at the 1 day and 60 day sampling times, respectively. Glyphosate was found at the 6 hour time in the processed soil extractable radioactivity with an average value of approximately 21 % AR. It is level increased to approximately 43 % AR in the processed soil extractable radioactivity of day 32. At experimental end (120 days) glyphosate accounted for approximately 13 % AR in the processed soil extractable radioactivity.

In the processed water and soil extracts unknown metabolites reached maximum levels less than 5 % AR (maximum: 2.4 % AR in the soil and 3.0 % AR in the total test system) of the applied radioactivity. The major metabolite of glyphosate, namely AMPA (aminomethylphosphonic acid), was identified by HPLC- and TLC- chromatographic analysis using a known reference item. AMPA was identified in the soil and water specimens of the test system and reached a maximum level of below 5 % AR in the water phases. In the processed soil extractable radioactivity AMPA underwent a plateau value of approximately 20 % AR by day 60 to 120. Further, glyphosate was converted in both anaerobic compartments of the test system into unknown degradates of low concentration (3.0 % AR maximum fraction in the total test system equivalent to 0.17 mg/kg parent equivalents).

F. KINETICS

No DT_{50} were calculated in the study. New kinetic calculations based on recent guidance were not provided due to the supporting character of the study.

III. CONCLUSIONS

Glyphosate degraded rapidly in the water phase of the test system.

The major degradation products of glyphosate produced under anaerobic conditions were AMPA and carbon dioxide. AMPA was found in the water specimens and predominantly in the soil phases of the test system.

AMPA reached a maximum level of below 5 % AR in the water phase in the processed soil extractable radioactivity AMPA underwent a plateau value of approximately 20 % of the applied radioactivity (% AR) to the test system. The evolved CO_2 accounted for 20 % AR at experimental end. Unknown degradation products of low concentration (3.0 % AR maximum fraction in the total test system equivalent to 0.17 mg/kg parent equivalents) were formed in the flooded soil system.

The appearance of AMPA, the formation of bound residues, and the formation of carbon dioxide reflect the anaerobic degradation pathway of glyphosate.

Assessment and conclusion by applicant:

The study provides information on the degradation behavior of glyphosate in soil under established thus strict anaerobic conditions. Such application to strictly anaerobic conditions (50 days) is not in agreement with the current guideline.

Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

The study design does not follow OECD 307 but follows the one defined in SETAC 1995. No deviation from SETAC 1995 is identified.

The study is considered as supportive.

[redacted] *2000*

Data point:	CA 7.1.1.2/004
Report author	[redacted]
Report year	2000
Report title	The degradation of [^{14}C]-Glyphosate in soil under anaerobic conditions
Report No	18201
Guidelines followed in study	SETAC (1995)
Deviations from current test guideline	From OECD 307: - Application of test substance to samples following establishment of strict anaerobic conditions (no aerobic incubation phase prior flooding)
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled test material

Identification: [^{14}C]-glyphosate

Lot No.: C-2417.2

Specific activity: 12.35 µCi/mg)

Radiochemical purity: 99.2 %; 100.0 % (HPLC) 97.6 % (TLC)

Non-radiolabelled test compound

Identification: Glyphosate

Lot No.: GLP-9606-7189-A

Chemical purity: 99.92 % (impurities weight: [redacted], [redacted], [redacted], [redacted])

2. Soil:

A freshly collected sample of sandy loam was used. The soil was collected from the upper 20 cm layer of a grassland site by removing surface vegetation and bagging the top soil immediately below. Characterisation data is presented in the table below. Soil was sieved (2 mm) prior to use on the study and its moisture content determined.

Table 8.1.1.1-63: Characteristics of test soil

Parameter	
Soil	PT 200
Country	not indicated
Textural Class (USDA)	Loamy sand
Sand (50 µm – 2 mm) (%)	67.2
Silt (2 µm – 50µmm) (%)	16.1
Clay (< 2 µm) (%)	16.7
pH (KCl)	5.8

Organic carbon (%)	1.7
Organic matter (%)	2.9
Cation exchange capacity (meq/100 g)	18.2
Water Holding Capacity at 0 bar (%)	65.3
Water Holding Capacity at 1/3 bar (%)	19.0
Microbial biomass prior to study initiation (mg C/100 g oven dry soil equivalent)	67 (3.9% OC)

B. STUDY DESIGN

1. Experimental conditions

Samples of soil (20, including 4 for contingency purposes; ca 50 g oven dry equivalent) were weighed into previously silanised Erlenmeyer flasks (250 mL capacity). Milli-Q grade water was added to each flask to create a layer of water (ca 3 cm depth; ca 135 g) over the soil. The depth of water of 3 cm was maintained for the duration of the study. Additionally, 2 units were prepared in Erlenmeyer flasks fitted with side-arm attachments. A platinum combination redox electrode was placed in each side-arm to allow in situ redox measurements at the base of the soil during the incubation period. A stream of moist, O_2-free nitrogen, at a flow rate of ca 5-15 mL min^{-1}, was passed over the surface of each sample. The gas mixture leaving each flask was passed through a series of 3 traps. The first trap was a safety trap to prevent back flow, the second contained ethanediol to trap non-specific ^{14}C-organic volatiles and the third trap contained ethanolamine to trap liberated $^{14}CO_2$. Air leaving each incubation unit were combined and passed over a copper II oxide catalyst at ca 800°C to oxidise any radioactivity to $^{14}CO_2$ (which was subsequently trapped in ethanolamine). Connections between traps and incubation flasks were made using a combination of glass connectors and PVC tubing.

The flooded soils were pre-incubated under an atmosphere of nitrogen for 39 days in the dark at a nominal temperature of 20°C. During the pre-incubation period the redox potential of the two control units was measured and the establishment of anaerobic conditions was confirmed when a redox potential of less than 200 mV was obtained.

Separate stock solutions of [^{14}C]-glyphosate and non-radiolabelled glyphosate were prepared in Milli-Q grade water and aliquots of each stock, containing 4.98 mg of [^{14}C]-glyphosate and 7.62 mg of non-radiolabelled glyphosate respectively, transferred to a volumetric flask and filled up to 5 mL with Milli-Q grade water Test solution (100 µl), containing 0.252 mg of glyphosate was applied dropwise to the surface of the water in each incubation flask. The application rate was 5.04 mg per kg soil (oven dry equiv.). The radioactive application to each sample was determined as 7.41 µCi. Following test material application, the samples were re-connected to the continuous gas flow system. The samples were then incubated in the dark at a nominal temperature of 20°C for up to 120 days.

2. Sampling

Duplicate incubates were sampled immediately following application of test solution, 3, 7, 14, 30, 60, 90 and 120 days. At each sampling interval, the redox potential of the soil and pH of the surface water were recorded.

Traps were sampled and replenished at regular intervals throughout the incubation period. Trapping solutions associated with the catalytic converter were stored at ambient temperature and not analysed further.

3. Analytical procedures

Surface waters were separated from soils by careful decanting. The soil residues were transferred into separate Nalgene® centrifuge bottles and extracted with 0.5 M ammonium hydroxide (3 x ca 100 mL; ca 1 h) and end-over-end shaking. After shaking, the extract was separated from the residue by centrifugation (ca 3500 r.p.m.; ca 30 min) and the amount of radioactivity in the supernatant determined by liquid scintillation counting. Surface water and soil extracts were subjected to HPLC and TLC analyses.

Following extraction, the radioactivity remaining in the soil was determined by combustion analysis in order to quantify residual radioactive content. The organic matter in selected extracted residues (Flask 21, 90 DAT and Flask 23, 120 DAT) was then fractionated. Each sample was extracted with 0.5 M sodium hydroxide (2 x ca 20 mL) by shaking (ca 30 min) and sonication (ca 5 min). The extracts were separated from the residue by centrifugation (ca 1000 r.p.m.; ca 15 min) and the radioactive content of the soil (humin) was determined by combustion analysis. The pH of the combined sodium hydroxide extracts was adjusted to ca 1 using concentrated hydrochloric acid and stirring, to precipitate out the humic acid fraction. The sample was then centrifuged and the supernatant, which contained the fulvic acid fraction, removed. The humic acid fraction was quantified by oxidation of the precipitate.

Following decanting, aliquots of each surface water were submitted for liquid scintillation counting followed by HPLC and TLC analyses. Following trap sampling, aliquots of each solution were submitted for liquid scintillation counting. After removal of samples from the flasks, the flasks were soaked in acetone to remove any residual radioactivity. Aliquots of each apparatus wash were submitted for liquid scintillation counting.

Radiolabelled glyphosate and its degradation products extracted from soil and present in the surface water were characterised and quantified by HPLC with TLC as confirmatory method. For each individual sample, an aliquot (ca 10% by volume) of each of its extracts was combined. For HPLC analysis, the pH of a sub-sample of each combined extract and surface water was adjusted to ca 2-3 using concentrated phosphoric acid, prior to chromatographic analysis. Quantification of radioactivity was determined by collecting fractions of HPLC column eluate (1 min intervals) and submitting these for liquid scintillation counting. Reference substances (glyphosate, AMPA, MAMPA and HMPA) were used to determine the order of elution and standard retention times.

Further preparation of samples for TLC analysis was required to optimise chromatographic resolution. To an aliquot of each pH adjusted combined extract sample, 0.1 M EDTA (50 pl) was added and the solution sonicated prior to chromatographic analysis. For the surface water samples, an aliquot of the original sample was mixed with 0.5 M ammonium hydroxide, the pH adjusted to ca 2-3 using concentrated phosphoric acid and the sample centrifuged. 0.1 M EDTA was added to a sub-sample of the supernatant and the sample sonicated prior to chromatographic analysis. For TLC analysis aliquots of each sample extract and surface water were applied to a Polygram lonex-25 SA-Na TLC plate (Macherey-Nagei, Germany) which was then developed in 0.015 M potassium dihydrogen phosphate (adjusted to ca pH 2.4 with concentrated phosphoric acid): methanol (9:1, v/v). Following chromatography, the areas of radioactivity present on TLC plates were quantified using a Molecular Dynamics phosphor imager. Standards were visualised using ninhydrin spray reagent. The limit of quantification for determination of radioactivity is 30 d.p.m. above the background (not given). No detailed information on the limit of detection (LOD) and limit of quantification (LOQ) is provided.

4. Determination of degradation rates

The mean percent of applied radioactivity present as glyphosate from the HPLC data in the surface water and the total flooded system (sum of the surface water and soil) was plotted against the incubation time at each sampling interval using the Timme, Frehse and Laska model (Bayer AG, Monheim, Germany). The model has not been subjected to full GLP validation at Inveresk, but has been validated by Bayer AG. DT_{50} values for the surface water and the total system were obtained by selecting the curve that gave the best fit to the data.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]-glyphosate and metabolites in soil extracts are summarised below for the sandy loam soil.

Table 8.1.1.1-64: Recovery of radioactivity of [^{14}C]-glyphosate applied to sandy loam under anaerobic conditions (expressed as percent of applied radioactivity)

Sampling Interval	Flask Number	Percentage of Applied Radioactivity Recovered as:

		Water	Soil Extracts	^{14}C-Organic Volatiles	$^{14}CO_2$	Non-extractable Residue	Apparatus Wash	Total
0 DAT	8	92.59	8.04	NS	NS	1.63	ND	102.27
	9	93.83	7.35	NS	NS	1.37	ND	102.56
	Mean	93.21	7.70	-	-	1.50	-	102.41
3 DAT	10	45.40	40.00	ND	0.07	9.95	0.04*	95.45
	11	49.34	39.08	ND	0.05	8.76	0.02*	97.25
	Mean	47.37	39.54	—	0.06	9.35	0.03	96.35
7 DAT	12	31.71	50.69	ND	0.12	12.27	0.05*	94.83
	13	33.31	50.62	ND	0.08	13.37	0.02*	97.40
	Mean	32.51	50.66	-	0.10	12.82	0.03	96.12
14 DAT	14	16.42	57.02	ND	0.21	18.37	ND	92.03
	15	19.30	61.42	ND	0.20	21.54	0.01*	102.47
	Mean	17.86	59.22	-	0.20	19.96	0.01	97.25
30 DAT	16	14.98	60.57	0.02	0.66	18.64	0.02*	94.89
	17	15.33	61.56	0.02	0.71	18.23	0.02*	95.86
	Mean	15.15	61.06	0.02	0.68	18.44	0.02	95.38
60 DAT	18	12.74	66.29	0.02	0.74	20.56	0.02*	100.37
	19	12.01	70.46	0.21	0.57	20.16	0.02*	103.43
	Mean	12.38	68.37	0.11	0.66	20.36	0.02	101.90
90 DAT	20	10.91	62.88	0.22	0.93	22.76	0.02*	97.71
	21	9.98	64.04	0.02	0.77	25.48	0.02*	100.30
	Mean	10.44	63.46	0.12	0.85	24.12	0.02	99.00
120 DAT	22	10.53	64.80	0.04	0.79	20.87	0.01*	97.05
	23	10.40	64.79	0.02	0.95	20.89	0.01*	97.07
	Mean	10.46	64.79	0.03	0.87	20.88	0.01	97.06

NS = No sample
ND = Not detected
*= Results calculated from data less than 30 d.p m. above background

Table 8.1.1.1-65: Characterisation of radioactivity in water following application of [^{14}C]-glyphosate under anaerobic conditions (expressed as percent of applied radioactivity) with HPLC

Sampling Interval	Flask Number	Component as a percentage of applied radioactivity		
		Glyphosate	AMPA	HMPA
0 DAT	8	87.04	5.00	0.54
	9	88.41	4.76	0.68
	Mean	87.73	4.88	0.61
3 DAT	10	40.91	4.49	ND
	n	47.04	2.30	ND
	Mean	43.98	3.40	-
7 DAT	12	30.55	1.16	ND
	13	31.75	1.56	ND
	Mean	31.15	1.36	-
14 DAT	14	15.65	0.77	ND
	15	18.36	0.53	0.41
	Mean	17.01	0.65	0.21
30 DAT	16	14.98	ND	ND
	17	14.88	0.45	ND
	Mean	14.93	0.23	-
60 DAT	18	12.74	ND	ND
	19	11.78	0.23	ND
	Mean	12.26	0.12	-

90 DAT	20	10.91	ND	ND
	21	9.98	ND	ND
	Mean	10.45	-	-
120 DAT	22	10.30	0.23	ND
	23	10.12	0.28	ND
	Mean	10.21	0.26	-

NS = No sample
ND = Not detected
*= Results calculated from data less than 30 d.p m. above background

Table 8.1.1.1-66: Characterisation of radioactivity in soil extract following application of [^{14}C]-glyphosate under anaerobic conditions (expressed as percent of applied radioactivity) with HPLC

Sampling Interval	Flask Number	Component as a Percentage of Applied Radioactivity	
		Glyphosate	AMPA
0 DAT	8	7.28	0.76
	9	6.62	0.73
	Mean	6.95	0.75
3 DAT	10	34.42	5.58
	11	34.51	4.57
	Mean	34.47	5.08
7 DAT	12	43.72	6.97
	13	44.65	5.97
	Mean	44.19	6.47
14 DAT	14	50.13	6.89
	15	55.14	6.28
	Mean	52.04	6.59
30 DAT	16	52.90	7.67
	17	53.74	7.82
	Mean	53.32	7.75
60 DAT	18	59.02	7.27
	19	62.34	8.12
	Mean	60.68	7.70
90 DAT	20	56.06	6.82
	21	57.47	6.57
	Mean	56.77	6.70
120 DAT	22	57.31	7.49
	23	58.09	6.70
	Mean	57.70	7.10

NS = No sample
ND = Not detected
*= Results calculated from data less than 30 d.p m. above background

Table 8.1.1.1-67: Characterisation of radioactivity in soil/water system following application of [^{14}C]-glyphosate under anaerobic conditions (expressed as percent of applied radioactivity) with HPLC

Sampling interval	Flask Number	Component as a percentage of applied radioactivity		
		Glyphosate	AMPA	HMPA
0 DAT	8	94.32	5.76	0.54
	9	95.03	5.49	0.68
	Mean	94.68	5.63	0.61
3 DAT	10	75.33	10.07	ND
	11	81.55	6.87	ND
	Mean	78.44	8.47	—

7 DAT	12	74 27	8.13	ND
	13	76.40	7.53	ND
	Mean	75.34	7.83	-
14 DAT	14	65.78	7.66	ND
	15	73.50	6.81	0.41
	Mean	69.64	7.24	0.21
30 DAT	16	67.88	7.67	ND
	17	68.62	8.27	ND
	Mean	66.25	7.97	-
60 DAT	18	71.76	7.27	ND
	19	74.12	8.35	ND
	Mean	72.94	7.81	-
90 DAT	20	66.97	6.82	ND
	21	67.45	6.57	ND
	Mean	67.21	6.70	-
120 DAT	22	67.61	7.72	ND
	23	68.21	6.98	ND
	Mean	67 91	7.35	-

ND = Not detected

B. MASS BALANCE

The mean recovery of applied radioactivity from flooded soil up to and including 120 DAT ranged from 95 % to 102 %.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The total levels of radioactivity extracted from the soil increased from 8 % at zero time to 59 % at 14 DAT and remained around this level for the remainder of the incubation period.

As the total levels of extractable radioactivity increased with time, a concomitant decrease in the levels of radioactivity present in the surface water resulted. At zero time, 93 % of applied radioactivity was associated with the surface water and levels decreased to 18 % by 14 DAT. Beyond 14 DAT, levels of radioactivity in the surface water declined more slowly, accounting for 10 % at study termination.

Radioactivity associated with the non-extractable residue increased form 2 % at zero time to 20 % at 14 DAT and remained around this level for the remainder of the incubation period. Following extraction, the organic matter from single replicates from 90 DAT and 120 DAT was fractionated into humin, humic acid and fulvic acid Radioactivity associated with the humin, fulvic acid and humic acid accounted for up to 9, 10 and 5 %, respectively.

D. VOLATILE RADIOACTIVITY

Radioactivity recovered as $^{14}CO_2$ as non-specific ^{14}C-volatiles and as washings in the apparatus was very low (< 1 %).

E. TRANSFORMATION OF THE TEST ITEM

At zero time, levels of glyphosate in the total flooded test system accounted for 95 % of applied radioactivity. As the incubation progressed, levels of parent compound declined, accounting for 68 % at study termination. In addition to parent compound, low levels of AMPA and HMPA were detected in samples at intervals throughout the study, accounting for up to 8 and 1 % of applied radioactivity, respectively.

F. KINETICS

For test system, estimated DT_{50} is 1699 days, based on square root 2nd order. For water, estimated DT_{50} is 3 days, based on square root 2nd order.

New kinetic calculations based on recent guidance were not provided due to the supporting character of the study.

III. CONCLUSIONS

In conclusion, following incubation in a flooded sandy loam soil, glyphosate disappeared quickly from the aqueous phase of the test system into the soil. Glyphosate slowly degraded to AMPA under anaerobic conditions.

Assessment and conclusion by applicant:

The study provides information on the degradation behavior of glyphosate in soil under established strict anaerobic conditions. Such application to strictly anaerobic conditions (50 days) is not in agreement with the current guideline.

Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

The study design does not follow OECD 307 but follows the one defined in SETAC 1995. No deviation from SETAC 1995 is identified.

The study is considered as supportive.

[redacted] *2004*

Data point:	CA 7.1.1.2/001
Report author	[redacted]
Report year	2004
Report title	[14C]-Glyphosate: Anaerobic Soil Metabolism (Rate and Route of Degradation in a Sandy Loam Soil)
Report No	SNN/05
Guidelines followed in study	None
Deviations from current test guideline	From OECD 307: - No information on soil history - Application of test substance 50 days after flooding (no aerobic incubation phase prior flooding) - Uncertainty regarding the establishment of anaerobic conditions - Discrepancies between chromatograph labelling and characterisation of radioactivity in soil extracts
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, considered as supplementary information in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled Test Material:

Identification: glyphosate, phosphonomethyl-2-^{14}C

Lot No.: 102K9424

Specific activity: 164.28 MBq/mg (4.44 µCi/mg)

Radiochemical purity: 97.0 %

Non-radiolabelled Test Material

Identification: glyphosate

Lot No.: 90K37441

Chemical purity: 96 %

2. Soil:

Soil was sieved to ≤2 mm. The soil was received immediately before testing and was air dried before sieving. Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-68: Characteristics of test soil

Parameter	Results
Soil	Manningtree A
Country	UK
Textural Class (MAFF)	Sandy loam
Sand (%)	66
Silt (%)	26
Clay (%)	8
pH (medium not indicated)	6.5
Organic carbon (%)	1.0
Cation exchange capacity (meq/100 g)	7.9
Maximum Water Holding Capacity (% m/m)	36.4
Water Holding Capacity at 1/3 bar (% m/m)	18.2
Bulk Density (disturbed) (g/cm^3)	1.4
Microbial biomass (mg C/100 g): Before application (0 DAT) Study end (120 DAT)	 16.55 (1.7% OC) 18.49

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of cylindrical glass vessels of 250 mL capacity filled with soil flooded with purified water to a depth of 3 cm. Each vessel was equipped with separate glass-flow system in a series as follows: pre-test system was a Dreschel bottle with sintered stem for uniform gas dispersion containing water to humidify gas flow. This was connected to a glass tube in the test vessel bringing the gas flow just below the water surface. Behind the test vessel an empty bottle was connected to prevent transfer of trapping solutions to the test vessel followed by 3 trapping bottles containing (a) ethyl digol for trapping organic volatiles, (b) 1 M aqueous potassium hydroxide solution with phenolphthalein indicator for trapping CO_2, and (c) ethanolamine/2-ethoxyethanol (1/3, v/v as backup CO_2 trap).

50 g of sieved soil (dry weight equivalents, ca. 52 g wet weight) and 70 mL of purified water were added to each test vessel. To establish anaerobic conditions, the flooded samples were purged with a stream of moist oxygen free nitrogen. Anaerobic conditions were monitored by regular measurement of the redox potential.

The test systems were acclimated for 50 days at test conditions (20 °C) prior to application of test item.

A test solution of [^{14}C]-glyphosate with a concentration of 6.96 mg/mL (38.67 mg diluted in 5.56 mL distilled water) was prepared. Aliquots of 100 µL diluted to 25 mL with distilled water were analysed by LSC. 200 µL of the [^{14}C]-glyphosate solution was applied to each test system. Dose checks confirmed that each test vessel received 0.21 mg [^{14}C]-glyphosate. Based on dose checks, the actual application rate was 4.8 mg/kg soil, corresponding to a field use rate of 3600 g a.s./ha (based on 5 cm depth and 1.5 g/cm^3 bulk density). After application the test vessels (except 0 DAT) were closed with trap attachments.

Test systems were incubated under anaerobic conditions in the dark at 20 ± 2 °C for 120 days in maximum.

2. Sampling

Duplicate test systems were processed and analysed 0, 1, 3, 7, 14, 30, 59, 91 and 120 days after treatment (DAT). All samples were processed on the day of sampling. Trapping media were analyzed and replaced at each sampling interval, then at weekly intervals during the first month and about 10-day intervals thereafter.

3. Analytical procedures

At each sampling interval, the soil and water phase were separated by decanting the water from the test vessel. The total volume of the water layer and concentration of radioactivity in the water was measured. The water was then stored at -15°C until chromatographic analysis.

Soil samples were extracted five times: three times with 0.5 M ammonium hydroxide and 2 times with 1 M hydrochloric acid. Extracts and soil were separated by centrifugation. The volume of each extract was measured and aliquots were analysed by LSC.

Water samples and soil extracts were further analysed by TLC and HPLC.

Radiolabelled components on thin-layer chromatograms were detected and quantified using prelayed cellulose TLC plates, layer thickness 0.25 mm. The developing system was Butanol:Water:Acetic acid with 6:5:2 v/v.

The Radio-HPLC isocratic method used was a Hamilton PRP-X400 cation exchange column run with an aqueous mobile phase at pH 1.9 (5mM potassium phosphate). No limit of quantification (LOQ) or limit of detection (LOD) are given but lowest reported values are 0.1 % AR.

Test item and metabolites were identified by comparison with reference items, however test items are not reported.

The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively. The presence of CO_2 in the potassium hydroxide traps was confirmed by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

For characterisation of unextractable radioactivity selected samples of extracted soils containing >10 % AR were further extracted with 0.5M NaOH solution for 18-24 hours on a rotary shaker at ambient temperature. After centrifugation, the aqueous layer was decanted. The soil debris was rinsed with further 0.5 M NaOH and these solutions combined with the initial 0.5 M NaOH extract for determination of radioactivity by LSC. After air drying, the radioactivity in the soil debris (humin fraction) was determined by combustion/LSC.

The 0.5M NaOH extract was adjusted to pH 1 with concentrated HCl and stored at room temperature for 18-24 hours. After centrifugation, the precipitate was washed with 1M HCI, the solution was combined with the pH 1 extract (fulvic acid fraction) and analyzed with LSC. The radioactivity of the precipitate (humic acid fraction) was measured by combustion/LSC.

The additional approach with an exaggerated application rate is not reported in this summary as no results on the characterisation of radioactivity is given in the report which was the main purpose for this additional setup.

3. Determination of degradation rates

The DT_{50} and DT_{90} were determined using PCModfit version 3.0. The kinetic data were characterised by a non-compartmental analysis (NCA).

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]-glyphosate and metabolites under anaerobic conditions in a sandy loam soil are summarised below for soil and water extracts.

Table 8.1.1.1-69: Recovery of radioactivity in water and soil incubated under anaerobic conditions (expressed as percent of applied radioactivity) following the application of [^{14}C]-glyphosate

		DAT								
Fraction	Replicate	0	1	3	7	14	30	59	91	120
Water	A	97.7	62.6	35.6	32.5	19.6	10.2	7.1	6.1	4.8
	B	96.7	63.9	44.8	26.8	14.4	11.4	9.1	9.6	6.1
	Mean	*97.2*	*63.3*	*40.2*	*29.7*	*17.0*	*10.8*	*8.1*	*7.9*	*5.5*
Carbon Dioxide	A	<0.1	0.4	0.3	0.3	5.2	10.0	14.3	27.8	31.1
	B	<0.1	0.5	0.3	0.3	1.1	11.9	19.0	9.2	20.3
	Mean	*<0.1*	*0.5*	*0.3*	*0.3*	*3.2*	*11.0*	*16.7*	*18.5*	*25.7*
Volatile organic compounds	A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	<0.1	<0.1	<0.1	<0.1	0.3	<0.1	<0.1	<0.1	<0.1
	Mean	*<0.1*	*<0.1*	*<0.1*	*<0.1*	*0.2*	*<0.1*	*<0.1*	*<0.1*	*<0.1*
Total extractable residues	A	0.6	29.4	51.6	50.6	58.7	61.1	58.4	52.4	45.6
	B	0.9	27.2	42.0	62.3	67.0	58.7	52.0	66.8	63.5
	Mean	*0.8*	*28.3*	*46.8*	*56.5*	*62.9*	*59.9*	*55.2*	*59.6*	*54.6*
Non-extractable Residues	A	<0.1	2.3	3.9	6.7	5.0	9.5	11.3	8.5	9.9
	B	<0.1	1.1	5.0	6.5	5.7	8.2	11.5	8.0	11.3
	Mean	*<0.1*	*1.7*	*4.5*	*6.6*	*5.4*	*8.9*	*11.4*	*8.3*	*10.6*
Mass balance	A	98.3	94.7	91.4	90.1	88.5	90.8	91.1	94.8	91.4
	B	97.6	92.7	92.1	95.9	88.2	90.2	91.6	93.6	101.2
	Mean	*98.0*	*93.7*	*91.8*	*93.0*	*88.4*	*90.5*	*91.4*	*94.2*	*96.3*

DAT: days after treatment

Table 8.1.1.1-70: Characterisation of radioactivity in water and soil extracts incubated under anaerobic conditions following treatment with [^{14}C]-glyphosate (expressed as percent of applied radioactivity), HPLC analysis

Water										
		DAT								
Compound	Replicate	0	1	3	7	14	30	59	91	120
Glyphosate	A	93.1	59.4	34.3	21.2	10.6	6.7	0.1	0.3	2.7
	B	93.3	59.9	42.4	25.4	12.5	4.4	1.0	0.5	4.5
	Mean	*93.2*	*59.7*	*38.4*	*23.3*	*11.6*	*5.6*	*0.6*	*0.4*	*3.6*
AMPA (P2)	A	1.8	1.3	0.6	5.3	5.1	1.4	5.1	3.1	1.2
	B	1.8	1.7	0.9	0.7	0.7	2.7	3.6	7.5	1.0
	Mean	*1.8*	*1.5*	*0.8*	*3.0*	*2.9*	*2.1*	*4.4*	*5.3*	*1.1*
P3 (15 min)	A	nd	nd	nd	4.5	2.5	1.5	1.3	0.9	0.6
	B	nd	nd	nd	0.3	0.5	3.4	3.0	1.1	nd
	Mean	-	-	-	*2.4*	*1.5*	*2.5*	*2.2*	*1.0*	*0.6*
Others[1]	A	2.7	1.9	0.7	1.5	1.3	0.6	0.5	1.8	0.4
	B	1.5	2.3	1.1	0.4	0.7	0.8	1.6	0.5	0.6
	Mean	*2.1*	*2.1*	*0.9*	*1.0*	*1.0*	*0.7*	*1.1*	*1.2*	*0.5*
Soil										
		DAT								
Compound	Replicate	0	1	3	7	14	30	59	91	120
Glyphosate	A	0.1	4.0	25.8	11.1	18.7	34.9	nm	11.6	nm
	B	0.1	17.4	16.9	29.8	19.5	nm	15.3	15.4	1.7
	Mean	*0.1*	*10.7*	*21.4*	*20.5*	*19.1*	*34.9*	*15.3*	*13.5*	*1.7*
AMPA (P2)	A	0.5	20.3	8.6	26.8	18.7	18.7	nm	15.0	nm
	B	0.6	5.6	9.1	19.4	19.5	nm	18.1	25.7	44.2
	Mean	*0.6*	*13.0*	*8.9*	*23.1*	*19.1*	*18.7*	*18.1*	*20.4*	*44.2*
P3 (15 min)	A	nd	nd	12.9	3.5	2.8	nd	nm	20.4	nm
	B	nd	nd	7.3	4.7	10.3	nm	11.0	9.4	9.9
	Mean	-	-	*10.1*	*4.1*	*6.6*	-	*11.0*	*14.9*	*9.9*
Others[1]	A	<0.1	5.1	4.3	9.2	18.7	7.4	7.6	5.4	nm
	B	0.2	4.2	8.7	8.5	17.7	nm	nm	16.4	7.7
	Mean	*0.2*	*4.7*	*6.5*	*8.9*	*18.2*	*7.4*	*7.6*	*10.9*	*7.7*

DAT: days after treatment
nd: not detected
nm: not measured
[1] Regions of radioactivity which cannot be assigned to a designated peak

B. MASS BALANCE

Mass balances ranged from 90.5 % to 98.0 % of applied radioactivity except day 14 where the recovery was 88.4 % AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 120 DAT from 97.2 to 5.5 % AR.

In parallel, soil extractable residues increased until 14 DAT from 0.8 and 62.9 % AR and subsequently slightly decreased to 54.6 % AR at 120 DAT.

The amount of non-extractable residues (NER) increased from 0 DAT to 59 DAT from <0.1 to 11.4 % AR and subsequently slightly decreased to 10.6 % AR at 120 DAT.

Fractionation of non-extractable residues into fulvic acid, humic acid and humin fractions in a representative soil sample resulted in ca. 65 % fulvic acid, 2 % AR humic acid and 30 % AR humins.

D. VOLATILE RADIOACTIVITY

Formation of $^{14}CO_2$ increased steadily during the experimental period. Maximum amounts of carbon dioxide reached at study end (120 DAT) were 25.7 % AR. Organic volatiles determined were ≤0.2 % AR.

E. TRANSFORMATION OF THE TEST ITEM

In water, the amount of glyphosate steadily decreased from 0 DAT to 120 DAT from 93.2 to 3.6 % AR. Decrease from water was paralleled by an increase of glyphosate extractable from soil from 0 DAT to 30 DAT from 0.1 to 34.9 % AR and subsequently decrease to 1.7 % AR at 120 DAT.

The metabolite AMPA was found predominantly in soil extracts where it reached a maximum amount of 44.2 % AR at 120 DAT. In water, AMPA was found with maximum 5.3 % AR at 91 DAT.

In the soil extracts, an unknown peak (P3) was observed at levels above 10 % AR (max. 14.9 % AR at 91 DAT). In water, amounts of P3 were ≤2.5 % AR. For the current submission, further attempts were made for identification (see statement below).

No other compounds were detected above 5 % AR at any time.

In soil extracts, up to 18.2 % AR could not be assigned to a distinct peak in chromatographic analysis. In water, unassigned radioactivity was ≤2.1 % AR.

F. KINETICS

Estimated DT_{50} and DT_{90} in water are 15.1 and 49.9 days. Estimated DT_{50} in soil are 19.3 and 64.1 days.

New kinetic calculations based on recent guidance were not provided due to the supporting character of the study.

III. CONCLUSIONS

Glyphosate was rapidly degraded in an anaerobic water/soil system. Glyphosate degraded to AMPA which was then mineralised to carbon dioxide.

Assessment and conclusion by applicant:

The study provides information on the degradation behavior of glyphosate in soil under strict anaerobic conditions in the laboratory. Such application to strictly anaerobic conditions (50 days) is not in agreement with the current guideline. Further considerations on identification of the unknown compound P3 is given below as well as examples for discrepancies in peak identification.

The study is considered as invalid.

The following additional expert statement was provided by the applicant.

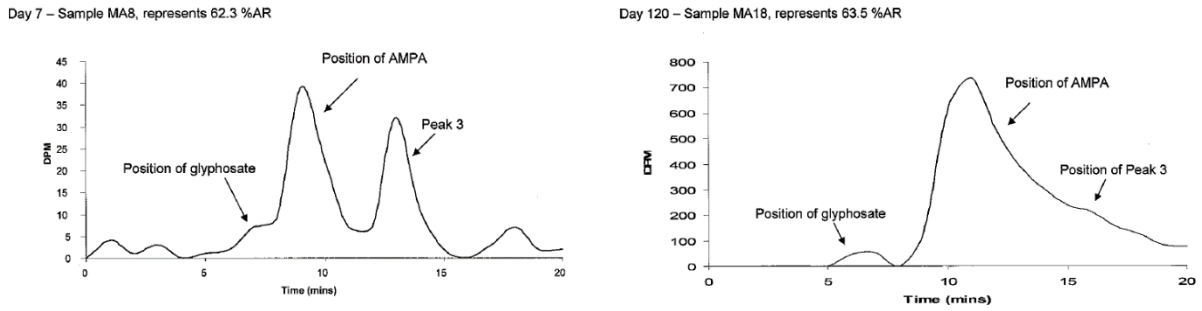
Expert statement to [redacted] (2004, CA 7.1.1.2/001): [^{14}C]-Glyphosate: Anaerobic Soil Metabolism (Rate and Route of Degradation in a Sandy Loam Soil)

This statement compiles additional information on the finding of unknown component "P3" in the study occurring at a maximum of 14.9 % AR after 91 days of incubation.

The Certificate of Analysis in the report identifies the test item as the single radiolabelled compound glyphosate-(phosphonomethyl-^{14}C) which is the monosodium salt of the acid active. The radio-HPLC isocratic method used (Hamilton PRP-X400-poly (styrene-divinyl-benzene) sulfonate cation-exchange column) has an aqueous mobile phase at pH 1.9 which is specifically used for glyphosate. The strong cation exchange column separates glyphosate and AMPA according to the overall positive charge of these molecules. The order of elution is based upon the ionic form of the molecule under the specific acidic pH conditions and the more positive a molecule, the longer the retention time. Hence, glyphosate elutes first, followed by AMPA. Both, glyphosate and AMPA, are present as zwitterions at pH 1.9.

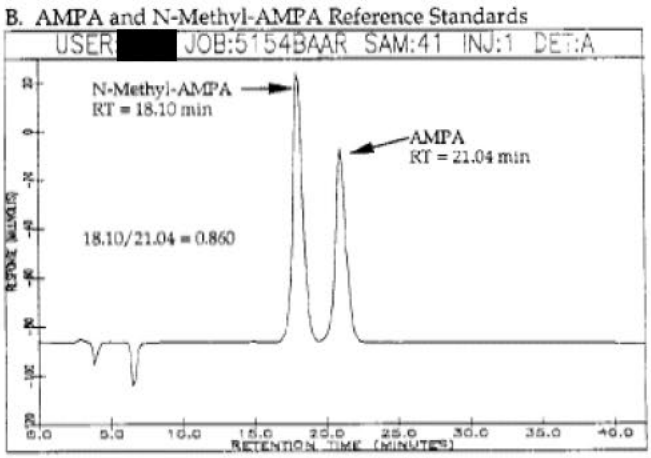
The unknown radio-peak "P3" could supposedly be the amino acid sarcosine or N-methyl-AMPA based on the position of the ^{14}C-label in the glyphosate test material (glyphosate-phosphonomethyl-2-C^{14}), where N-methyl-AMPA would be a zwitterion as well at pH 1.9, and sarcosine would be a cation at this pH. This could potentially mean that N-methyl-AMPA would co-elute with AMPA whereas sarcosine would definitely elute after AMPA as indicated in the chromatograph (however in left figure probably glyphosate 9-11 min, AMPA at 13 min and Peak 3 at 18 min if comparing to %AR reported for 7 DAT in results table). Peak identification is not clear in all available chromatographs of soil extracts. Positions in graph of 7 DAT do not agree with the findings in the table in summary above. Similarly, the peak identification in soil at 120 DAT seems rather speculative. With the peak at 18 min being supposedly "P3" on 7 DAT, this peak would be less prominent than indicated by the values in the table. The elution time of the indicated peak is later than elution times of glyphosate or AMPA.

Figure 8.1.1.1-13: Representative HPLC radio chromatogram following analysis of soil extract ([redacted] 2004 Anaerobic Soil Metabolism study)



In order to have some confirmation on the identity of the potential degradation product of glyphosate, verification was seeked in similarly conducted analyses and found in a soybean metabolism study on glyphosate where N-methyl AMPA was identified ([redacted], 1994, CA 6.2.1/022). The chromatogram in Figure 8.1.1.1-14, shows reference standards of AMPA and N-methyl AMPA under cation exchange chromatography conditions in phosphate buffer at pH 2.0 and shows N-methyl AMPA eluting earlier than AMPA.

Figure 8.1.1.1-14: Comparison of HPLC retention times of AmPA and N-methyl AMPA (CX HPLC/Refractive Index Detection Chromatogram; [redacted], 1994, CA 6.2.1/022)



The amino acid sarcosine would be another option based on the position of the ^{14}C-label. However, sarcosine has not been found in GLP soil degradation studies and was rarely found in literature and if only in highly specific conditions, e.g. the presence of certain bacterial strains or the absence of phosphate. It might be, that in anaerobic conditions given specific circumstances, the C-P lyase pathway would be triggered to provide phosphate and this was the case in [redacted] (2004, CA 7.1.1.2/001). It would be unclear why sarcosine was not faster degraded to glycine, but this degradation step might be slowed down in anaerobic conditions. It would also remain unclear why sarcosine was never found in other anaerobic studies with similar conditions (SETAC 1995 protocol).

The tentative identification shows that peak "P3" was most likely not N-methyl-AMPA (due to the elution time). No further identification was possible. Overall, the available chromatographs on soil extracts are difficult to read and the labelling in the graphs show discrepancies to the characterisation of the single components of glyphosate, AMPA and "P3" in %AR. The study is considered as invalid to address the data point due to study design and issues in residue identification. The findings would therefore not be relevant for the current submission.

Assessment and conclusion by RMS:

No information on soil history is provided.

The study design does not follow OECD 307 but is close to the one defined in SETAC 1995. The soil was flooded and acclimated during 50 days before substance application. The study could therefore provide supportive information. However RMS notes that measured redox potential in soil is 71.8/120.9 mV at day 2 and is still 60.2/74.1 mV at day 91. Next measure at day 120 is -136.8/-147.7 mV. Therefore, it cannot be ensured that anaerobic conditions are really established, except at 120 days.

In addition, as highlighted by the applicant, there are issues on residues identification. As a consequence, RMS considers that the study is not acceptable.

[redacted] *1987*

Data point:	CA 7.1.1.2/005
Report author	[redacted]
Report year	1987
Report title	SC-0224: Anaerobic soil metabolism study: fate of the carboxymethyaminomethylphosphonic acid moiety
Report No	PMS-217
Guidelines followed in study	Guideline Subdivision N, Section 162-2
Deviations from current test guideline	From OECD 307: - No determination of biomass and no information on soil history - Soil samples aerated with pure oxygen during aerobic incubation - Duration of study only 66 days instead of 120 days (degradation not >90% at test end)

	- Two sampling dates for aerobic and anaerobic phase, respectively, first sample in anaerobic conditions after 30 days whereas soil was flooded after 3 days - No measurement of oxygen and redox potential - Mass balance based on recovery of 0 DAT - Total recovery below 90 % AR during anaerobic conditions - No chromatograms examples available
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C-CMAMP] SC-0224 (trimethylsulfonium carboxymethyl)

Lot No.: WRC-7615-29-01

Specific activity: 30 µCi/mg

Radiochemical purity: 98.1 % (after purification)

2. Soil:

Soil was sieved to ≤ 2 mm. Moisture content of the air-dried soil was determined to be 1.97 g H_2O/100 g based on weight loss from 4-5 g samples of soil -heated for 10 min in a microwave oven.

Table 8.1.1.1-71: Characteristics of test soils

Parameter	Results
Soil	Sorrento
Country	IT
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	53.2
Silt (2 µm – 50µmm) (%)	34.4
Clay (< 2 µm) (%)	12.4
pH (water)	6.9
Organic carbon (%)[1]	1.28
Organic matter (%)	2.2
Cation exchange capacity (meq/100 g)	17.6
Half saturation [2]	21%
Bulk Density (disturbed) (g/cm^3)	1.43
Soil moisture adjusted to 75% field capacity	42g water/100g soil

[1] Calculated from organic matter according to OC = OM x 0.58

[2] Not given if volume metric or gravimetric value

B. STUDY DESIGN

1. Experimental conditions

200 g of air-dried soil were placed into each of 10 one-L biometer flasks. Using a volumetric pipet 10 mL of the soil treatment solution were slowly and uniformly applied to the surface of the soil in each biometer flask. Three additional flasks, two containing 200 g untreated soil and one containing no soil, were set up as controls. Soil moisture was adjusted to 75 % of field capacity (field capacity – 42 g water / l00 g soil) by adding 47.8 mL water to the soil or each glyphosate trimesium treated flask and 57.8 mL to the soils of the two control flasks. Two trapped flasks were set aside for immediate analysis as 0 time samples. The remaining flask was then fitted with a polyurethane foam plug in the

flask bridge. The sidearm of each flask was then filled with 1.0 M sodium hydroxide. All flasks were placed in an environmental chamber maintained at 23 °C and in total darkness for the duration of the study. The flasks were maintained initially under aerobic conditions, all being connected to a gas distribution line of oxygen. Pressure was maintained under pressure by connecting the oxygen line to a "U" tube containing mineral oil.

After three days of incubation, anaerobic conditions were achieved by flooding each soil vessel with water (200 mL) and substituting nitrogen for oxygen in the gas suppling system.

2. Sampling

Duplicate test systems were processed and analysed 0, 3, 33 and 66 days after treatment (DAT). NaOH solutions were collected and replaced with fresh solution at each soil sampling interval.

3. Analytical procedures

Each aerobic soil was transferred into 250 mL polypropylene centrifuge bottles and extracted with 1.0 M ammonium hydroxide (two times, approximately 150 mL each extraction). Each extraction step was conducted by hand shaking followed by separation of soil and extract by centrifugation at 10000 x G).Each extract was decanted and immediately neutralized to pH 7 with HCL to prevent base hydrolysis of [^{14}C]glyphosate trimesium to AMPA and radio-assayed by LSC. Each anaerobically incubated soil plus flood water was transferred equally into two 250 mL polypropylene centrifuge bottles and centrifuged. The flood water was decanted and radioassayed. The soils were extracted with ammonium hydroxide.

The ammonium, hydroxide soil extracts and the flood water, were reduced to dryness using high-vacuum rotary evaporation, and the residues were re-dissolved in 10 mL water for analysis.

Aliquots of the NaOH traps and ethyl acetate extracts were radio-assayed. The occurrence of $^{14}CO_2$ in the alkali traps was determined by $BaCl_2$ precipitation. $BaCl_2$ was added to aliquots of composited trap solutions represent in the collection intervals 0 to 68 days. The NaOH trap samples were analysed for ^{14}C both before and after $BaCl_2$ treatment by counting 0.1 mL aliquots.

The soil extracts and floodwaters were purified by cation exchange micro-column chromatography prior to metabolite characterisation via TLC. The purification step was needed to remove soil cations which were shown to interfere with the movement of glyphosate trimesium on the cation-exchange TLC plates used in this study. Column chromatography was performed using Dowex G 50W-X8 resin (hydrogen form, 200-400 mesh; Bio-Rad, Richmond, CA). The column was then rinsed with purified water added dropwise until the pH of the eluted water reached 7.0. Each soil extract/floodwater was then applied to the column and eluted with 5 mL purified water. Fractions were collected (200~400 µL each and radioassayed using one-µL aliquots counted by LSC. The column was washed with 1.0N HC1 (5 mL) then rinsed with water prior to application of the next sample. The ^{14}C in each sample emerged approximately between 3 mL and 3.5 mL total elution volume. Fractions containing this peak were analysed by TLC.

II. RESULTS AND DISCUSSION

A. DATA

Over the 66 day duration of the study, 43 % AR of the applied ^{14}C was recovered from NaOH traps, confirmed to be $^{14}CO_2$ by precipitation as barium salts (> 98 % AR of the trapped ^{14}C). No ^{14}C was retained by the polyurethane foam traps.

The bound ^{14}C decreased from 33 % AR at 0 time to 24 % AR by the end of the study at 66 days. Floodwater contained 2-3 % AR.

Radioactive mass balance and distribution of [^{14}C]-glyphosate trimesium and metabolites in soil extracts are summarised below.

Table 8.1.1.1-72: Distribution of radioactivity under aerobic and anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity[1])

Compound		DAT			
	Replicate	0	3	33	66
		aerobic conditions		anaerobic conditions	

Extractable	Mean	66.70	37.78	15.82	16.02
Bound	Mean	33.30	29.82	30.00	23.59
CO_2	Mean	-	23.86	39.53	43.21
Floodwater	Mean	-	-	2.72	2.45
Total mass balance	Mean	100.00	91.47	88.06	85.27

DAT: days after treatment
[1]Recoveries based on recovery of 0 DAT

Table 8.1.1.1-73: Characterisation of radioactivity in soil extracts under aerobic and anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

TLC[1] AREA	aerobic conditions		anaerobic conditions	
IDENTITY	0 DAT	3 DAT	33 DAT	66 DAT
AMPA	0.33	0.13	0.50	NS
CMAMP	65.81	37.34	15.26	15.97
"Area D"[1]	0.56	0.31	0.06	0.05
ORIGIN	NS	NS	NS	NS
TOTAL	66.70	37.78	15.82	16.02

[1] Corresponds to the section of the TLC plate directly below glyphosate (presumably a tailing effect of CMAMP).
NS not significant

Table 8.1.1.1-74: Characterisation of radioactivity in water following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

TLC[3] AREA	33 DAT	66 DAT
IDENTITY	%	%
"Area A"[1]	0.02	NS
AMPA	0.24	0.52
CMAMP	1.73	1.38
"Area D"[2]	0.40	0.24
ORIGIN	0.33	0.31
TOTAL	2.72	2.45

[1] Corresponds to the least polar section of TLC plate
[2] Corresponds to the section of the TLC plate directly below CMAMP (presumably a tailing effect of CMAMP).
NS not significant

B. MASS BALANCE

The overall distribution of ^{14}C recovered from [^{14}C]-glyphosate trimesium treated soils in the range of 85 % AR to 100 % AR using the ^{14}C recovery from 0 time soil as the basis for dosage determination. The recovery of ^{14}C from 0 time represented 93.0 % AR of the theoretical applied.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The ammonium hydroxide-soluble ^{14}C fraction proved to be short-lived, declining from an initial level of about 67 % AR at 0 time to approximately 38 % AR at 3 days and by 16 % AR after 66 days.

D. VOLATILE RADIOACTIVITY

43 % AR of the applied ^{14}C was recovered from NaOH traps at the last study day and confirmed to be $^{14}CO_2$ by precipitation as barium salts (> 98 % AR of the trapped ^{14}C). No ^{14}C was retained by the polyurethane foam traps.

E. TRANSFORMATION OF THE TEST ITEM

Results of the TLC characterisation of the soil extracts show that unchanged [^{14}C]-glyphosate was the single major component of the soil extractable fraction and the metabolite AMPA occurred as a minor component. At all sampling intervals [^{14}C]-glyphosate accounted for between 96-99 % AR. The determined half-life of [^{14}C]-glyphosate was approximately 3 days.

TLC analysis of floodwater showed that the ^{14}C fraction consisted of mainly unchanged [^{14}C]-glyphosate (about 69 % AR). The remaining floodwater ^{14}C was in the form of the metabolite AMPA (9 % at DAA 33, 21 % AR at DAA 66.) and unresolved material more polar than [^{14}C]-glyphosate trimesium (below 1 % AR).

F. KINETICS

In view of the low number of datapoints and since supporting information, a kinetic evaluation according to current guidance was not performed.

III. CONCLUSIONS

This study has shown that [^{14}C]-glyphosate trimesium is very rapidly and extensively metabolised in anaerobic soil.

Assessment and conclusion by applicant:

The study provides information on the degradation behavior of glyphosate trimesium in soil under anaerobic conditions. The study is considered to have supportive character based on the low number of sampling points. Beyond the fact that this low number does not allow for the conclusion on trends in degradation from the route perspective, the study is not kinetically evaluable to derive degradation rates.

The study is therefore considered as invalid to contribute adequately to the degradation behavior of glyphosate residues in soil under anaerobic conditions.

Assessment and conclusion by RMS:

Several deviations from OECD 307 are identified.

Soil biomass was not determined, no information on soil history is reported, soil samples were aerated with pure oxygen during aerobic incubation, duration of study was limited to 66 days instead of 120 days (and degradation not >90% at test end), only 2 sampling were done during the anaerobic phase, the first sample in anaerobic conditions was done after 30 days whereas soil was flooded after 3 days, there was no measurement of oxygen and redox potential to confirm that anaerobic conditions were reached, mass balance was based on recovery of 0 DAT, total recovery was slightly below 90 % AR during anaerobic conditions and no examples of chromatograms are available.

The study is not considered acceptable.

[redacted], *1972*

Data point:	CA 7.1.1.2/006
Report author	[redacted]
Report year	1972
Report title	The degradation and metabolism of MON-0573 in soil
Report No	269
Guidelines followed in study	U.S. Department of Agriculture (ARS, Pesticides Regulation Division): Pesticide Registration (PR) Notice 70-15 "Guidelines For Studies to Determine the Impact of Pesticides on the Environment." June 23, 1970
Deviations from current test guideline	From OECD 307: - mixed aerobic/anaerobic design strongly beyond actual standards and guidelines in soil degradation testing, i.e. soil suspended in aqueous solution during incubation and application of the test substance - work-up of aliquots only instead of complete soil samples - closed system without air exchange - incubation at 30 °C - soil history, sampling and storage not reported
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not mentioned in RAR (2015) but not accepted in DAR (2001)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: aerobic/anaerobic soil metabolism, degradation in water Test item: [^{14}C] glyphosate, phosphonomethyl-label (97 % radiochemical purity), 1-glycine label (96 % radiochemical purity), 2-glycine label (99 % radiochemical purity) Test soils (soil type): Ray (silt loam), Drummer (silty clay loam), Lintonia (sandy loam), Norfolk (sandy loam) pH: 6.5, 7.0, 6.0, 5.7 (method not stated) Organic matter: 1.0 %, 6 %, 1 %, 1 % The total study included various tests including aerobic and anaerobic degradation (samples water-logged) in non-sterile and sterilized soil (soil Ray only). Tests with exaggerated application rates performed for identification of metabolites (soil Ray). This summary focuses on the results of aerobic degradation tests. Application rate: 109 to 126 mg/kg for the different labels, 1000 mg/kg for metabolite identification with test substance applied to water phase, i.e. not applied directly to soil Test design: 5 g soil suspended in 100 mL water, continuously agitated by shaking; 100 g soil and 1000 mL for large scale tests Volatiles trapping: CO_2: ascarite trap Organic volatiles: no trapping Incubation: 30 °C, continuous shaking, soil flooded/suspended Sampling: 0, 1, 3, 7, 14, 21, 28 days after treatment (DAT) for soil Ray, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77, 84, 91, 105 and 112 DAT for soil Norfolk, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77 and 84 DAT for soil Drummer, 0, 1, 3, 7, 14, 21, 28 and 35 DAT for soil Lintonia, single samples collected per soil and sampling interval Workup: taking of an aliquot of the soil-water suspension, centrifugation, washing of soil with water, lyophilisation of soil, threefold extraction with 0.5 N aqueous NH_4OH solution at ambient temperature Determination of radioactivity: Extracts: LSC NER: combustion/LSC Volatiles: ascarite treated with HCl, trapping in 0.25 N NaOH, LSC Identification of radioactive residues: TLC/radiodetection co-chromatography with reference items, ^{1}H and ^{31}P-NMR
Short description of results:	Recovery of radioactivity: 68.7 – 109.8 % AR for all glyphosate labels and soils at the day of experiment termination Mineralization: 46.8 to 55.3 % AR for soil Ray, 5.8 to 9.3 % AR for soil Norfolk, 34.7 to 41.4 % AR for soil Drummer, 14.3 % AR for soil Lintonia (for all soils at termination) Other volatiles: not measured Extractable radioactivity: 2.7 to 22.9 % AR at 28 DAT for soil Ray, 65.4 to 81.8 % AR at 112 DAT for soil Norfolk, 12.0 to 19.6 % AR at 84 DAT for soil Drummer, 18.3 % AR at 35 DAT for soil Lintonia Non-extractable radioactivity: 8.5 to 40.3 % AR at 28 DAT for soil Ray, 4.6 to 13.5 % AR at 112 DAT for soil Norfolk, 16.7 to 33.9 % AR at 84 DAT for soil Drummer, 2.6 % AR at 35 DAT for soil Lintonia Transformation of test item (TLC analysis): Glyphosate: 0.2 to 7.4 % AR at 14 DAT and not detected at 28 DAT for soil Ray, 45.6 to 80.1 % AR at 14 DAT and 0.8 to

	16.3 % AR at 112 DAT for soil Norfolk, 12.5 to 25.5 % AR at 14 DAT and 7.6 to 15.7 % AR at 84 DAT for soil Drummer, 69.5 % AR at 14 DAT and 59.5 % AR at 35 DAT for soil Lintonia AMPA: 8.5 % AR at 14 DAT and 4.4 % AR at 28 DAT for soil Ray; 0.5 % AR at 14 DAT and 1.7 % AR at 28 DAT for soil Norfolk, 1.8 % AR at 14 DAT, 8.4 % R at 56 DAT and 8.3 % AR at 84 DAT for soil Drummer, 6.9 % AR at 14 DAT and 6.6 % AR at 35 DAT for soil Lintonia (phosphonomethyl-label only for all soils) No unknown metabolites were observed at >5 % AR.
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid due to the following deficiencies: - mixed aerobic/anaerobic design strongly beyond actual standards and guidelines in soil degradation testing, i.e. soil suspended in aqueous solution during incubation and application of the test substance - work-up of aliquots only instead of complete soil samples - closed system without air exchange - incubation at 30 °C - soil history, sampling and storage not reported

Assessment and conclusion by RMS:

RMS agrees with the deviations identified above.

The study is not considered acceptable.

B.8.1.1.1.3. Soil photolysis (laboratory studies)

The molar decadic absorption coefficient (ε) of glyphosate is << 10 L mol^{-1} cm^{-1} at wavelengths >295 nm. Therefore, direct photolysis is not expected to significantly contribute to degradation of glyphosate in soil.

However photodegradation of glyphosate was investigated in 6 existing studies. No new study was provided for the renewal. An updated kinetic evaluation of the valid results has also been provided.

Table 8.1.1.1-75: List of existing studies on soil photolysis with glyphosate

Annex point	Study	Study type	Substance(s)	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021
CA 7.1.1.3/003	[redacted], 1993	Soil photolysis	Glyphosate	Valid	Acceptable
CA 7.1.1.3/002	[redacted], 1996	Soil photolysis	Glyphosate	Valid	Supportive
CA 7.1.1.3/004	[redacted], 1989	Soil photolysis	Glyphosate	Valid	Not acceptable
CA 7.1.1.3/005	[redacted], 1983	Soil photolysis	Glyphosate-trimesium	Invalid	Not acceptable
CA 7.1.1.3/006	[redacted], 1978	Soil photolysis	Glyphosate	Invalid	Not acceptable
CA 7.1.1.3/007	[redacted], 1972	Soil photolysis	Glyphosate	Invalid	Not acceptable

Within the search for peer reviewed scientific literature (2010-2020), no article was identified that would provide information relevant to this data point.

[redacted], 1993

Data point:	CA 7.1.1.3/003
Report author	[redacted]
Report year	1993
Report title	Photodegradation study of ^{14}C-Glyphosate on soil

Report No	315764
Guidelines followed in study	U.S. EPA 161-3
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - only single sample data is available - performed at 22°C - LOD/LOQ not reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]glyphosate
Lot No.: CFA.745 C6
Specific activity: 12.3 MBq/mg (333 mCi/g)
Radiochemical purity: > 99.3 %

2. Soil:

The selected soil was air-dried and sieved to ≤2 mm. Before the start of the experiment, the untreated soil was stored in concrete cylinders in the open. Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-76: Characteristics of test soil

Parameter	Results
Soil	Les Evouettes II
Country	Switzerland
Textural Class (USDA)	Loam / silt loam
Sand (50 µm – 2 mm) (%)	38.0
Silt (2 µm – 50µmm) (%)	50.7
Clay (< 2 µm) (%)	11.3
pH [2]	6.1
Organic carbon (%)	1.40
Organic matter (%) [1]	2.41
Cation exchange capacity (meq/100 g)	15.5
Maximum Water Holding Capacity (%)	55.3
Field capacity (%)	40.2
40 % MWC (g/100 g soil)	22.1
Bulk Density (dry weight basis) (g/cm^3)	0.856
Microbial biomass / Total plate counts	
At the start of the experiments	2.2 x 10^5 / g soil
At 30 DAT of incubation (illuminated plate)	1.4 x 10^5 / g soil
At 31 DAT of incubation (dark control plate)	0.6 x 10^5 / g soil

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Calculated from organic carbon according to OM = OC / 0.58
[2] Buffer medium not indicated

B. STUDY DESIGN

1. Experimental conditions

For illumination, the soil thin-layer plates were placed in a metal-chamber with a matt-black interior covered with a quartz plate. The metal-chamber beneath the photolysis apparatus was cooled by means of a waterbath, allowing maintenance of constant temperature. The light source was a Hanau Suntest CPS apparatus equipped with a xenon burner 1.1 kW and a UV filter system simulating natural sunlight (300-

800 nm). Radiation intensity was measured at regular time intervals and on average the light intensity was 93 Klux. The temperature was continuously monitored and remained constant (22 ± 1 °C) except for the transition period. The system was continuously ventilated with air by means of a membrane pump. The air in the metal-chamber was saturated by placing moistened filter paper against the walls. Additionally, the incoming air was moistened by bubbling through a flask containing saturated $NaHSO_4$. The outcoming air was passed through a CO_2-trapping system (NaOH) and through an ethylene glycol trap.

Dark soil samples were placed in an all-glass chamber under exclusion of light and incubated in an air-conditioned room at a temperature of 22 ± 2 °C. Air was ventilated by means of a membrane pump and trapped as described for the illuminated set-up.

100 g of sieved soil was mixed with 75 mL of bidistilled water. After homogenization (4 minutes) the soil thin-layer plates were prepared by applying the slurry to the surface of 16 clean, pre-weighed glass-plates (5 x 10 cm) using a TLC-plate coater adjusted to a layer-thickness of about 1.0 mm.

Based on a target dose of 8.4 mg/kg soil (3.6 kg a.s./ha, dry weight based), the average soil weight per soil plate (2.472 g) and a target application volume of 1.5 mL, an aliquot of 1010 µl (346.4 µg) stock solution was made up to 25 mL with bidistilled water. The application solution proved to contain 13.7 µg/mL of [^{14}C]glyphosate. Based on the concentration of test item in the application solution and the average soil weight per treatment are, 1.52 mL containing 20.8 µg [^{14}C]glyphosate were applied to each plate.

Test systems were incubated for 30 days using a 12 hours light/dark cycle for irradiated samples.

2. Sampling

After incubation, samples were weighed, left at room temperature for about 2 hours and re-weighed to get information on the moisture content of the incubated soil. No difference in moisture content between illuminated and dark control soil plates was found.

The soil was sampled at time intervals of 0, 3, 7, 14, 21 and 30 days (dark controls: 31 DAT instead of 30 DAT). The soil was stored at -20 °C until analyses. $^{14}CO_2$ and ^{14}C-volatiles were measured at each sampling interval except for 0 DAT for both illuminated and dark samples.

For test on vitality of microbial biomass, one sample (about 5 g soil) at 0 DAT and two samples (about 5 g each) at 30 DAT (illuminated soil) and 31 DAT (dark control) was collected.

3. Analytical procedures

The air-dried soil samples (about 2-3 g) were extracted 3 times with 0.5 M NH_3 (about 4-6 mL/g soil) by shaking for 30 minutes at room temperature. For time intervals 30 DAT/31 DAT, additional extractions with H_2O and 0.1N HCl were performed. An exhaustive extraction with refluxing methanol/0.5M NH_3 (8+2, v/v) at 70 °C was performed for time intervals 21 DAT and 30 DAT/31 DAT; these additional extractions were performed to show that extraction of radioactivity was complete. After each extraction, samples were centrifuged for 10 minutes at 1900 g, the supernatant decanted and filtered through a filter paper. The radioactivity in each extract was determined by Liquid Scintillation Counting (LSC). The NH_3-extracts were combined and directly analysed by TLC. Thereafter, extracts were stored at -20 °C until HPLC. Remaining soil was air-dried, homogenized and the non-extracted radioactivity determined by combustion of aliquots (about 200-500 mg) and LSC.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/radiodetection method were not reported.

The amount of volatiles was determined by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in soil extracts are summarised below.

Table 8.1.1.1-77: Mass balance for [^{14}C]glyphosate in irradiated samples (expressed as percent of applied radioactivity)

Compound	Sampling intervals (days) 0	3	7	14	21	30
Extracted						
Room temperature						
1. 0.5M NH_3	74.6	65.0	60.2	54.9	53.2	43.3
2. 0.5M NH_3	16.1	18.9	17.0	17.6	16.2	19.4
3. 0.5M NH_3	4.2	5.7	5.2	5.9	5.3	6.1
- H_2O	n.d.	n.d.	n.d.	n.d.	n.d.	2.2
- 0.1N HCl	n.d.	n.d.	n.d.	n.d.	n.d.	1.3
Subtotal	94.9	89.6	82.4	78.4	74.7	72.3
Reflux at 70 °C						
- MeOH/0.5M NH_3 (8+2, v/v)	n.d.	n.d.	n.d.	n.d.	0.3	1.0
Subtotal	94.9	89.6	82.4	78.4	75.0	73.3
Non-extracted	7.5	13.3	14.6	19.4	17.3	15.5
Cumulative volatiles						
- NaOH trapped	n.d.	4.3	6.3	8.6	11.3	14.6
- Ethylene glycol trapped	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
Total	102.4	107.2	103.3	106.4	103.6	103.4
Total mean ± SD	104.4 ± 1.9					

n.d. = not determined, SD = Standard deviation

Table 8.1.1.1-78: Mass balance for [^{14}C]glyphosate in dark control samples (expressed as percent of applied radioactivity)

Compound	Sampling intervals (days) 0	3	7	14	21	31
Extracted						
Room temperature						
1. 0.5M NH_3	74.6	67.0	63.5	61.5	60.5	52.2
2. 0.5M NH_3	16.1	16.4	18.7	18.7	18.0	21.2
3. 0.5M NH_3	4.2	5.2	5.5	5.6	6.0	6.4
- H_2O	n.d.	n.d.	n.d.	n.d.	n.d.	2.4
- 0.1N HCl	n.d.	n.d.	n.d.	n.d.	n.d.	1.2
Subtotal	94.9	88.6	87.7	85.8	84.5	83.3
Reflux at 70 °C						
- MeOH/0.5M NH_3 (8+2, v/v)	n.d.	n.d.	n.d.	n.d.	0.4	0.6
Subtotal	94.9	88.6	87.7	85.8	84.9	83.9
Non-extracted	7.5	13.0	15.0	15.5	17.4	16.5
Cumulative volatiles						
- NaOH trapped	n.d.	3.9	5.0	5.1	5.2	5.4
- Ethylene glycol trapped	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
Total	102.4	105.5	107.7	106.4	107.5	105.8
Total mean ± SD	106.6 ± 1.0					

n.d. = not determined, SD = Standard deviation

Table 8.1.1.1-79: Characterisation of extractable radioactivity following treatment with [^{14}C]glyphosate in irradiated samples (expressed as percent of applied radioactivity)

Compound	Sampling interval (Days) 0	3	7	14	21	30
Glyphosate	94.9	75.7	65.3	64.8	60.3	60.5
AMPA (M2)	n.d.	7.4	8.2	5.2	7.4	6.5
M3 [1]	n.d.	3.6	5.0	4.8	4.3	2.5
M4 [2]	n.d.	2.9	3.9	3.6	2.7	2.8
Total	94.9	89.6	82.4	78.4	74.7	72.3

n.d. = not detected

[1] Tentatively identified as (N-methyl-N-phosphono-methyl)-glycine

[2] Tentatively identified as hydroxymethylphosphonic acid

Table 8.1.1.1-80: Characterisation of extractable radioactivity following treatment with [^{14}C]glyphosate in dark control samples (expressed as percent of applied radioactivity)

Metabolite	Sampling interval (Days)					
Code	0	3	7	14	21	31 [1]
Glyphosate	94.9	82.5	83.8	82.9	80.8	79.6
AMPA	n.d.	6.1	3.9	2.9	3.7	3.7
Total	94.9	88.6	87.7	85.8	84.5	83.3

n.d. = not detected

Table 8.1.1.1-81: Amount of [^{14}C]glyphosate in irradiated soil samples after correction for the degradation in the dark (expressed as percent of applied radioactivity initially applied to each plate)

	Sampling interval (Days)					
	0	3	7	14	21	30/31 [1]
I	94.9	75.7	65.3	64.8	60.3	60.5
II	94.9	82.5	83.8	82.9	80.8	79.6
III	94.9	88.1	76.4	76.8	74.4	75.8

I: Amount of 14C-Glyphosate in irradiated samples
II: Amount of 14C-Glyphosate in dark controls
III: Amount of 14C-Glyphosate in irradiated samples after correction for its degradation in the dark (III = 94.9 % - (II - I))
[1] Irradiated: 30 days; dark controls: 31 days

B. MASS BALANCE

Total recovery of radioactivity ranged from 102.4 % AR to 107.2 % and from 105.5 to 107.7 % AR in irradiated and dark control samples, respectively.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Extracted radioactivity decreased from 94.9 to 73.3 % AR and from 88.6 to 83.9 % AR in irradiated and dark control samples, respectively.

Non-extracted radioactivity was 7.5 % AR at 0 DAT and was at similar levels in irradiated and dark control samples from DAT 3 to study end. NER increased to 19.4 % AR (14 DAT) and to 17.4 % AR (21 DAT) in irradiated and dark control samples, respectively, and decreased then to 15.5 and 16.5 % AR at study end (30 DAT in irradiated, 31 DAT in dark control samples).

D. VOLATILE RADIOACTIVITY

In irradiated samples high amounts of $^{14}CO_2$ were evolved, increasing from 4.3 % AR (3 DAT) to 14.6 % AR at 30 DAT (cumulative levels), while the cumulative levels of $^{14}CO_2$ were similar during 31 days of incubation in the dark, ranging from 3.9 (3 DAT) to 5.4 % AR (31 DAT). Organic volatiles determined were <0.05 % AR for irradiated and dark control samples at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

The occurrence of higher amounts of $^{14}CO_2$ in irradiated samples as compared to the dark controls indicated that glyphosate could be mineralized by the process of photolysis.

E. TRANSFORMATION OF THE TEST ITEM

In irradiated samples, the amount of glyphosate decreased from 94.9 % AR to 60.5 % AR (30 DAT). From 3 DAT on, besides glyphosate three radioactive fractions, M2 (AMPA), M3 and M4 were detected, with M3 and M4 tentatively identified as (N-methyl-N-phosphono-methyl)-glycine) and hydroxymethylphosphonic acid, respectively. AMPA was detected with a maximum amount of 8.2 % AR at 7 DAT, with similar amounts at other sampling times, ranging from 5.2 (14 DAT) to 7.4 % AR (3 DAT/21 DAT). The amount of radioactive fraction M3 increased from 3 DAT (3.6 % AR) to 5.0 % AR at 7 DAT and, thereafter, decreased to 2.5 % AR at 30 DAT. Radioactive fraction M4 had similar levels of radioactivity from 3 DAT to 30 DAT, ranging from 2.7 % (21 DAT) to 3.9 % AR (7 DAT). No other metabolites were detected above 5 % AR at any time.

In dark control samples, the amount of glyphosate decreased from 94.9 % AR to 79.6 % AR (31 DAT). Except for 0 DAT, one radioactive fraction (AMPA) was detected throughout the incubation interval, with a maximum amount of 6.1 % AR at 3 DAT. Thereafter, the amounts were somewhat smaller, ranging from 3.9 % (7 DAT) to2.9 % AR (14 DAT).

A different metabolite pattern was found after irradiation as compared to the dark control during 30 days if incubation. Radioactive fraction M2 occurred in both, irradiated and dark control samples. Therefore, radioactive fractions M3 and M4 were specific photolytic products of [^{14}C]glyphosate on soil.

F. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. The new evaluation is reported under [redacted] (2020, CA 7.1.1.3/0001).

III. CONCLUSIONS

The present data indicated that the degradation of [^{14}C]glyphosate on soil under irradiation conditions simulating natural sunlight (light/dark cycle: 12 hours) proceeded faster than in the dark.

After TLC-analyses of the extracted radioactivity from irradiated soil plates, mainly parent compound (M1) was found at all time intervals. With increasing irradiation time, one major (M2) and two minor radioactive fractions (M3 and M4) were detected. Radioactive fraction M2 was proven to be identical to aminomethylphosphonic acid (Ref. B), also designated AMPA. Radioactive fractions M3 and M4 were tentatively identified to be (N-methyl-N-phosphono-methyl)-glycine (Ref. D) and hydroxymethylphosphonic acid (Ref. E), respectively.

In conclusion, taking into account the specific occurrence of $^{14}CO_2$ in the irradiated samples as compared to the dark controls, the present data showed that glyphosate could be slowly mineralized by the process of photolysis.

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]glyphosate on soil surfaces was examined using an artificial light source at an application rate of 3.6 kg/ha soil. Mass balances ranged from 102.4 to 107.7 % of applied radioactivity (% AR).

The study is considered valid to address the data point.

Assessment and conclusion by RMS:

The study is well performed. No metabolite was observed at concentrations >5% except for AMPA. LOQ/LOD are not reported, and no replicates are available but this does not impact the reliability of the study.

The study is acceptable.

[redacted], 1996

Data point:	CA 7.1.1.3/002
Report author	[redacted]
Report year	1996
Report title	[P-Methylene-14C]Glyphosate acid: Photodegradation in/on soil by natural sunlight
Report No	547W-1
Guidelines followed in study	U.S. EPA 161-3
Deviations from current test guideline	SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - material balance 80.0 to 97.6 % AR

	- tests were not conducted with an artificial irradiation source, but samples exposed to natural sunlight of 250-700 nm range - temperature was 25°C
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [P-Methylene - ^{14}C]Glyphosate Acid ([^{14}C]PMG)
Lot No.: WRC Ref. 15617-06-02
Specific activity: 42.7 mCi/mmole
Radiochemical purity: 97.3 %

2. Soil:

Upon arrival at the testing facility, the sandy loam soil used in the study was sieved to ≤2 mm. The soil was maintained at approximately 8 °C in an incubator until experimental start of the study. No chemicals had been applied to the soil the three years before use for experiment, except for applications of diazinon and malathion the year before start of the study.

Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-82: Characteristics of test soil

Parameter	Results
Soil	Visalia (KOFO1A)
Country	CA, USA
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	71.2
Silt (2 µm – 50µmm) (%)	20.0
Clay (< 2 µm) (%)	8.8
pH (medium not indicated)	8.3
Organic carbon (%) [1]	0.46
Organic matter (%)	0.80
Cation exchange capacity (meq/100 g)	8.14
Water Holding Capacity at 0.33 bar (%)	11.92
Water Holding Capacity at 15 bar (%)	4.18
Bulk Density (disturbed) (g/cm^3)	1.46
Microbial biomass [2] [Colony forming units (CFU)/g soil] Total aerobic bacteria Total actinomycetes Total fungi	 5.050 x 10^6 2.050 x 10^6 0.009 x 10^6

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Calculated from organic matter according to OC = OM x 0.58
[2] tested within a week of experimental start date

B. STUDY DESIGN

1. Experimental conditions

The test system consisted of thin soil layers placed in specially designed and temperature controlled round chambers (50 mm diameter, 20 mm height) made of quartz for light exposed samples and borosilicate glass covered with aluminium foil to prevent irradiation for dark control samples. Six extra soil containers were prepared. Duplicate containers of both light exposed and dark control samples were sealed with a screw cap fitted with a Teflon septum. The sample containers were submerged in a bath containing deionized water at an approximate 30° angle with respect to the horizon to maximize irradiation during periods of

strong sunlight intensity. The water was circulated using a Lauda ™ Constant Temperature Circulator and maintained at approximately 25.0 ± 1.0 °C. Two small submersible pumps were placed in the bath to prevent local temperature differences. The temperature was acquired each 10 seconds using Type T thermocouples. Three thermocouples were used, one was placed in the water bath and one each placed inside and attached to the bottom of the irradiated and dark containers before adding the soil slurry.

Volatiles from each individual container were trapped by inserting a needle with tubing attached to a series of traps connected to a water aspirator pump (no flow through system). The traps consisted of one ethylene glycol trap (50 mL) to collect organic volatiles and two 10 % NaOH traps to account for carbon dioxide. Samples were weighed following each intermittent trapping to assure that moisture content was maintained at 75% of soil water holding capacity at 1/3 bar. After intermittent trapping the punctured septa were replaced by new ones, and the sealed containers placed back into the water bath. Since some radiocarbon recoveries were low and large amounts of $^{14}CO_2$ were produced, additional trapping experiments were conducted at day 20 and 30 samplings after purging and trapping the headspace gases. Acidic phosphate buffer (5 mL of ~ pH 2.0) was injected through each septum, the containers were connected to the trapping system, and the mixtures vortexed to release $^{14}CO_2$ adsorbed to the moist soil.

The equivalent of 3.1 g of dry soil was weighed into each sample container. Deionized water (3 mL) was added to each dish to form slurries; slurries were allowed to dry and form thin soil layers (1-2 mm) on the bottom of the containers.

The dosing solution was prepared by adding aqueous [^{14}C]glyphosate stock solution (0.238 mL, 870 µg) to 2.562 mL of deionized water. Aliquots (100 µL) of the dosing solution were applied as evenly as possible to each of the previously prepared soil containers by using a glass syringe. Deionized water (177 µL) was then added to achieve 75 % water holding capacity at 1/3 bar. Aliquots of the dosing solution taken prior to, during and after the application process were radio assayed by LSC to determine the applied radiocarbon. The final concentration of test substance in the soil was 10.19 µg/g corresponding to 11 kg/ha (10 lb/acre).

Test systems were incubated for 30 days at 75 % of the maximum water holding capacity at 1/3 bar. Cloud cover data were compiled. The exposure phase was carried out in [redacted], CA at latitude [redacted], longitude [redacted], between October 18 and November 17, 1995. Sunlight intensity and cumulative energy (250 – 700 nm range) were measured and recorded at 20 minute intervals throughout the study using an International Light Radiometer. The mean total light energy was 7.02 W min/cm^2, with the cumulative light energy of 217.6 W min/cm^2.

2. Sampling

Duplicate test systems were sampled 0, 2, 6, 12, 20 and 30 days after treatment (DAT). At day 2 and following sample times, duplicate light-exposed and control samples were removed from the water bath. Traps with ethylene glycol and 10 % NaOH were sampled at all these occasions except 0 DAT. Intermittent trapping of the headspace was performed once a week starting approximately one week after dosing. Trapping solutions and soil extracts were analysed by LSC on the day of collection. Extracts were analysed by HPLC within 24 hours of sampling, with the exception of 2 DAT samples, which were analysed after three days. All samples were frozen when not in use.

3. Analytical procedures

At each sampling time the soils were transferred from the containers into pre-weighed Teflon centrifuge tubes (50 mL) by rinsing the containers with 1 M aqueous KH_2P0_4 (15 mL) adjusted to ~ pH 2.0 with concentrated H_3PO_4. The mixture was shaken for ten minutes with a Wrist Action Shaker. After centrifugation (5,000 rpm, 10 minutes) the supernatant was separated from the residue, and the residue extracted once more with the extraction solvent (total of 15 mL) in the same manner as the first extraction. The supernatants were combined, the volumes recorded, and aliquots (3 x 1 mL) radio assayed by Liquid Scintillation Counting (LSC). For HPLC analyses, subsamples of each replicate sample were filtered and aliquots of the filtrates were co-injected with solutions of mixed analytical reference standards glyphosate and AMPA.

The limit of detection (LOD) for individual degradates in the HPLC radio chromatograms were determined by the dpm injected and the liquid scintillation counting detection limit. As an example a limit of 0.3 % AR is given for a background of 30 dpm and a sample size of 10,000 dpm injected of a matrix containing 5 ppm.

[^{14}C]glyphosate and its metabolites were analysed by HPLC of soil extract aliquots. Structural assignment was based on co-elution of ^{14}C-peaks with reference substances by HPLC and confirmed by one-dimensional TLC co-migration of ^{14}C-spots with reference substances.

Bound ^{14}C-residues present at ≥ 10 % AR were further characterized in selected samples (30 DAT replicate A and B light exposed extracted soil). Humic and fulvic acids residue were determined by extracting samples twice with 0.1 M NaOH (15 mL) by shaking for 24 hours under nitrogen using a wrist action shaker. After centrifugation the combined extract was acidified to pH 1 by adding a few drops of 6N HCl and humic acid allowed to precipitate overnight in an ice bath. The humic acid fraction (pellet) was separated from the fulvic acid fraction (supernatant) by centrifugation (2,000 rpm for 5 min). The volume of total supernatant was determined and aliquots (3 x 500 µl) taken for radioassay by LSC. The pellet (humic acid fraction) was redissolved in a minimal volume of 0.1M NaOH solution and the radiocarbon quantified by LSC of aliquots (3 x 200 µL).

The amount of volatiles was determined by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents of 6 DAT samples. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in soil extracts are summarised below. Fractionation of non-extractable residues into fulvic acid, humic acid in humin fractions is also presented.

Table 8.1.1.1-83: Overall mass balance

Days after application		% applied ^{14}C in soil extract		^{14}C non-extracted in residue soil		^{14}C as total volatiles		Total recovery	
conditions		light-exposed	dark control	light-exposed	dark control	light-exposed	dark control	light-exposed	dark control
0 d	Rep A	91.6	91.6	3.0	3.0	n.d.	n.d.	94.7	94.7
	Rep B	92.4	92.4	3.1	3.1	n.d.	n.d.	95.6	95.6
2 d	Rep A	70.9	70.4	6.3	6.1	12.2	16.5	89.4	93.0
	Rep B	65.3	73.2	6.2	6.4	18.8	18.0	90.3	97.6
6 d	Rep A	49.8	51.5	6.4	6.2	25.7	32.8	81.8	90.6
	Rep B	48.4	43.7	7.2	6.6	30.6	36.4	86.2	86.7
12 d	Rep A	40.7	37.9	13.0	6.1	37.8	40.2	91.5	84.1
	Rep B	40.1	40.1	14.8	6.7	29.1	37.4	84.0	84.1
20 d	Rep A	36.2	36.3	16.2	6.1	37.4	24.4	89.8	66.8 [1]
	Rep B	41.8	36.3	19.0	6.5	28.5	43.5	89.4	86.3
30 d	Rep A	25.3	28.8	36.1	7.4	30.3	28.2	91.7	64.4 [1]
	Rep B	26.8	29.2	31.0	7.2	31.5	43.6	89.2	80.0

n.d. = not determined

[1] Significant losses of $^{14}CO_2$. These numbers were not considered for range of mass balance.

Table 8.1.1.1-84: Distribution of [^{14}C]glyphosate and its degradates in extracts

Days after application		% applied ^{14}C in soil extract		Glyphosate		AMPA		Degradate [1]		Others	
conditions		light-exposed	dark control	light-exposed	dark control	light-exposed	dark control	light-exposed	dark control	light-exposed	dark control
0 d	Rep A	91.6	91.6	88.69	88.69	2.49	2.49	0.21	0.21	0.21	0.21
	Rep B	92.4	92.4	89.60	89.60	1.89	1.89	0.17	0.17	0.74	0.74
2 d	Rep A	70.9	70.4	57.82	57.17	12.58	12.07	0.00	0.61	0.50	0.56
	Rep B	65.3	73.2	50.99	60.93	13.67	11.83	0.64	0.34	0.00	0.09
6 d	Rep A	49.8	51.5	26.34	30.16	22.08	20.85	1.38	0.00	0.00	0.49

	Rep B	48.4	43.7	26.62	19.51	20.69	23.47	1.02	0.72	0.08	0.00
12 d	Rep A	40.7	37.9	10.85	10.32	26.82	26.36	3.02	1.22	0.00	0.00
	Rep B	40.1	40.1	10.48	13.64	27.54	25.46	2.07	0.99	0.00	0.00
20 d	Rep A	36.2	36.3	6.36	7.57	26.60	27.36	3.15	1.38	0.09	0.00
	Rep B	41.8	36.3	8.05	5.92	30.10	28.70	3.65	1.60	0.00	0.09
30 d	Rep A	25.3	28.8	3.41	2.78	18.61	25.00	2.57	1.02	0.71	0.00
	Rep B	26.8	29.2	2.91	4.36	21.06	23.61	2.83	1.24	0.00	0.00

n.d. = not determined

[1] Significant losses of $^{14}CO_2$. These numbers were not considered for range of mass balance.

Table 8.1.1.1-85: Fractionation of 30 DAT post extracted soil

Days after application	% applied		
	Fulvic acid	Humic acid	Humin
30 d Rep A	6.6	4.3	25.2
30 d Rep B	6.0	2.9	22.1
Average	6.3	3.6	23.7

B. MASS BALANCE

Material balances ranged from 84.0 to 95.6 % of applied radioactivity (% AR) (single values, n = 12) for light exposed samples and from 80.0 to 97.6 % AR for dark control samples (single values, n = 10). Since the amounts of extracted and bound radiocarbon were usually consistent between replicates, losses of radiocarbon that occurred after 2 DAT were attributed to the rapid and steady formation of large amounts of $^{14}CO_2$. This caused some leakage from the headspace of the sample containers resulting in lower recoveries in some replicates. Intermittent purging of the headspace at 7 DAT intervals helped to mitigate the losses, but did not completely solve the problem in the dark-control samples.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 30 DAT from 92.0 % AR to 26.1 % AR (mean of two replicates) in light exposed samples and from 92.0 % AR to 29.0 % AR in dark control samples (mean of two replicates).

The amount of non-extractable residues (NER) increased from 0 DAT to 30 DAT from 3.1 % AR (mean of two replicates) to 33.6 % AR (mean of two replicates) in light exposed samples and to 7.3 % AR (mean of two replicates) in dark control samples. Light exposed extracted soils from 30 DAT (replicates A and B) were therefore selected for additional extraction using 01.M NaOH for characterization of bound ^{14}C-residues. Only 3.6 and 6.3% of applied dose (average of replicates) were associated with the humic and fulvic acid fractions, respectively.

D. VOLATILE RADIOACTIVITY

Total volatiles trapped at the end of the test period amounted to 30.9 % AR and 35.9 % AR in irradiated and darck control samples (both values mean of two replicates). [^{14}C]-PMG degraded rapidly to $^{14}CO_2$, with maximum values of 32.9 % AR and 36.7 % AR (each mean of two replicates) at 12 DAT in the light exposed and dark control samples, respectively. At study end at 30 DAT, 29.5 and 30.1 % AR were detected as $^{14}CO_2$ (each mean of two replicates) in light exposed and dark control samples, respectively, due to significant losses of $^{14}CO_2$. Considering unaccounted ^{14}C as CO_2, more CO_2 was formed in the dark-control than in the light-exposed samples (53.1 % AR and 34.2 % AR (each mean of two replicates) in dark control and light exposed samples, respectively). Radiocarbon found in the light exposed and dark control ethylene glycol traps reached a maximum of 1.9 and 5.8 % AR (each mean of two replicates) at 20 DAT and

30 DAT, respectively, and was not further characterized. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

[^{14}C]glyphosate rapidly degraded in the light exposed and dark control soil samples and represented only 3.2 and 3.6 % AR (mean of two replicates), respectively, at study end at 30 DAT0. The major degradate detected in light exposed and dark control soil extracts after 30 days was AMPA. AMPA reached a maximum of 28.4 and 28.0 % AR (mean of replicates) respectively, in light exposed and dark control soil samples at 20 DAT0, and represented 19.8 and 24.3 % AR (mean of replicates) for light exposed and dark control soil extracts, respectively, at 30 DAT. An unidentified degradate, designated "Degradate 1", reached maximum values of 3.4 and 1.5 % AR, respectively, in light exposed and dark control soil samples, respectively, at 20 DAT. With the exception of AMPA no other degradates above 5 % AR were detected. ^{14}C as total volatiles reached a maximum of 33.5 and 38.8 % AR (each mean of two replicates) in light exposed and dark control soil samples at 12 DAT, respectively.

F. KINETICS

New kinetic calculations based on more recent guidance will not be provided as this study is only delivering supplemental information. The half-life of glyphosate was calculated to be 6.5 days (R^2 = 0.940) for the light exposed and 6.6 days (R^2 = 0.922) for the dark control samples, using pseudo first order kinetics.

III. CONCLUSIONS

A study of the photodegradation of [P-Methylene-^{14}C]Glyphosate Acid ([^{14}C]PMG) in natural sunlight on sandy loam soil was conducted for 30 days at about 25 °C. Dark control samples were maintained concurrently to account for non photolytic degradation processes.

Radiocarbon recoveries ranged from 84.0 to 95.6 % of applied radioactivity (% AR) (single values, n = 12) for light exposed samples and from 80.0 to 97.6 % AR for dark control samples (single values, n = 10). Small losses of radiocarbon occurred throughout the study, due to the rapid and steady formation of $^{14}CO_2$. Up to 32.9 % AR and 36.7 % AR in the light exposed and dark control NaOH traps was recovered as $^{14}CO_2$ at 12 DAT. Glyphosate rapidly degraded in both, light exposed and dark control, representing only 3.2 and 3.6 % AR (mean of two replicates), respectively, at study end at 30 DAT.

The major product detected in light exposed and dark control soil extracts was AMPA, which reached a maximum of 28.4 and 28.0 % AR (mean of replicates) respectively, in light exposed and dark control soil samples at 20 DAT, and represented 19.8 and 24.3 % AR (mean of replicates) for light exposed and dark control soil extracts, respectively, at 30 DAT. An unidentified degradate, designated "Degradate 1", reached maximum values of 3.4 and 1.5 % AR, respectively, in light exposed and dark control soil samples, respectively, at 20 DAT. No degradates other than AMPA were detected at >3.7% AR at any time. A pattern of steady increase of the major terminal metabolite $CO_2$and the rise and slight decline of the metabolite AMPA was clearly established.

The only significant difference between light exposed and dark control samples was increased post extraction soil residues in irradiated samples. The unextracted radiocarbon in the dark control soil reached 7.3 % AR at 30 DAT while amounts in light exposed soil reached 33.6 % AR at 30 DAT. Additional extractions with 01.M NaOH showed that 3.6, 6.3 and 23.7 % AR was associated with the humic acid, fulvic acid and humin fractions, respectively.

Exposure of glyphosate treated soil to light had no effect on the degradation rate of glyphosate or extractable residues found.

The results of this study indicate that photolysis in/on soil is not likely to be a significant route of dissipation for glyphosate compared to rapid microbial degradation in soil.

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]glyphosate on soil surfaces under natural sunlight was examined for 30 days using a field application rate of 11 kg/ha (10 lb/acre) soil. Mass balances ranged from 80.0 to 97.6 % AR.

As natural sunlight was used for the experiment instead of preferred artificial light, the study is considered as supportive information.

Assessment and conclusion by RMS:

Mass balance was below 90% AR at some sampling dates in the irradiated samples and in the dark control. It is particularly low for one of the replicates at 20 d and at 30 d (66.8 and 64.4% AR, respectively).

Natural sunlight was used. Although artificial sunlight is generally preferred, all relevant information on the sunlight conditions (sunrise, sunset, cloud cover, light intensity and total light energy) are reported in the study.

The study is considered as supportive.

[redacted], *1989*

Data point:	CA 7.1.1.3/004
Report author	[redacted]
Report year	1989
Report title	Photodegradation of [^{14}C]Glyphosate in/on soil by natural sunlight
Report No	153W
Guidelines followed in study	U.S. EPA 161-3
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - tests were not conducted with an artificial irradiation source, but samples exposed to natural sunlight - no constant temperature - no information on the soil origin/stockage/transport before use
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]glyphosate (N-phosphonomethylglycine)
Lot No.: not indicated
Specific activity: 8.08 mCi/mmol
Radiochemical purity: 98.9 %

2. Soil:

Soil was sieved to ≤2 mm. The soil was stored in a freezer (-20 °C) upon reception, prior to use. Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-86: Characteristics of test soil

Parameter	Results
Soil	KP8 Composite
Country	Kentucky, USA
Textural Class (USDA)	Sandy loam

Sand (50 µm – 2 mm) (%)	74
Silt (2 µm – 50µmm) (%)	16
Clay (< 2 µm) (%)	10
pH (water)	7.6
Organic carbon (%) [1]	0.9
Organic matter (%)	1.6
Cation exchange capacity (mgq/100 gm)	6

[1] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

The test was performed in flow-through systems connected to three traps, one containing ethylene glycol and two containing 10 % NaOH solution for collection of volatile organic compounds and carbon dioxide, respectively. The test system consisted of thin layers of soil in petri dishes placed in temperature controlled stainless steel chambers. Aliquots of the soil (3.1 g) were weighed into 50 mm petri dishes. Distilled water (3 mm) was added to each dish and the slurries were allowed to dry, forming a uniform layer on the bottom of the petri dishes. At dosing, the soil surface (19.6 cm^{-2} per petri dish) was treated with aliquots (200 µl) of a glyphosate stock solution (4.725 mg [^{14}C]glyphosate plus 21.28 mg unlabelled glyphosate) in a circular pattern. The volume of water in the dosing solution was calculated to provide 75 % of the soil water holding capacity. The amount of glyphosate applied to each petri dish was equivalent to an application rate of 4.48 kg glyphosate/ha (4.0 lb/acre).

After dosing, petri dishes were placed in temperature controlled stainless steel chambers: one set was covered with dark material to prevent exposure of dark control samples to light, the other with quartz glass plates for the light exposed samples. Sample chambers were exposed to natural sunlight at 37.45° N ad longitude 122.26° W (Richmond, California) from February 24 through March 27, 1989 corresponding to 31 days of incubation. Sunlight intensity and cumulative sunlight energy were measured and recorded at 10 minute intervals throughout the study.

Each chamber was equipped with a coolant (Prestone antifreeze:water (1:1)); temperature was continuously monitored at 10 minute intervals using thermocouples attached to the soil surface in both irradiated and dark conditions. The temperature range was 15.6 to 30.7 °C in the light exposed samples, and 15.8 to 28.5 °C in the dark control samples.

Humidified air was drawn through each sample chamber and then consecutively through three traps, one with ethylene glycol and the other two with 10 % NaOH solution for trapping of volatile organic compounds and carbon dioxide, respectively.

2. Sampling

Duplicate test systems were processed and analysed 0, 3, 7, 11, 20 and 31 days after treatment (DAT).

All soil samples were processed on the day of sampling. Trapping solutions were collected for analysis and replaced with fresh solutions at the same sampling times.

3. Analytical procedures

At each sampling interval, soil samples were extracted twice with 0.5 N KOH (1x 20 mL, 1 x 15 mL) by vortexing and subsequent centrifugation. Extracts were combined and the total volume recorded; then, aliquots (3 x 0.5 mL) were analysed for radioactivity by liquid scintillation counting (LSC). Extracted soil samples were dried and aliquots (2 x 500 mg) analysed for unextracted radiocarbon by combustion followed by LSC. Glyphosate and its potential degradates were identified by HPLC. Identities of degradates were confirmed by TLC.

Soil samples in which > 9 % AR remained bound after extraction with 0.5 N KOH as determined by combustion, were re-extracted to reduce the radiocarbon level in soil. Aliquots of the soil samples (0.25 g) were shaken on a wrist action shaker for one hour with 0.03 M Na_2EDTA (20 mL). Radiocarbon was measured and selected extracts were analysed by HPLC.

The limit of detection (LOD) and limit of quantification (LOQ) for both chromatographic methods (HPLC, TLC) were 0.5 % AR and 0.1 % AR, respectively. LOD and LOQ for the radiodetection method were not reported. [^{14}C]Carbon dioxide was trapped in sodium hydroxide solutions. Its presence was confirmed by precipitation with barium chloride.

II. RESULTS AND DISCUSSION

A. DATA

The radioactive overall mass balance and distribution of glyphosate and metabolites in soil extracts are summarised below.

Table 8.1.1.1-87: Mass balance for [^{14}C]glyphosate in irradiated and dark control samples (expressed as percent of applied radioactivity)

Sample description/ Replicate	**Extractable**	**Unextracted [^{14}C] in soil**			**Volatiles**		**Total** [2]
		Original unextracted	Extracted with EDTA	Residual unextracted	NaOH	Ethylene glycol	
Day 0							
Irradiated (1)	103.1	1.9	- [1]	1.9	< LOQ	< LOQ	105.0
Irradiated (2)	106.5	3.6	- [1]	3.6	< LOQ	< LOQ	110.2
Dark Control (1)	102.5	2.8	- [1]	2.8	< LOQ	< LOQ	105.2
Dark Control (2)	93.2	3.2	- [1]	3.2	< LOQ	< LOQ	96.3
Day 3							
Irradiated (1)	94.2	9.6	4.9	4.7	1.3	0.06	105.1
Irradiated (2)	96.9	9.7	4.9	4.8	1.3	0.06	107.9
Dark Control (1)	96.9	10.1	4.8	5.3	4.1	0.01	111.2
Dark Control (2)	96.1	8.6	- [1]	8.6	4.1	0.01	108.9
Day 7							
Irradiated (1)	98.2	10.4	4.2	6.2	1.7	0.15	110.4
Irradiated (2)	95.1	9.9	5.3	4.6	1.7	0.15	106.8
Dark Control (1)	91.6	6.4	- [1]	6.4	4.8	0.02	102.8
Dark Control (2)	89.2	9.6	3.7	5.9	4.8	0.02	103.5
Day 11							
Irradiated (1)	95.6	6.8	- [1]	6.8	1.9	0.24	104.5
Irradiated (2)	96.6	5.8	- [1]	5.8	1.9	0.24	104.6
Dark Control (1)	93.1	7.4	- [1]	7.4	5.1	0.05	105.6
Dark Control (2)	93.6	5.1	- [1]	5.1	5.1	0.05	103.9
Day 20							
Irradiated (1)	87.6	10.1	9.4	0.7	2.3	0.34	100.4
Irradiated (2)	85.6	13.8	6.4	7.4	2.3	0.34	102.1
Dark Control (1)	84.8	11.8	5.8	6.0	5.8	0.08	102.5
Dark Control (2)	83.2	10.3	5.7	4.6	5.8	0.08	99.4
Day 31							
Irradiated (1)	91.5	11.5	10.4	1.1	4.0	0.5	107.4
Irradiated (2)	90.7	14.8	5.1	9.7	4.0	0.5	110.0
Dark Control (1)	87.5	10.5	6.5	4.0	6.6	0.09	104.7
Dark Control (2)	85.0	13.2	5.1	8.1	6.6	0.09	104.9

[1] Soil samples were not re-extracted as <9 %AR remained bound after extraction with 0.5N KOH
[2] There may be slight discrepancies due to rounding errors

Table 8.1.1.1-88: Characterisation of extractable radioactivity following treatment with [^{14}C]glyphosate in irradiated and dark control samples (expressed as percent of applied radioactivity)

Sample description/ Replicate	**Glyphosate**	**AMPA**	**Unknowns**
Day 0			
Irradiated (1)	101.6	1.5	0.0
Irradiated (2)	104.8	1.6	0.1
Dark Control (1)	100.8	1.6	0.0
Dark Control (2)	91.7	1.4	0.0
Day 3			

Irradiated (1)	85.6	8.3	0.2
Irradiated (2)	88.3	8.4	0.3
Dark Control (1)	90.1	6.8	0.1
Dark Control (2)	89.9	6.2	0.1
Day 7			
Irradiated (1)	88.9	9.3	0.2
Irradiated (2)	85.6	9.5	0.2
Dark Control (1)	84.3	7.3	0.0
Dark Control (2)	81.9	7.2	0.1
Day 11			
Irradiated (1)	85.9	9.7	0.2
Irradiated (2)	85.9	10.7	0.2
Dark Control (1)	83.7	9.3	0.1
Dark Control (2)	85.3	8.3	0.1
Day 20			
Irradiated (1)	76.8	10.8	0.3
Irradiated (2)	75.1	10.5	0.3
Dark Control (1)	76.2	8.7	0.1
Dark Control (2)	74.7	8.5	0.1
Day 31			
Irradiated (1)	78.9	12.6	0.5
Irradiated (2)	77.2	13.3	0.7
Dark Control (1)	78.6	8.8	0.1
Dark Control (2)	74.3	10.7	0.1

B. MASS BALANCE

Radiocarbon recoveries averaged 105.1 % based on nominal applied radioactivity (% AR). Single values ranged from 96.3 to 111.2 % (% AR). Recoveries averaged 89.5 ± 4.0 % (average ± SD, n = 12) and 89.3 ± 5.9 % (average ± SD, n = 10) for light exposed and dark control samples, respectively.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 20 DAT from 103.1/106.5 to 87.6/85.6 % AR in irradiated soil and from 102.5/93.2 to 84.8/83.2 % AR in dark control soil followed by a slight increase at 31 DAT to 91.5/90.7 and 87.5/85.0 % in irradiated and dark control samples, respectively.

Non-extractable ^{14}C increased over the study period in both light exposed and dark control samples, from 0 DAT to 31 DAT from 1.9/3.6 to 11.5/14.8 % AR in irradiated soil and with similar amounts in dark control samples (from 2.8/3.2 at 0 DAT to 10.5/13.2 % AR at 31 DAT). Soil samples in which >9 % AR remained bound following extraction with 0.5N KOH were re-extracted with 0.03N Na_2EDTA. HPLC analysis of a representative light exposed extract indicated that the bound material was glyphosate and AMPA. Although the low [^{14}C] concentration and high Na_2EDTA concentration in the dark control extracts precluded HPLC analysis, it is highly probable that the extracted radiocarbon was likewise comprised of glyphosate and AMPA.

D. VOLATILE RADIOACTIVITY

Formation of $^{14}CO_2$ increased during the experimental period. Maximum amounts of carbon dioxide reached at study end (31 DAT) were 4.0 % AR in irradiated soil and 6.6 % AR in dark control. No radiocarbon was detected in the ethylene glycol traps at levels >0.5 % AR. $^{14}CO_2$ evolved during the study was quantitated as sodium carbonate and its identity confirmed by precipitation with barium chloride.

E. TRANSFORMATION OF THE TEST ITEM

The major degradation product observed in both light and dark samples was the AMPA derivative of glyphosate. Formation of carbon dioxide was observed in both light exposed and dark control samples with slightly higher yields in the latter. AMPA appears to generally form in somewhat greater amounts in the light (31 DAT: maximum amounts of 12.6/13.3 % AR and 8.8/10.7 % AR in light exposed and dark control samples, respectively). However, the combined amounts of both degradation products (AMPA and CO_2)

are essentially constant between irradiated and dark control samples. No other metabolites were detected above 0.7 % AR at any time.

F. KINETICS

New kinetic calculations based on more recent guidance will not be provided as this study is only delivering supplemental information. DT_{50} values for glyphosate, based upon a linear extrapolation to the first order model, were 90.2 days (R = 0.82) in sunlight and 96.3 days (R = 0.86) in the dark.

III. CONCLUSIONS

The photodegradation of [^{14}C]]glyphosate on soil surfaces to its AMPA derivative is not a photochemically accelerated process. A significant difference in degradation rates for light exposed and dark control samples was not obtained.

The results of this study support that the degradation of glyphosate to AMPA on soil is microbially induced.

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]glyphosate on soil surfaces was examined at an application rate of 4.48 kg/ha soil under natural sunlight. Mass balances ranged from 96.3 to 111.2 % of applied radioactivity (% AR).

As natural sunlight was used for the experiment instead of preferred artificial light, the study is considered as supportive information.

Assessment and conclusion by RMS:

Natural sunlight was used. Although artificial sunlight is generally preferred, all relevant information on the sunlight conditions (sunrise, sunset, cloud cover, light intensity and total light energy) are reported in the study.

However, more details would be required on the soil used for the experiment. No information on the soil history, storage or transport are reported in this study. In addition, the temperature was not constant throughout the study (15.6 to 30.7°C for the light exposed samples).

The study is not acceptable.

[redacted], *1983*

Data point:	CA 7.1.1.3/005
Report author	[redacted]
Report year	1983
Report title	The photodegradation of SC-0224 applied to soil
Report No	PMS-137
Guidelines followed in study	None
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - test duration not clear - samples exposed during day (192 hours) and frozen over night - tests were not conducted with an artificial irradiation source, but samples exposed to natural sunlight
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Analytical SC-0224, trimethylsulfonium carboxymethylaminomethylphosphonate, consisting of 57.04 % SC-0224 and 40.4 % water

Lot No.: WRC-7466-14-01

Chemical purity: 95.7 % on an anhydrous basis

2. Soil:

A Felton loam sand soil was selected as test soil and sieved to 500 µ (0.5 mm). Characteristics of the test soil are presented in the table below.

Table 8.1.1.1-89: Characteristics of test soil

Parameter	**Results**
Soil	Felton
Country	Not indicated
Textural Class	Loamy Sand
Sand (1.0 – 2.0 mm µm – 2 mm) (%)	0.1
Course sand (0.5 – 1.0 mm)	16.0
Medium fine and very fine sand (0.05 – 0.5 mm)	72.0
Silt (0.002 mm – 0.05 mm) (%)	6.8
Clay (< 0.002 mm) (%)	5.1
pH [2]	5.4
Organic carbon (%) [1]	1.5
Organic matter (%)	2.6
Cation exchange capacity (meq/100 g)	10.9

[1] Calculated from organic matter according to OC = OM x 0.58

[2] Medium not stated

B. STUDY DESIGN

1. Experimental conditions

5 g of the selected soil was weighed into 9 cm (diameter) pyrex petri plates. The addition of 3.0 - 3.5 mL deionized water helped to spread the soil into a thin, even layer in each plate. The soil in uncovered plates dried overnight. Due to its sandy nature, the dry soil did not adhere well to the plate and was thus dampened by spraying it with a small amount of water. Subsequently, the soil was treated with 1 mL of the SC-0224 solution (non-labelled test item), sprayed on the soil holding a DVilbiss sprayer 5.1 - 7.6 cm above the plate. To ensure that the entire dose reached the soil, each application was rinsed through the sprayer with 0.5 mL water, also sprayer onto the soil.

The application solution of SC-0224 contained 2.61 x 10^{-2} g analytical SC-0224 (100 mL H_2O)$^{-1}$ and accordingly 1 mL of the solution sprayed on the surface of a thin layer of 5 g soil in a petri plate resulted in a concentration of 30 mg SCC-0224/kg soil.

Following treatment, all samples were covered with a box and allowed to partially dry overnight. Subsequently, the samples to be illuminated were set uncovered on a bench in an outdoor area exposed to sunlight. Dark controls were grouped according to total exposure time and each group was loosely wrapped in aluminium foil. Temperature and light meter readings were taken throughout the day; thermometers were located both inside and outside the soil. Each evening all samples were covered and frozen (- 20 °C) until the following exposure day. Dark controls were handled expediently to minimize exposure to light. No temperature and moisture control was taking place, the graphical temperature plot shows variances of 20 to 40 °C.

Soil in petri plates were incubated under outdoor conditions for 192 hours of exposure in the daytime.

2. Sampling

Quadruplicate samples were collected after 0, 6, 12, 18, 36, 48, 96, 144 and 192 hours of incubation, i.e. the time where samples were frozen during night-time was excluded. Sampling of quadruplicates allowed

the separate analysis of glyphosate anion and trimesium (TMS) cation, each in duplicates. Following treatment, samples from 0 hours were immediately frozen until analysis.

3. Analytical procedures

The anion was extracted with 0.5 M NH_4OH, filtered, concentrated to dryness and derivatized with 9-fluorenylmethyl chloroformate. Samples were analysed using HPLC with a strong anion exchange column and a variable wavelength fluorescence detector. The solvent system was 0.02 M borate buffer (pH 9.0, flow rate 2 mL min^{-1}). This analysis procedure detected both glyphosate and its photolyte, aminomethylphosphonate (AMPA). The theoretical maximum concentration of AMPA that could form (13.6 ppm (13.6 mg/L)) was used to calculate the percent of AMPA found. Samples were not analysed for other photolytes. The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC method were 0.01 and 0.1 mg/L, respectively.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.1-90: Characterisation of extractables following treatment with SC-0224 in illuminated and dark control samples (expressed as % of nominal amount of glyphosate applied, all values are means of two replicates if not indicated otherwise)

Illumination length (h)	glyphosate		AMPA		glyphosate + AMPA
	Illuminated	Dark	Illuminated	Dark	(Illuminated)
0	98.8	93.2	4.8	<0.7 [1,2]	103.6
6	92.8	93.2	4.1	1.5	96.9
12	92.0	93.2 [1]	5.2	<0.7 [1,2]	97.2
18	83.1	85.3	6.3	<0.7 [1,2]	89.4
36	74.9	91.6	9.6	<0.7 [1,2]	84.5
48	75.9	91.1	12.5	<0.7 [1,2]	88.4
96	64.3	87.0 [1]	16.2 [1]	8.1 [1]	80.5
144	62.6	89.4	19.9 [1]	<0.7 [1,2]	82.5
192	59.7	84.1	24.3 [1]	<0.7 [1,2]	84.0

[1] Only one of the duplicates was analysed
[2] AMPA concentration was less than the LOQ of 0.1 mg/L (i.e. 0.7 % of the total possible theoretical concentration)

B. DEGRADATION OF THE TEST ITEM

In illuminated samples, the sum of the % glyphosate anion and % AMPA constituted recoveries between 80.3 to 103.6 % of the nominally applied glyphosate (mean of two replicates). Material balance cannot be determined. Over the course of the study, glyphosate decreased from initially 98.8 % of nominally applied to 59.7 % after 192 h of irradiation. The amount of AMPA (molar base, relative to glyphosate nominally applied) increased during the study period towards a maximum amount of 24.3 % at study end (192 h) in irradiated samples. Recovery of glyphosate in dark control samples declined gradually throughout the experiment, from 93.2 % to 84.1 % of the glyphosate applied. There was no corresponding formation of AMPA in the dark controls.

Trimesium recovery generally was between 55 and 69 % except for two samples (96 h and 144 h) in both the illuminated and dark control samples where duplicates were not analysed. Throughout the study, the resulting recoveries in dark controls and illuminated samples parallel each other with no apparent overall decrease in concentration. This indicates that no photodegradation of TMS occurred throughout the study period. Recovery of trimesium from 0 DAT samples was between 64 and 70 % for dark control and illuminated samples, respectively, and recovery of trimesium from the soil fortified just prior to analysis resulted in recoveries between 62.5 – 72 % of that added. Apparently an instantaneous, chemical breakdown of trimesium occurs in soil, described also in other experiments in soil and aquatic/sediment systems. In summary, approximately 35 % of trimesium was lost instantly from both illuminated and dark control samples. Subsequently, recovery was stable indicating no photodegradation on trimesium.

C. KINETICS

New kinetic calculations based on more recent guidance will not be provided as this study is not considered valid to describe the photolytic behaviour of glyphosate.

Assessment and conclusion by applicant:

The photodegradation of unlabelled glyphosate (applied as analytical trimethylsulfonium carboxymethylaminomethylphosphonate SC-0224) on soil surfaces was examined at a concentration of 300 mg SC-0224/kg soil. The duration of the study was 192 hours, discontinued each evening when all samples were covered and frozen until the following exposure day.

Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

Considering the major deviation to guideline in study conditions (mostly temperature during the night and duration of the study) the study is not acceptable.

[redacted], *1978*

Data point:	CA 7.1.1.3/006
Report author	[redacted]
Report year	1978
Report title	Photodegradation and anaerobic aquatic metabolism of Glyphosate, N-Phosphono-Methylglycine
Report No	MSL-0598
Guidelines followed in study	None
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - study duration of 3 days - temperature not measured in the study – indicated as 54°C on soil surface by applicant - important basic data not available (e.g. LOD/LOQ, amount of soil used)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]glyphosate (PMG)
Lot No.: not indicated
Specific activity: 10.12 mC/mM
Radiochemical purity: 98 – 99 % (TLC)

2. Soil:

Soils were sieved to ca. 0.6 mm. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-91: Characteristics of test soil

Parameter	Results
Soil	Ray silt loam
Country	not indicated
Textural Class	Silt loam
Sand (%)	4.6
Silt (%)	84.2
Clay (%)	10.0
pH (medium not stated)	8.1
Organic carbon (%) [1]	0.7

Organic matter (%)	1.2

[1] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

Sieved soil was slurried with water to prepare test plates (20 x 20 cm). The plates were divided into four sections and trimmed so that each section was 8 x 8 cm. The plates were exposed to a 275-watt GE sunlamp for 72 hours in order to eliminate microbial degradation of glyphosate.

Each section of the plate was treated with 717 µg of a mixture of 5 µg of [^{14}C]glyphosate and 712 µg of unlabelled glyphosate. This treatment is equivalent to 4.5 kg/ha (4 lb/acre). Following treatment, sections were exposed to artificial sunlight. An additional section was treated, covered with aluminium foil and placed under the sunlamp to serve as control.

The lamp was placed 15 cm above the soil surface so that the greatest intensity of light, 1500 watts/m², as determined by a Radiometer, was at the centre of the plate and the intensity of the plate at the extreme corners was equal.

2. Sampling

The prepared sections were exposed to artificial sunlight for 0, 24 and 72 hours.

3. Analytical procedures

After the appropriate exposure period, the soil from the exposed and control sections was scraped from the plate and extracted two times with 0.5 N NH_4OH. Radioactivity was quantified by LSC and degradation of glyphosate analysed by HPLC and TLC. One section of the plate was treated and extracted immediately to determine the recovery of ^{14}C-activity at zero time.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/TLC//LSC were not reported.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.1-92: Degradation over time following treatment with [^{14}C]glyphosate in light exposed and dark control samples (expressed as % of initial amount)

Exposure	TLC	HPLC
Dark control (72 h)	2.2	2.3
Irradiated (24 h)	4.6	6.6
Irradiated for (72 h)	5.2	8.3

B. MASS BALANCE

The total recovery of ^{14}C-activity extracted from soils was 100.2, 102.7, 102.7 and 105.7 % for the zero time control, the 72 h control, the 24 h irradiated soil and the 72 h exposed soil, respectively. In view of these recoveries it is evident that there was no loss of ^{14}C-activity indicating that glyphosate is not volatilized from the dry soil surface.

C. EXTRACTABLE RESIDUES

The degradation of [^{14}C]glyphosate in soil extracts was relatively slow, with 4.6/6.6 % degradation after 24 hours and 5.2/8.3 % degradation after 72 hours of irradiation determined by TLC/HPLC analyses, respectively. Degradation of glyphosate was lower in dark control samples, with 2.2 and 2.3 % degradation based on TLC and HPLC results, respectively.

E. KINETICS

In view of the low number of data points, a kinetic assessment is not feasible.

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]glyphosate on soil surfaces was examined under simulated sunlight at a rate of 4.5 kg/ha soil. The study duration was only 3 days. The temperature on the soil surface was 54 °C. Mass balances ranged from 100.2 to 105.7 % AR. Limited information on the soil given in the study report.

Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

Very shallow information are provided in the study report (lack of soil history, conditions of transport/storage…). In addition, major deviations are observed in this study.

The study duration is very short (3 days). The applicant mentions that the temperature on the soil surface was 54°C but RMS could find no indication of any temperature during the study. However, the report indicates that degradations were slow "under the combined effects of sunlight and heat", seemingly confirming that a high temperature was observed on the soils surface. The sunlamp was placed 15cm above the soil surface with a high intensity of 1500 W/m² which would probably explain the high soil surface temperature. It is not reported whether radiations below 290 nm were cut-off.

The study is not acceptable.

[redacted], *1972*

Data point:	CA 7.1.1.3/007
Report author	[redacted]
Report year	1972
Report title	MON-0573, Residue and metabolism. Part 2: The photolysis, run-off, and leaching of MON-0573 on or in soil
Report No	258
Guidelines followed in study	US PR Notice 70-15 (1970)
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - study duration of 48 hours - temperature on soil surface 30-31°C - application rate unclear - important basic data not available (e.g. amount of soil used, light intensity). - tests were conducted with an artificial irradiation source, but samples exposed to UV light of 100-380 nm range
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]MON-0573
Lot No.: not indicated
Specific activity: 8.06 mc/mMol (analysis prior to experimental start)
Radiochemical purity: 96.0 % (analysis prior to experimental start)

2. Soil:

All soils were sieved to ca. 0.6 mm. Characteristics of the test soils are presented in the table below.

Table 8.1.1.1-93: Characteristics of test soils

Parameter	Results		
Soil	Ray	Drummer	Norfolk
Country			
Textural Class	Silt loam	Silty clay loam	Sandy loam
Sand (%)	6.0	2.0	86.0
Silt (%)	83.2	55.4	11.0
Clay (%)	9.6	36.8	2.3
pH [2]	6.5	7.0	5.7
Organic carbon (%) [1]	0.6	3.5	0.6
Organic matter (%)	1.0	6.0	1.0

[1] Calculated from organic matter according to OC = OM x 0.58
[2] Buffer medium not indicated

B. STUDY DESIGN

1. Experimental conditions

Sieved soil (400, 300 and 300 g for soils Norfolk, Ray and Drummer, respectively) and water (107, 135 and 176 mL for soils Norfolk, Ray and Drummer, respectively) were mixed and formed a slurry on TLC plates of 0.75 mm thickness. Following spreading onto TLC plates by using a Shandon spreader, the soil slurry was allowed to dry overnight.

A stock solution was prepared by dissolving 46.75 mg of [^{14}C]glyphosate in 46.75 mL of 0.1 M NH_4HCO_3. Each of the three 2 cm bands located on the origin (3 cm from the bottom) was spotted with 10 µL of the stock solution, containing each 1,050,000 dpm corresponding to 10 µg [^{14}C]glyphosate. Separate sheets of aluminium foil were used to cover the left and middle 2 cm bands of glyphosate on the three soil TLC´s.

All three plates were then placed under a black light fluorescent fixture so that the exposed 2 cm bands were ca. 2.5 cm directly below one of the fluorescent tubes. The black light utilised was a 91 cm fluorescent fixture equipped with three 40 watt GE-F4OBL fluorescent tubes. After a 24 hours exposure to UV light, the aluminium foil was removed from the middle band and an additional 24 hours UV exposure was carried out. As a result, the three 2 cm bands were exposed to UV light for 0, 24 and 48 hours, respectively.

A recording thermometer with a probe next to the soil TLC´s indicated the temperature ranged from 30 – 31°C during UV exposure.

2. Sampling

The 2 cm band of soil treated with glyphosate were exposed to UV light for 0, 24 and 48 hours.

3. Analytical procedures

Following development of soil TLC plates with water in a horizontal chromatography chamber, the soil TLC´s were allowed to dry horizontally overnight before evaluation with a Beta Camera. After evaluation of the first development, the soil TLC´s were developed with water a second time as before.

II. RESULTS AND DISCUSSION

A. TRANSFORMATION OF THE TEST ITEM

After UV exposure of [^{14}C]-glyphosate on soil for 48 hours there were no significant degradation products that were moved from the origin after two developments of the soil TLC´s with water.

B. KINETICS

In view of the low number of data points, a kinetic assessment is not feasible.

III. CONCLUSIONS

Irradiation of three soil TLC plates for 48 hours failed to give any soil mobile decomposition products. Photolysis is not considered to be a major cause of breakdown of glyphosate on soil.

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]-glyphosate on soil surfaces was examined using an artificial light source according to a pertinent guideline at the time of conduct. In view of current guidelines there are several deviations. The application rate is not clear. The study duration was only 48 hours. The temperature on the soil surface ranged from 30 to 31 °C. Limited information on the soil, preparation of soil layers, amount of soil used, light intensity and application procedure given in the study report.

Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

Considering the above mentioned deviations, the study is not acceptable.

B.8.1.1.1.4. Summary on the route of degradation of glyphosate in soil

Under laboratory aerobic conditions, reliable information on the route of degradation of glyphosate are available from 11 soils. The extent of mineralisation was high with a maximum amount of 70.6 % AR after 121 days (mean of replicates). The formation of non-extractable residues reached a maximum amount of 21.6 % AR (mean of replicates) after 90 days. The major degradation product observed in soil under aerobic conditions is aminomethylphosphonic acid (AMPA). AMPA was found with a maximum occurrence of 42.4% AR in laboratory studies.

Under anaerobic laboratory conditions, the degradation of glyphosate slowed down while the degradation pathway remained identical to that under aerobic conditions. The only major degradation product observed was AMPA with a maximum occurrence of 29.7 % AR after 84 days of anaerobic incubation. Mineralisation was negligible under anaerobic conditions and non-extractable residues increased by a maximum of 10 % AR during the anaerobic incubation phase.

A single laboratory soil photolysis study is considered as reliable and shows that degradation of glyphosate in soil is slightly enhanced by irradiation. Mineralisation reached 14.6% AR after 30 days under irradiated conditions against 5.4% AR in the dark control. The extent of non-extractable residues was similar in irradiated and dark control, with maximum of 19.4% AR after 14 days and 17.4% AR after 21 days, respectively. The major degradation product observed was AMPA with a maximum occurrence of 8.2 % AR after 7 days in irradiated samples (6.1 % AR after 3 days in dark control samples). No further photolytic degradation products were observed at levels above 5 % AR.

The proposed degradation pathway of glyphosate in soil is available below.

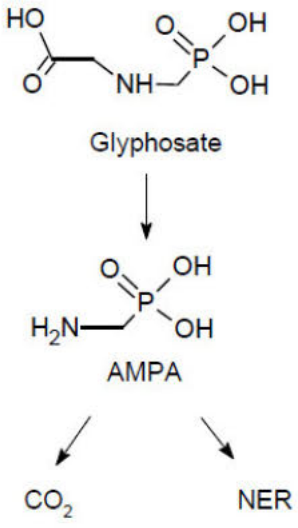


Figure 8.1.1.1-15: Proposed degradation pathway of glyphosate in soil

B.8.1.1.2. *Rate of degradation in soil*

B.8.1.1.2.1. Rate of degradation in soil – aerobic conditions

Glyphosate

All study summaries for glyphosate applied studies are reported under point B.8.1.1.1. The results of the studies were kinetically evaluated according to the current EU guidance (FOCUS, 2006, 2014) to derive degradation rates for glyphosate and AMPA for comparison with trigger values and as endpoints for input in modelling ([redacted] 2020a).

In the scientific literature review for glyphosate (2010-2019), eleven articles were identified to provide further information relevant to the data point.

The kinetic evaluation from [redacted] 2020a is presented below. Please note that for easier reading, RMS comments on the kinetic evaluation provided by the applicant is reported soil by soil in the study summary. In most cases, additional fittings were done by RMS and are also presented within the study summary (but easily identifiable in blue boxes). Some of the soils were kinetically fitted by applicant but were not considered as reliable for deriving endpoints by RMS (see section 8.1.1.1). These fits were presented under appendix 1 in order to leave below only the relevant kinetics and ease the reading.

The RMS evaluation presented below is based on the recommendations of FOCUS Kinetic guidance. RMS also tried to take into account the recent practice in other active substance dossiers as it has evolved since the Kinetic guidance was published.

Despite the rules given in the FOCUS Kinetic guidance, some decisions on kinetic fits still needs to be based on expert judgement and thus can be subject to discussions. General principles considered by RMS are clarified below, and might help to understand some choices made by the RMS.

<u>For parent substance glyphosate</u>

For modelling endpoints:

Parent-only fits were first evaluated.

SFO is usually preferred for modelling because FOCUS models use first-order kinetics. However glyphosate was shown to have a biphasic degradation in most of the soils, with SFO not being sufficiently acceptable for modelling. Recommendations of FOCUS Kinetic guidance were followed to determine the appropriate biphasic model, in particular by considering whether <10% AR remained as glyphosate at the end of the study.

FOCUS Kinetic guidance proposes some approaches to derive modelling endpoints when biphasic kinetic is selected (*i.e.* use of DT_{90FOMC}/3.32, use of slow-phase from DFOP or HS). These endpoints can be used for modelling of parent glyphosate alone.

However, it is also clearly indicated that "these corrected DT_{50} values can only be used to simulate the leaching of a parent compound, and must not be used to simulate the fate of the parent and a metabolite in a linked model run (i.e. the formation of the metabolite is directly calculated from the degradation of the parent). Information on how to proceed in this situation can be found in Chapter 8". Indeed, these corrected DT_{50} values are worst-case for the parent, but not necessarily for the metabolite simulation.

The FOCUS guidance also states that "If the SFO model is not appropriate for the parent, and the FOMC model is shown to be more appropriate as outlined in section 6.3.1 (indicating a bi-phasic degradation pattern), the parent should then be fitted with an appropriate non-SFO model that may be implemented in environmental models, as recommended in Section 7.1.2. The option of back-calculating a half-life from a bi-phasic DT_{90} is limited to modelling of the parent alone, and is not appropriate for deriving the kinetic endpoints of metabolites".

These points were kept in mind by the RMS when selecting the kinetic for modelling endpoint for the pathway fit. Therefore, in RMS opinion, since FOMC cannot be implemented in FOCUS models, this kinetic is not suitable for parent-metabolite modelling endpoint. Therefore, when biphasic kinetic was

required for parent, DFOP was systematically preferred (providing that it is visually and statiscally acceptable) for the pathway fit.

For metabolite AMPA

For parent-applied studies, in some cases no clear decline of AMPA was observed, but generally a plateau was reached. RMS considered that even when no decline is observed, it is still possible to derive reliable endpoint for metabolites. Indeed, the formation phase of the metabolite also include degradation of the metabolite and the kinetic tools can derive endpoints.

RMS first checked whether the experimental points were well described by the fitted curve (visual assessment). If it was not the case, the fit was rejected. If the visual assessment was acceptable but t-test was not acceptable, as a conservative approach the fit was generally selected since this approach allows to take into account the potential higher persistence of the metabolite in these soils.

Data point:	CA 7.1.2.1.1/001
Report author	[redacted]
Report year	2020a
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from aerobic laboratory soil degradation studies
Report No	112148-001
Guidelines followed in study	FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance 2014: - initial concentration was not corrected for radiochemical purity
GLP	Not applicable
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

The purpose of this assessment was to conduct a kinetic modelling evaluation for glyphosate and its major soil metabolite AMPA using results from laboratory soil degradation studies. The aim of the evaluation was to derive the following endpoints:

- Trigger endpoints to be used as triggers for higher-tier environmental fate studies
- Modelling endpoints for use in calculating predicted environmental concentrations.

The degradation of glyphosate under aerobic conditions was investigated in eight laboratory studies ([redacted], 1993, [redacted], 1995, [redacted], 1996, [redacted], 1996, [redacted] 2010, [redacted], 1992; [redacted], 1993).

The test soils covered a broad range of soil types and pH values. A total number of 20 soil degradation experiments under dark aerobic conditions were conducted for up to 364 days. Incubation temperatures were between 8 and 25 °C. The soils moisture content ranged between 20 and 50 % MWHC. Additionally, degradation of glyphosate was tested under sterile conditions or with reduced application rate ([redacted], 1992, dose groups D and E, respectively).

1. Data pre-processing

The standard procedures recommended by FOCUS (2006, 2014) were followed to adjust the experimental data for kinetic modelling. Replicate samples were available for all of the studies except [redacted] (1996) and [redacted] (1993).

All measured data points derived from the study reports were included in the kinetic evaluation even if the material balance of single measurements dropped below the level of 90 % of the applied radioactivity as either the material balances were close to 90 % or lower material balances could be attributed to potential loss of $^{14}CO_2$ during the experiments (soil Speyer 2.1 of study [redacted], 1993; soil Drusenheim of study [redacted], 2010).

For experiments exceeding the recommended duration of 120 days (e.g. [redacted], 1993; [redacted], 1995; [redacted], 2010) all data points were included for kinetic evaluation as microbial biomass measurements and ongoing decline of glyphosate concentrations indicated that microbial degradation still occurred.

The initial amounts of glyphosate were set to the value of the material balance at day 0, thus assigning all radioactivity observed at day 0 to the parent compound and assuming that no degradation processes have yet taken place. Accordingly, the initial amounts of the metabolites were set to 0.

It is recommended that values below the LOD should be replaced by half the LOD (FOCUS; 2006, 2014). Processed residue data are presented along with the kinetic results for easier reading..

2. Kinetic models and analysis

Kinetic models

Four kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model), double-first-order-in-parallel (DFOP) and Hockey-stick (HS) (FOCUS; 2006, 2014). The HS model was tested only in cases where none of the other models were able to provide a visually and statistically reliable fit.

In a first step, parent-only fits were conducted to determine the appropriate kinetic model for the parent for trigger and modelling endpoint. For the parent compound, the best-fit model was accepted for deriving trigger endpoints, while the DT_{50} calculated from SFO model was preferably selected as modelling endpoint. If SFO did not provide an acceptable fit, modelling endpoints were derived from an appropriate bi-phasic model. If 10 % of the initial concentration was reached within the experimental period, the DT_{50} was back-calculated from DT_{90} as $DT_{50} = DT_{90}/3.32$. Otherwise, the DT_{50} was derived from the slow-phase degradation rate of the DFOP or HS model.

In a second step, metabolite AMPA was included in the fits. For the metabolite, pathway fits were conducted using the previously selected kinetic model for trigger and modelling endpoints for the parent determination and SFO for the metabolite.

In general, kinetic endpoints for parent and metabolite were derived from acceptable pathway fits. In cases where no reliable pathway fit could be established, kinetic endpoints for the parent were derived from the corresponding parent-only fit, and decline fits were conducted for the metabolite, starting from the maximum observed concentration. The respective day was defined as 0 days after maximum concentration, and later time points were adjusted accordingly.

Optimisation

The kinetic analyses were conducted using the software CAKE 3.3.

The data were directly fitted with the complete dataset and unconstrained initial concentration (M0) for the parent substance. Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M0), degradation model parameters k, α, β, g or tb, depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. By default, the initial amount of metabolite was fixed to 0. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

If a pathway fit did not yield visually and/or statistically reliable results, the kinetic model was further optimised by fixing one or more of the model parameters to either the value derived from a reliable parent-only fit (e.g. M0, k-rates) or to values derived from previous pathway fits with unbound parameters (e.g. ff). A stepwise fixing procedure has been applied in these cases which is further described in the results chapter for the respective pathway fits.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ 2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered

A statistical measure of the quality of a fit is given by the χ^2-test. The χ^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, for parent compounds, it is recommended that if the χ^2-error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. The guidance is less clear for metabolites due to the complexity of the curve fitting for multiple components, and so this criterion is a little more relaxed.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised degradation rate constants (k) of the SFO, DFOP and HS kinetic models were significantly different from zero at a chosen significance level of 5 %. For the FOMC kinetic model, only the confidence interval of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a 95 % confidence interval on the estimated parameters. It should be relatively tight and not contain 0 to be considered statistically robust.

3. Normalisation

Modelling endpoints (DT_{50} values) derived from kinetic analyses have to be normalised to the soil moisture content at field capacity (pF2) and a temperature of 20 °C to be used in environmental fate models.

Moisture correction was carried out by multiplying the respective DT_{50} values by a moisture correction factor. A Walker exponent of 0.7 was used for the correction. The gravimetric water content during the study (θact) was calculated using the soil water characteristics that were given in the respective study reports except for [redacted] (1995) for which the FOCUS default value of 27 g/100 g was used. For the gravimetric water content at pF2 (θref) the default values for the relevant soil types as given by FOCUS (2000) were used.

A temperature correction was necessary for all experiments which were not conducted at 20 °C. A Q_{10} value of 2.58 was used for the correction.

II. RESULTS

[redacted] *2010a, Gartenacker*

Table 8.1.1.2-1: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (2010a), soil Gartenacker

Time (d)	Glyphosate (% AR)	AMPA (% AR)
Gartenacker ([redacted] 2010a)		
0	100.1[1]	0.0[2]
0	99.2[1]	0.0[2]
3	71.1	4.3
3	69.2	4.6
6	58.1	7.0
6	56.6	7.2
10	44.4	8.2
10	43.4	8.0
20	33.3	11.0
20	29.2	13.7
34	17.6	11.5
34	18.0	12.7
55	10.5	14.9
55	9.3	14.5
90	4.5	12.1
90	4.7	12.3
112	3.0	9.9
112	3.4	10.2
132	2.3	8.8
132	2.7	7.8

[1] Set to material balance
[2] Amounts of metabolites set to 0 at day 0

Table 8.1.1.2-2: Kinetic models and statistics for soil Gartenacker ([redacted] 2010a) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.5	k: 0.0604	13.1	k: <0.001	k: 0.0488	k: 0.072	11.5	38.1
FOMC	Good	98.3	α: 1.2563 β: 11.2442	4.6	-[1]	β: 7.6729	β: 14.815	8.3	59.1
DFOP	Good	99.4	k_1: 0.2486 k_2: 0.0305 g: 0.4446	3.0	k_1: <0.001 k_2: <0.001	k_1: 0.1837 k_2: 0.0259	k_1: 0.313 k_2: 0.035	7.9	56.2

Applicant's conclusion

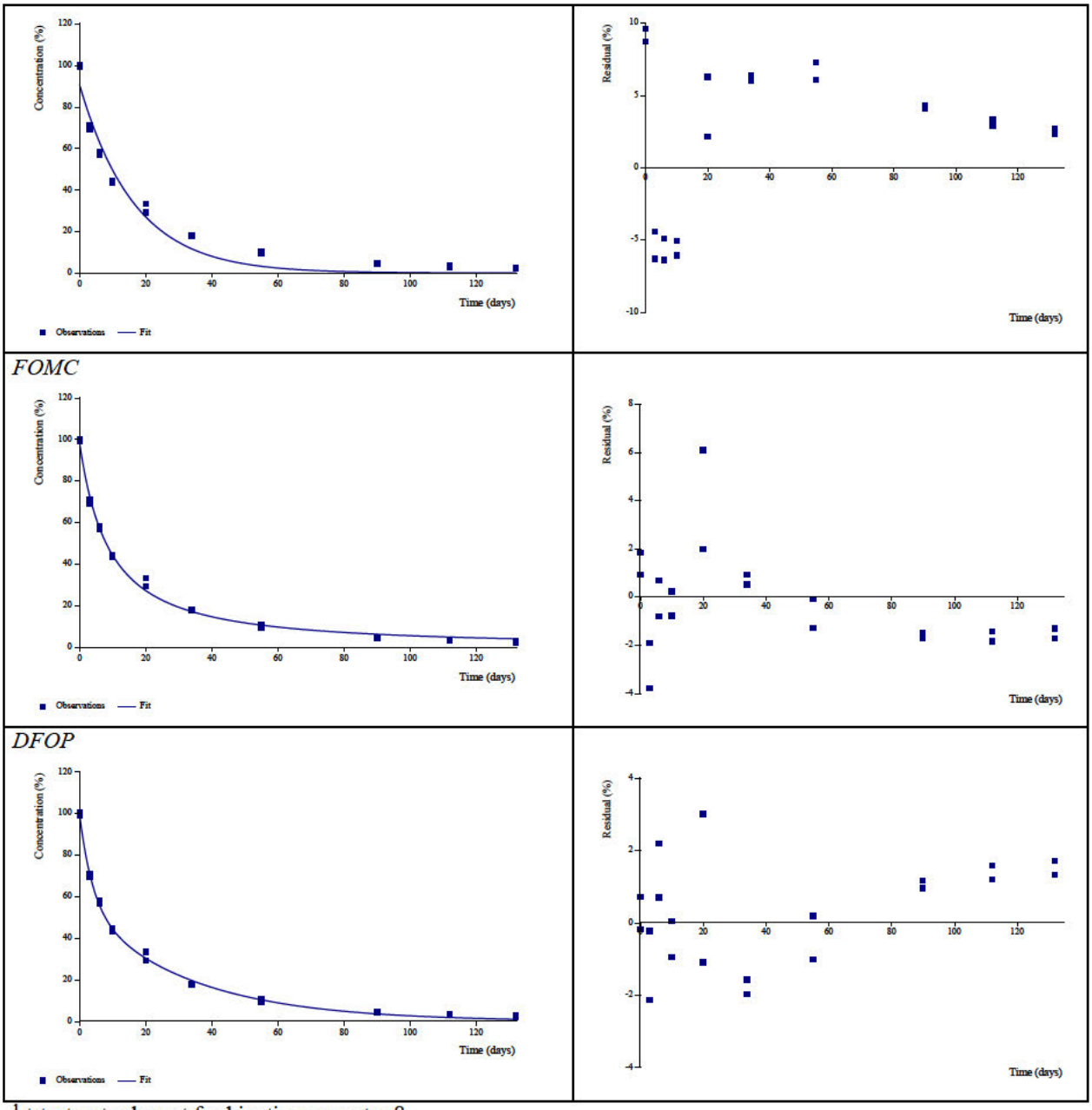
Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits but the DFOP model provides the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

RMS conclusion

Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.

SFO



[1] t-test not relevant for kinetic parameter β

Table 8.1.1.2-3: Kinetic models and statistics for soil Gartenacker of study [redacted] (2010a) -Pathway fits (parent and metabolite)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	99.0	k_1: 0.2501 k_2: 0.0314 g: 0.4307	3.1	k_1: <0.001 k_2: <0.001	k_1: 0.185 k_2: 0.0269	k_1: 0.315 k_2: 0.036	8.1	55.4	-
AMPA: SFO	Acceptable	-	k: 0.0058	8.2	k: <0.001	k: 0.0042	k: 0.007	119	396	0.183 (±0.009)
Applicant's conclusion										

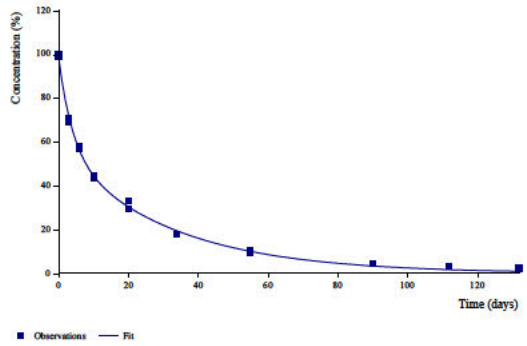
Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

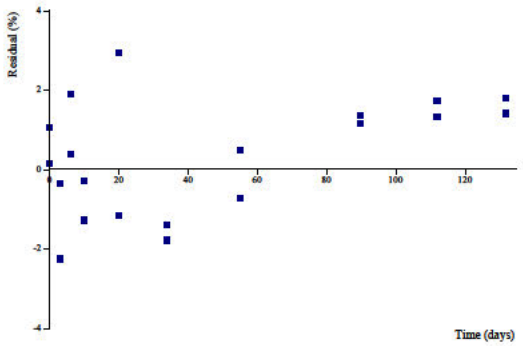
Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

RMS conclusion

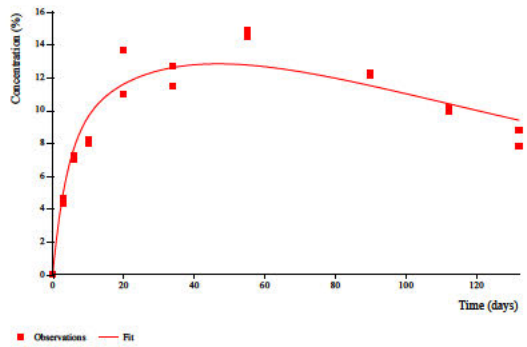
As mentionned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.

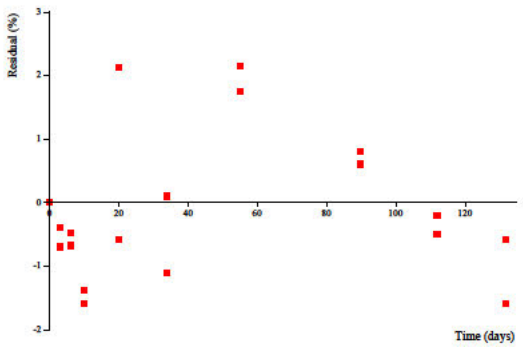
Glyphosate (DFOP)





AMPA (SFO)





RMS additional fittings – Gartenacker, [redacted] 2010a

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 96.3%)
100.1	96.4
99.2	95.5

Parent-only fittings (Gartenacker, [redacted] 2010a)

Kinetic model	Visual/ residual	M0	Kinetic para-meters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	87.94	k: 0.05724	Yes		11.8	12.1	40.2	0.9798
FOMC	Good	94.76	α: 1.366 β: 13.64		No No	4	9.02	60	0.9967
DFOP	Good	95.71	k1: 0.2111 k2: 0.02928 g: 0.4504	Yes Yes		2.83	8.62	58.2	0.9982
RMS conclusion	SFO does not well describe the overall degradation of glyphosate, residuals are not randomly distributed, with a systematic error from day 20. SFO is not considered suitable for trigger nor for modelling endpoint.								

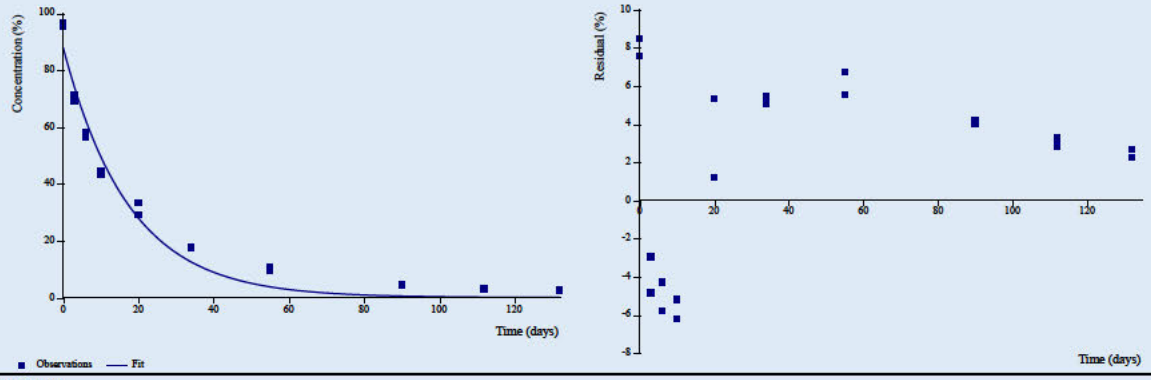
Biphasic kinetics provide better fits. Visual and statistical results are good for both FOMC and DFOP kinetics.

Trigger endpoints: DFOP model is selected as it provides the best fit (lowest χ^2 value, better description of M0, lower extent of residuals compared to FOMC).

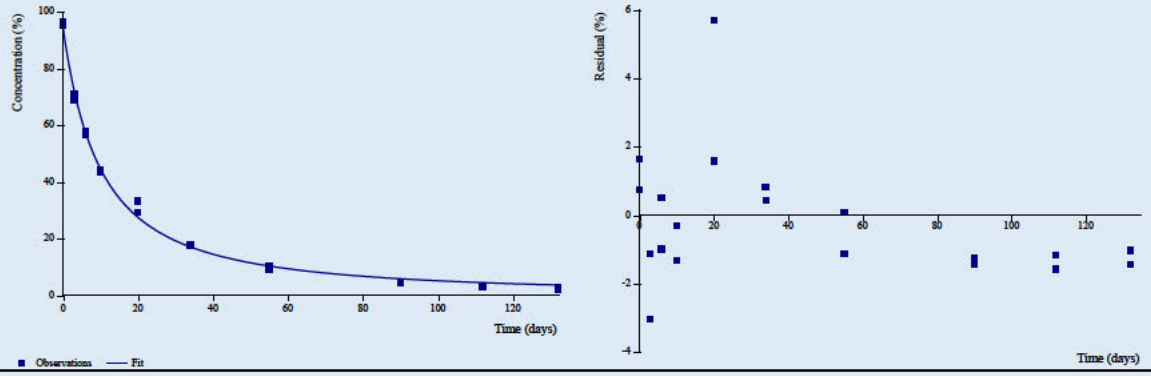
Modelling endpoint: Glyphosate represented less than 10% AR at the end of the study. Therefore FOMC can be selected for modelling of parent-only. However, it is reminded that FOMC kinetic cannot be directly implemented in FOCUS models, and the use of $DT_{90}/3.32$ is not suitable when metabolites are included in the degradation pathway for modelling (see more justification in the introduction).
In this case, DFOP kinetic is fully acceptable, and even provide a better χ^2 error and slightly better description of M0. The extent of residuals is lower with DFOP kinetics. As a consequence, RMS considers that DFOP should be selected for modelling endpoint in pathway fit.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
FOMC acceptable for parent-only modelling.
DFOP to be used in pathway fit for modelling endpoints.

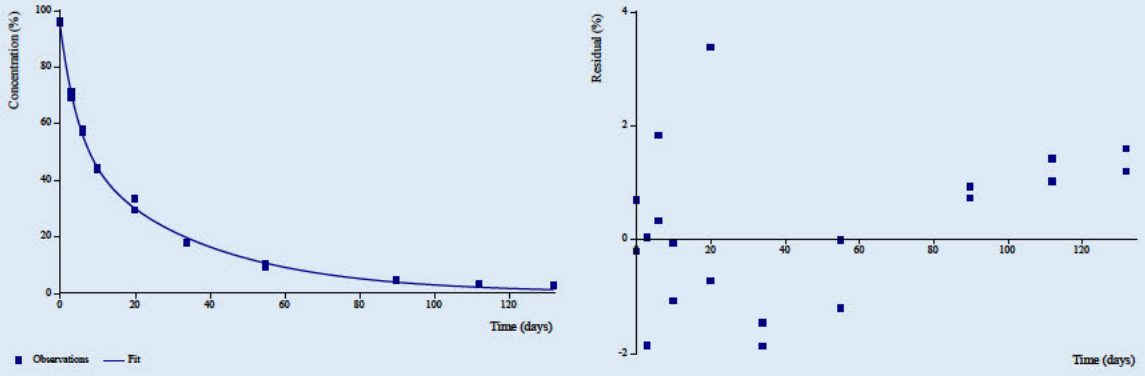
SFO



FOMC



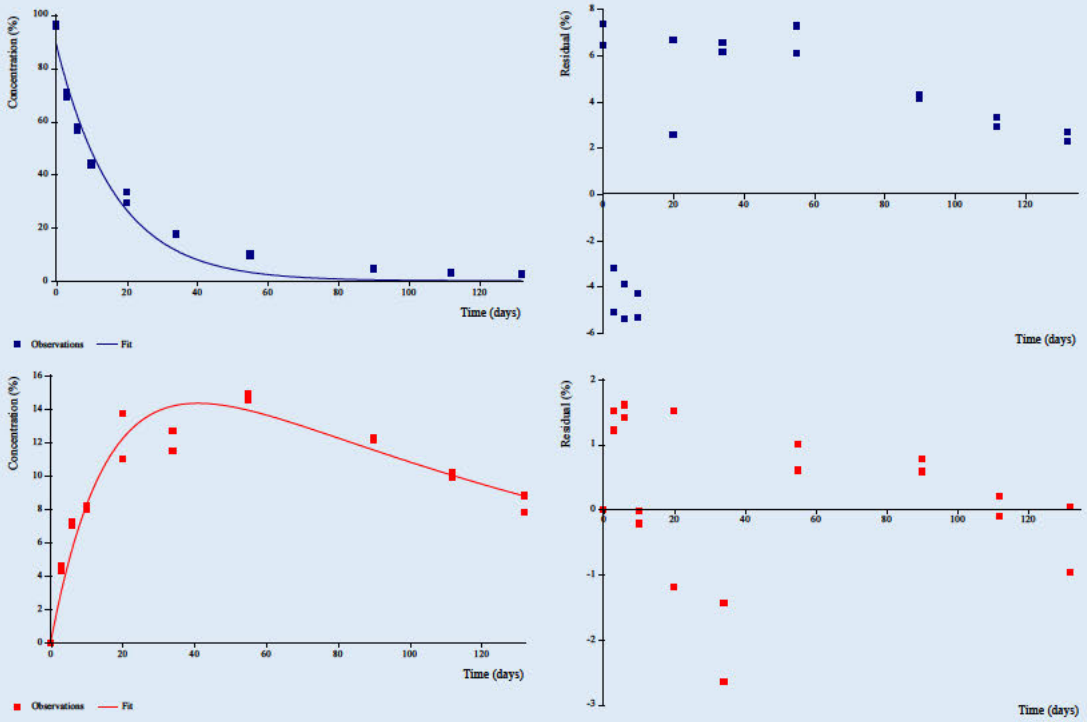
DFOP



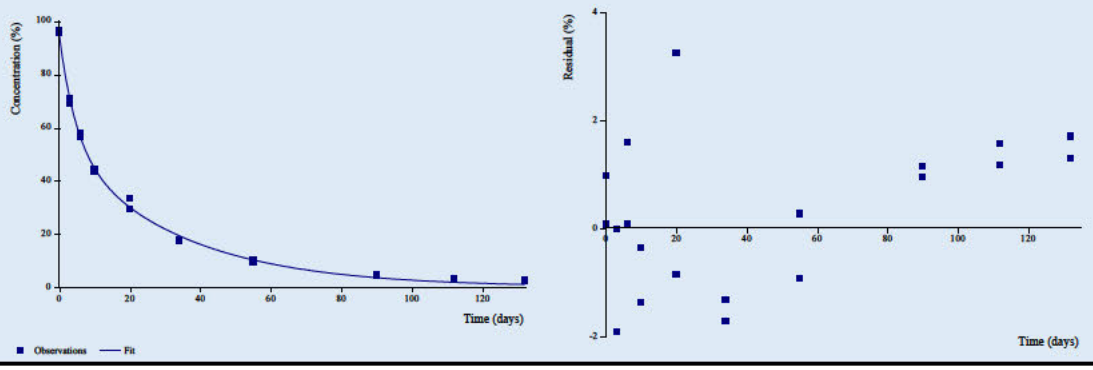
Parent-metabolite fittings (Gartenacker, [redacted] 2010a)

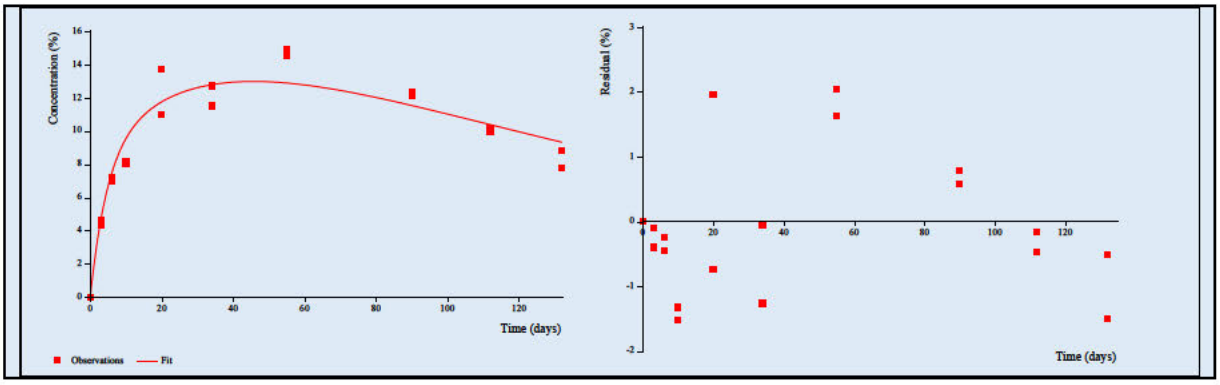
	Kinetic model	Visual/residual	Kinetic para-meters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.06031	11.9	Yes	11.5	38.2		0.9817
AMPA	SFO	Acceptable	k: 0.006672	8.36	Yes	104	345	0.2114	0.9401
Glyphosate	DFOP	Acceptable	k_1: 0.2138 k_2: 0.03023 g: 0.4345	2.88	Yes Yes	8.79	57.3		0.9982
AMPA	SFO	Acceptable	k: 0.006181	7.59	Yes	112	373	0.1955	0.9424
RMS conclusion	Although SFO-SFO provides a good description of the degradation of metabolite AMPA, it is proposed that DFOP-SFO kinetics is selected since it significantly improves the description of the degradation of glyphosate. It is noted that the impact on endpoints (DT_{50} / ffm) for AMPA is minor. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA								

SFO – SFO kinetics



DFOP – SFO kinetics





[redacted], *2010b: Drusenheim, Pappelacker, 18 Acres*

Table 8.1.1.2-4: Processed residue data of glyphosate and its metabolite AMPA in [redacted] 2010b

Time (d)	Glyphosate (% AR)	AMPA (% AR)	Time (d)	Glyphosate (% AR)	AMPA (% AR)	Time (d)	Glyphosate (% AR)	AMPA (% AR)
Drusenheim			**Pappelacker**			**18-Acres**		
0	102.2[1]	0.0[2]	0	102.2[1]	0.0[2]	0	101.3[1]	0.0[2]
0	100.9[1]	0.0[2]	0	102.0[1]	0.0[2]	0	99.5[1]	0.0[2]
1	64.9	9.6	1	77.1	4.2	8	73.9	3.3
1	66.2	7.7	1	77.2	3.9	8	73.9	3.4
3	43.5	15.0	3	59.0	7.4	14	69.4	3.9
3	44.1	15.1	3	58.1	7.9	14	73.1	2.9
8	18.3	21.2	8	27.4	14.5	21	65.6	6.4
8	18.1	21.1	8	29.2	13.7	21	65.3	7.2
14	10.2	19.7	14	19.1	14.2	41	55.9	9.1
14	10.8	18.9	14	29.6	12.2	41	54.4	8.5
27	4.9	17.5	27	10.1	13.7	63	47.0	11.7
27	3.3	15.9	27	18.2	13.2	63	49.3	12.0
48	1.6	9.5	48	4.5	13.6	91	44.7	13.3
48	1.5	9.8	48	9.1	15.4	91	46.7	13.2
70	1.1	6.2	70	2.3	10.4	120	42.1	14.3
70	0.9	6.1	70	2.9	11.6	120	41.3	12.1
			91	2.0	10.0			
			91	1.8	9.5			
			120	2.0	9.1			
			120	2.2	9.0			

[1] Set to material balance

[2] Amounts of metabolites set to 0 at day 0

Slightly grey boxes were residues for which the mass balance was below 90%

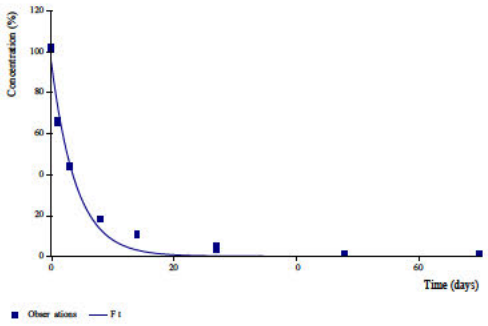
Drusenheim soil

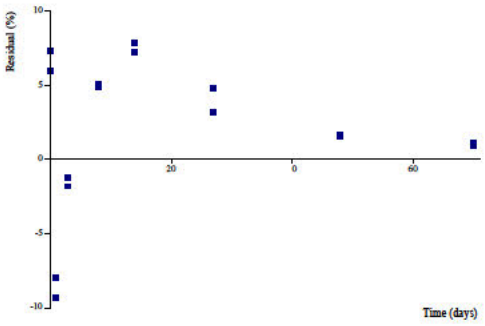
Table 8.1.1.2-5: Kinetic models and statistics for soil Drusenheim ([redacted] 2010b) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Drusenheim									
SFO	Poor	95.0	k: 0.2463	13.6	k: <0.001	k: 0.1939	k: 0.299	2.8	9.3
FOMC	Good	100.6	α: 1.271 β: 2.863	4.9	-[1]	β: 1.878	β: 3.849	2.1	14.7

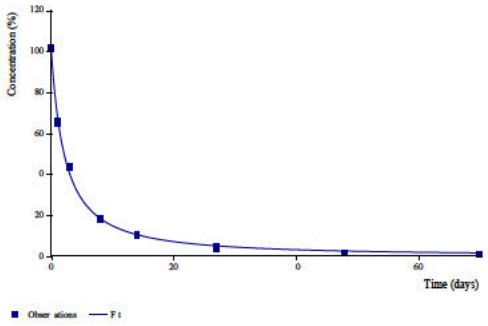
DFOP	Good	101.5	k_1: 1.295 k_2: 0.1403 g: 0.3711	4.8	k_1: <0.001 k_2: <0.001	k_1: 0.6895 k_2: 0.1145	k_1: 1.9 k_2: 0.166	2.0	13.1
Applicant's conclusion		Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides the best visual fit (residues at the last five sampling dates) and a similar χ^2 error compared to DFOP. Thus, the FOMC model is selected as the best-fit model for parent-only fit. As 10 % of the initial concentration was reached within the experimental period, the FOMC model can also be used for derivation modelling endpoints. Conclusion: FOMC to be used in pathway fit for trigger endpoints FOMC to be used in pathway fit for modelling endpoints							
RMS' conclusion		Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.							

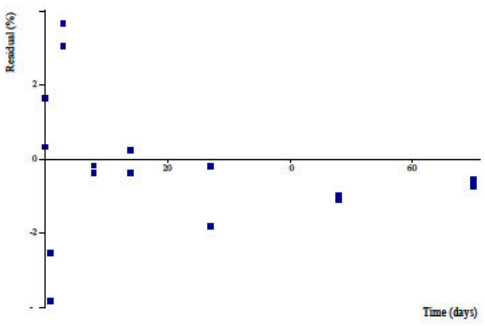
SFO



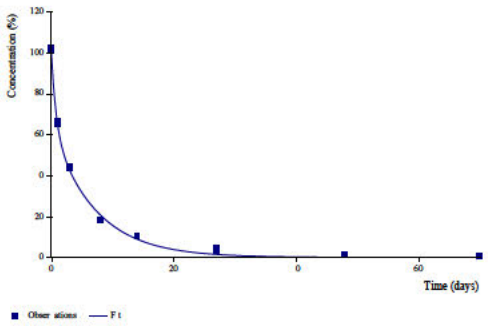


FOMC





DFOP



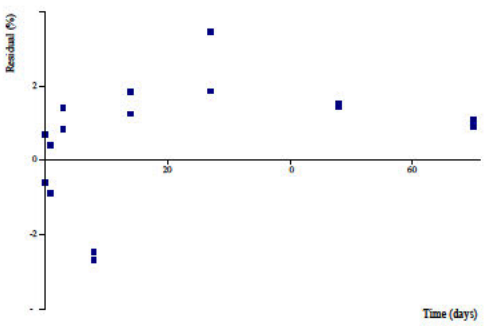
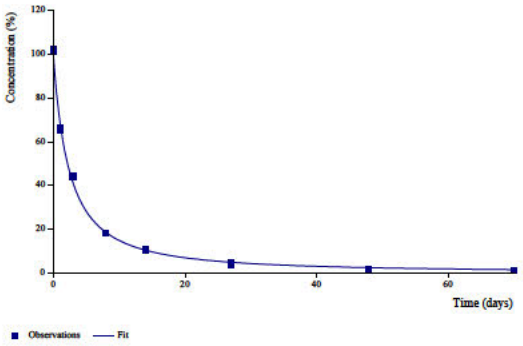


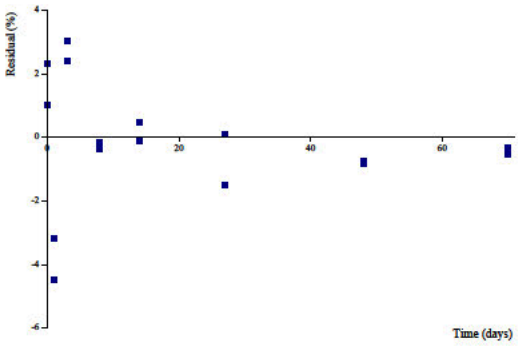
Table 8.1.1.2-6: Kinetic models and statistics for soil Drusenheim of study [redacted] (2010b) - Pathway fits (parent and metabolite)

Kinetic model		M_0				Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff

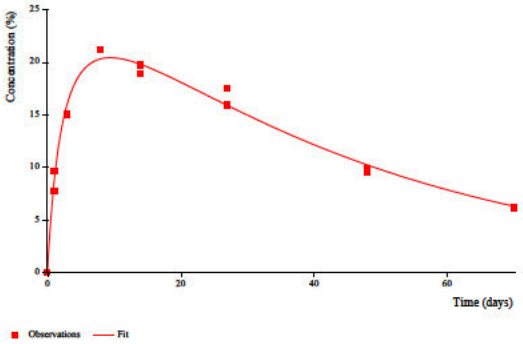
	Visual assess-ment		Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)					(± std. dev.)
Glypho-sate: FOMC	Good	99.9	α: 1.362 β: 3.26	5.0	-[1]	β: 2.279	β: 4.241	2.2	14.4	-
AMPA: SFO	Good	-	k: 0.0236	3.8	k: <0.001	k: 0.0210	k: 0.026	29.4	97.7	0.285 (±0.009)
Applicant's conclusion		Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: FOMC-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA								
RMS' conclusion		Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.								

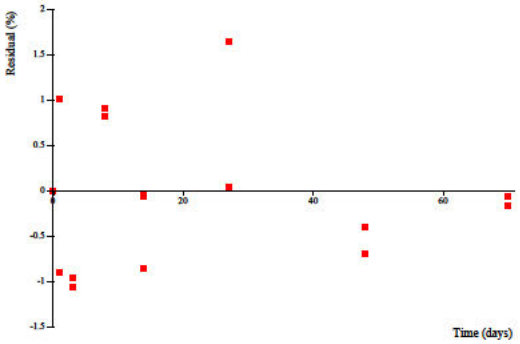
Glyphosate (FOMC)





AMPA (SFO)





RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 96.3%)
102.2	98.42
100.9	97.17

Parent-only fittings (Drusenheim, [redacted] 2010b)

Kinetic model	Visual/ residual	M0	Kinetic para-meters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	91.89	k: 0.2321	Yes		12.3	2.99	9.92	0.9848

FOMC	Good	96.88	α: 1.379 β: 3.471		No No	4.18	2.27	15	0.9979
DFOP	Good	97.7	k1: 1.104 k2: 0.137 g: 0.364	Yes Yes		4.8	2.23	13.5	0.9981

RMS conclusion

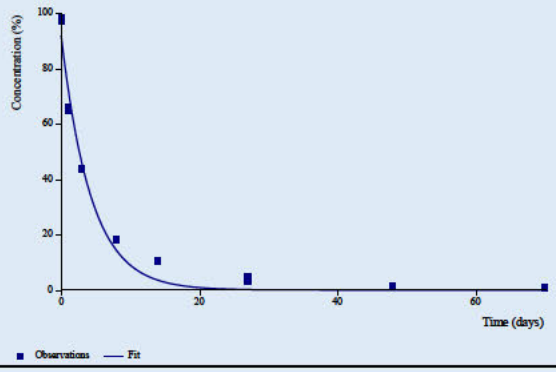
SFO does not well describe the overall degradation of glyphosate, residuals are not randomly distributed, with a systematic error from day 8. SFO is not considered suitable for trigger nor for modelling endpoint.
Biphasic kinetics provide better fits. Visual and statistical results are good for both FOMC and DFOP kinetics.

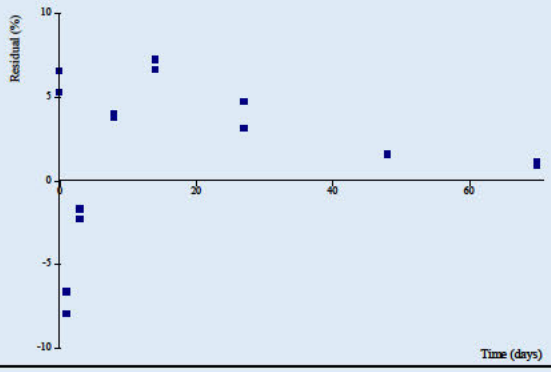
Trigger endpoints: FOMC is considered as best-fit based on chi2-error and better description of the 3 last points.

Modelling endpoint: Glyphosate represented less than 10% AR at the end of the study. Therefore FOMC can be selected for modelling of parent-only. However, it is reminded that FOMC kinetic cannot be directly implemented in FOCUS models, and the use of $DT_{90}/3.32$ is not suitable when metabolites are included in the degradation pathway for modelling (see more justification in the introduction).
In this case, DFOP kinetic is acceptable. Although it is acknowleged that the 3 last points are underestimated, the fit is statistically and visually acceptable. As a consequence, RMS considers that DFOP should be selected for modelling endpoint in pathway fit.

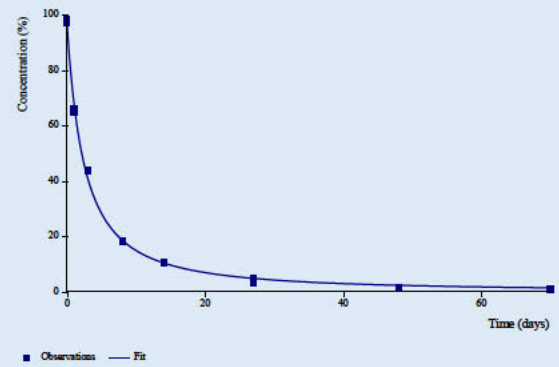
Conclusion: FOMC to be used in pathway fit for trigger endpoints
FOMC acceptable for parent-only modelling.
DFOP to be used in pathway fit for modelling endpoints.

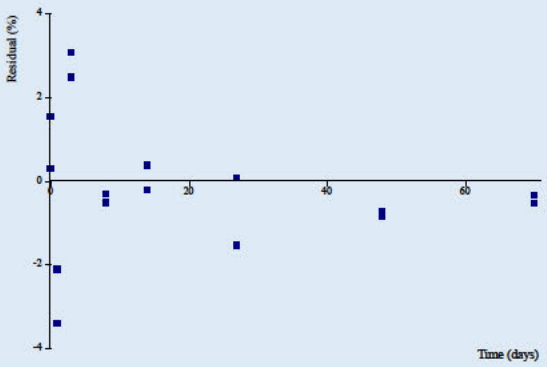
SFO



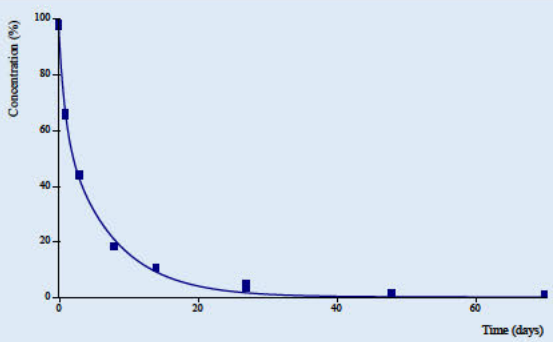


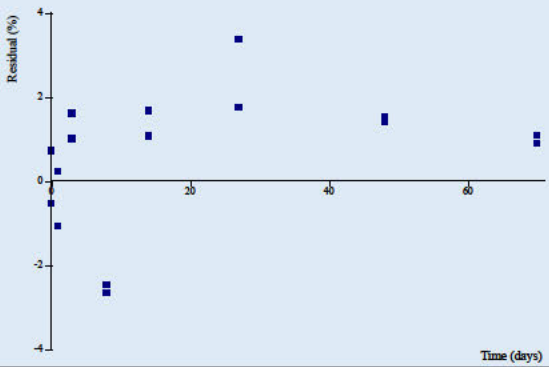
FOMC





DFOP

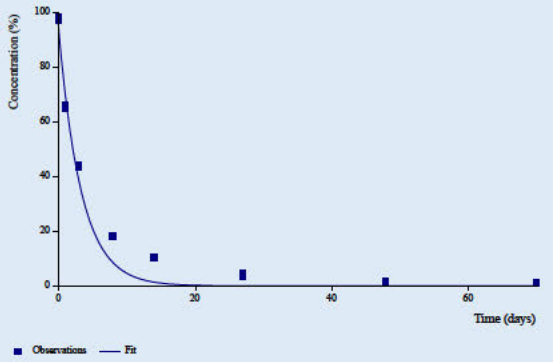


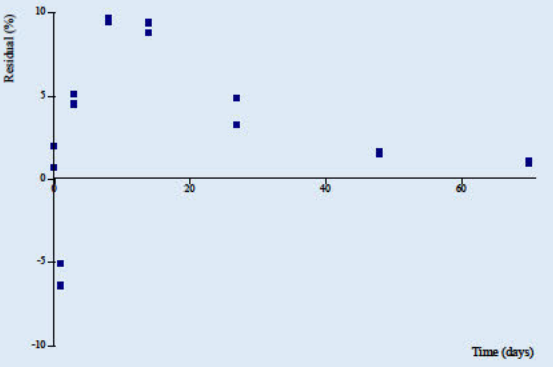


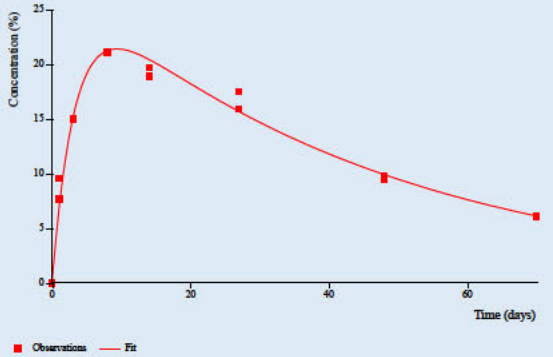
Parent-metabolite fittings (Drusenheim, [redacted] 2010b)

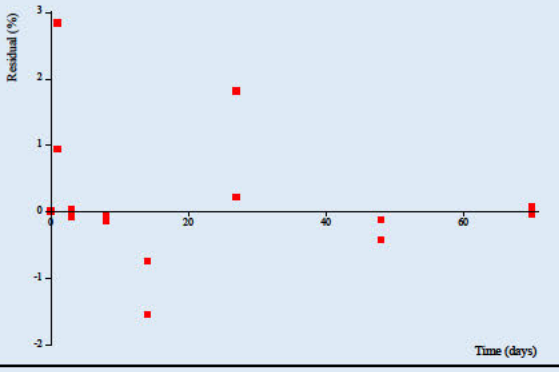
	Kinetic model	Visual/residual	Kinetic parameters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.302	14.8	Yes	2.3	7.63		0.986
AMPA	SFO	Acceptable	k: 0.02185	5.31	Yes	31.7	105	0.2723	0.981
Glyphosate	FOMC	Acceptable	α: 1.414 β: 3.635	4.2	CI does not contain 0	2.3	14.9		0.998
AMPA	SFO	Acceptable	k: 0.02421	3.51	Yes	28.6	95.1	0.3	0.988
Glyphosate	DFOP	Good	k1: 0.9889 k2: 0.1375 g: 0.3704	4.9	Yes Yes	2.27	13.4		0.998
AMPA	SFO	Good	k: 0.02489	3.93	Yes	27.98	92.5	0.2974	0.986
RMS conclusion	Although SFO-SFO provides a good description of the degradation of metabolite AMPA, it is proposed that FOMC-SFO and DFOP-SFO kinetics are selected respectively for trigger and modelling endpoints since they significantly improve the description of the degradation of glyphosate. It is noted that the impact on endpoints (DT_{50} / ffm) for AMPA is minor. Conclusion: FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA DFOP-SFO to be used for deriving modelling endpoints for glyphosate and AMPA								

SFO – SFO kinetics

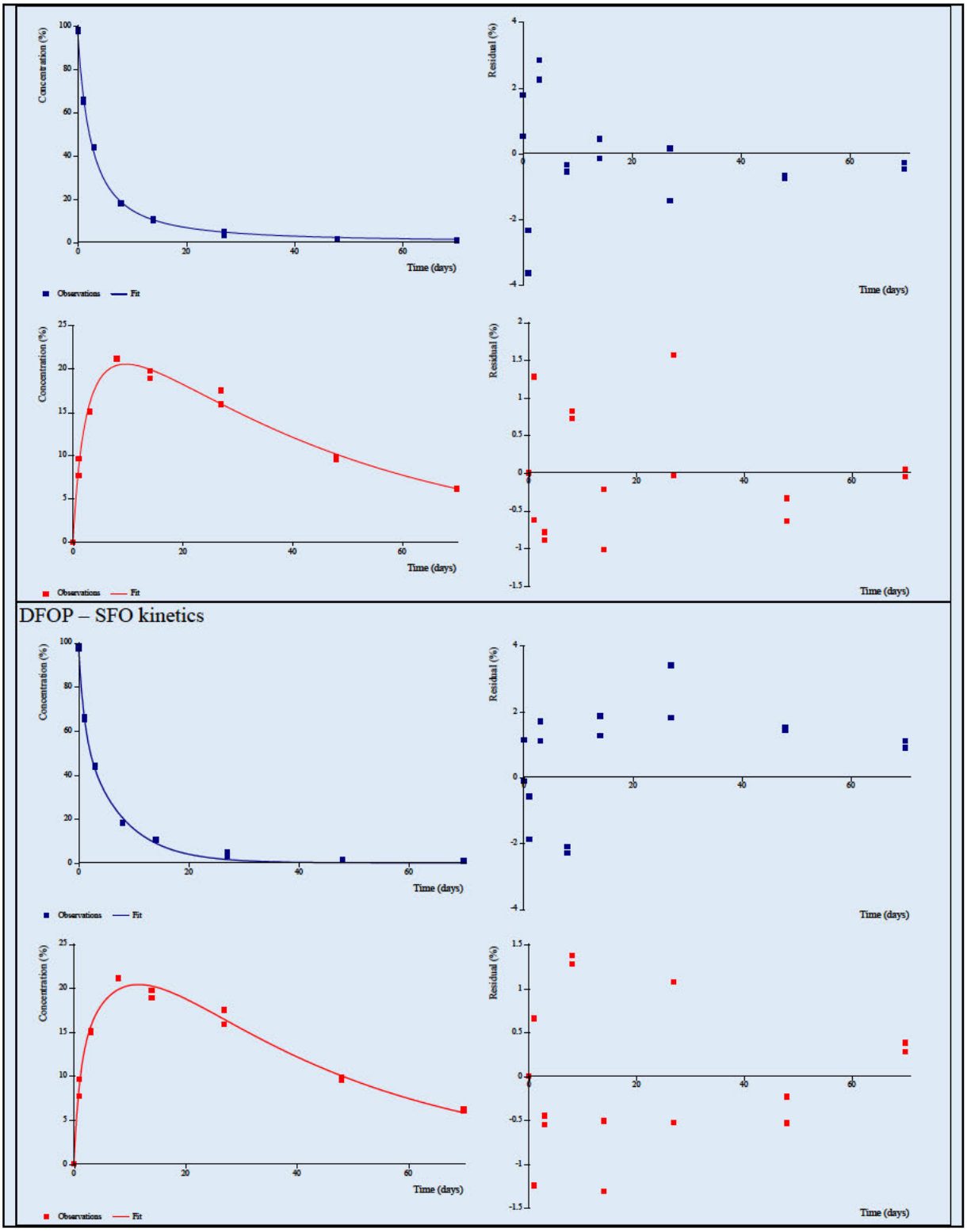








FOMC – SFO kinetics



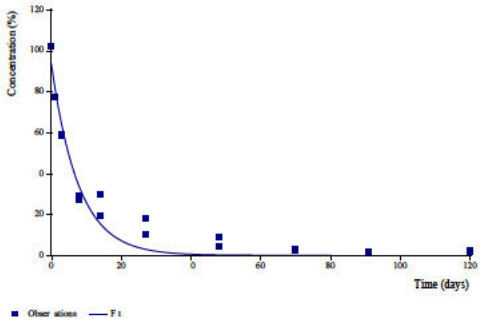
Pappelacker soil

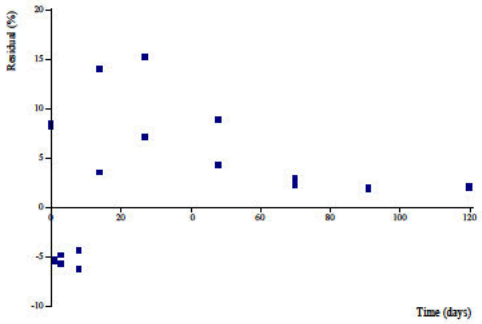
Table 8.1.1.2-7: Kinetic models and statistics for soil Pappelacker, [redacted] (2010b) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Pappelacker									

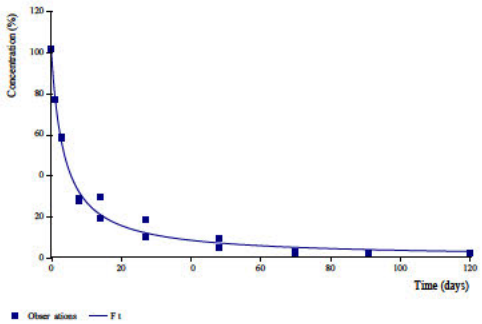
SFO	Poor	93.8	k: 0.1283	16.3	k: <0.001	k: 0.0977	k: 0.159	5.4	18.0
FOMC	Good	101.1	α: 1.022 β: 3.827	6.1	-[1]	β: 1.946	β: 5.708	3.7	32.6
DFOP	Good	100.8	k_1: 0.3443 k_2: 0.0335 g: 0.6505	5.5	k_1: <0.001 k_2: <0.001	k_1: 0.232 k_2: 0.0193	k_1: 0.457 k_2: 0.048	3.6	37.3
Applicant's conclusion		Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit and the lowest χ^2 error. Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints							
RMS' conclusion		Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.							

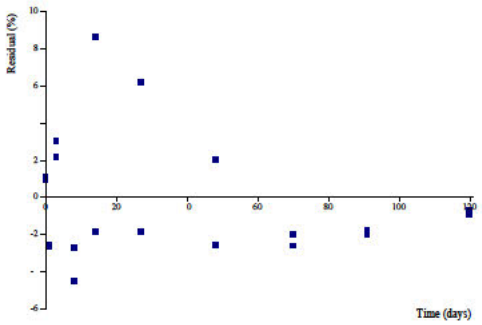
SFO



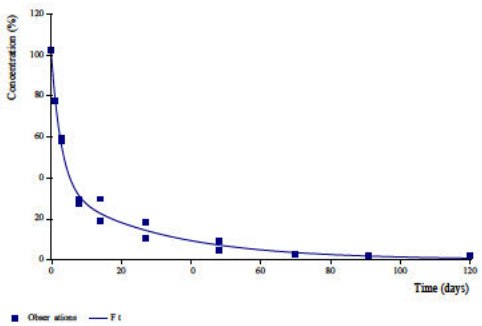


FOMC





DFOP



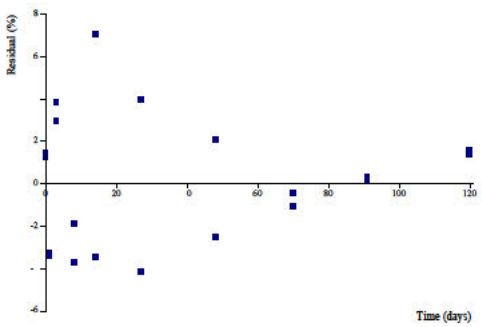
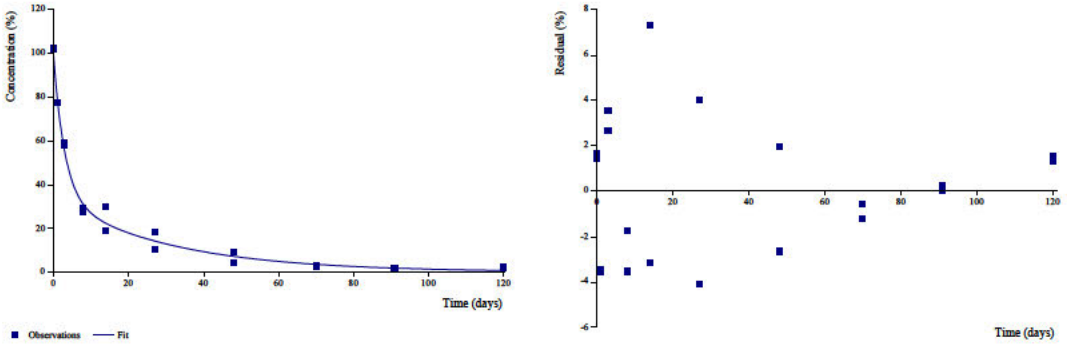


Table 8.1.1.2-8: Kinetic models and statistics of pathway fits for soil Pappelacker of study [redacted] (2010b) Pathway fits (parent and metabolite)

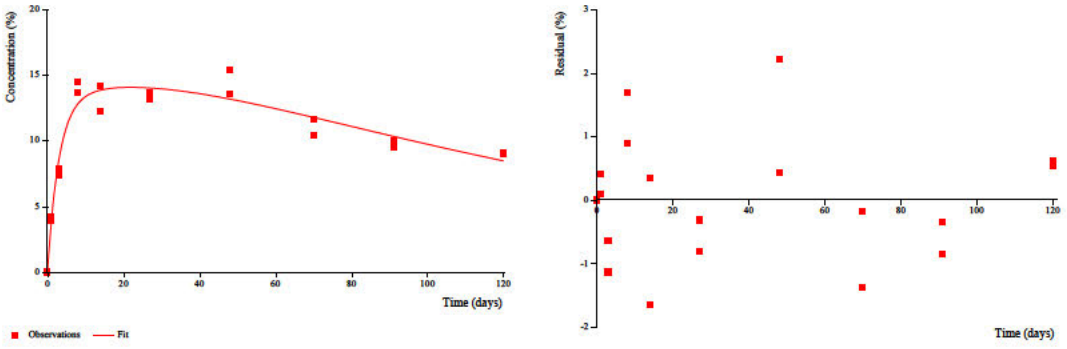
Kinetic model	Visual assess-ment	M0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)

Glypho-sate: DFOP	Good	100.6	k1: 0.3322 k2: 0.0325 g: 0.6609	5.5	k1: <0.001 k2: <0.001	k1: 0.2464 k2: 0.0199	k1: 0.418 k2: 0.045	3.6	37.6	-
AMPA: SFO	Good	-	k: 0.0076	6.2	k: <0.001	k: 0.006	k: 0.009	90.9	302	0.192 (±0.009)
Applicant's conclusion		Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA								
RMS' conclusion		As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.								

Glyphosate (DFOP)



AMPA (SFO)



RMS additional fittings – Pappelacker ([redacted] 2010b)

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 96.3%)
102.2	98.4
102.0	98.2

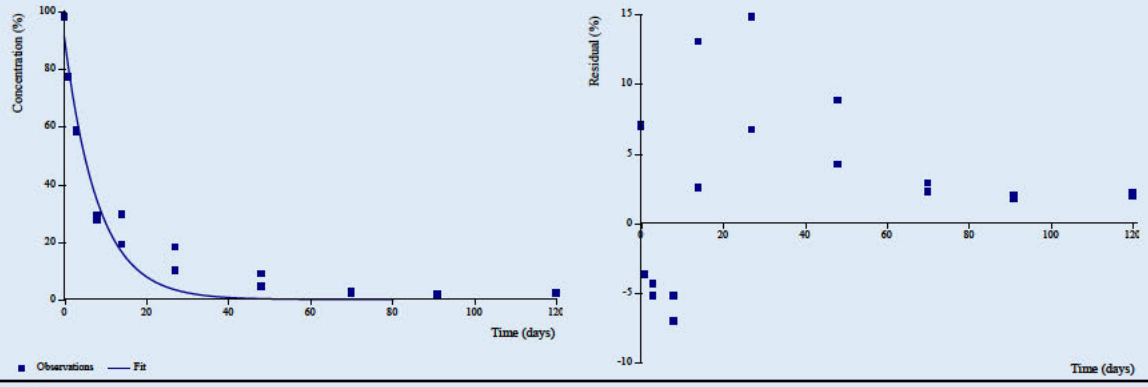
Parent-only fittings (Pappelacker, [redacted] 2010b)

Kinetic model	Visual/ residual	M0	Kinetic parameters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	91.3	k: 0.1221	Yes		12.7	5.68	18.9	0.9607
FOMC	Good	98.14	α: 0.9695 β: 3.799		No No	4.46	3.97	37	0.988

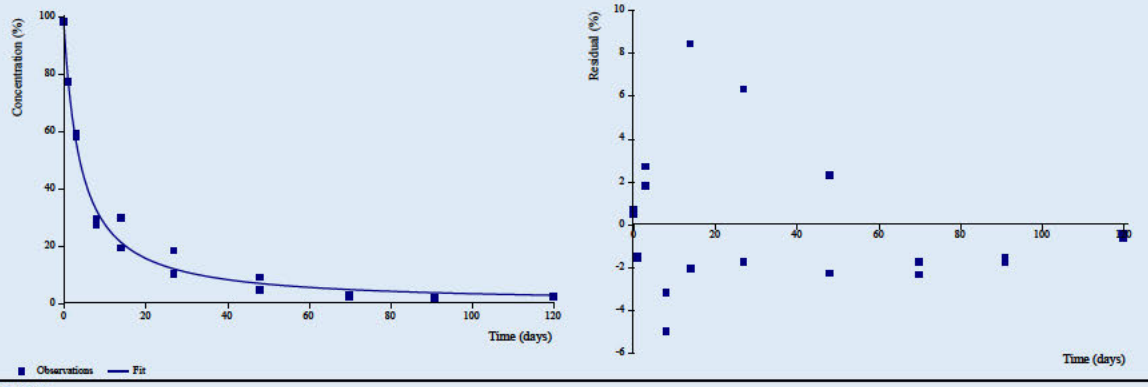
DFOP	Good	97.51	k1: 0.3092 k2: 0.0318 g: 0.6571	Yes Yes		4.26	3.9	38.8	0.9892

RMS conclusion	SFO does not well describe the overall degradation of glyphosate, residuals are not randomly distributed, with a systematic error from day 14. SFO is not considered suitable for trigger nor for modelling endpoint. Biphasic kinetics provide better fits. Visual and statistical results are good for both FOMC and DFOP kinetics. For trigger endpoints, DFOP model is selected as it provides the best fit (lowest χ^2 value, lower extent of residuals compared to FOMC). For modelling endpoint: Glyphosate represented less than 10% AR at the end of the study. Therefore FOMC can be selected for modelling of parent-only. However, it is reminded that FOMC kinetic cannot be directly implemented in FOCUS models, and the use of DT_{90}/3.32 is not suitable when metabolites are included in the degradation pathway for modelling (see more justification in the introduction). In this case, DFOP kinetic is fully acceptable, and even provide a slightly better χ^2 error. The extent of residuals is lower with DFOP kinetics. As a consequence, RMS considers that DFOP should be selected for modelling endpoint in pathway fit. **Conclusion:** DFOP to be used in pathway fit for trigger endpoints FOMC acceptable for parent-only modelling DFOP to be used in pathway fit for modelling endpoints

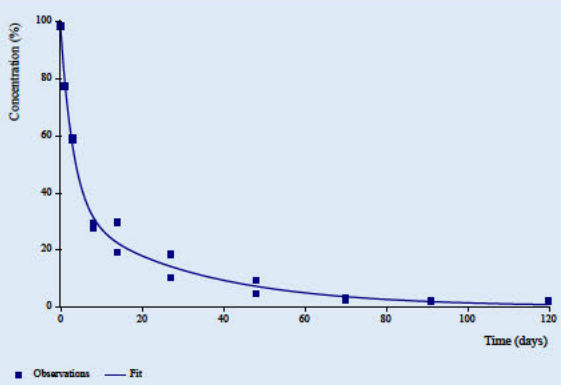
SFO

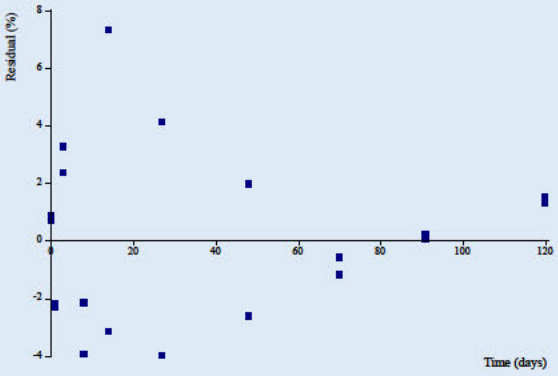


FOMC



DFOP

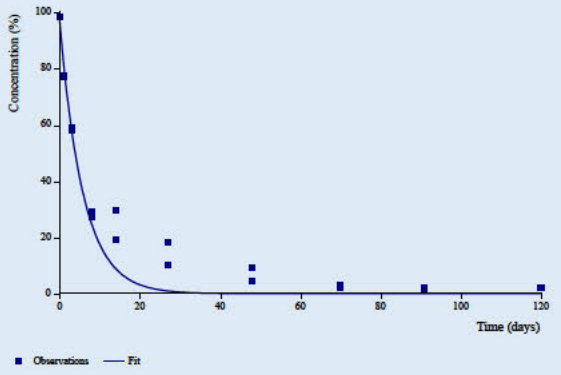


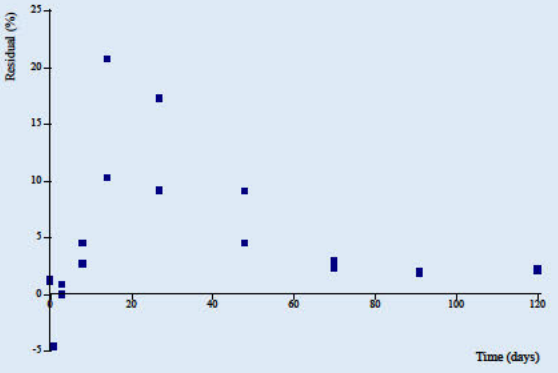


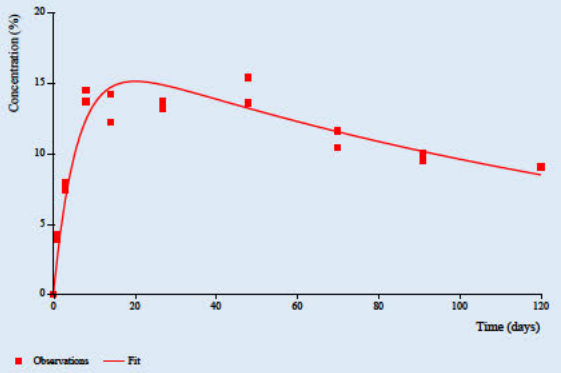
Parent-metabolite fittings (Pappelacker, [redacted] 2010b)

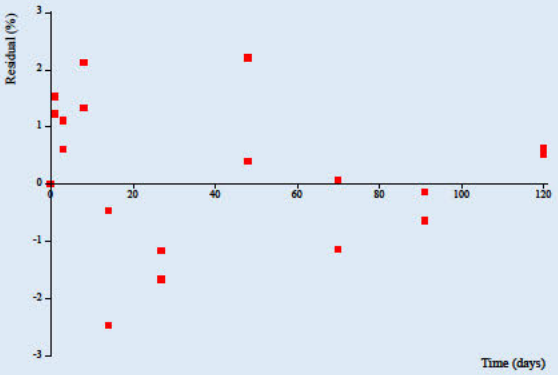
	Kinetic model	Visual/ residual	Kinetic parameters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.181	16.1	Yes	3.83	12.7		0.9789
AMPA	SFO	Acceptable	k: 0.004879	9.19	No 0.068	142	472	0.17	0.9482
Glyphosate AMPA	DFOP	Good	k1: 0.3125 k2: 0.03172 g: 0.6584	4.95	Yes Yes	3.85	38.7		0.9926
	SFO	Good	k: 0.007863	6.24	Yes	88.2	293	0.2004	0.9565
RMS conclusion		Although SFO-SFO provides a good description of the degradation of metabolite AMPA, it is proposed that DFOP-SFO kinetics is selected since it significantly improves the description of the degradation of glyphosate Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA							

SFO – SFO kinetics

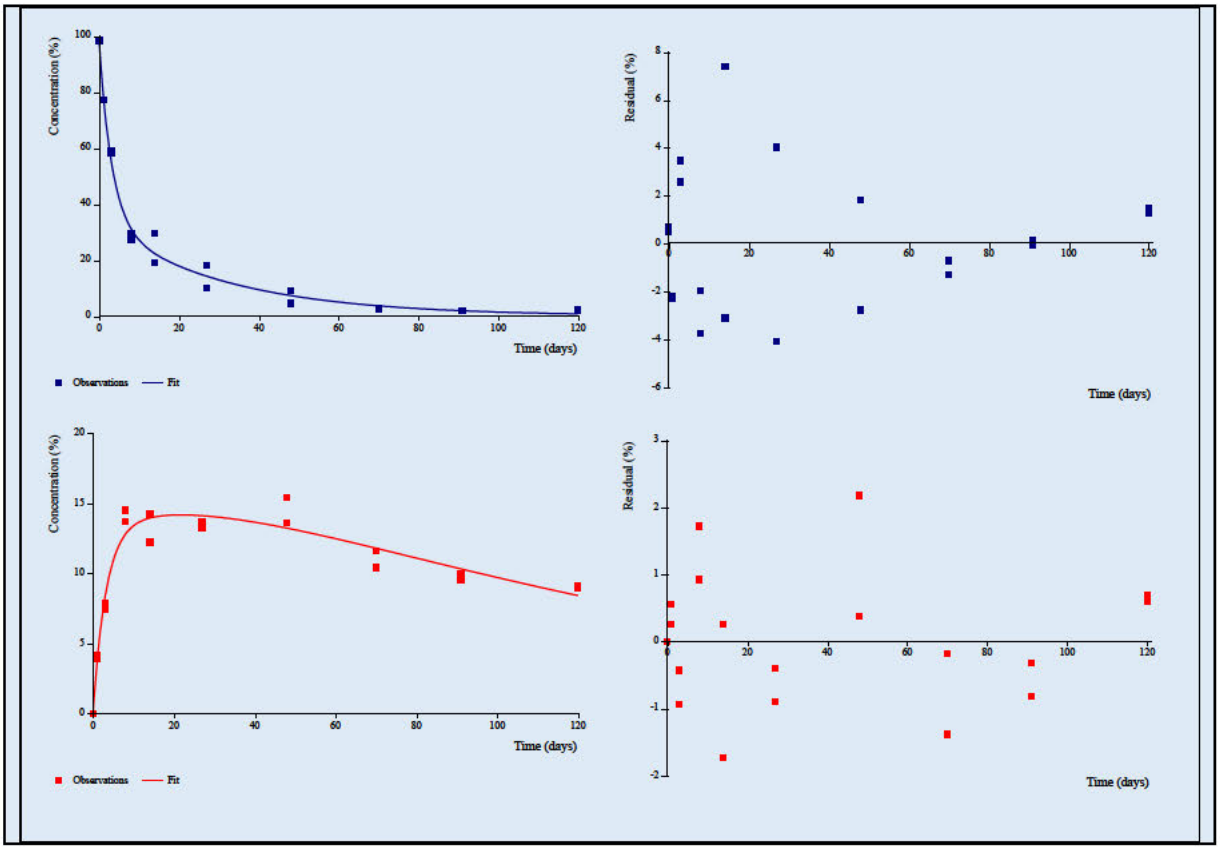








DFOP – SFO kinetics



18 Acres soil

Table 8.1.1.2-9: Kinetic models and statistics for soil 18-Acres of study [redacted] (2010b) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
18 Acres									
SFO	Poor	84.1	k: 0.0076	9.9	k: <0.001	k: 0.0053	k: 0.01	90.9	302
FOMC	Good	100.1	α: 0.2605 β: 4.522	2.0	-[1]	β: 2.626	β: 6.418	60.2	>1000
DFOP	Good	99.7	k_1: 0.1125 k_2: 0.0040 g: 0.3458	2.9	k_1: <0.001 k_2: <0.001	k_1: 0.0630 k_2: 0.0026	k_1: 0.162 k_2: 0.005	67.7	473
Applicant's conclusion		Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides the best visual fit (residues at the last two sampling dates) and the lowest χ^2 error. Thus, the FOMC model is selected as the best-fit model for parent-only fit. As 10 % of the initial concentration was not reached within the experimental period, the DFOP model is selected for derivation of modelling endpoints. Conclusion: FOMC to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints							
RMS' conclusion		Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.							
SFO									

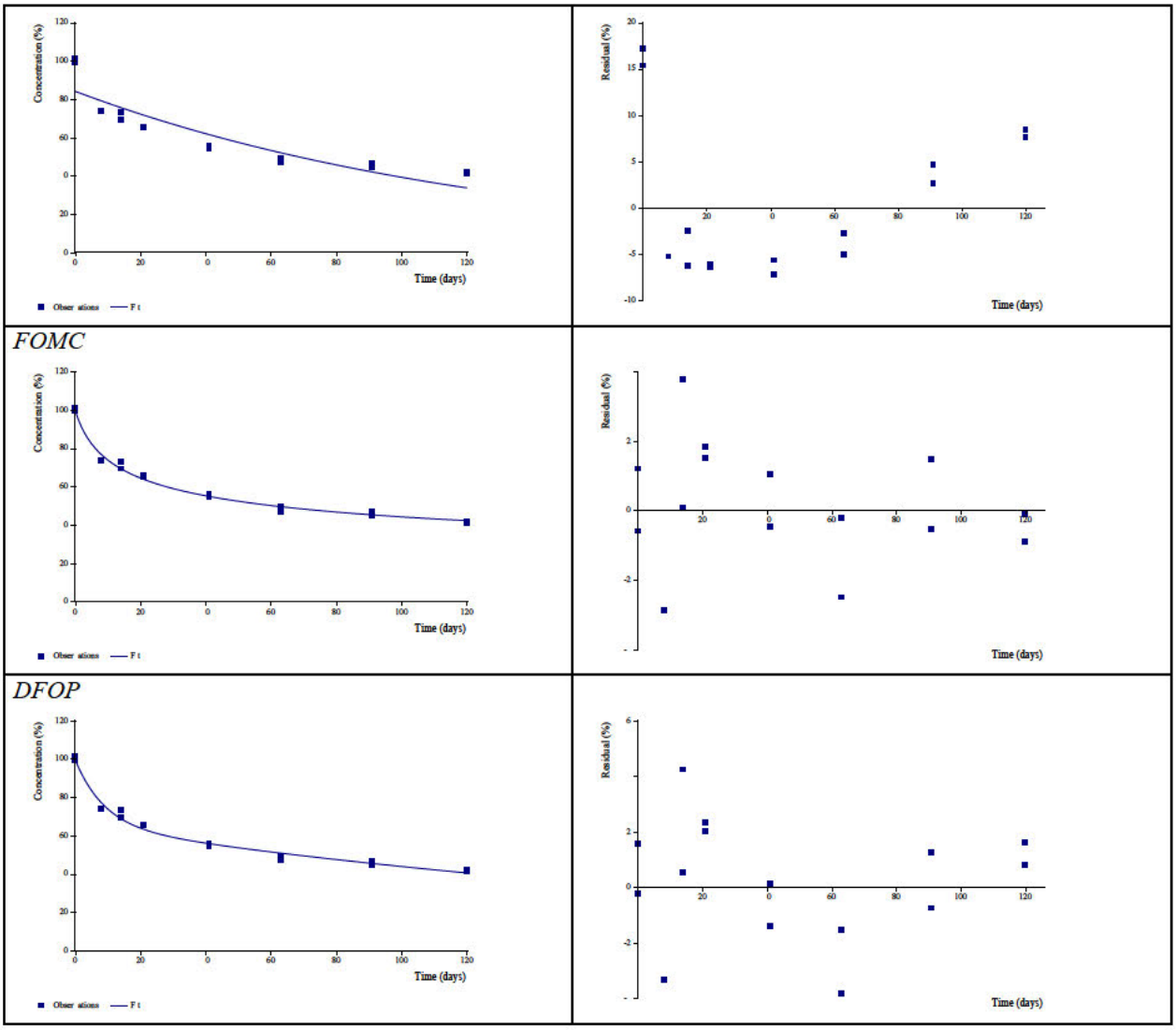
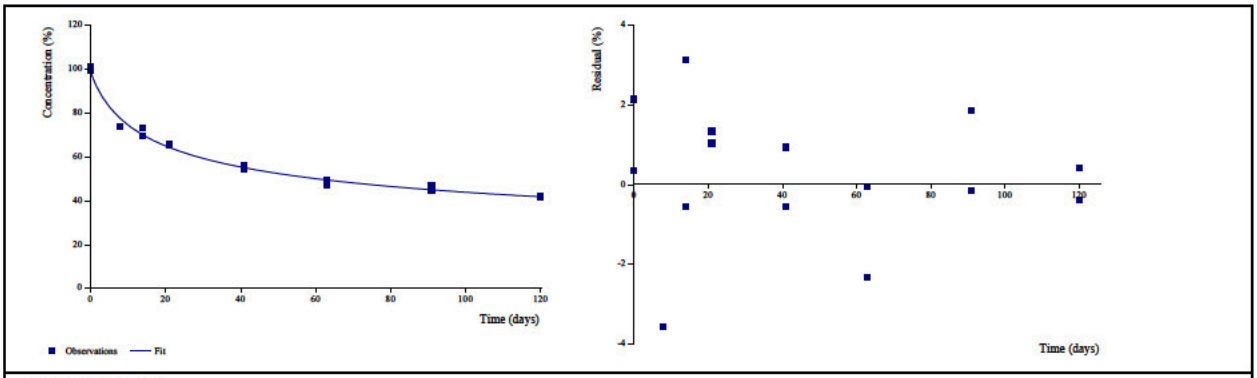
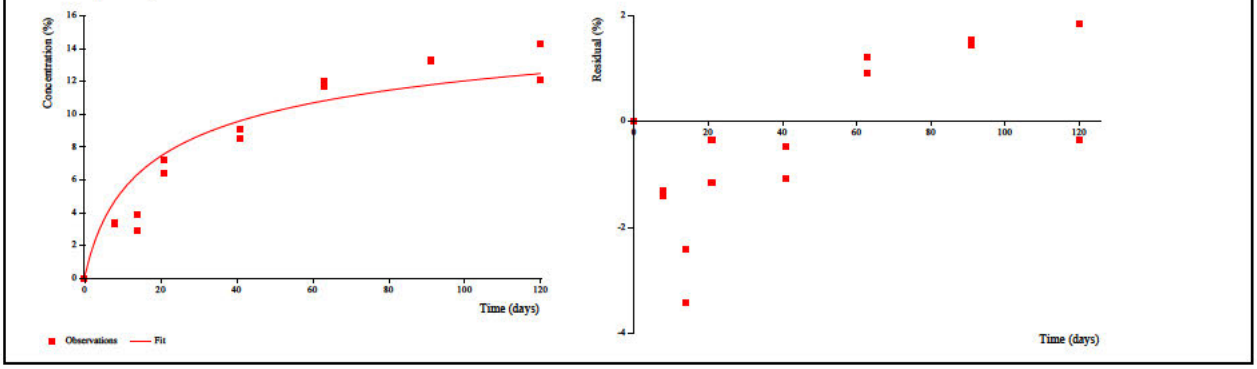


Table 8.1.1.2-10: Kinetic models and statistics for soil 18-Acres of study [redacted] (2010b) - Pathway fits (parent and metabolite)

endpoints	Kinetic model	Visual assess-ment	M0	Kinetic para-meters	χ2 er-ror (%)	Prob > t (5 % level)	CI contain 0?	DT_{50} (d)	DT_{90} (d)	ff
Trigger	Glypho-sate: FOMC	Good	99.2	α: 0.2789 β: 5.624	2.1	-1	No	61.9	>1000	-
	AMPA: SFO	Accep-table	-	k: <0.0001	13.6	k: 0.5	-	>1000	>1000	0.217
Applicant's conclusion	The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable - Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate - No trigger endpoints can be derived for AMPA									
RMS conclusion	As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.									
Glyphosate (FOMC)										


Concentration (%)
Time (days)
Observations
Fit
Residual (%)
Time (days)

AMPA (SFO)


Concentration (%)
Time (days)
Observations
Fit
Residual (%)
Time (days)

Modelling	Glypho-sate: DFOP	Good	97.5	k1: 0.0817 k2: 0.0037 g: 0.3569	3.3	k1: <0.001 k2: <0.001	-	68.9	504	-
	AMPA: SFO	Accep-table	-	k: <0.0001	12.0	k: 0.5	-	>1000	>1000	0.225 (±0.027)

Applicant's conclusion

The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable.

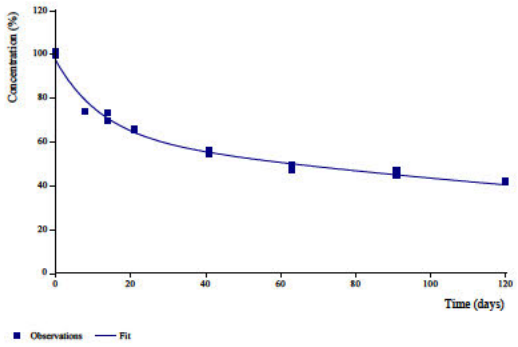
Conclusion: Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate

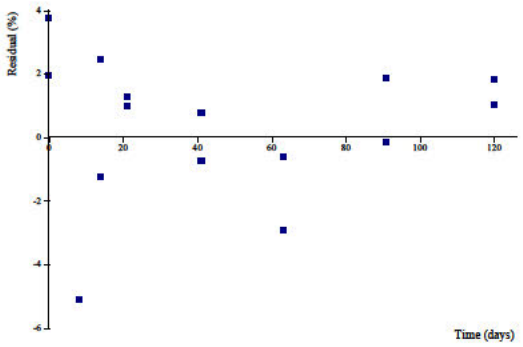
No trigger endpoints can be derived for AMPA

RMS conclusion

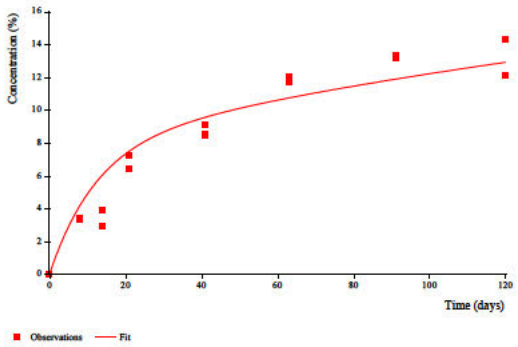
As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.

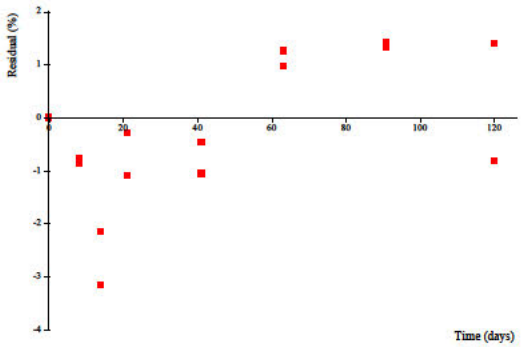
Glyphosate (DFOP)





AMPA (SFO)





RMS additional fittings – 18 Acres ([redacted] 2010b)

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 96.3%)
101.3	97.6
99.5	95.8

Parent-only fittings (18 Acres, [redacted] 2010b)

Kinetic model	Visual/ residual	M0	Kinetic para-meters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	82.72	k: 0.007324	Yes		8.73	94.6	314	0.8415
FOMC	Good	96.36	α: 0.2737 β: 6.071		No No	1.86	70.3	>10 000	0.9907
DFOP	Acceptable	95.86	k1: 0.08923 k2: 0.003598 g: 0.3425	Yes Yes		2.63	76.3	523	0.9862

RMS conclusion

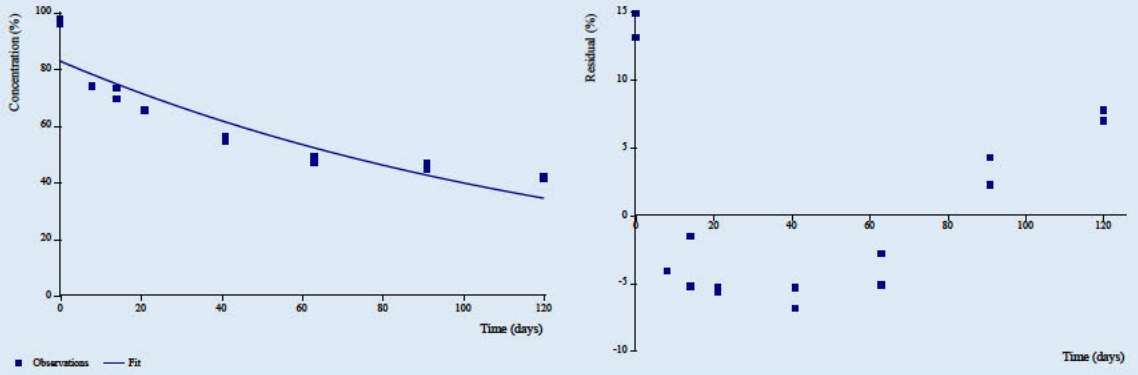
SFO does not well describe the overall degradation of glyphosate, residuals are not randomly distributed, and M0 is significantly underestimated. SFO is not considered suitable for trigger nor for modelling endpoint.
Degradation of glyphosate is best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits.

For trigger endpoints: FOMC results in the lowest chi^2 error, but the DT_{90} > 10000 days seems to be overconservative considering all other results. RMS also notes that the visual fits and the extent of residuals for both FOMC and DFOP are very similar. RMS proposes that DFOP is selected for trigger endpoints.

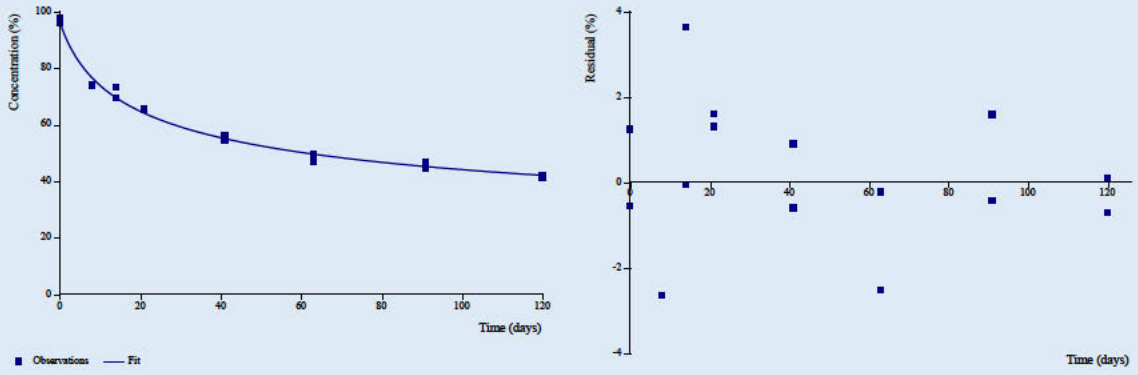
For modelling endpoints: Glyphosate still represented 40% AR at the end of the study. DFOP is therefore selected.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

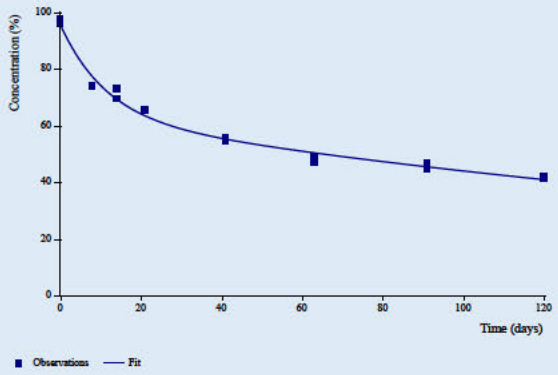
SFO

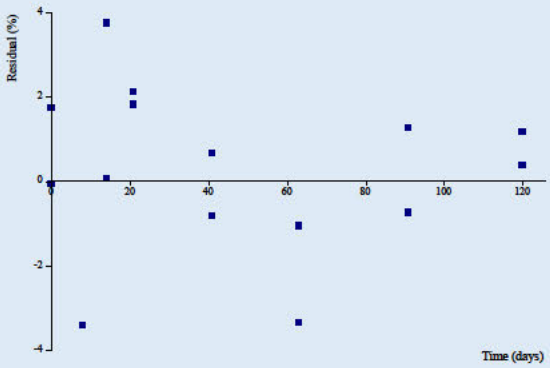


FOMC



DFOP

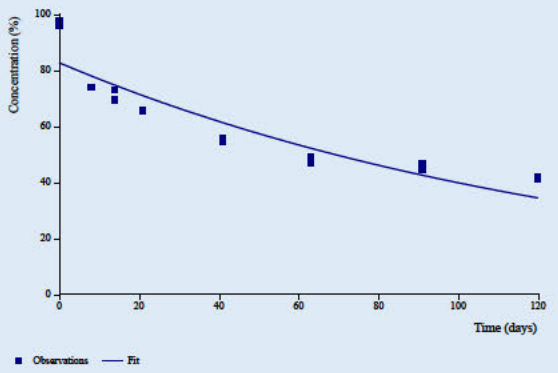


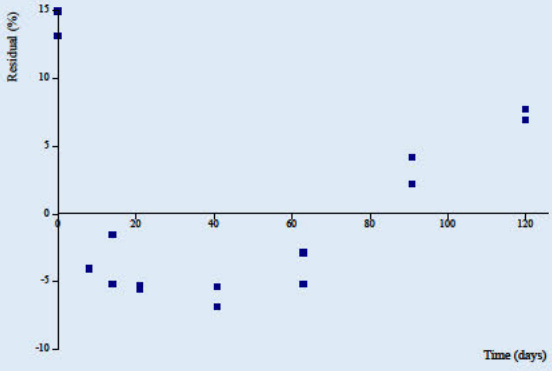


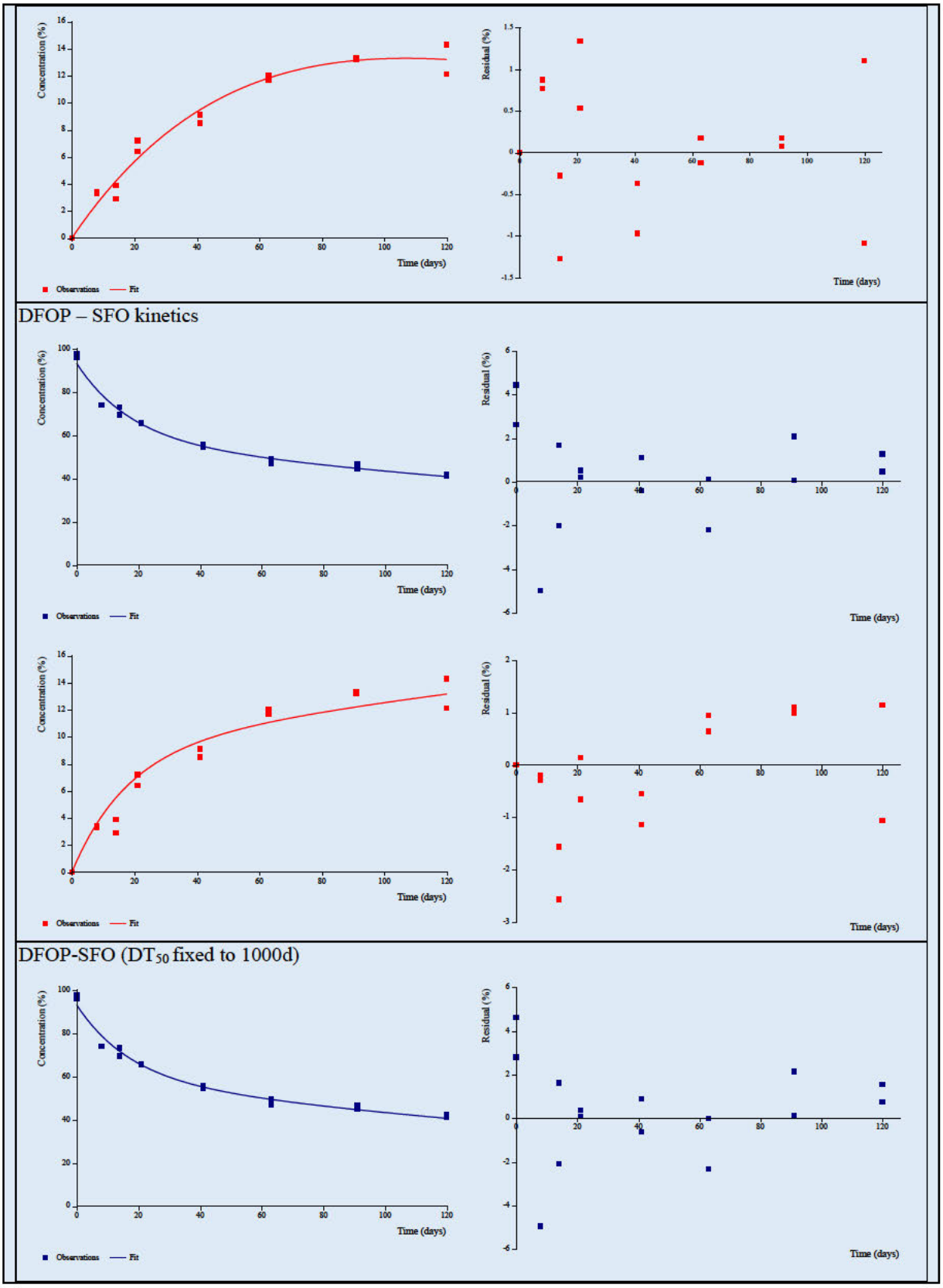
Parent-metabolite fittings (18 Acres, [redacted] 2010b)

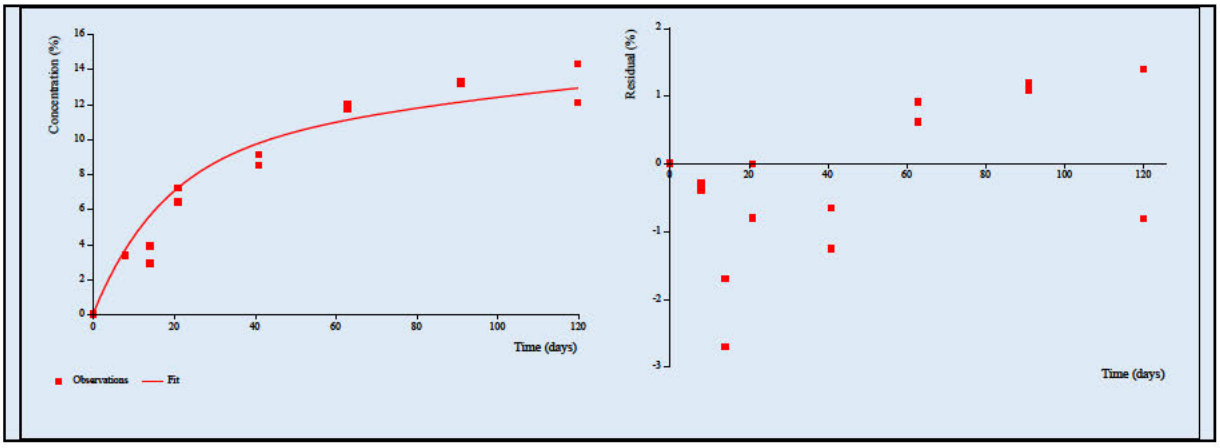
	Kinetic model	Visual/ residual	Kinetic parameters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Gly.	SFO	Acceptable	k: 007306	8.73	Yes	94.9	315		0.8414
AMPA	SFO	Good	k: 0.01176	5.62	Yes	59	196	0.5645	0.9761
Gly.	DFOP	Good	k1: 0.05753 k2: 0.002946 g: 0.3764	3.32	Yes	77.9	621		0.9798
AMPA	SFO	Acceptable	k: 0.5.92 x 10^{-24}	9.07	No (0.5)	> 10 000	> 10 000	0.2514	0.959
Gly.	DFOP	Good	k1: 0.05856 k2: 0.003146 g: 0.3644	3.39	Yes Yes	78.6	588		0.979
AMPA	SFO fixed	Good	k: 0.00069	9.2	Fixed	1000	3320	0.2618	0.9547
RMS conclusion	DFOP kinetics better describe the degradation of glyphosate. The DFOP-SFO kinetics also seems to better describe the degradation of AMPA. Indeed, in the SFO-SFO fit, it seems that a kind of plateau is reached for AMPA, which is not necessarily consistent with the measured data; since 40% glyphosate still remains at the end of the study, it cannot be excluded that AMPA degradation is overestimated with SFO-SFO. However the DFOP-SFO fit results in overconservative DT_{50}/DT_{90} (outside the range of other available values) for AMPA. RMS performed an additional fitting, fixing the DT_{50} of AMPA to 1000 d since no decline in the residues was observed during the study, and the estimated DT_{50} for AMPA was > 10000 d. The visual fit of this option is still good, with the statistical parameters acceptable. The predicted concentrations well describe the experimental concentrations of AMPA. Taking a default value of 1000 days allows to account for the higher persistence of AMPA that seems to be observed in this soil. **Conclusion:** DFOP-SFO (fixed DT_{50} of 1000 days for AMPA) to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									

SFO – SFO kinetics






Concentration (%)
Time (days)
Observations
Fit
Residual (%)
Time (days)
DFOP – SFO kinetics
DFOP-SFO (DT50 fixed to 1000d)



[redacted], *1996: Soil B*

Table 8.1.1.2-11: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1996), soil B

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
0	100.3[2]	0.0[3]
0	99.2[2]	0.0[3]
1	50.5	16.7
1	50.5	15.4
3	36.6	16.6
3	36.4	16.1
7	20.0	20.8
7	19.6	20.8
14	11.3	20.5
14	13.6	19.6
30	6.3	21.4
30	5.4	20.7
63	7.8	11.5
63	2.4	16.2
90	2.1	14.5
90	2.1	13.6
121	1.9	11.9
121	2.1	14.7

1 Soil extracts were analysed using both HPLC and TLC method. As analysis of the ammonia extracts by TLC and HPLC showed the chromatographic profiles to be very similar at each sampling interval, results of both methods were used as analytical replicates and were averaged for kinetic analysis.
2 Set to material balance
3 Amounts of metabolites set to 0 at day 0

Table 8.1.1.2-12: Kinetic models and statistics for soil Soil B of study [redacted] (1996) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	CI contains 0	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.3	k: 0.3155	25.6	k: <0.001	-	2.2	7.3
FOMC	Good	99.4	α: 0.6566 β: 0.6408	6.9	-	No	1.2	20.7
DFOP	Acceptable	99.7	k_1: 1.722 k_2: 0.0937 g: 0.5492	9.0	k_1: <0.001 k_2: <0.001	-	1.0	16.1

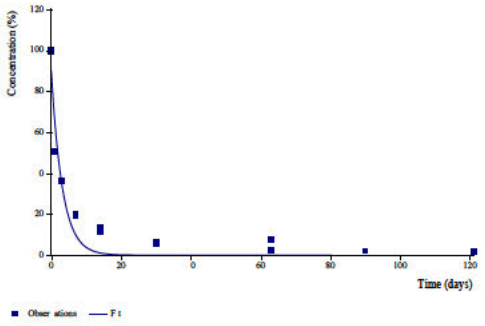
Applicant's conclusion
Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides the best visual fit (M_0 as well as the residues at the last five sampling dates) and the lowest χ^2 error. Thus,

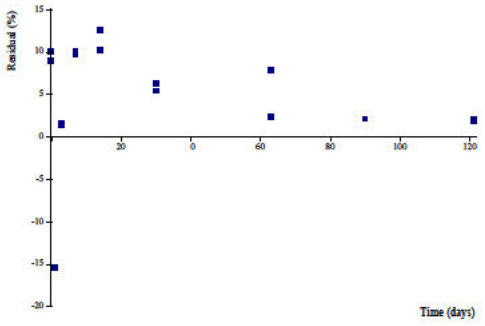
the FOMC model is selected as the best-fit model for parent-only fit. As 10 % of the initial concentration was reached within the experimental period, the FOMC model can also be used for derivation modelling endpoints.
Conclusion: FOMC to be used in pathway fit for trigger endpoints
FOMC to be used in pathway fit for modelling endpoints

RMS conclusion

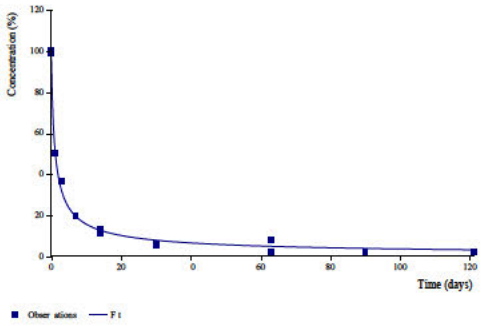
Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. In addition, RMS used only the HPLC results instead of mean of HPLC and TLC. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.

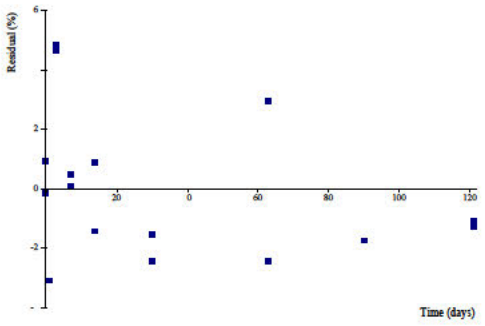
SFO



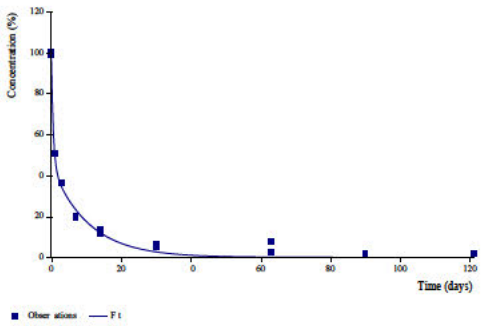


FOMC





DFOP



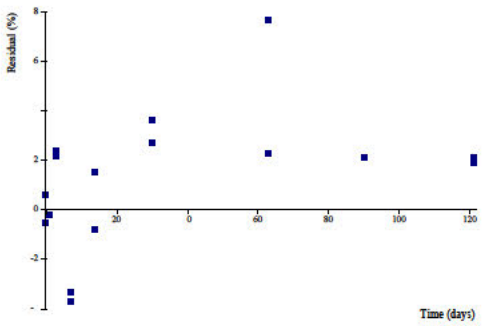


Table 8.1.1.2-13: Kinetic models and statistics for soil Soil B of study [redacted] (1996) - Pathway fit (parent and metabolite)

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	99.9	α: 0.6314 β: 0.571	7.0	-[1]	β: 0.354	β: 0.788	1.1	21.3	-

AMPA: SFO	Accep-table	-	k: 0.007	8.9	k: <0.001	k: 0.0049	k: 0.009	99.4	330	0.264 (±0.014)

Applicant's conclusion

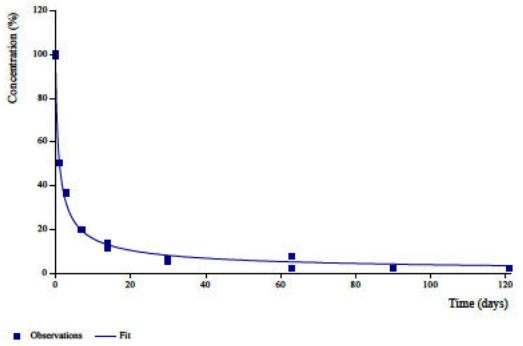
Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

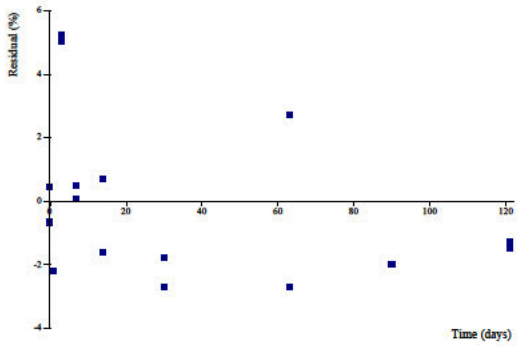
Conclusion: FOMC-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

RMS conclusion

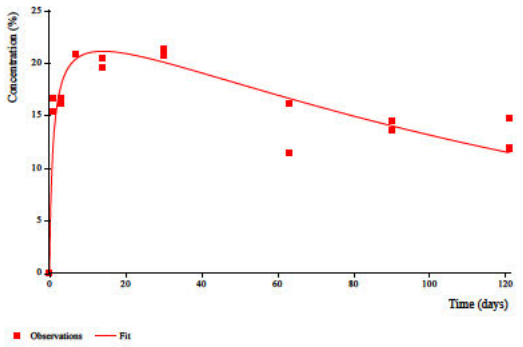
As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. In addition, RMS used only the HPLC results instead of mean of HPLC and TLC. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.

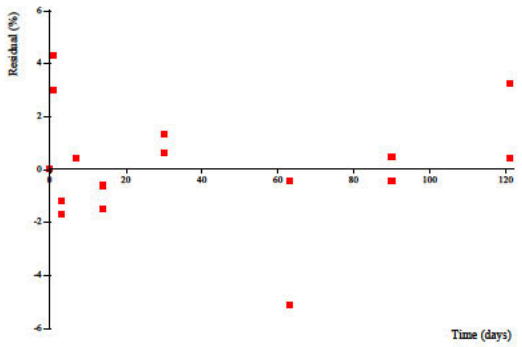
Glyphosate (FOMC)





AMPA (SFO)





RMS additional fittings - Soil B ([redacted] 1996)

RMS performed additional fittings considering only HPLC results at all sampling time instead of a mean from HPLC and TLC results (see comments under point B.8.1.1.1). Additionally, T0 for glyphosate was calculated considering material balance at T0 corrected by the radioactive purity of the substance in this study (99.2%).

Processed residue data used for kinetic fitting

DAT	Glyphosate (applicant – mean TLC and HPLC)	AMPA (applicant – mean TLC and HPLC)	Glyphosate (RMS - HPLC)	AMPA (RMS - HPLC)
0	100.3	0.0	99.5	0
0	99.2	0.0	98.4	0
1	50.5	16.7	44.7	20.6
1	50.5	15.4	45.6	19.1
3	36.6	16.6	34.4	17.4
3	36.4	16.1	34	17.3
7	20.0	20.8	18.6	22.6
7	19.6	20.8	18.6	22.1
14	11.3	20.5	11.4	19.7
14	13.6	19.6	13.5	19.7
30	6.3	21.4	6	21.9
30	5.4	20.7	5	21.3

63	2.4	16.2	2.6	16.3
90	2.1	14.5	2.9	14.4
90	2.1	13.6	2.6	13.9
121	1.9	11.9	1.8	13
121	2.1	14.7	2	15.8

Parent-only fittings (Soil B, [redacted], 1996)

Kinetic model	Visual/ residual	M0	Kinetic parameters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	89.71	k: 0.3685	Yes		29	1.88	6.25	0.9308
FOMC	Acceptable	95.65	α: 0.5994 β: 0.4408		No No	8.55	0.96	20.1	0.9932
DFOP	Acceptable	98.94	k1: 2.127 k2: 0.0916 g: 0.5739	Yes Yes		7.98	0.8	15.8	0.9962

RMS conclusion

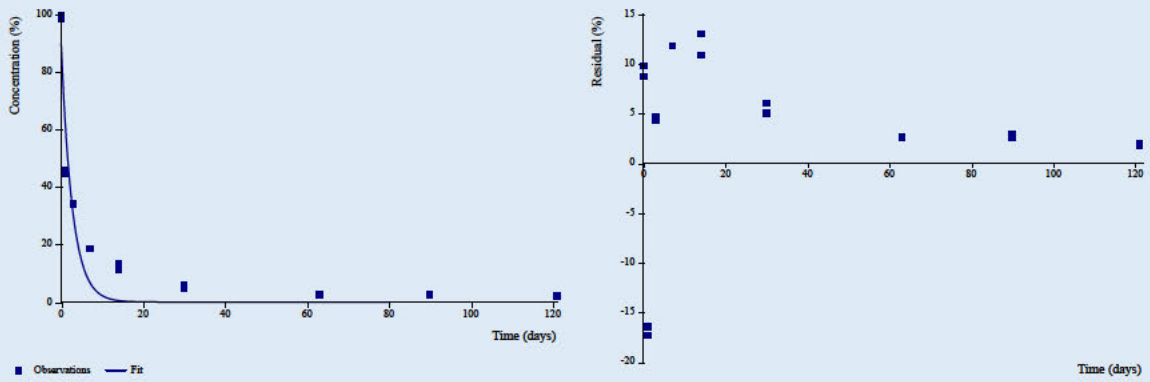
SFO poorly describes the degradation of glyphosate, with a poor M0 estimation; residuals are not randomly distributed, with a systematic error from day 7. SFO is not considered suitable for trigger nor for modelling endpoint.
Biphasic fittings provide better fits. Visual and statistical results are good for both FOMC and DFOP kinetics.

For trigger endpoints: visual fit is better for FOMC than for DFOP, since DFOP underestimates the concentrations from day 30. However it is highlighted that glyphosate already represented less than 10% AR at this time. With DFOP, M0 is better described, chi2 error is slightly better, and the extent of residuals is also slightly lower than for FOMC. It it therefore proposed that DFOP is selected for trigger endpoint. It is highlighted that considering the DT_{90}obtained for this soil, the choice of the kinetic model will have no impact on the estimation of persistence of glyphosate.

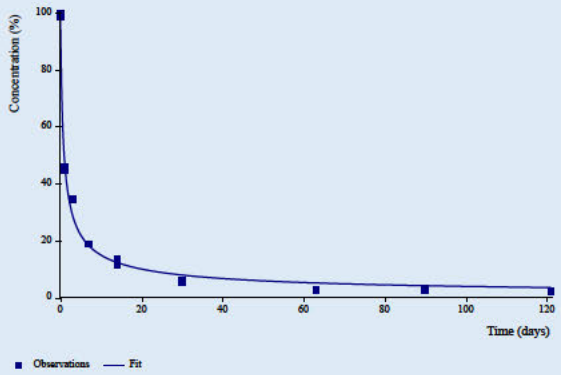
For modelling endpoints: Glyphosate represented less than 10% AR at the end of the study. Therefore FOMC can be selected for modelling for parent-only. However, it is reminded that FOMC kinetic cannot be directly implemented in FOCUS models, and the use of DT_{90}/3.32 is not suitable when metabolites are included in the degradation pathway for modelling (see more justification in the introduction).
In this case, DFOP is considered visually and statistically acceptable, as detailed above. As a consequence, RMS considers that DFOP should be selected for modelling endpoint in pathway fit.

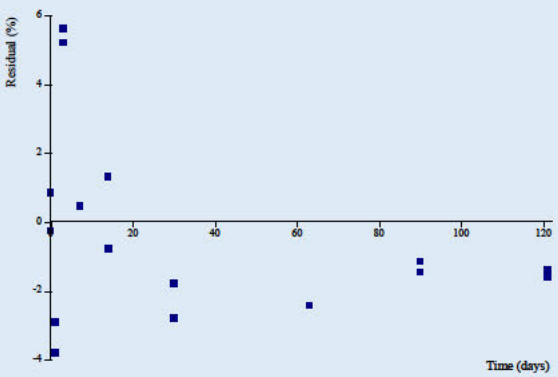
Conclusion: DFOP to be used in pathway fit for trigger endpoints
FOMC acceptable for parent-only modelling
DFOP to be used in pathway fit for modelling endpoints.

SFO

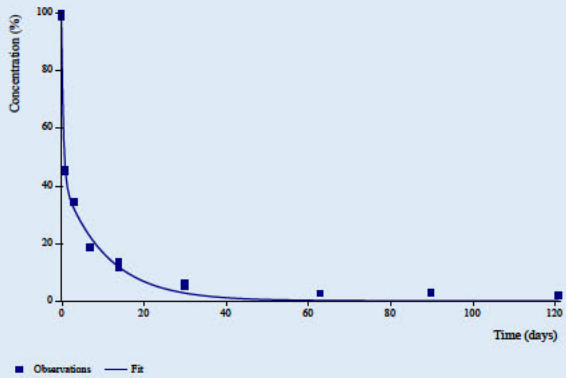


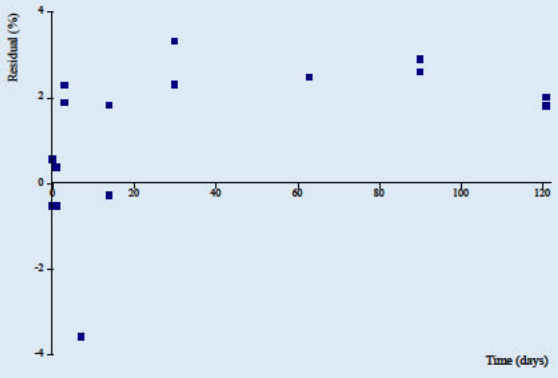
FOMC





DFOP

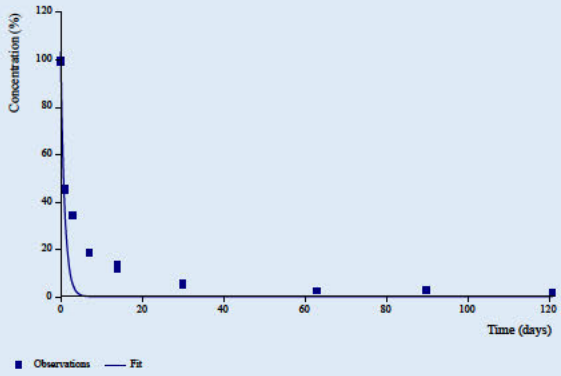


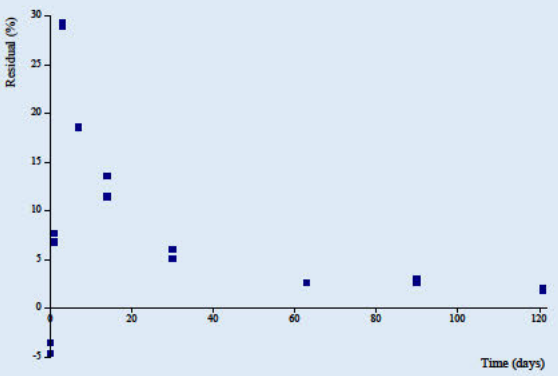


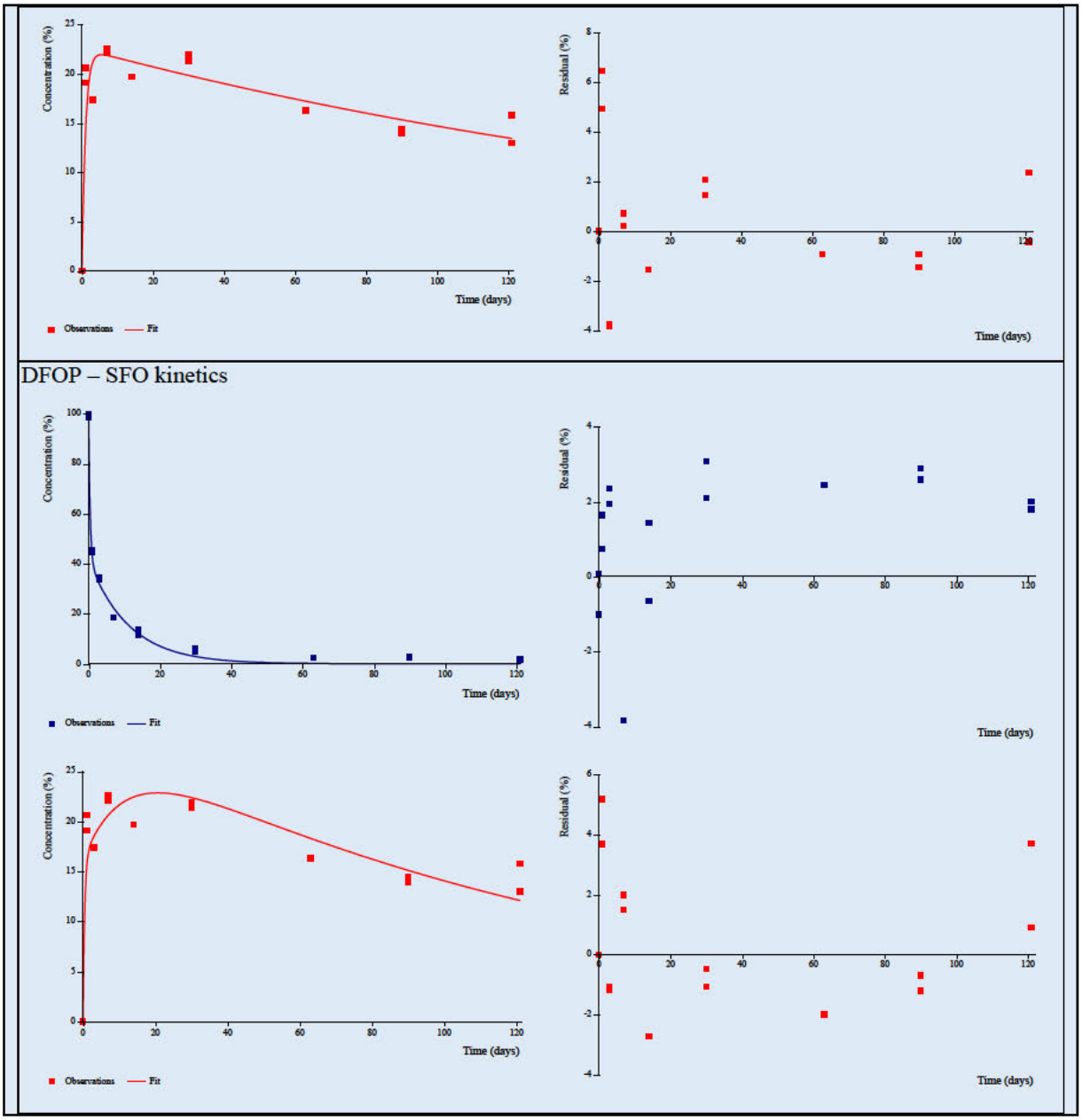
Parent-metabolite fittings (Soil B, [redacted], 1996)

Compounds	Kinetic model	Visual/ residual	Kinetic parameters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Gly.	SFO	Poor	k: 1.07	42.7	Yes	0.65	2.15		0.9137
AMPA	SFO	Good	k: 0.004209	11.1	Yes	165	547	0.2157	0.8601
Gly.	DFOP	Acceptable	k1: 2.306 k2: 0.08875 g: 0.58	8.16	Yes Yes	0.74	16.2		0.9959
AMPA	SFO	Good	k: 0.007187	10.1	Yes	96.4	320	0.2793	0.8904
RMS conclusion	Although SFO-SFO provides a good description of the degradation of metabolite AMPA, it is proposed that DFOP-SFO kinetics is selected since it significantly improves the description of the degradation of glyphosate. RMS notes that the degradation of AMPA may be slightly overestimated with this kinetic. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA								

SFO – SFO kinetics







[redacted], *1995: Arrow*

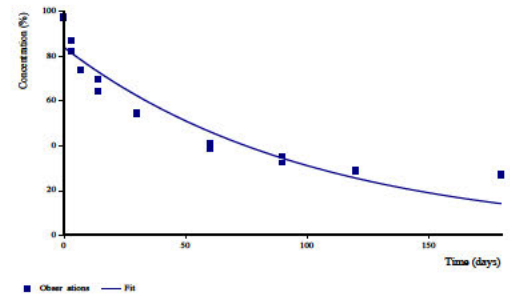
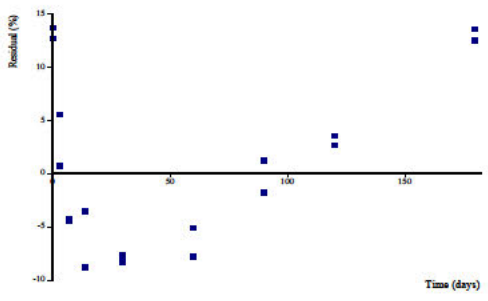
Table 8.1.1.2-14: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1995)

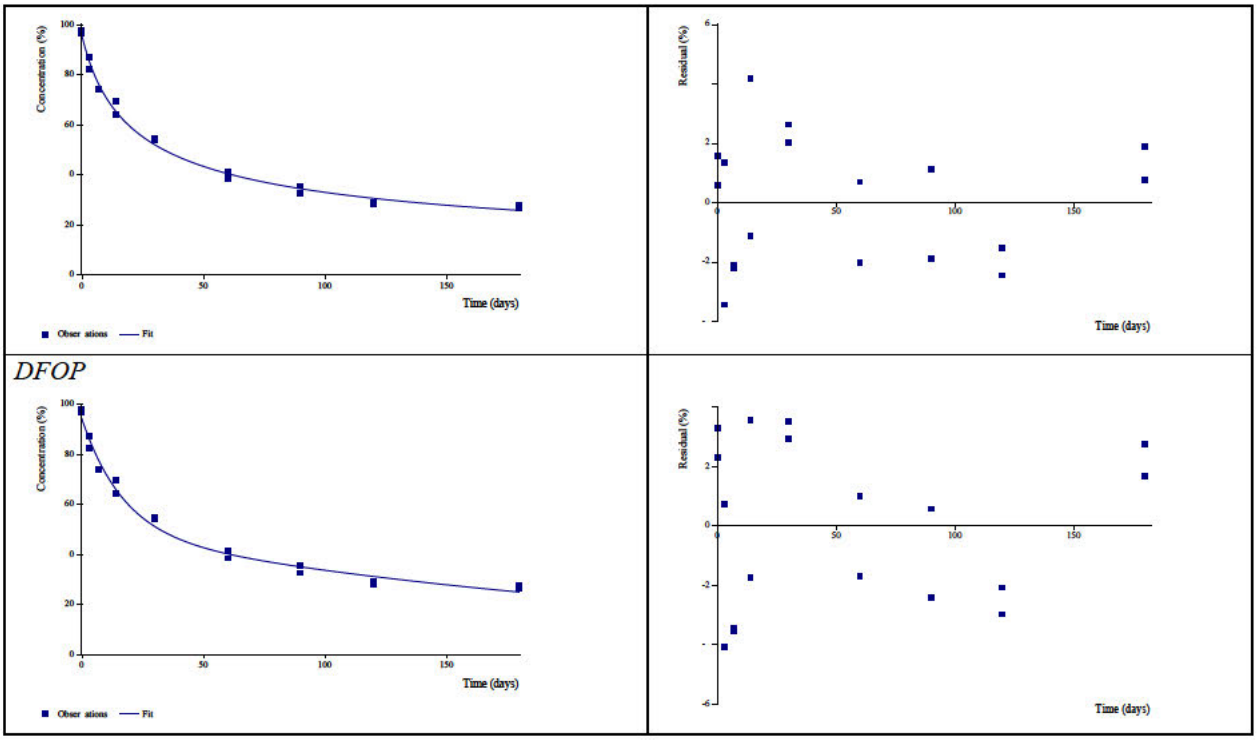
Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	97.6[1]	0.0[2]
0	96.6[1]	0.0[2]
3	87.0	3.9
3	82.2	3.1
7	74.0	6.9
7	73.9	6.6
14	64.2	10.4
14	69.5	8.3
30	54.0	14.4
30	54.6	13.7
60	41.1	22.1
60	38.4	22.3
90	32.5	27.5
90	35.5	25.4

Time (d)	Glyphosate (% AR)	AMPA (% AR)
120	28.1	28.0
120	29.0	26.6
180	26.5	25.8
180	27.6	25.3

[1] Set to material balance
[2] Amounts of metabolites set to 0 at day 0

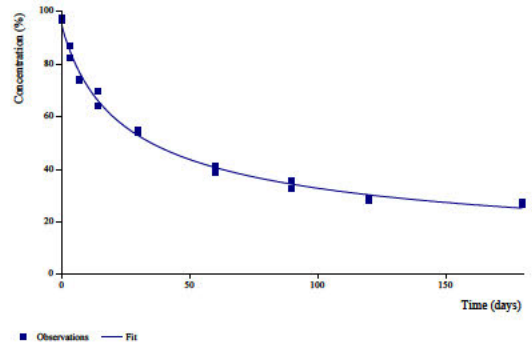
Table 8.1.1.2-15: Kinetic models and statistics for soil Arrow of study [redacted] (1995) - Parent-only fits

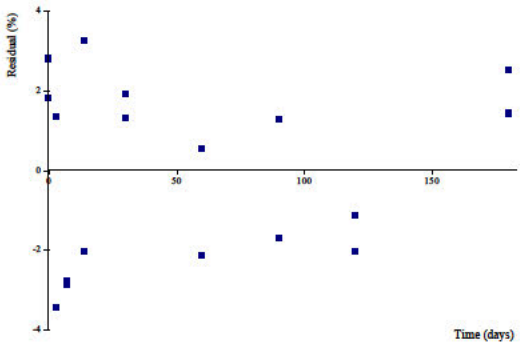
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	84.0	k: 0.0099	10.9	k: <0.001	k: 0.0076	k: 0.012	69.8	232
FOMC	Good	96.0	α: 0.4539 β: 10.47	2.3	-[1]	β: 6.312	β: 14.63	37.8	1660
DFOP	Good	94.3	k_1: 0.0595 k_2: 0.0037 g: 0.4852	3.6	k_1: <0.001 k_2: <0.001	k_1: 0.0345 k_2: 0.0017	k_1: 0.085 k_2: 0.006	37.4	440
Applicant's conclusion		Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides a slightly better visual fit (M_0 as well as the residues at the last two sampling dates) and the lowest χ^2 error. Thus, the FOMC model is selected as the best-fit model. As 10 % of the initial concentration was not reached within the experimental period, the DFOP model is selected for derivation of modelling endpoints. Conclusion: FOMC to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints							
RMS conclusion		The radioactive purity of glyphosate in this study was reported to be >99% therefore no adjustments were needed and the data used by the applicant are considered appropriate. RMS agrees that SFO is not considered suitable for trigger nor for modelling endpoint. For persistence endpoint, FOMC is considered as best-fit based on chi2-error, better description of M0 and 2 last points (although the extent of residual is slightly higher than for DFOP). For modelling endpoint: Gyphosate still represents more than 10% AR at the end of the study, RMS agrees with the applicant that DFOP should be used in pathway fit for modelling endpoint. Conclusion: FOMC to be used in pathway fits for trigger endpoints DFOP to be used in pathway fits for modelling endpoints							
SFO									
FOMC									



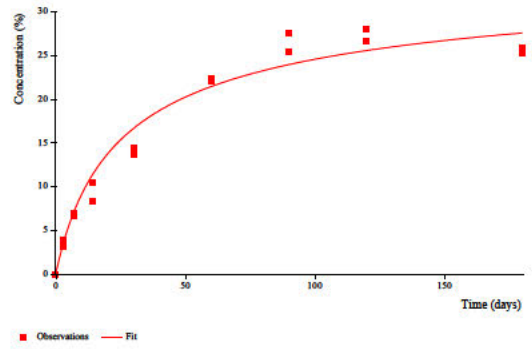
Kinetic models and statistics for soil Arrow of study [redacted] (1995) - Pathway fits (Parent and metabolites)

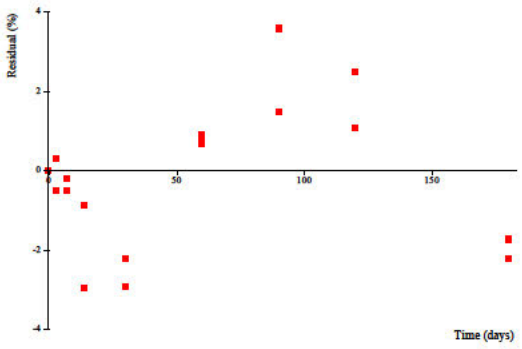
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Trigger endpoints										
Glypho-sate: FOMC	Good	94.8	α: 0.496 β: 13.22	2.5	-[1]	β: 8.158	β: 18.28	40.3	1360	-
AMPA: SFO	Poor	-	k: 0	8.3	k: 0.5	k: -0.0013	k: 0.001	>10 000	>10 000	0.395 (±0.028)
Applicant's conclusion	The degradation of glyphosate is well described by the pathway fit. For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak. Conclusion: - Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate - No trigger endpoints can be derived for AMPA									
RMS conclusion	The degradation of glyphosate is still well described. For AMPA, RMS agrees with the applicant that the experimental points are not well described, since the predicted curve still increases while measured values indicate that a plateau was reached. As a consequence, in this case RMS agrees that no reliable trigger endpoint can be derived for AMPA. For glyphosate, endpoints from parent only fit should be selected for trigger endpoints.									
Trigger : Glyphosate (FOMC)										





Trigger : AMPA (SFO)

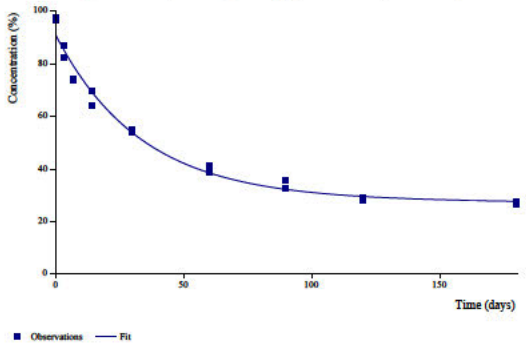


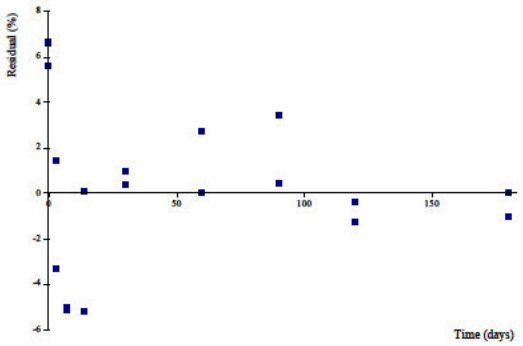


Modelling endpoints – initial fitting

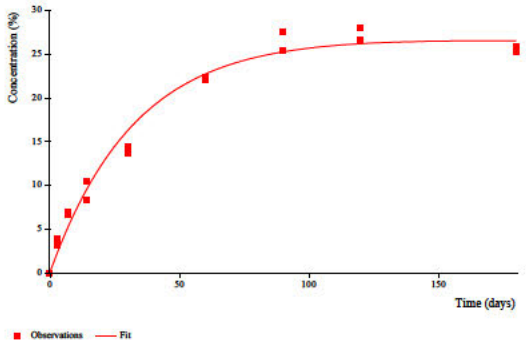
Glyphosate: DFOP	Poor	91.0	k_1: 0.0301 k_2: 0.0003 g: 0.6852	4.7	k_1: <0.001 k_2: 0.436	k_1: 0.0210 k_2:-0.0031	k_1: 0.039 k_2: 0.004	42.8	3860	-
AMPA: SFO	Acceptable	-	k: 0.0003	5.9	k: 0.325	k: -0.0011	k: 0.002	2260	7510	0.437 (±0.033)
Applicant's conclusion		For the parent, the visual fit is poor (M_0 is underestimated compared to parent-only DFOP fit) and the parameter k_2 is not significantly different from zero. As the residue data of AMPA are acceptably described, the fitting was repeated with initial parameters for parent (M_0, k_1, k_2 and g) fixed to results from parent-only DFOP fit.								
RMS conclusion		The visual fit for glyphosate remains good. However, it is agreed that M0 is underestimated compared to the parent-only fit, and t-test for k_2 fails. It is also noted that the inclusion of AMPA in the pathway results in DFOP DT_{90} which is very different from the parent only fit. Considering that t-test is not acceptable for parent in this pathway fit, the option proposed by the applicant to provide an additional fit with fixed parameters for parent is considered suitable. Please refer to next fit.								

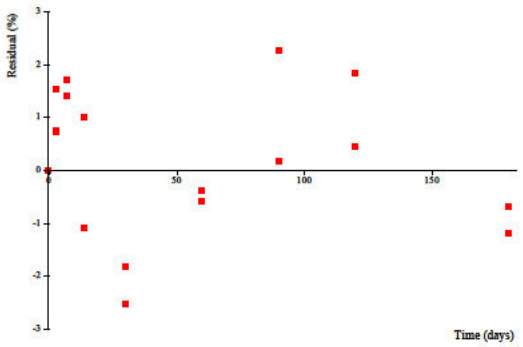
Modelling Initial fitting: Glyphosate (DFOP)





Modelling Initial fitting: AMPA (SFO)



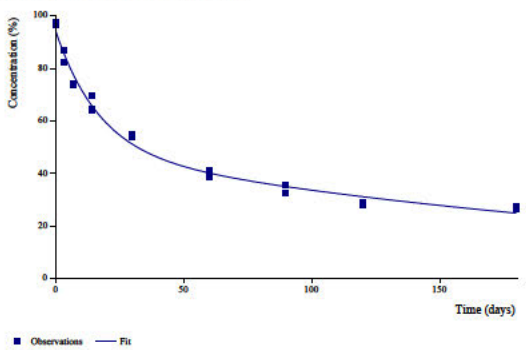


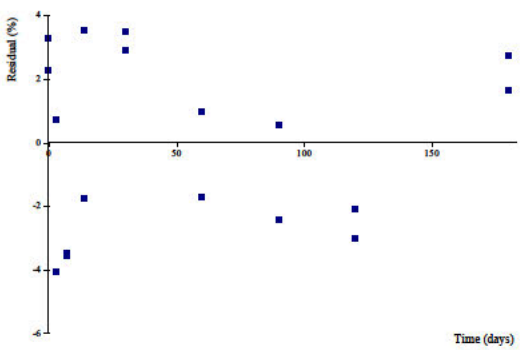
Modelling endpoints – Repeated fitting: initial parameters for parent fixed to results from parent-only fit

Glypho-sate: DFOP	Good	fixed to 94.3	fixed to k1: 0.0595 k2: 0.0037 g: 0.4852	3.1	-1	-1	-1	37.5	440	-
AMPA: SFO	Poor	-	k:<0.0001	9.3	k: 0.5	k: -0.0013	k: 0.001	>1000	>1000	0.398 (±0.023)

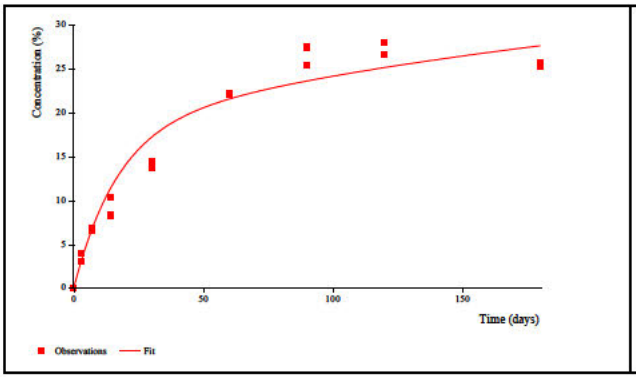
Applicant's conclusion	For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak. **Conclusion:** Parent-only DFOP fit to be used for deriving modelling endpoints for glyphosate No modelling endpoints can be derived for AMPA
RMS conclusion	RMS agrees with the applicant's analysis: the experimental points for AMPA are not well described, since the predicted curve still increases while measured values indicate that a plateau was reached. No reliable modelling endpoint can be derived for AMPA. For glyphosate, endpoints from parent only fit should be selected for modelling endpoints.

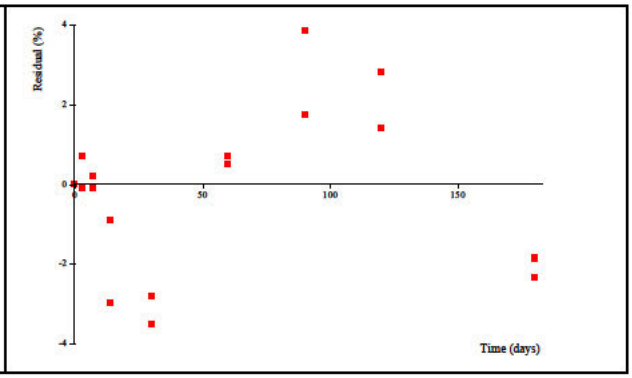
Modelling Repeated fitting: Glyphosate (DFOP) – fixed initial parent parameters





Modelling Repeated fitting: AMPA (SFO)





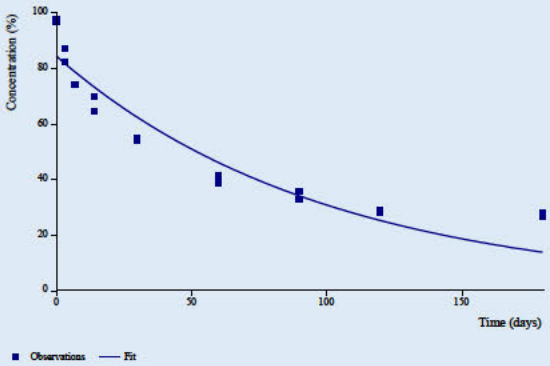
RMS additional fittings – [redacted] (1995), Arrow soil

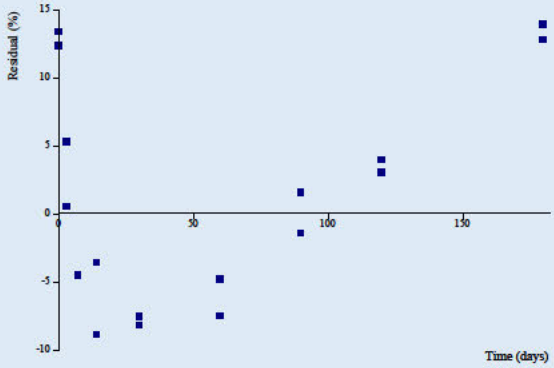
RMS performed additional SFO-SFO fittings when not provided by the applicant. In case of no significant improvement of the biphasic-SFO fittings compared with the SFO-SFO fittings, the latter would be considered a better fit for modelling.

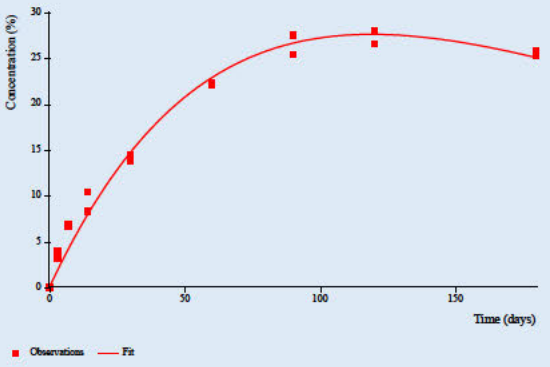
Parent-metabolite fittings - [redacted] (1995), Arrow soil

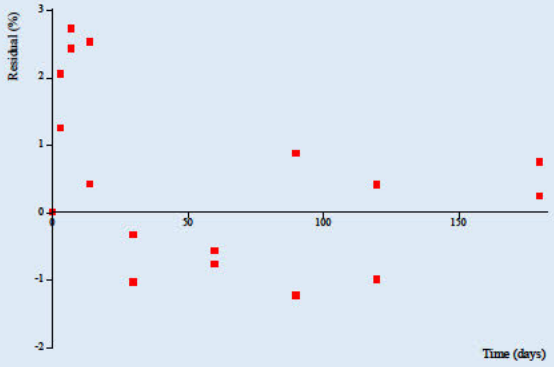
	Kinetic model	Visual/ residual	Kinetic para-meters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.009515	11.2	Yes	72.8	242		0.9051
AMPA	SFO	good	k: 0.00738	5.99	Yes	93.9	312	0.8124	0.9891
RMS conclusion	The degradation of glyphosate is not well described by SFO kinetics, with residuals not randomly distributed. The formation of AMPA is well described. A plateau seems to be reached during the study, but since there is no clear decline observed, it cannot be excluded that the predicted curve overestimates AMPA degradation. This fit should not be considered further.								

SFO – SFO kinetics









[redacted], *1993: Les Evouettes*

Table 8.1.1.2-16: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1993)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	98.6[1]	0.0[2]
3	65.6	6.2
3	69.0	6.4
7	49.5	14.9
7	58.6	11.5
14	48.9	12.2
14	38.7	13.5
28	36.7	19.7
28	36.1	21.9
56	24.3	21.1
56	25.4	22.7
84	19.4	28.3
84	19.6	30.4
112	16.3	28.3
112	21.8	26.9
168	9.4	16.6
168	10.8	21.7
252	8.3	17.7
252	8.4	18.8
364	7.4	21.2
364	6.0	21.4

[1] Set to material balance
[2] Amounts of metabolite set to 0 at day 0

Table 8.1.1.2-17: Kinetic models and statistics for soil Les Evouettes of study [redacted] (1993) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	69.0	k: 0.0171	24.9	k: <0.001	k: 0.0108	k: 0.023	40.5	135
FOMC	Good	96.5	α: 0.4936 β: 3.316	6.2	-[1]	β: 1.5133	β: 5.119	10.2	349
DFOP	Good	97.1	k_1: 0.2332 k_2: 0.0082 g: 0.5408	6.1	k_1: <0.001. k_2: <0.001	k_1: 0.1456 k_2: 0.0061	k_1: 0.321 k_2: 0.010	8.6	185
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide equally reliable and visually acceptable results but the least χ^2 error is provided by the DFOP model. Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints								
RMS conclusion	Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.								

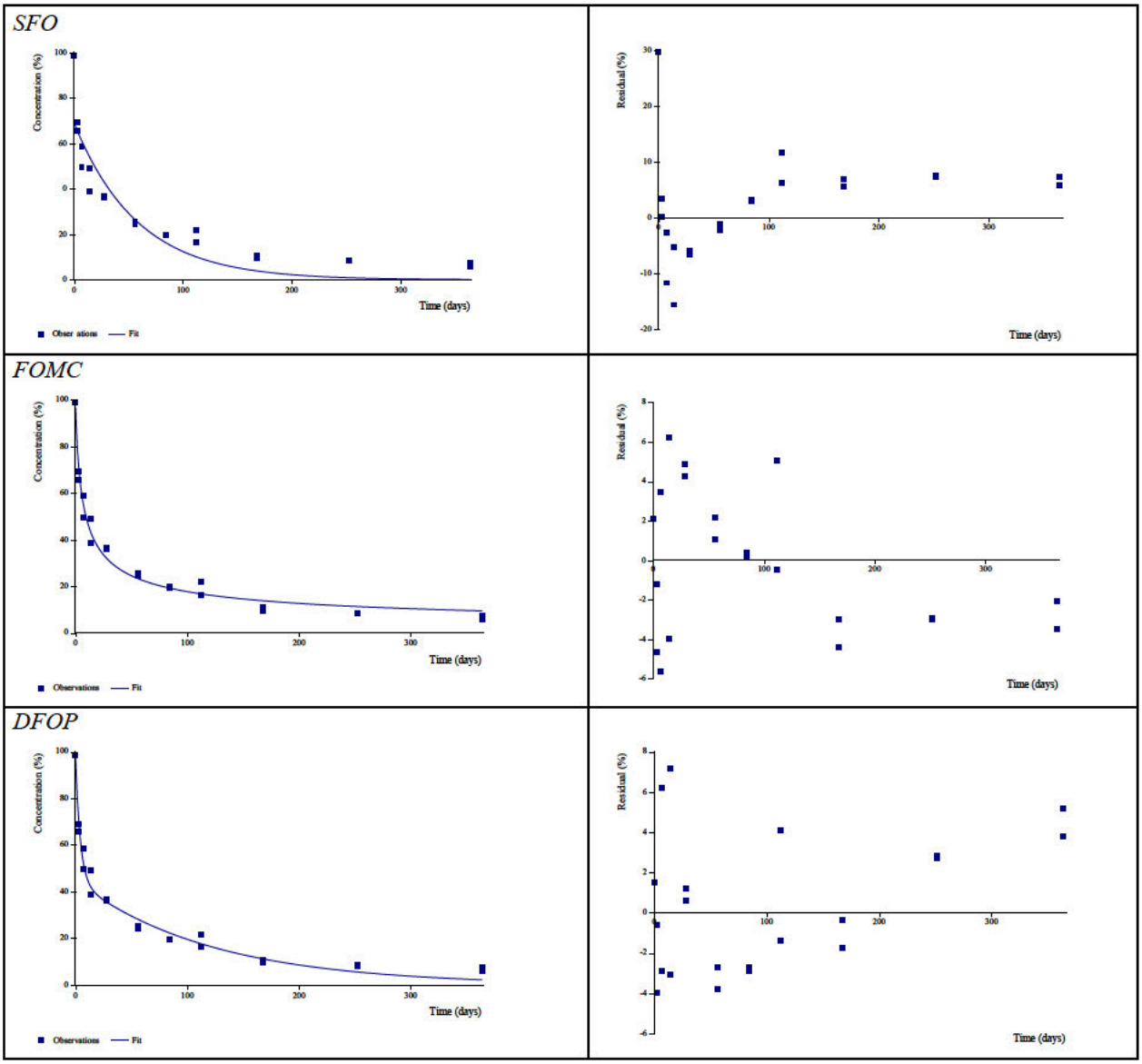
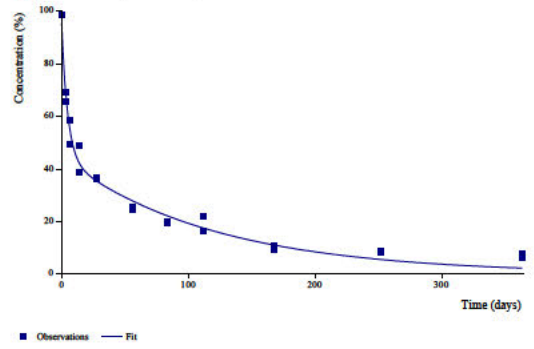
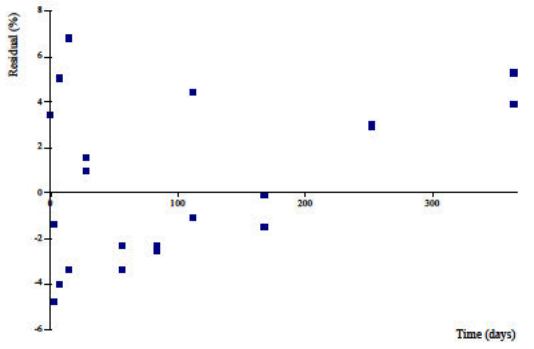


Table 8.1.1.2-18: Kinetic models and statistics for soil Les Evouettes of study [redacted] (1993) - Pathway fit (parent and metabolite)

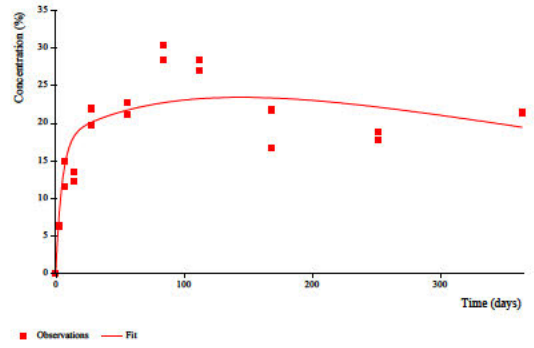
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	95.2	k_1: 0.2083 k_2: 0.0083 g: 0.5355	6.5	k_1: <0.001 k_2: <0.001	k_1: 0.1346 k_2: 0.0062	k_1: 0.282 k_2: 0.01	9.7	184	-
AMPA: SFO	Acceptable	-	k: 0.0016	15.4	k: 0.002	k: 0.0005	k: 0.003	424	>1000	0.346 (±0.033)
Applicant's conclusion	Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									
RMS conclusion	As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.									

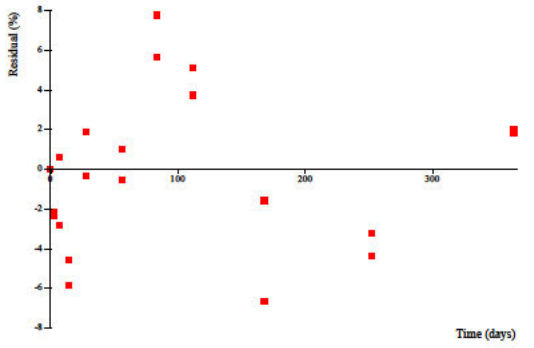
Glyphosate (DFOP)





AMPA (SFO)





RMS additional fittings - [redacted] (1993) - Les Evouettes

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

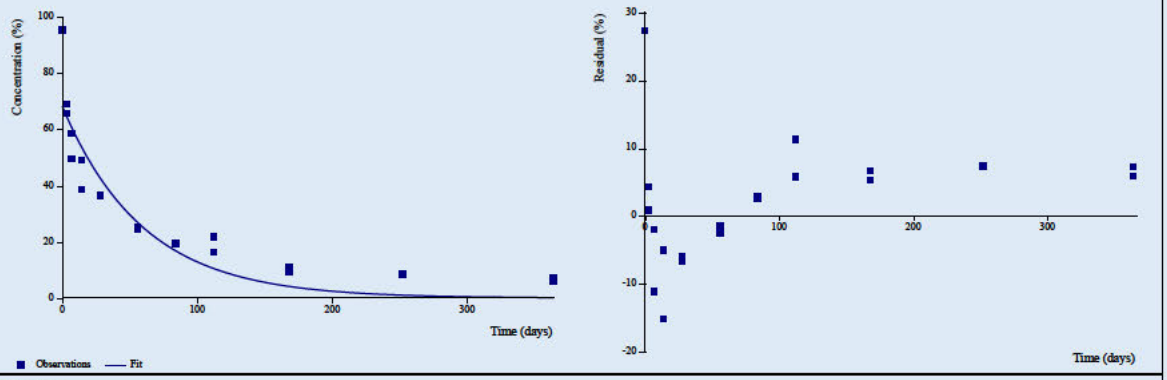
T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 96.6%)
98.6	95.3

Parent-only fittings - [redacted] (1993) - Les Evouettes

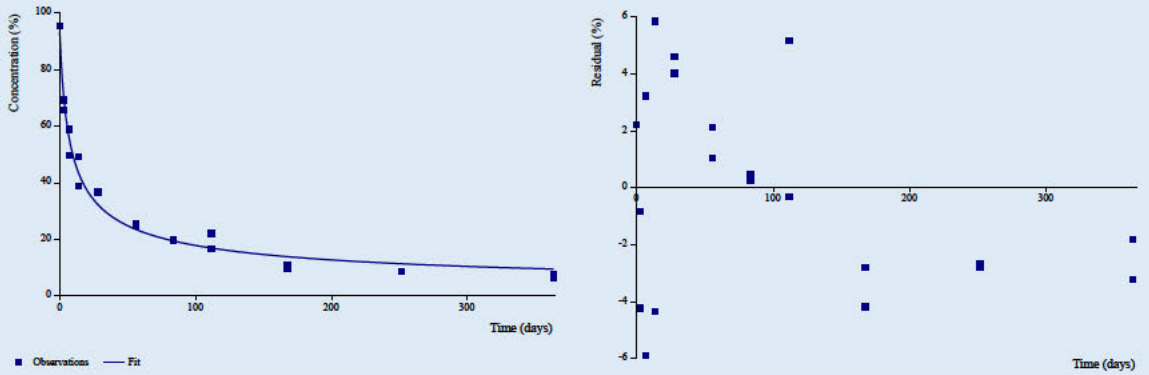
Kinetic model	Visual/ residual	M0	Kinetic para-meters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	68.08	k: 0.01667	Yes		23.5	41.6	138	0.8803
FOMC	Acceptable	93.11	α: 0.51 β: 3.96		No No	5.92	11.5	358	0.9798
DFOP	Acceptable	93.75	k1: 0.2084 k2: 0.008013 g: 0.5339	Yes Yes		5.88	9.81	192	0.9813
RMS conclusion	SFO does not well describe the overall degradation of glyphosate, residuals are not randomly distributed, with a systematic error from day 84, and M0 is significantly underestimated. SFO is not considered suitable for trigger nor for modelling endpoint. Biphasic kinetics provide better fits. Visual and statistical results are good for both FOMC and DFOP kinetics. For trigger endpoint: FOMC model is selected as it provides the best visual fit (chi2 is marginally better for DFOP, but the last data points are better described by FOMC). For modelling endpoint: Glyphosate represented less than 10% AR at the end of the study. Therefore FOMC can be selected for modelling of parent-only. However, it is reminded that FOMC kinetic cannot be directly implemented in FOCUS models, and the use of $DT_{90}/3.32$ is not suitable when metabolites are included in the degradation pathway for modelling (see more justification in the introduction).								

	In this case, since DFOP kinetic is fully acceptable, RMS considers that DFOP should be selected for modelling endpoint in pathway fit. **Conclusion:** : FOMC to be used in pathway fit for trigger endpoints FOMC acceptable for parent-only modelling DFOP to be used in pathway fit for modelling endpoints

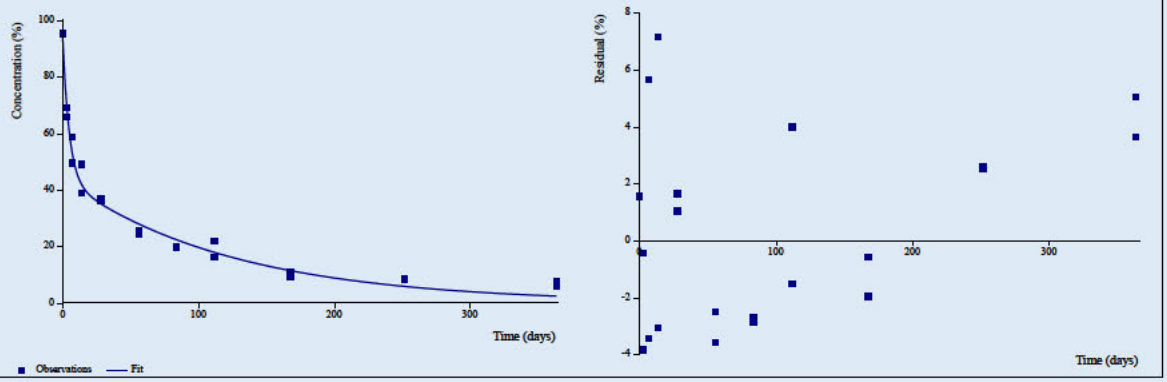
SFO



FOMC



DFOP

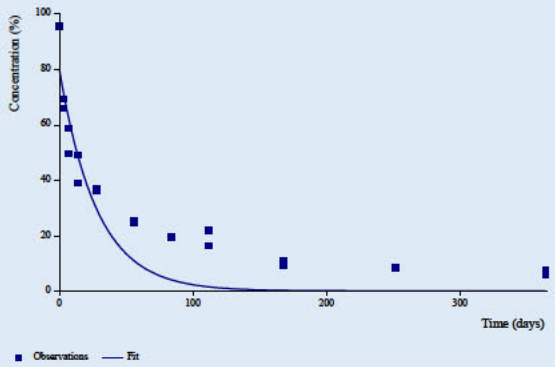


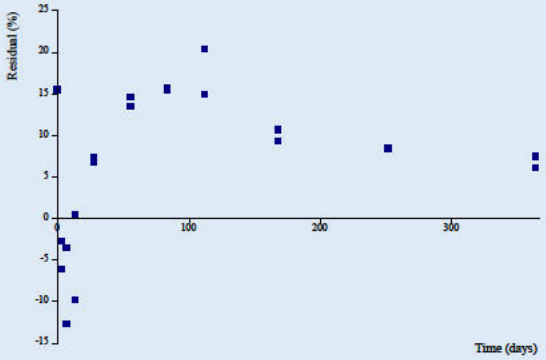
Parent-metabolite fittings - [redacted] (1993) - Les Evouettes

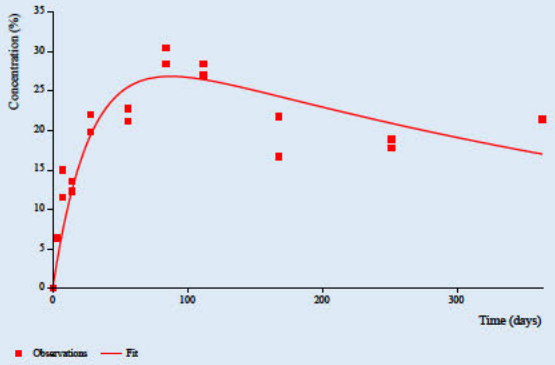
	Kinetic model	Visual/residual	Kinetic para-meters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.03564	25.5	Yes	17.7	58.8		0.9208
AMPA	SFO	Acceptable	k: 0.00185	14.9	Yes	412	1370	0.3939	0.834
Glyphosate	DFOP	Acceptable	k_1:0.184 k_2: 0.008016 g: 0.5313	6.28	Yes Yes	11	193		0.9808
AMPA	SFO	Acceptable	k: 0.001758	15	Yes	394	1310	0.3691	0.7798
RMS conclusion	RMS considers that due to scattering of the data, it is difficult to obtain a visually acceptable fit for AMPA. It is proposed that no endpoint is derived for AMPA for this soil. FOMC-SFO is not presented but would not provide additional relevant information in this case.								

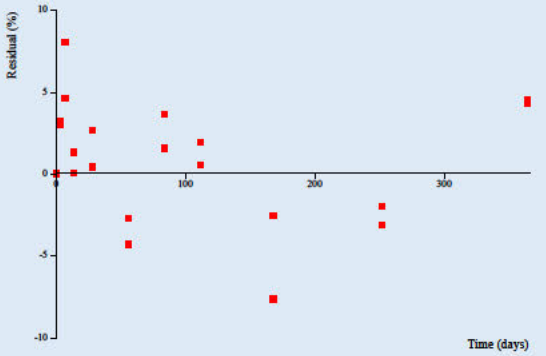
	For glyphosate, endpoints from parent only fit should be selected for both trigger and modelling endpoints.

SFO – SFO kinetics

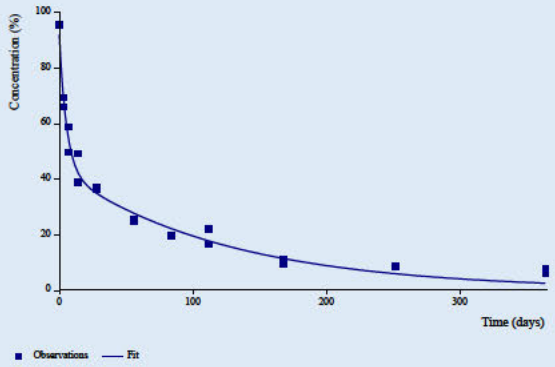


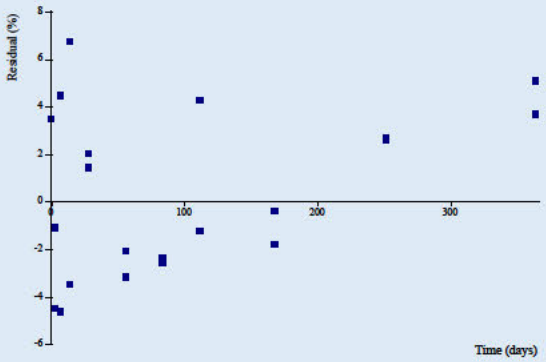


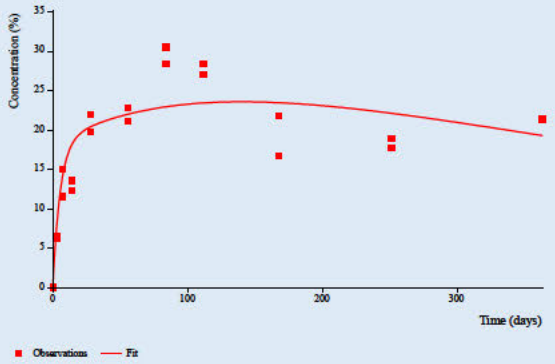


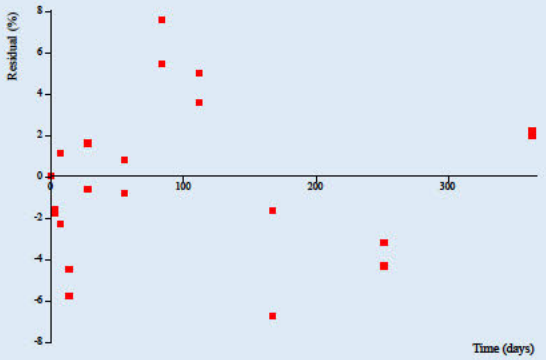


DFOP – SFO kinetics









[redacted] *(1993)*

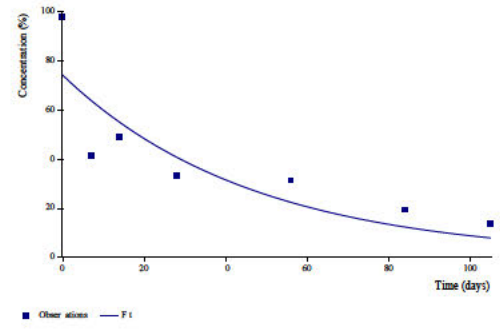
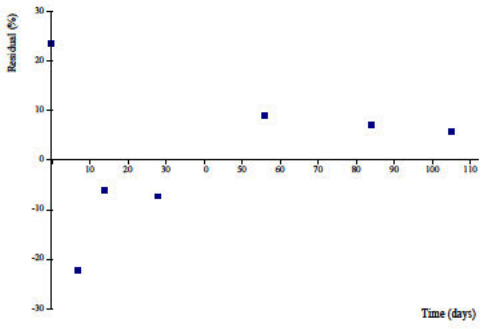
Table 8.1.1.2-19: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1993) and [redacted] (2002) – TLC results

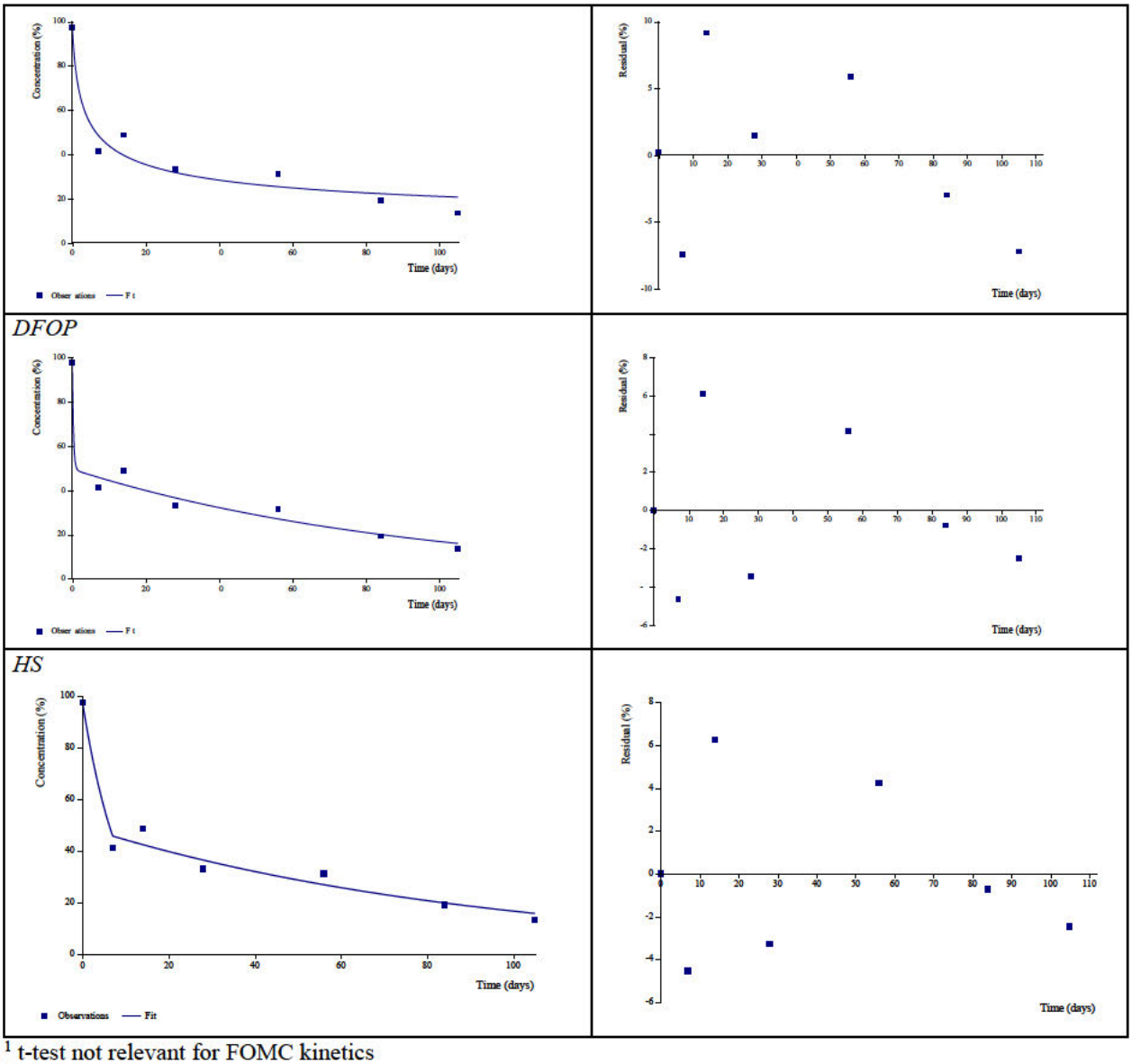
Time (d)	Glyphosate (% AR)	AMPA (% AR)	Glyphosate (% AR)	AMPA (% AR)
Speyer 2.2			**Speyer 2.3**	
0	97.6[1]	0.0[2]	92.3[1]	0.0[2]
7	41.4	42.4	39.4	13.6
14	48.8	31.4	19.7	25.1
28	33.3	33.1	5.5	25.1
56	31.3	34.6	4.3	18.9
84	19.3	33.9	3.0	18.5
105	13.5	35.4	2.5	12.1

[1] Set to material balance
[2] Amounts of metabolite set to 0 at day 0

Speyer 2.2. soil

Table 8.1.1.2-20: Kinetic models and statistics for soil Speyer 2.2 of study [redacted] (1993) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	74.2	k: 0.0214	26.7	k: 0.027	k: -0.0005	k: 0.043	32.3	107
FOMC	Good	97.4	α: 0.3322 β: 1.001	12.3	-[1]	β: -2.613	β: 4.615	7.1	>1000
DFOP	Good	97.6	k_1: 3.186 k_2: 0.0108 g: 0.4929	8.5	k_1: 0.493 k_2: 0.012	k_1: -540.8 k_2: 0.0027	k_1: 547.2 k_2: 0.019	1.7	151
HS	Good	97.6	k_1: 0.1079 k_2: 0.0108 t_b: 7.0[2]	7.8	k_1: <0.001 k_2: 0.005	k_1: 0.0721 k_2: 0.0045	k_1: 0.144 k_2: 0.017	6.4	151
Applicant's conclusion	Degradation of glyphosate was best described by bi-phasic models. As the FOMC and DFOP model did not provide statistically reliable parameters, the HS model has additionally been tested and provided the best fit with statistically reliable parameters. Conclusion: HS to be used in pathway fit for trigger endpoints HS to be used in pathway fit for modelling endpoints								
RMS conclusion	Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.								
SFO									
FOMC									



DFOP

HS

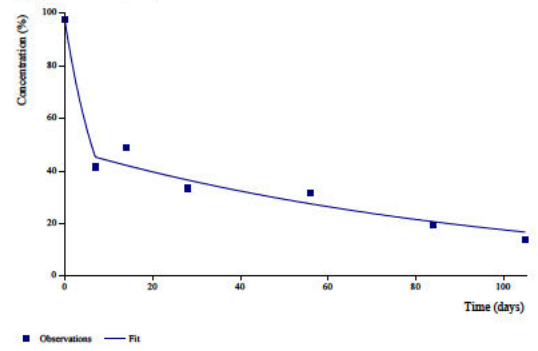
[1] t-test not relevant for FOMC kinetics

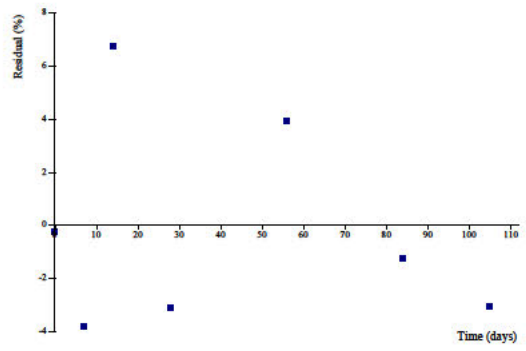
[2] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly

Table 8.1.1.2-21: Kinetic models and statistics for soil Speyer 2.2 of study [redacted] (1993) - Pathway fit (Parent and metabolite)

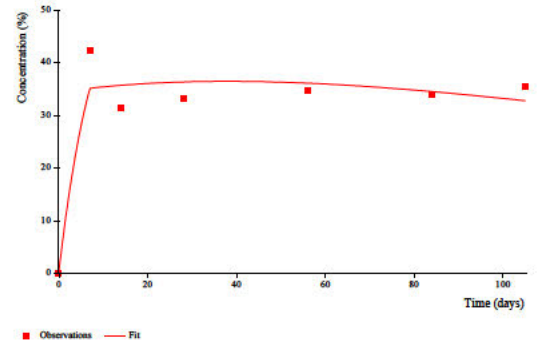
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: HS	Good	97.9	k_1: 0.1105 k_2: 0.0102 t_b: 7.0[1]	7.8	k_1: <0.001 k_2: <0.001	k_1: 0.0806 k_2: 0.0051	k_1: 0.14 k_2: 0.015	6.3	157	-
AMPA: SFO	Good	-	k: 0.0063	8.9	k: 0.016	k: 0.0007	k: 0.012	110	365	0.683 (±0.098)
Applicant's conclusion		Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: HS-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA								
RMS conclusion		As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.								

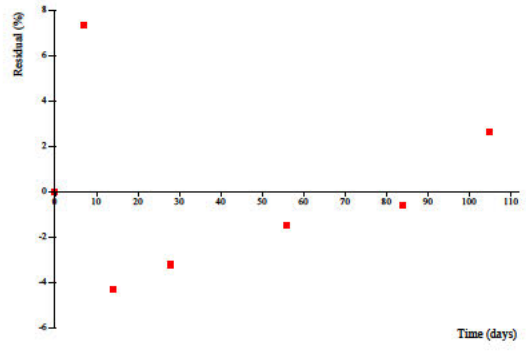
Glyphosate (HS)





AMPA (SFO)





[1] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly.

RMS additional fittings – [redacted] (1993) – Speyer 2.2

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 99.3%)
97.6	96.9

Parent only fits- [redacted] (1993) – Speyer 2.2

Compound	Kinetic model	M0	Visual/ residual	Kinetic parameters	χ^2 (%)	T test <0.05	CI contains 0	DT_{50} (d)	DT_{90} (d)
Glyphosate	SFO	73.71	Poor	k: 0.02119	26.4	Yes		32.7	109
	FOMC	96.7	Acceptable	α:0.333 β: 1.034	12.3		No Yes	7.25	1040
	DFOP	96.9	Good	k_1: 8.104 k_2: 0.01078 g: 0.4893	8.56	No (0.498) Yes		1.97	151

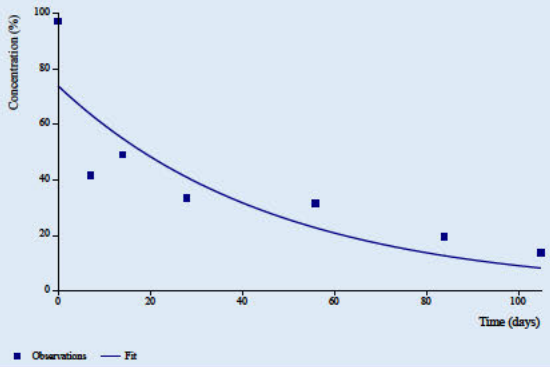
RMS conclusion;
SFO does not well describe the overall degradation of glyphosate (M0 underestimated, initial degradation underestimated, last points overestimated , high chi2-error). SFO is not considered suitable for trigger nor for modelling endpoint.
The biphasic fittings improve the description of glyphosate.

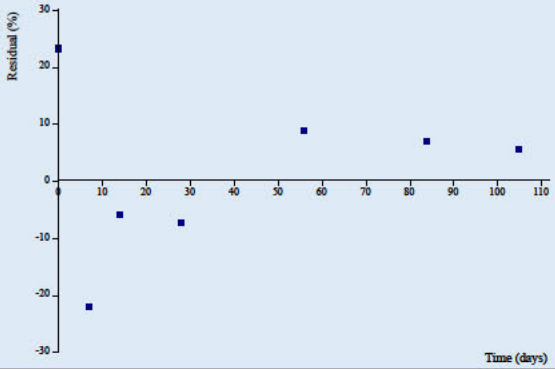
Trigger endpoints: DFOP provides the best visual fit and chi2-error is lower than for FOMC. It is noted that t-test for k1 DFOP fails. It is likely because the fast phase has terminated before sufficient measurements could be taken. In this specific case, RMS considers that this can be accepted.

Modelling endpoints: Glyphosate still represents more than 10% AR at the end of the study. Therefore, DFOP should be selected. As explained above, in this specific case it can be accepted that t-test for k1 fails.

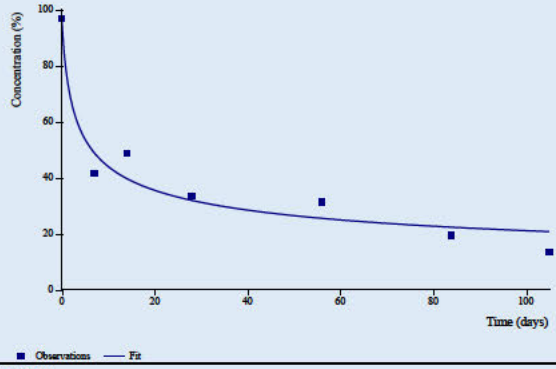
Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints.

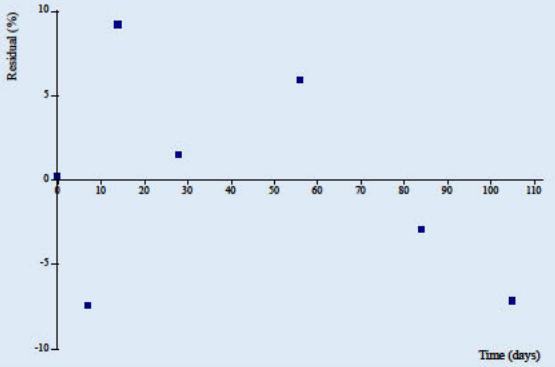
SFO



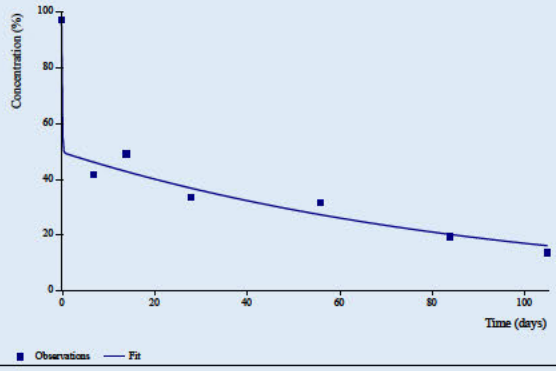


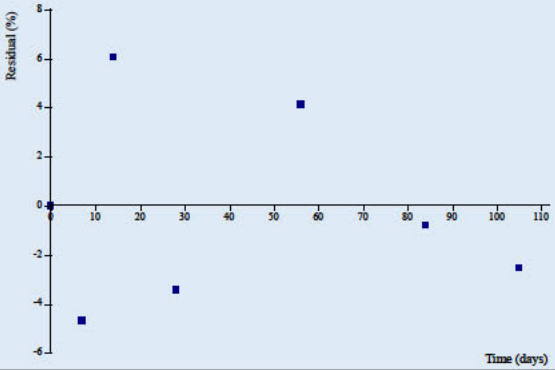
FOMC





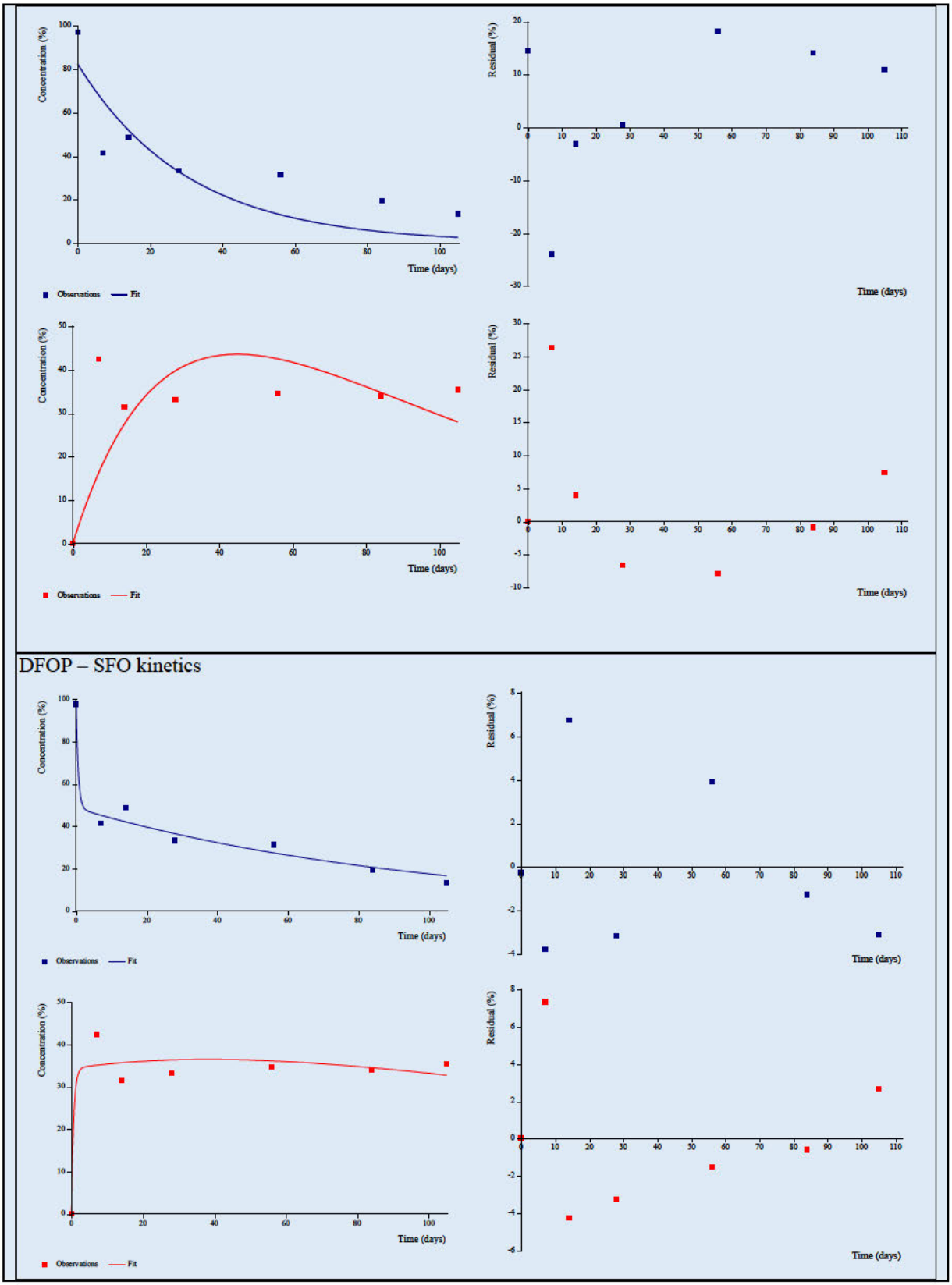
DFOP





Parent-metabolite fittings - [redacted] (1993) – Speyer 2.2

	Kinetic model	Visual/ residual	Kinetic para-meters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.03296	28.2	Yes	21	69.9		0.7769
AMPA	SFO	Poor	k: 0.01424	27.2	No (0.114)	48.7	162	1	0.4563
Glyphosate AMPA	DFOP	Good	k_1: 1.645 k_2: 0.01022 g: 0.5041	8.62	No (0.499) Yes	2.14	157		0.9794
	SFO		k: 0.006381	8.85	No (0.334)	109	361	0.6926	0.9189
RMS conclusion	RMS considers that due to the profile of the data obtained for AMPA, it is difficult to obtain a visually acceptable fit for AMPA. It is proposed that no endpoint is derived for AMPA for this soil. **Conclusion:** DFOP fit for trigger endpoints for glyphosate, from parent only fit DFOP for modelling endpoints for glyphosate, from parent only fit								
SFO – SFO kinetics									



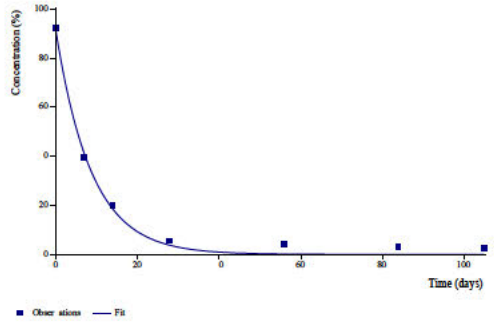
Speyer 2.3 soil

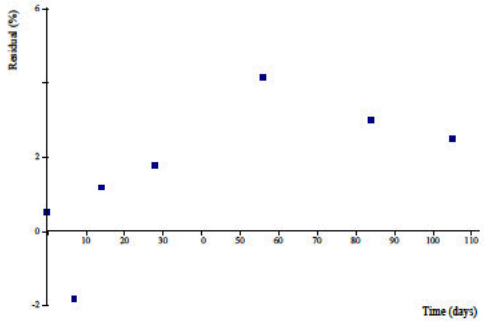
Table 8.1.1.2-22: Kinetic models and statistics for soil Speyer 2.3 of study [redacted] (1993) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

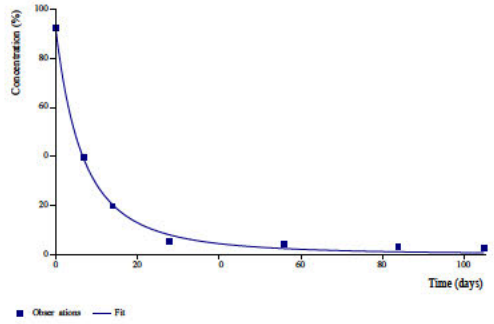
SFO	Poor	91.8	k: 0.1143	8.0	k: <0.001	k: 0.0943	k: 0.134	6.1	20.1
FOMC	Good	92.4	α: 2.467 β: 16.42	5.8	-[1]	β: -4.695	β: 37.53	5.3	25.3
DFOP	Good	92.2	k_1: 0.1296 k_2: 0.0056 g: 0.9474	2.5	k_1: <0.001 k_2: 0.201	k_1: 0.1104 k_2:-0.0126	k_1: 0.149 k_2: 0.024	5.8	22.2
Applicant's conclusion		Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (residues at the last four sampling dates) and the lowest χ^2 error. The parameter k_2 is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.9474). Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints							
RMS conclusion		Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.							

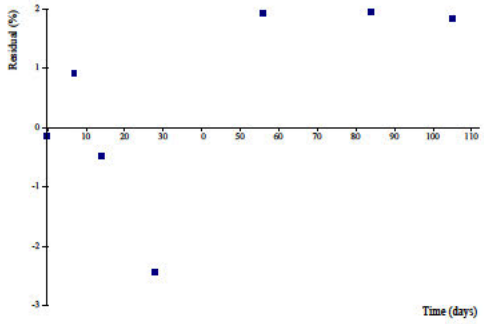
SFO



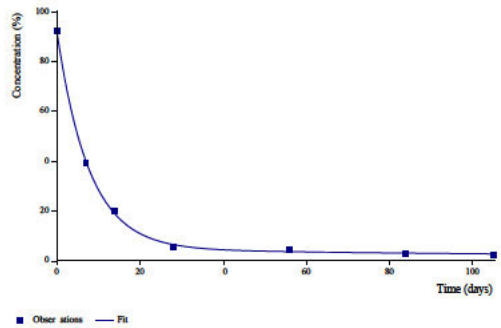


FOMC





DFOP



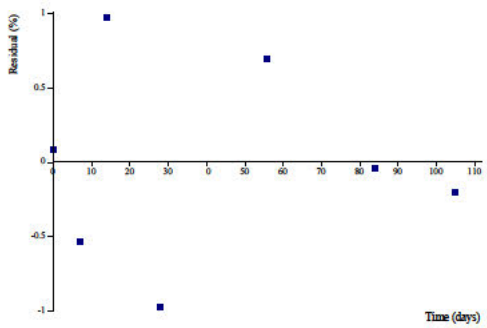
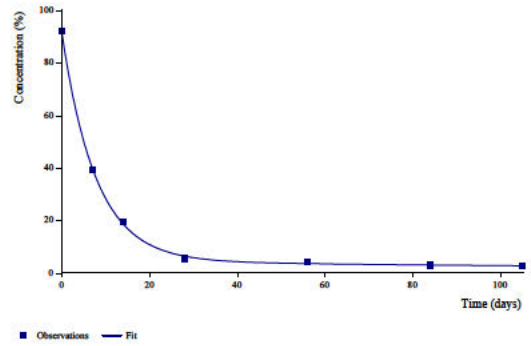
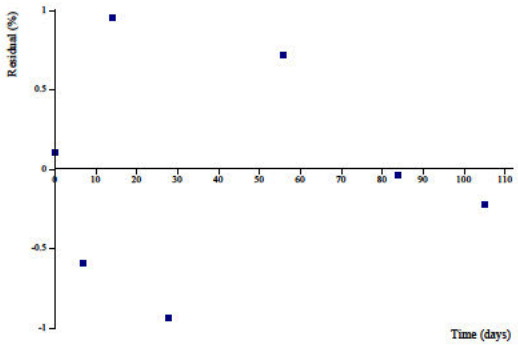


Table 8.1.1.2-23: Kinetic models and statistics for soil Speyer 2.3 of study [redacted] (1993) - Pathway fits (parent and metabolite)

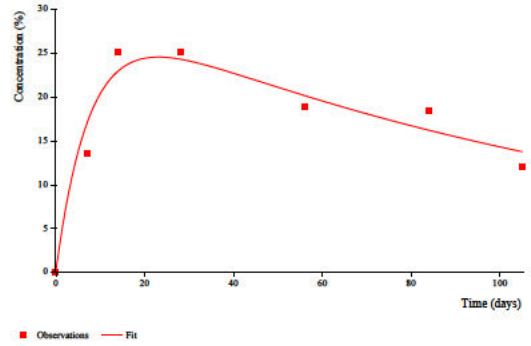
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	92.2	k_1: 0.1291 k_2: 0.0052 g: 0.9488	2.5	k_1: <0.001 k_2: 0.179	k_1: 0.1161 k_2:-0.0073	k_1: 0.142 k_2: 0.018	5.8	22.2	-
AMPA: SFO	Good	-	k: 0.0082	8.8	k: 0.002	k: 0.0035	k: 0.013	85.0	282	0.336 (±0.030)
Applicant's conclusion	The visual fits for glyphosate and AMPA are good and degradation parameters for AMPA are reliable. For glyphosate, the parameter k_2 is not significantly different from zero which again can be accepted as the overall degradation of glyphosate is dominated by k_1 as indicated by a high value for parameter g (0.9488), and the respective modelling endpoint is derived from DT_{90}/3.32 as 10 % of the initial concentration was reached within the experimental period. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									
RMS conclusion	As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.									

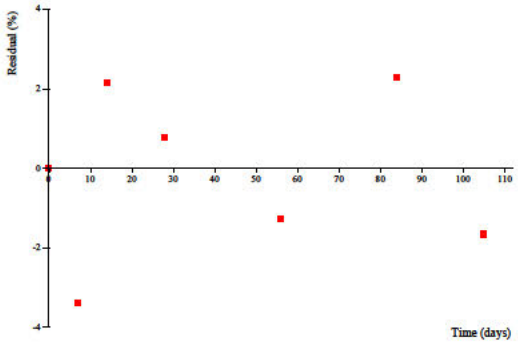
Glyphosate (DFOP)





AMPA (SFO)





RMS additional fittings – [redacted] (1993) – Speyer 2.3

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 99.3%)
92.3	91.7

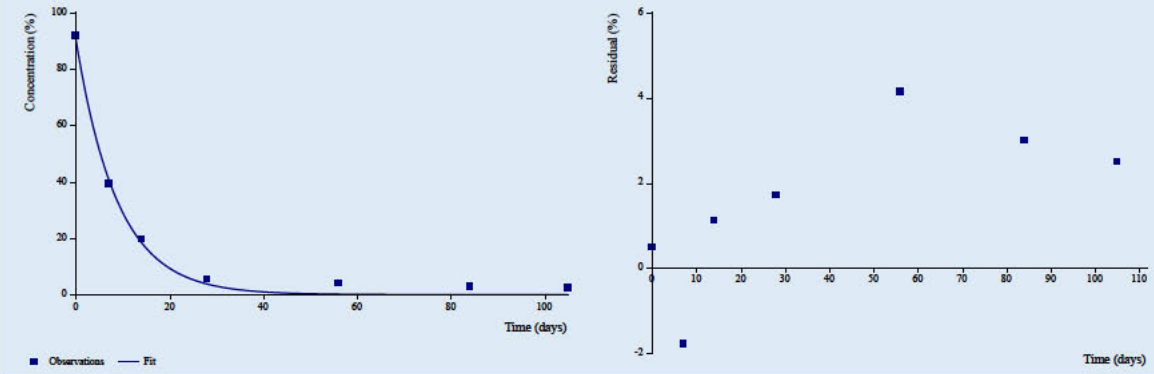
Parent only fits- [redacted] (1993) – Speyer 2.3

Compound	Kinetic model	M0	Visual/ residual	Kinetic parameters	χ^2 (%)	T test <0.05	CI contains 0	DT_{50} (d)	DT_{90} (d)
Glyphosate	SFO	91.21	Poor	k: 0.1136	8	Yes		6.1	20.3
	FOMC	91.85	Acceptable	α:2.493 β: 16.76	5.81		No Yes	5.37	25.4
	DFOP	91.62	Good	k_1: 0.1285 k_2: 0.005332 g: 0.9482	2.43	Yes No(0.208)		5.8	22.3

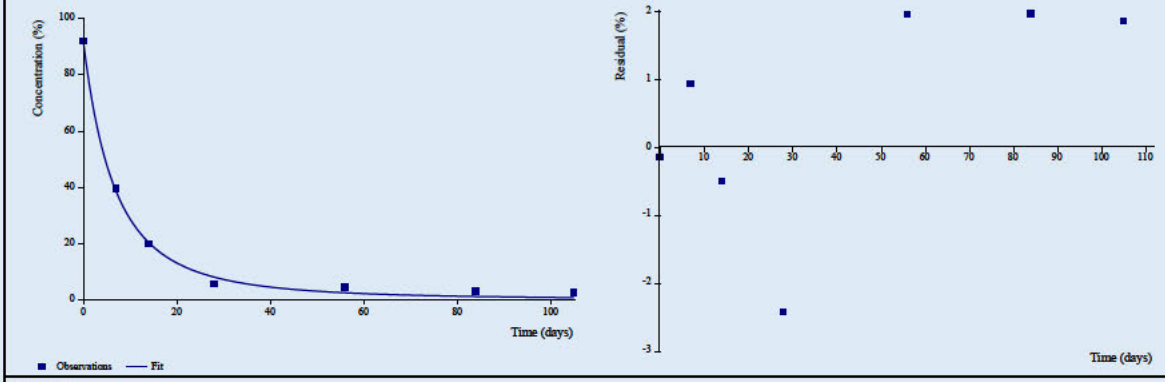
RMS conclusion;
SFO kinetics overestimate the degradation for the last points, but 90% degradation is reached before this underestimation. T-test is acceptable.
The biphasic fittings improve the description of glyphosate. However, with FOMC the last data points are still underestimated and confidence interval for beta includes 0. DFOP provides the best visual fit, with lowest chi2-error. However, k2 is statistically unreliable;in addition, the g parameter is very close to 1, indicating that most of the degradation is described by k_1 parameter. It is also noted that the difference in the degradation rates between SFO and DFOP is very low.
In this specific case, RMS considers that SFO is suitable.

Conclusion: SFO to be used in pathway fit for trigger endpoints
SFO to be used in pathway fit for modelling endpoints.

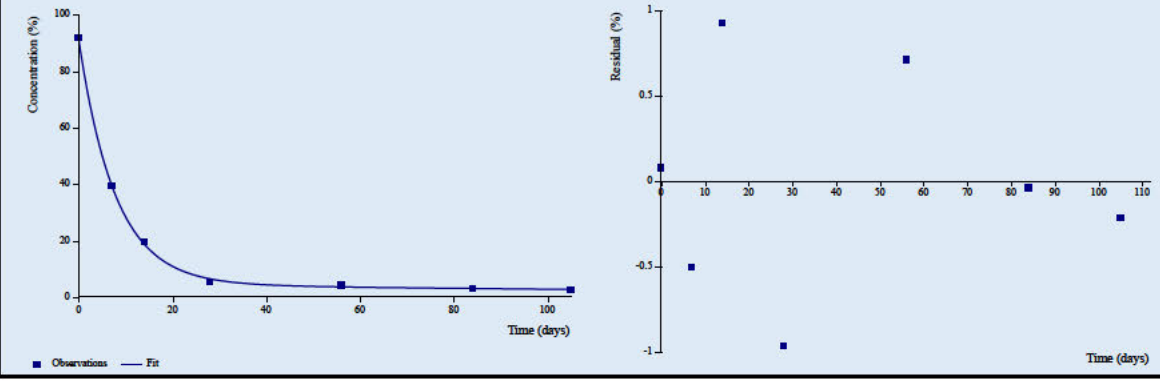
SFO



FOMC



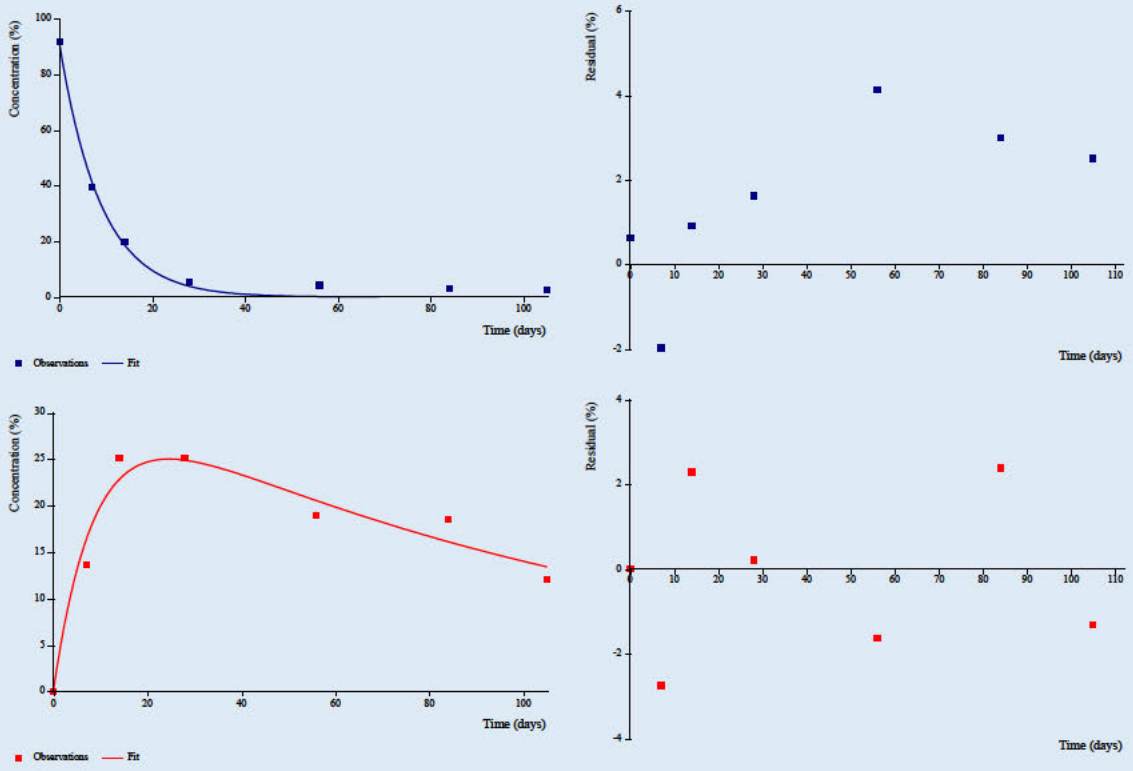
DFOP



Parent-metabolite fittings - [redacted] (1993) – Speyer 2.3

	Kinetic model	Visual/ residual	Kinetic para-meters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Acceptable	k: 0.1127	8.01	Yes	6.15	20.4		0.9977
AMPA	SFO	Good	k: 0.008753	8.24	Yes	79.2	263	0.3406	0.9509
RMS conclusion	The degradation of glyphosate and AMPA is quite well described for this soil. **Conclusion:** SFO-SFO fit to be used for trigger and modelling endpoints								

SFO – SFO kinetics



[redacted] *(1992)*

Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1992)

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]	Glyphosate (% AR)[1]	AMPA (% AR)[1]	Glyphosate (% AR)[1]	AMPA (% AR)[1]
Speyer 2.1, dose group A 20 °C, 40 % MWHC, 4 mg/kg			**Speyer 2.1, dose group B 20 °C, 20 % MWHC, 4 mg/kg**		**Speyer 2.1, dose group C 8 °C, 40 % MWHC, 4 mg/kg**	
0	99.2[2]	0.0[3]	100.1[2]	0.0[3]	98.9[2]	0.0[3]
0	99.1[2]	0.0[3]	97.6[2]	0.0[3]	98.3[2]	0.0[3]
2	53.7	7.1	57.7	10.0	66.7	5.6
2	65.0	9.4	58.6	9.2	77.3	5.1
4	54.6	15.3	48.6	15.7	76.5	7.7
4	55.0	15.3	45.0	15.7	68.8	6.8
8	46.9	18.6	34.1	15.3	68.9	9.9
8	41.8	17.4			69.3	8.2
16	35.0	25.5	31.3	16.6	65.7	13.7
16	32.0	24.2	28.5	23.1	61.3	14.2
33	21.0	29.2	20.9	27.0	54.3	16.5
33	21.2	29.8	21.0	28.0	59.9	18.2
64	13.1	33.9	11.4	28.3	35.6	17.8
64	13.3	29.7	12.9	26.6	37.4	19.4

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]	Glyphosate (% AR)[1]	AMPA (% AR)[1]	Glyphosate (% AR)[1]	AMPA (% AR)[1]
104	7.5	29.9	8.0	26.6	25.1	23.8
104	8.0	29.7	6.5	28.6	28.5	22.6

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]	Glyphosate (% AR)[1]	AMPA (% AR)[1]
Speyer 2.1, dose group D 20 °C, 40 % MWHC, 4 mg/kg, sterile			**Speyer 2.1, dose group E 20 °C, 40 % MWHC, 0.4 mg/kg**	
0	98.1[2]	0.0[3]	101.4[2]	0.0[3]
0	95.3[2]	0.0[3]	103.1[2]	0.0[3]
2	63.0	5.4	71.8	10.6
2	61.2	5.7	75.8	11.4
4	61.8	7.4	75.1	18.2
4	60.2	8.4	65.4	15.0
8	52.5	9.8	46.7	16.8
8	57.7	7.2	NaN	NaN
16	43.5	10.7	33.0	24.0
16	42.5	15.7	33.4	25.0
33[a]	47.4	11.6	23.0	27.4
33 [a]	30.2	17.4	24.1	31.0
64 [b]	23.9	21.1	12.7	30.8
64 [b]	24.3	19.6	12.9	32.1
104			7.6	33.7
104			6.9	27.3

[a] DAT 34 for sterile soil
[b] DAT 70 for sterile soil
[1] Residues are mean values of two solvent system (solvent system 1 and solvent system 5). As data in the two solvent systems are similar, mean values were calculated and used for kinetic analysis.
[2] Set to material balance
[3] Amounts of metabolites set to 0 at day 0

Speyer 2.1 soil, Group A

Table 8.1.1.2-24: Kinetic models and statistics for soil Speyer 2.1, dose group A (20° C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	80.5	k: 0.0592	20.2	k: <0.001	k: 0.0322	k: 0.086	11.7	38.9
FOMC	Good	98.0	α: 0.4984 β: 1.6154	7.2	-[1]	β: 0.5095	β: 2.7210	4.9	162
DFOP	Good	99.0	k_1: 0.7469 k_2: 0.0245 g: 0.4592	5.6	k_1: <0.001 k_2: <0.001	k_1: 0.3338 k_2: 0.0176	k_1: 1.16 k_2: 0.031	4.5	68.9
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits but the DFOP model provides the lowest χ^2 error. Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints								
RMS conclusion	Kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.								
SFO									

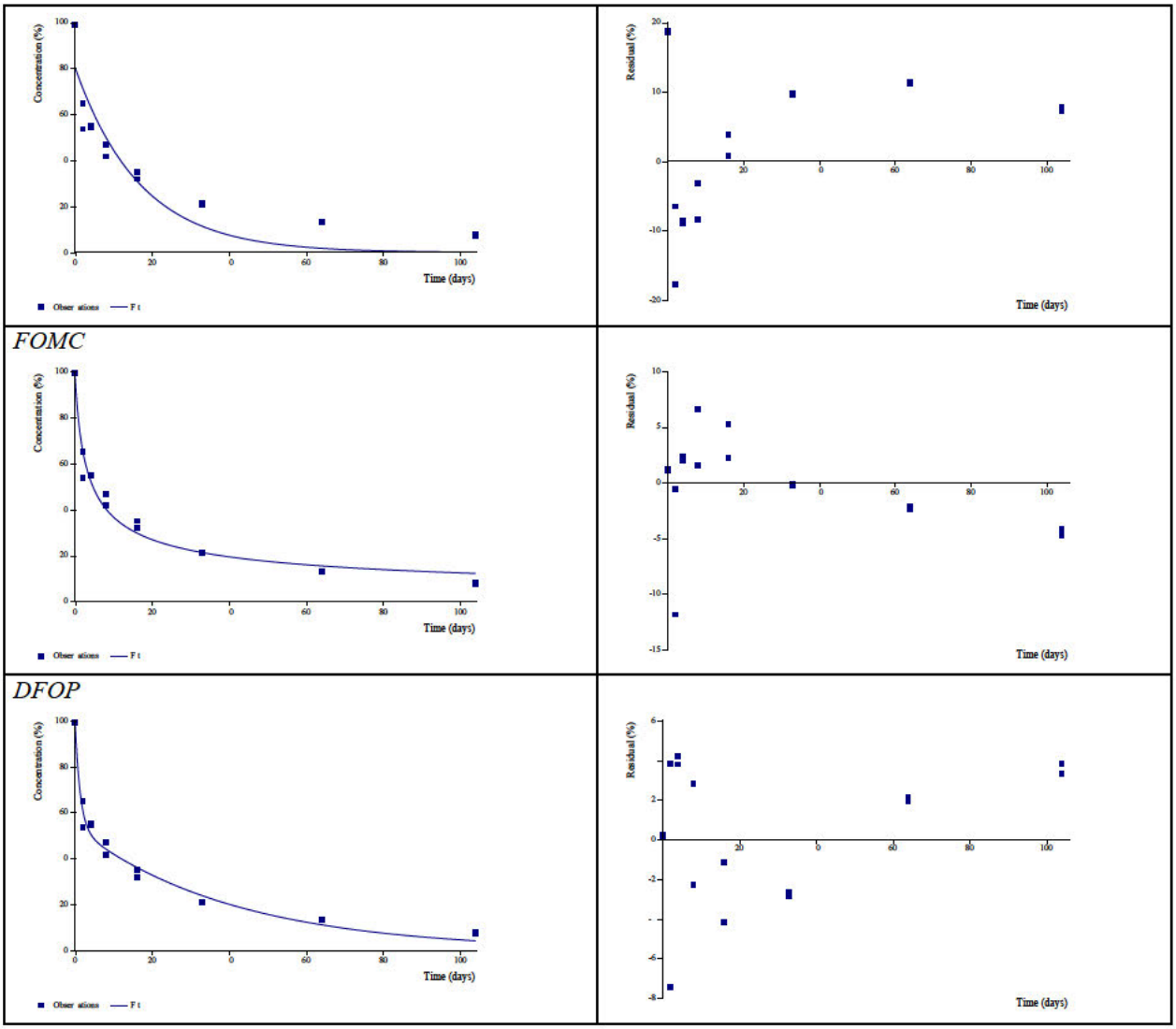
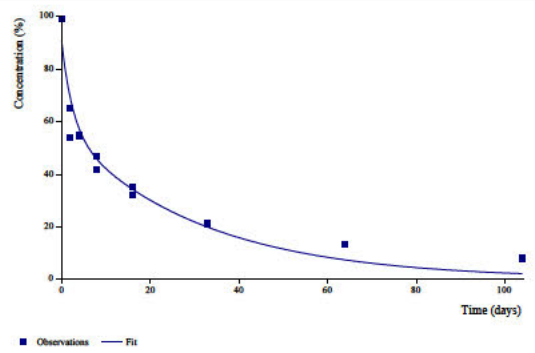
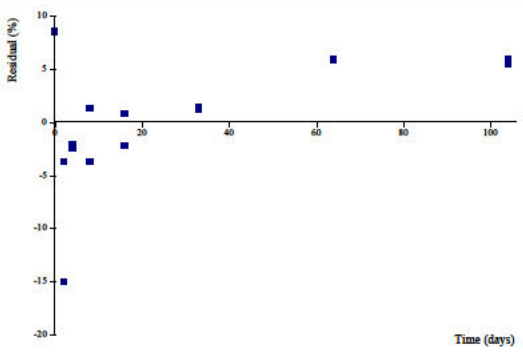


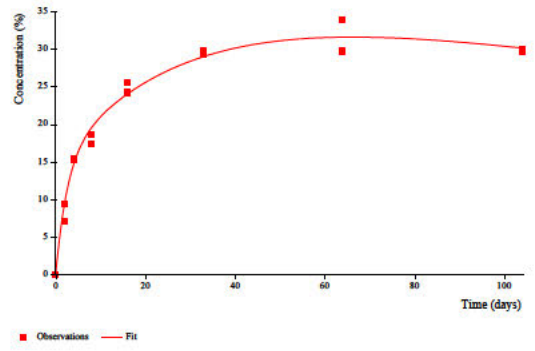
Table 8.1.1.2-25: Kinetic models and statistics for soil Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992) - Pathway fits (parent and metabolite)

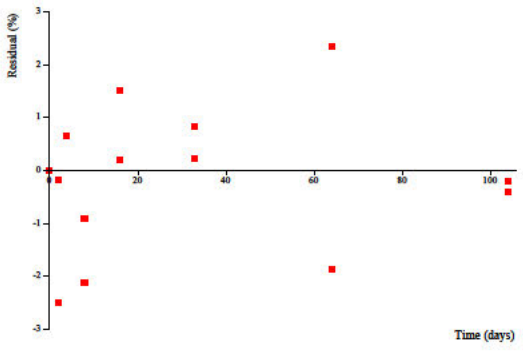
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting										
Glypho-sate: DFOP	Poor	90.6	k_1: 0.3946 k_2: 0.0322 g: 0.3697	12.0	k_1: <0.001 k_2: <0.001	k_1: 0.1762 k_2: 0.0202	k_1: 0.613 k_2: 0.044	8.1	57.2	-
AMPA: SFO	Good	-	k: 0.003	3.2	k: 0.002	k: 0.0011	k: 0.005	228	757	0.441 (±0.042)
Applicant's conclusion	For the parent, the visual fit is poor (M_0 is underestimated compared to parent-only DFOP fit). As the residue data of AMPA are well described, the fitting was repeated with initial parameters for parent (M_0, k_1, k_2 and g) fixed to results from parent-only DFOP fit.									
RMS conclusion	As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.									
Initial fitting: Glyphosate (DFOP)										





Initial fitting: AMPA (SFO)

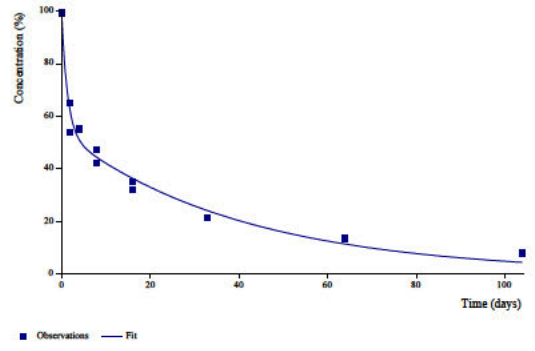


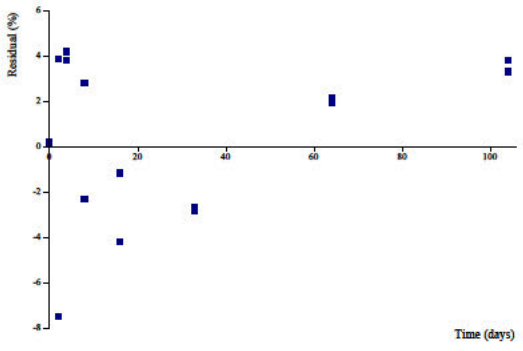


Repeated fitting: initial parameters for parent fixed to results from parent-only fit

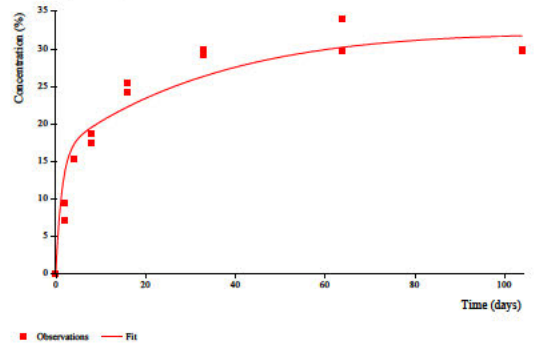
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 99.0	fixed to k_1: 0.7469 k_2: 0.0245 g: 0.4592	5.7	-[1]	-[1]	-[1]	4.5	68.9	-
AMPA: SFO	Poor	-	k: 0.0008	10.1	0.251	k: -0.0015	k: 0.003	906	>1000	0.357 (0.021)
Applicant's conclusion	For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak. Conclusion: Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate No trigger and modelling endpoints can be derived for AMPA									
RMS conclusion	As mentioned above, kinetic fittings have been redone by RMS since the applicant did not correct the initial concentration of glyphosate by the radiochemical purity. As a consequence, these fits from the applicant were not further considered by the RMS. Fits from RMS are presented below in blue box.									

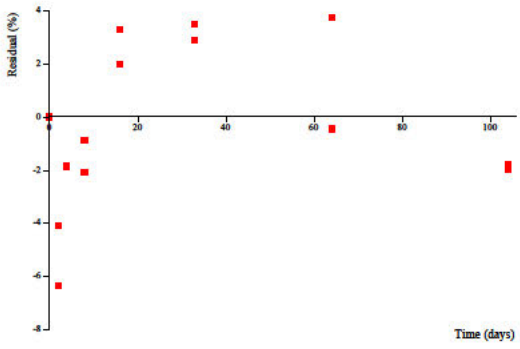
Glyphosate (DFOP) – fixed input parameters for parent from parent-only fit





AMPA (SFO)





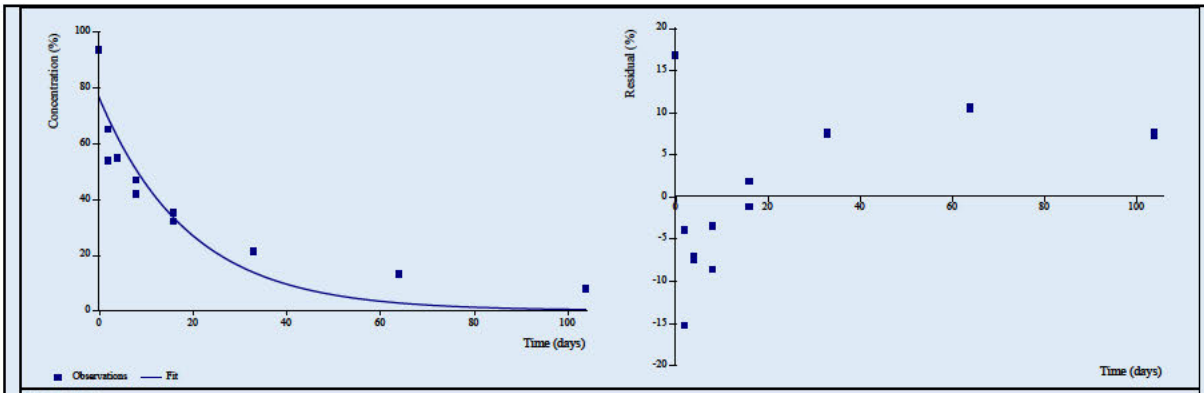
RMS additional fittings - Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992)

RMS performed additional fittings considering the same data as the applicant except for T0 for glyphosate, which was additionally corrected for radiochemical purity, as recommended in FOCUS guidance.

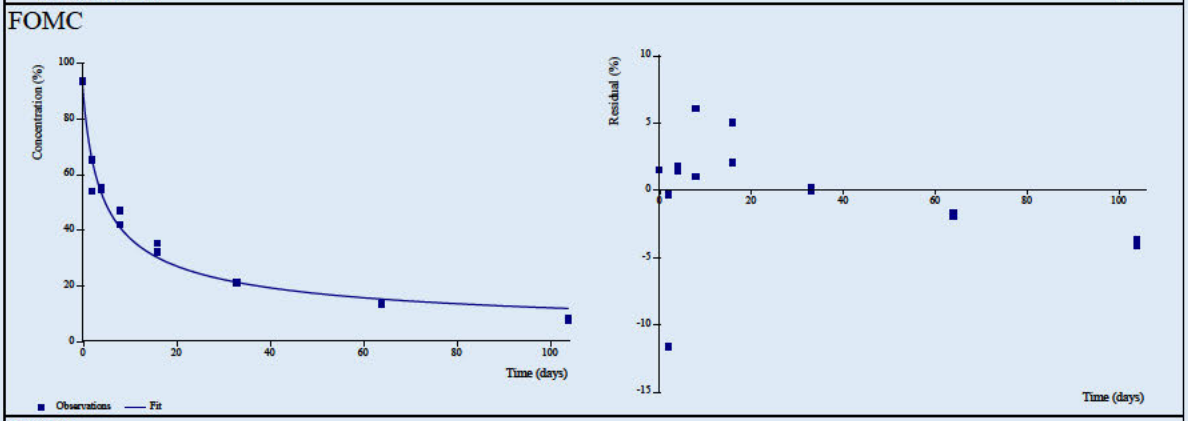
T0 for glyphosate considered by applicant (material balance)	T0 for glyphosate considered by RMS (material balance x Radioactive purity of 94.1%)
99.2	93.3
99.1	93.3

Parent-only fittings (Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992))

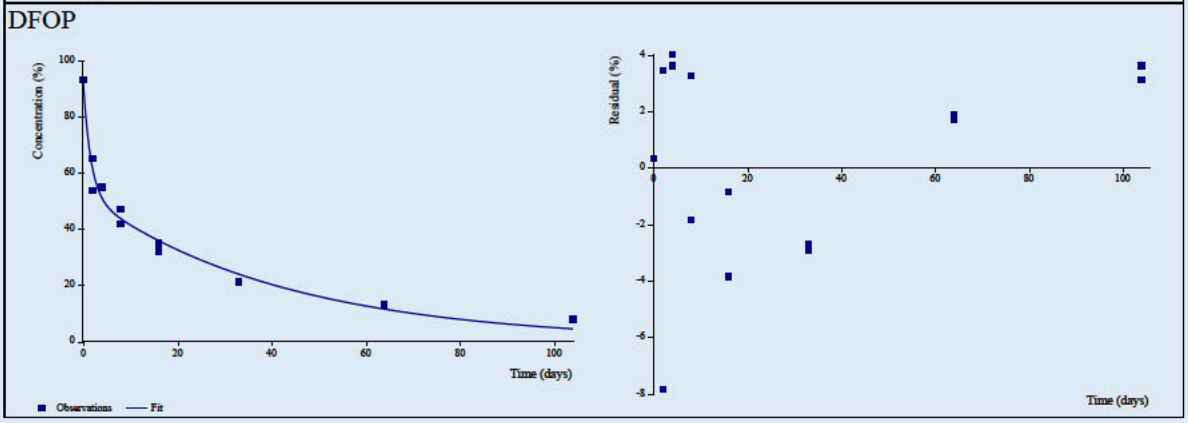
Kinetic model	Visual/ residual	M0	Kinetic para-meters	T test <0.05	CI contains 0	χ^2 (%)	DT_{50} (d)	DT_{90} (d)	R^2
SFO	Poor	76.56	k: 0.05224	Yes		18	13.3	44.1	0.8953
FOMC	Acceptable	91.81	α: 0.5353 β: 2.259		No No	6.76	5.99	165	0.978
DFOP	Acceptable	92.98	k1: 0.6289 k2: 0.02383 g: 0.4353	Yes Yes		5.48	5.97	72.6	0.9848
RMS conclusion	SFO does not well describe the overall degradation of glyphosate (M0 not well described, last points underestimated). SFO is not considered suitable for trigger nor for modelling endpoint. Biphasic kinetics provide better fits. Visual and statistical results are good for both FOMC and DFOP kinetics. Ttrigger endpoints, DFOP model is selected as it provides the best fit (lowest χ^2 value, lower extent of residuals compared to FOMC). Modelling endpoints: Glyphosate represented less than 10% AR at the end of the study. Therefore FOMC can be selected for modelling of parent-only. However, it is reminded that FOMC kinetic cannot be directly implemented in FOCUS models, and the use of DT_{90}/3.32 is not suitable when metabolites are included in the degradation pathway for modelling (see more justification in the introduction). In this case, DFOP kinetic is fully acceptable, and even provide a better χ^2 error and slightly better description of M0. The extent of residuals is lower with DFOP kinetics. As a consequence, RMS considers that DFOP should be selected for modelling endpoint in pathway fit. **Conclusion:** DFOP to be used in pathway fit for trigger endpoints FOMC acceptable for parent-only modelling DFOP to be used in pathway fit for modelling endpoints								
SFO									



FOMC

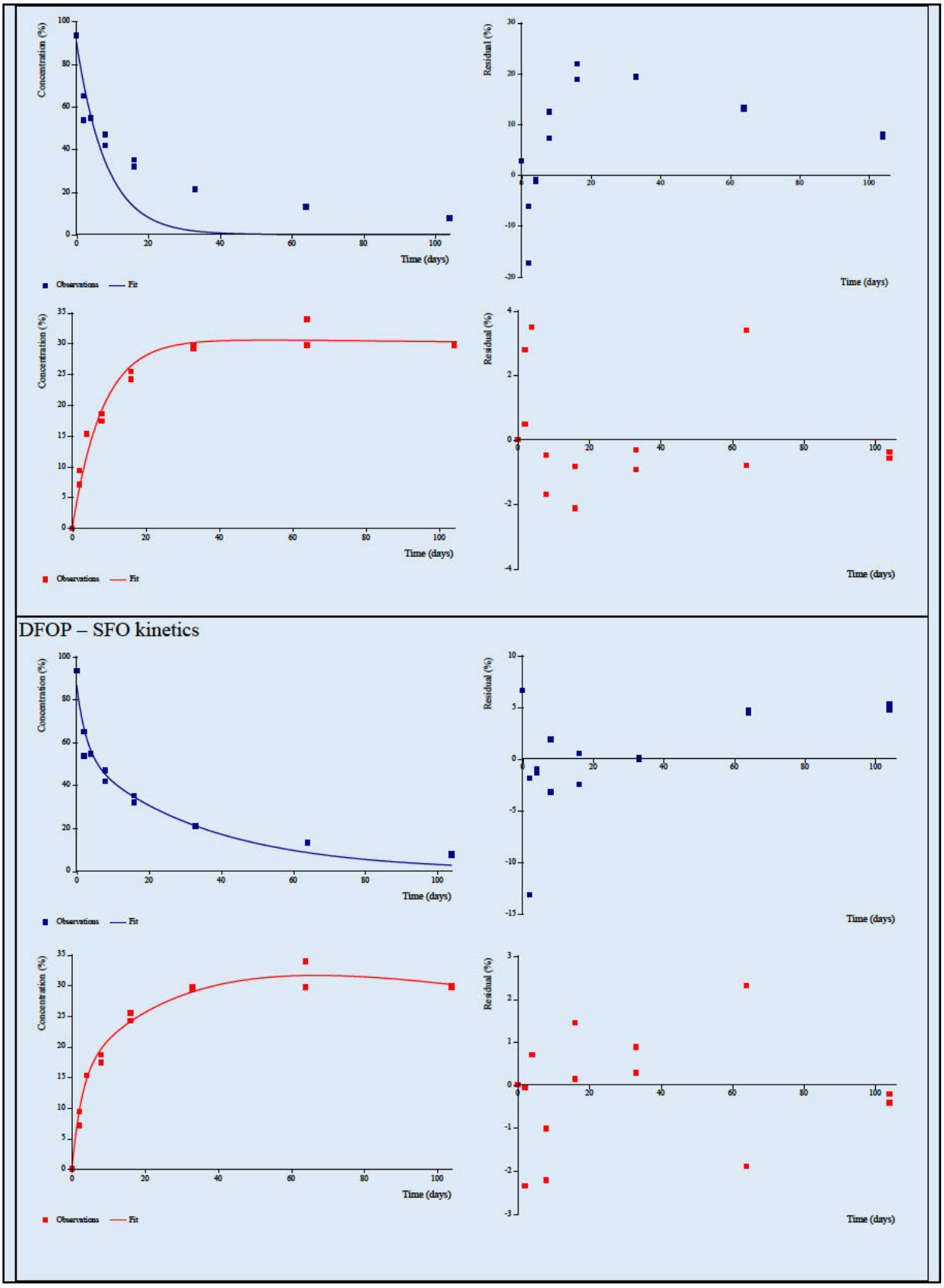


DFOP



Parent-metabolite fittings (Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992))

	Kinetic model	Visual/residual	Kinetic para-meters	χ^2 (%)	T test <0.05	DT_{50} (d)	DT_{90} (d)	ff	R^2
Glyphosate	SFO	Poor	k: 0.1207	24.5	Yes	5.75			0.9309
AMPA	SFO	Good	k: 0.000198	6.05	No (0.415)	3500	> 10 000	0.3408	0.9747
Glyphosate	DFOP	Acceptable	k1:0.3685 k2: 0.02889 g: 0.3702	9.72	Yes Yes	8.95	63.7		0.9688
AMPA	SFO	Good	k: 0.003459	3.18	Yes	200	666	0.4796	0.9871
RMS conclusion	With SFO-SFO, the visual fit for AMPA is good, but t-test is not met. Degradation of glyphosate is not well described. DFOP-SFO provides a better description of degradation of glyphosate, and the fit for AMPA is visually and statistically acceptable. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA								
SFO – SFO kinetics									

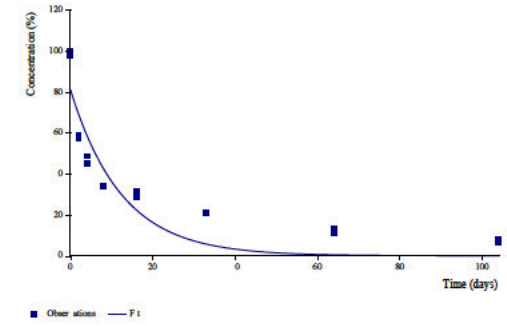


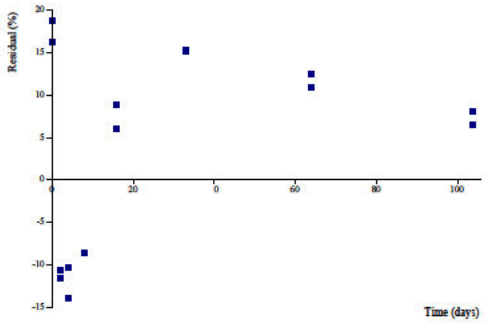
Speyer 2.1, group B

Table 8.1.1.2-26: Kinetic models and statistics for soil Speyer 2.1, dose group B (20° C, 20 % MWHC, 4 mg/kg), of study [redacted] (1992) - Parent-only fits

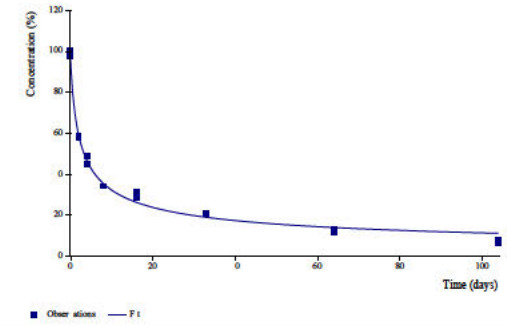
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	81.4	k: 0.0804	24.6	<0.001	k: 0.0362	k: 0.125	8.6	28.6
FOMC	Good	98.6	α: 0.4762 β: 1.0519	4.8	-[1]	β: 0.5975	β: 1.506	3.5	131
DFOP	Good	98.6	k_1: 0.5105 k_2: 0.0182 g: 0.5961	2.8	k_1: <0.001 k_2: <0.001	k_1: 0.4200 k_2: 0.0148	k_1: 0.601 k_2: 0.022	3.2	76.7
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (the residues at the last four sampling dates) and the lowest χ^2 error. Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints period								
RMS conclusion	Concentration at t0 for glyphosate should have been corrected for the radiochemical purity of 94.1%. In this case, fittings were not redone by the RMS since results for this soil incubated at 20° C, 20 % MWHC, 4 mg/kg will not be used directly in the risk assessment. Indeed, results from incubation on the same soil at more representative conditions (20° C, 40 % MWHC, 4 mg/kg) are available. RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.								

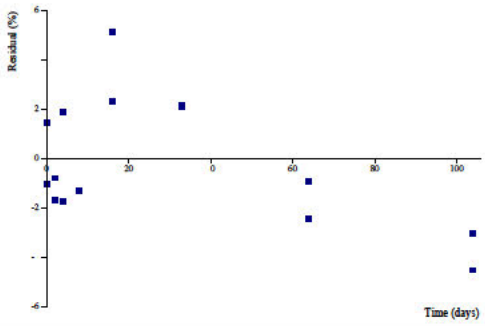
SFO



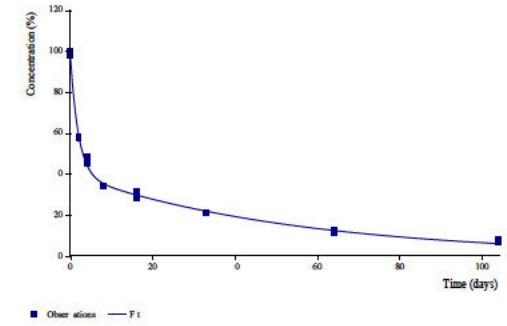


FOMC





DFOP



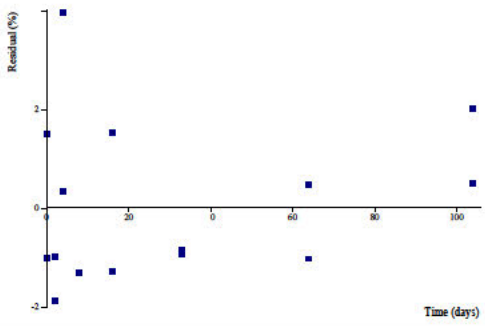
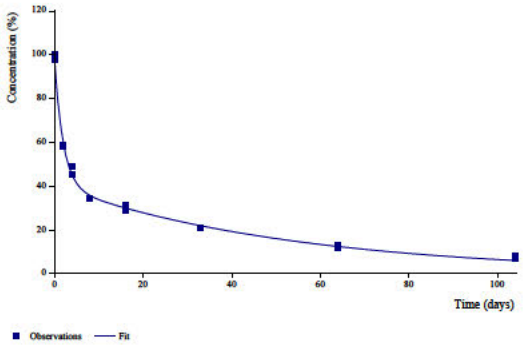
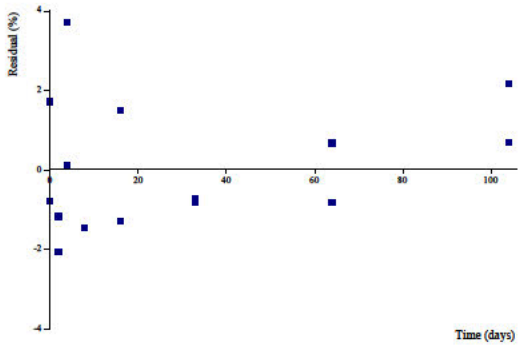


Table 8.1.1.2-27: Kinetic models and statistics for soil Speyer 2.1, dose group B (20 °C, 20 % MWHC, 4 mg/kg), of study [redacted] (1992) - Pathway fits (parent and metabolite)

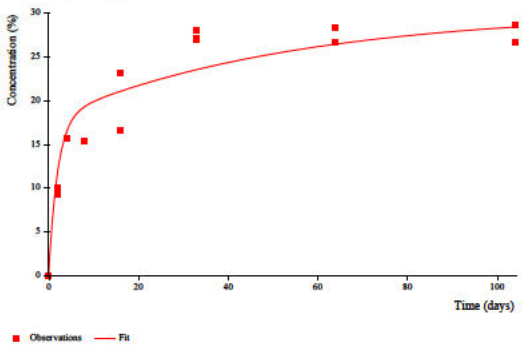
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	98.4	k_1: 0.5067 k_2: 0.0185 g: 0.5927	2.8	k_1: <0.001 k_2: <0.001	k_1: 0.4222 k_2: 0.0153	k_1: 0.591 k_2: 0.022	3.2	75.8	-
AMPA: SFO	Poor	-	k: <0.0001	9.3	k: 0.5	k: -0.0024	k: 0.002	>1000	>1000	0.306 (±0.020)
Applicant's conclusion	For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak. Conclusion: Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate No trigger and modelling endpoints can be derived for AMPA									
RMS conclusion	As explained above for the parent only fit, RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.									

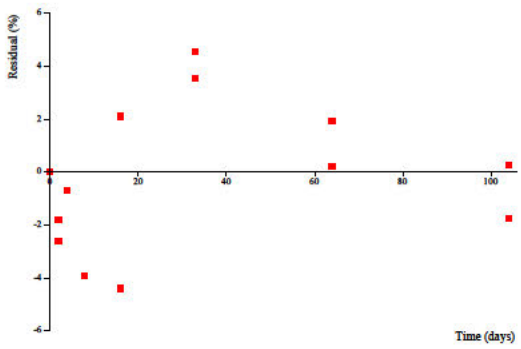
Glyphosate (DFOP)





AMPA (SFO)



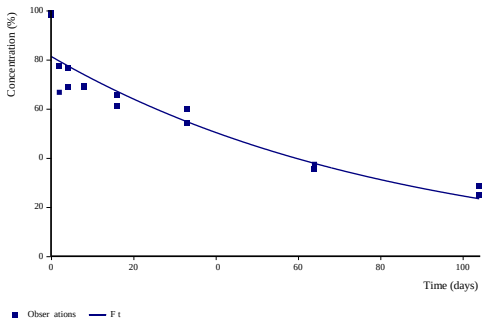


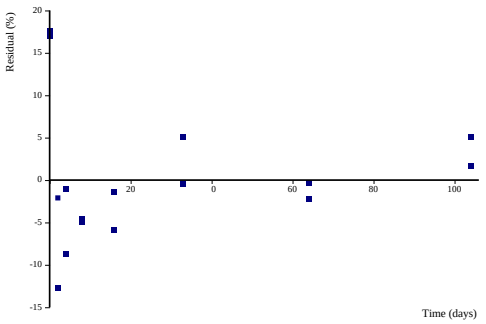
Speyer 2.1, group C

Table 8.1.1.2-28: Kinetic models and statistics for soil Speyer 2.1, dose group C (8° C, 40 MWHC, 4 mg/kg), of study [redacted] (1992) – Parent only fits

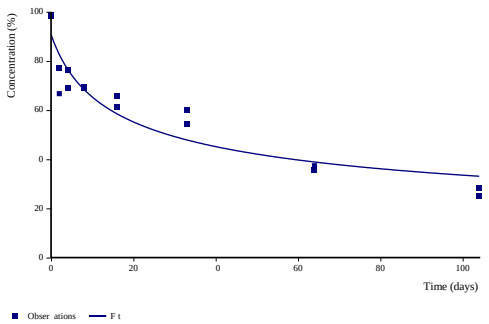
Kinetic model	Visual assessment	M_0	Parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	81.3	k: 0.0120	9.4	k: <0.001	k: 0.0083	k: 0.016	57.9	192
FOMC	Acceptable	90.8	α: 0.3595 β: 6.689	9.0	-[1]	β: -5.079	β: 18.46	39.3	>1000
DFOP	Good	98.6	k_1: 8.463 k_2: 0.0101 g: 0.2397	2.5	k_1: n.c.[2] k_2: <0.001	k_1: n.c.[2] k_2: 0.0085	k_1: n.c.[2] k_2: 0.012	41.6	201
HS	Good	98.6	k_1: 0.2841 k_2: 0.0101 t_b: 1.0[3]	2.3	k_1: <0.001 k_2: <0.001	k_1: 0.2149 k_2: 0.0085	k_1: 0.353 k_2: 0.012	41.6	201
Applicant's conclusion	Degradation of glyphosate was best described by bi-phasic models. As the FOMC and DFOP model did not provide statistically reliable parameters, the HS model has additionally been tested and provided the best fit with statistically reliable parameters. **Conclusion:** HS to be used in pathway fit for trigger endpoints HS to be used in pathway fit for modelling endpoints								
RMS conclusion	Concentration at t0 for glyphosate should have been corrected for the radiochemical purity of 94.1%. In this case, fittings were not redone by the RMS since results for this soil incubated at 8° C, 40 % MWHC, 4 mg/kg will not be used directly in the risk assessment. Indeed, results from incubation on the same soil at more representative conditions (20° C, 40 % MWHC, 4 mg/kg) are available. RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.								

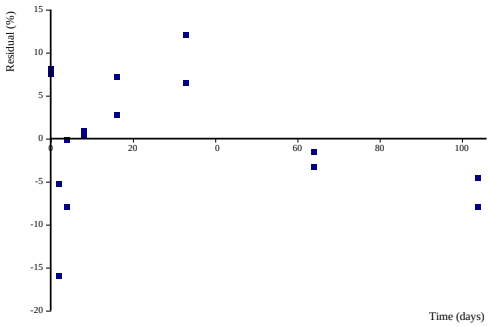
SFO



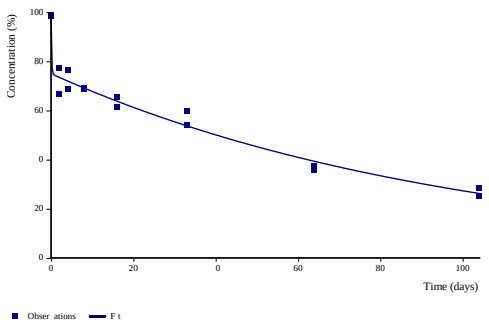


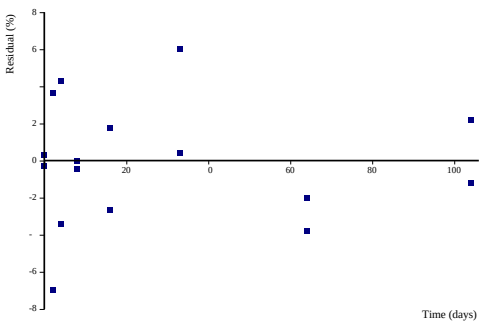
FOMC



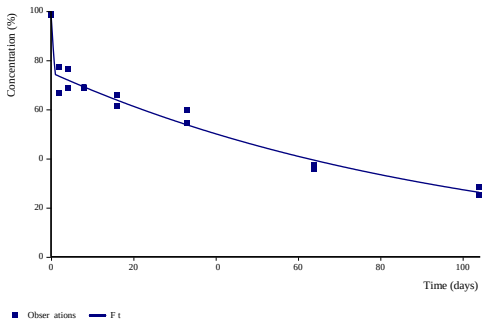


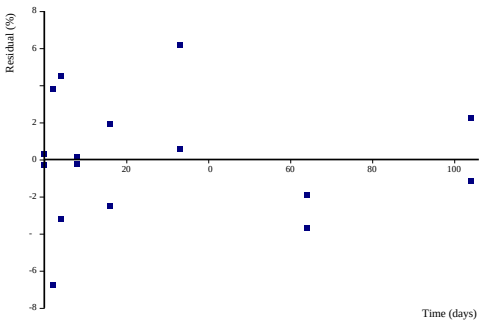
DFOP





HS





[1] t-test not relevant for kinetic parameter β

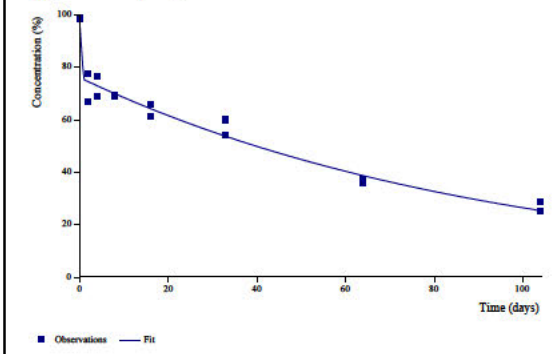
[2] Errors and t-test values could not be calculated because the covariance matrix could not be created

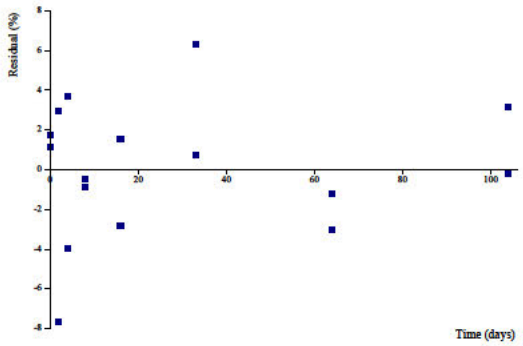
[3] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly

Table 8.1.1.2-29: Kinetic models and statistics for soil Speyer 2.1, dose group C (8 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992) - Pathway fits (parent and metabolite)

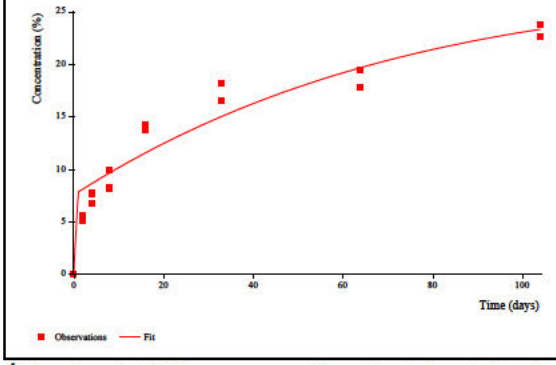
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: HS	Good	97.2	k_1: 0.2568 k_2: 0.0106 t_b: 1.0[1]	2.5	k_1: <0.001 k_2: <0.001	k_1: 0.1919 k_2: 0.0090	k_1: 0.322 k_2: 0.012	42.3	195	-
AMPA: SFO	Accep-table	-	k: 0.0013	10.4	k: 0.243	k: -0.0024	k: 0.0050	555	>1000	0.355 (±0.043)
Applicant's conclusion	The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable. Conclusion: Parent-only HS fit to be used for deriving trigger and modelling endpoints for glyphosate No trigger and modelling endpoints can be derived for AMPA									
RMS conclusion	As explained above for the parent only fit, RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.									

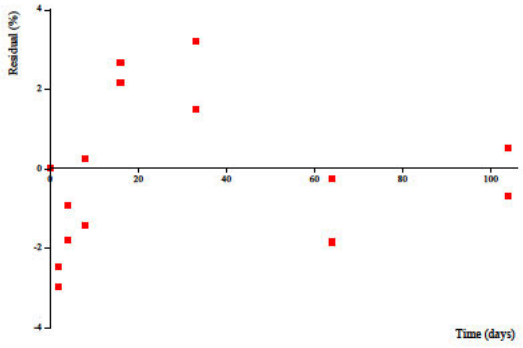
Glyphosate (HS)





AMPA (SFO)





[1] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly

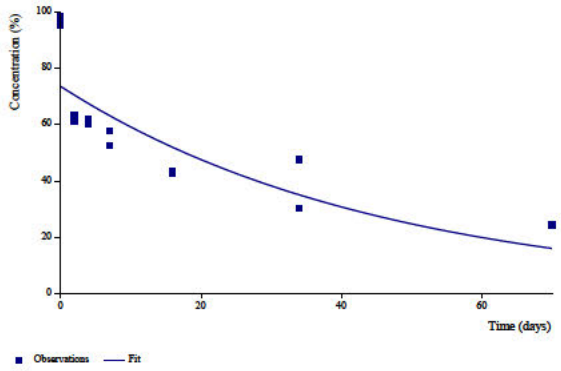
Speyer 2.1, group D

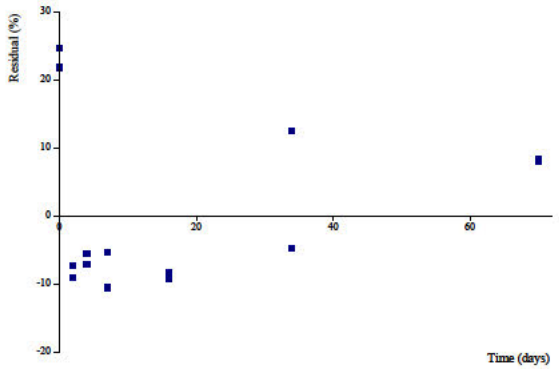
Table 8.1.1.2-30: Kinetic models and statistics for soil Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile), of study [redacted] (1992) - Parent-only fits

Kinetic model	Visual assessment	M_0	Parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	73.5	k: 0.0219	16.3	k: 0.001	k: 0.0097	k: 0.0340	31.7	105
FOMC	Good	96.2	α: 0.2475 β: 0.6142	5.6	-[1]	β: -0.1447	β: 1.3730	9.5	>1000

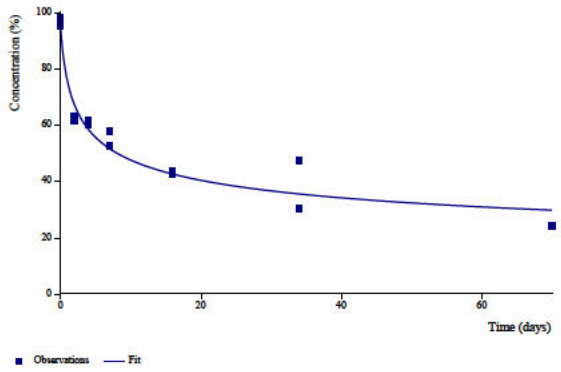
DFOP	Good	96.6	k_1: 1.029 k_2: 0.0137 g: 0.3794	4.4	k_1: 0.033 k_2: <0.001	k_1:-0.0857 k_2: 0.0086	k_1: 2.145 k_2: 0.019	15.8	134
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (the residues at the last two sampling dates) and the lowest χ^2 error. **Conclusion:** DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints								
RMS conclusion	Concentration at t0 for glyphosate should have been corrected for the radiochemical purity of 94.1%. In this case, fittings were not redone by the RMS since results for this sterile soil will not be used directly in the risk assessment. Indeed, results from incubation on the same non sterile soil are available. RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.								

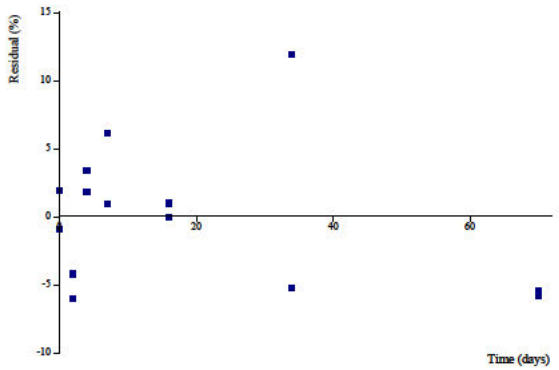
SFO



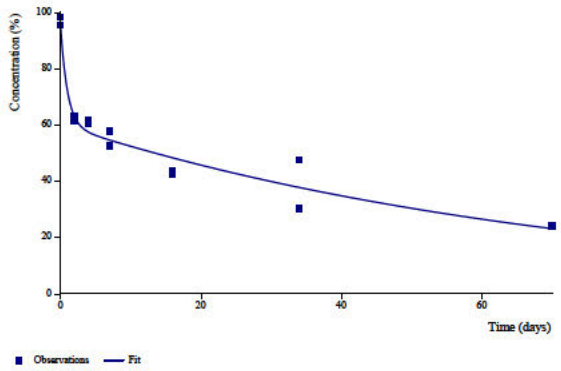


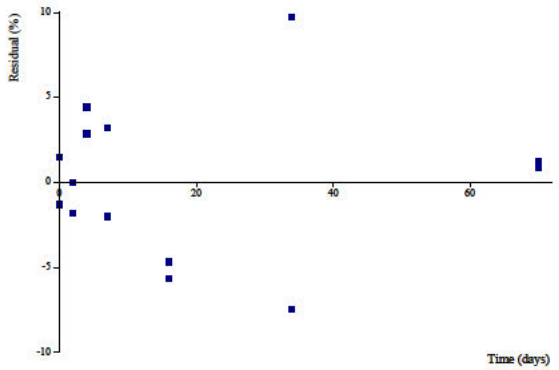
FOMC





DFOP





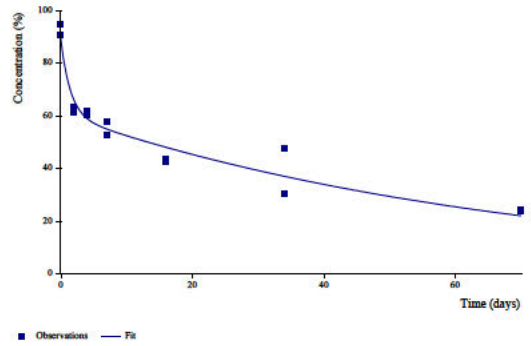
[1] t-test not relevant for kinetic parameter β

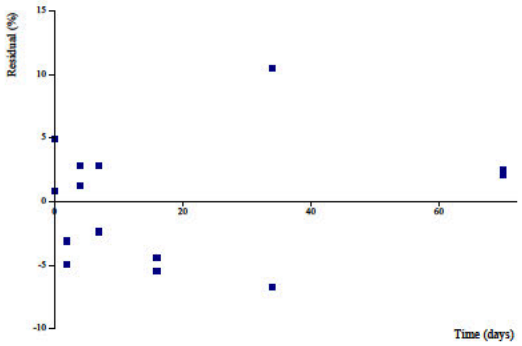
Table 8.1.1.2-31: Kinetic models and statistics for soil Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile), of study [redacted] (1992) - Pathway fits

Kinetic model		M_0				Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff

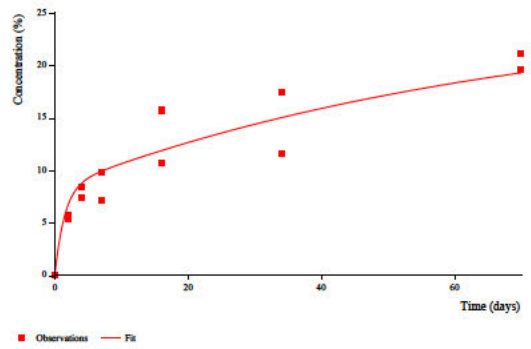
	Visual assess-ment		**Kinetic para-meters**	**χ^2 er-ror (%)**	**Prob > t (5 % level)**					**(± std. dev.)**
Glypho-sate: DFOP	Good	93.8	k_1: 0.7952 k_2: 0.015 g: 0.3468	5.4	k_1: 0.014 k_2: <0.001	k_1: 0.0957 k_2: 0.0096	k_1: 1.495 k_2: 0.020	17.8	125	-
AMPA: SFO	Accep-table	-	k:<0.0001	9.1	k: 0.5	k: -0.0066	k: 0.0070	>1000	>1000	0.261 (±0.039)
Applicant's conclusion	The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable. **Conclusion:** Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate No trigger and modelling endpoints can be derived for AMPA									
RMS conclusion	As explained above for the parent only fit, RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.									

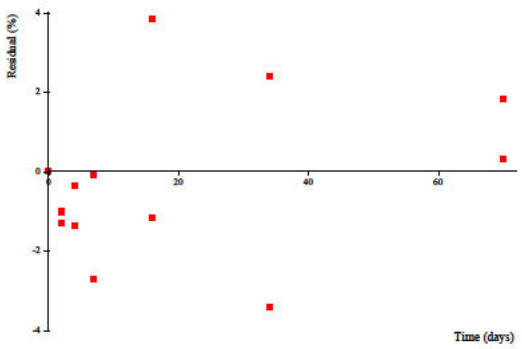
Glyphosate (DFOP)





AMPA (SFO)





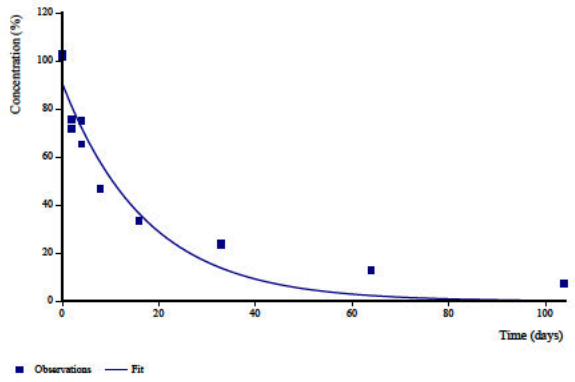
Speyer 2.1, group E

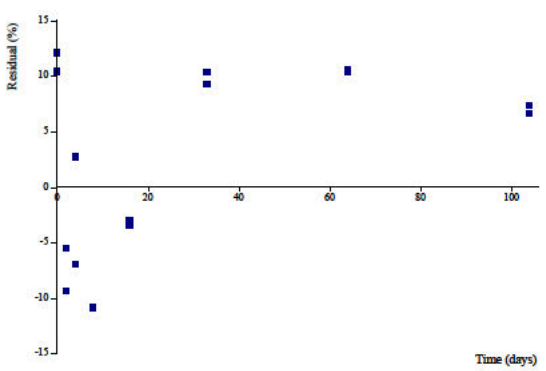
Table 8.1.1.2-32: Kinetic models and statistics for soil Speyer 2.1 (20° C, 40 MWHC, 0.4 mg/kg), dose group E, [redacted] (1992) - Parent-only fits

Kinetic model	**Visual assessment**	**M_0**	**Kinetic parameters**	**χ^2 error (%)**	**Prob > t (5 % level)**	**Lower CI (95 %)**	**Upper CI (95 %)**	**DT_{50} (d)**	**DT_{90} (d)**
SFO	Poor	91.0	k: 0.0573	14.5	k: <0.001	k: 0.0384	k: 0.076	12.1	40.2
FOMC	Good	101.0	α: 0.8032 β: 5.523	5.4	-[1]	β: 2.278	β: 8.767	7.6	91.6
DFOP	Good	100.5	k_1: 0.2004 k_2: 0.0177 g: 0.5861	5.7	k_1: <0.001 k_2: <0.001	k_1: 0.109 k_2: 0.0084	k_1: 0.292 k_2: 0.027	7.3	80.3
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide equally reliable and visually acceptable results. The DFOP model provides slightly lower residuals for the data points from day 8 onwards.								

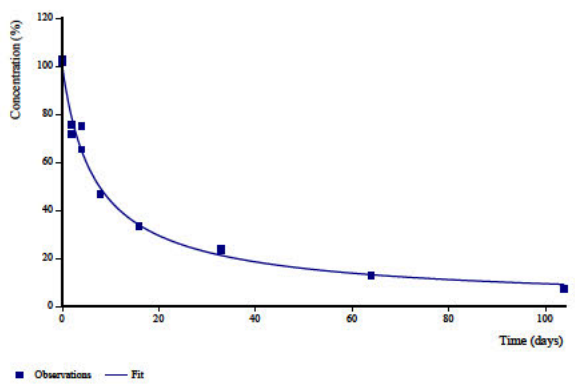
	Conclusion: DFOP to be used in pathway fits for trigger endpoints DFOP to be used in pathway fits for modelling endpoints
RMS conclusion	Concentration at t0 for glyphosate should have been corrected for the radiochemical purity of 94.1%. In this case, fittings were not redone by the RMS since results for this soil incubated at 20° C, 40 % MWHC, 0.4 mg/kg will not be used directly in the risk assessment. Indeed, results from incubation on the same soil at more representative conditions (20° C, 40 % MWHC, 4 mg/kg) are available. RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.

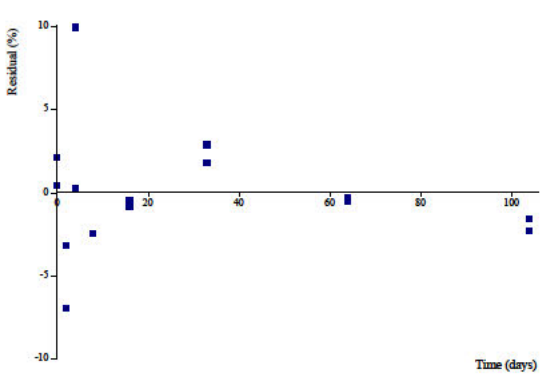
SFO



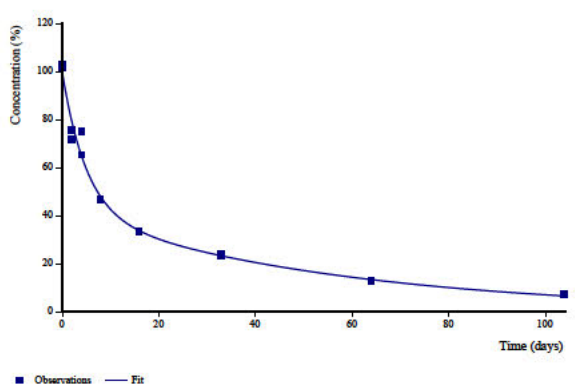


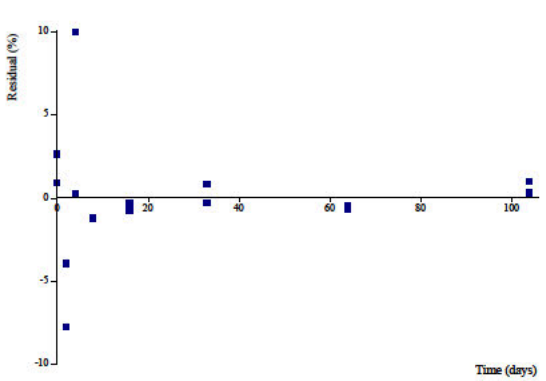
FOMC





DFOP





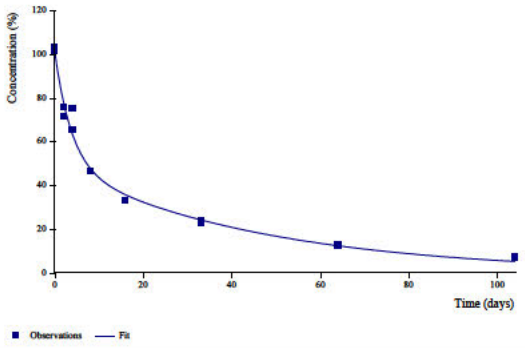
[1] t-test not relevant for kinetic parameter β

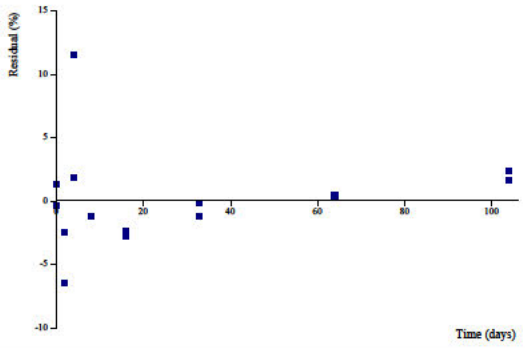
Table 8.1.1.2-33: Kinetic models and statistics for soil Speyer 2.1, dose group E (20 °C, 40 % MWHC, 0.4 mg/kg), of study [redacted] (1992) - Pathway fits (parent and metabolite)

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)

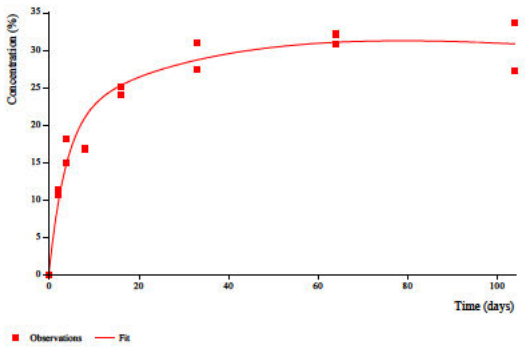
Glypho-sate: DFOP	Good	101.8	k_1: 0.2635 k_2: 0.0216 g: 0.5149	6.2	k_1: <0.001 k_2: <0.001	k_1: 0.16 k_2: 0.0131	k_1: 0.367 k_2: 0.03	7.0	73.2	-
AMPA: SFO	Good	-	k: 0.0024	6.4	k: 0.019	k: 0.0002	k: 0.005	283	940	0.393 (±0.030)
Applicant's conclusion	Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. **Conclusion:** DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									
RMS conclusion	As explained above for the parent only fit, RMS considers that no persistence and modelling endpoints need to be determined in this case. The evaluation from the applicant is not further reviewed.									

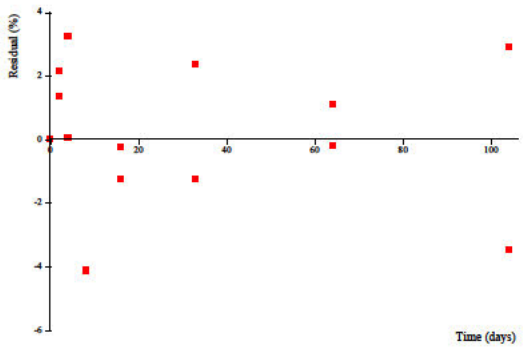
Glyphosate (DFOP)





AMPA (SFO)





Normalization data

Table 8.1.1.2-34: Temperature and moisture correction factors for normalisation of modelling endpoints (grey rows correspond to soils that were discarded by RMS)

Study	Soil type	Temperature		Moisture					Overall correction factor ($f_{overall}$)
		During study[1] (°C)	Correction factor (f_{temp}) (-)	During study[1] (% of MWHC)	Gravimetric water content at MWHC[1] (g / 100 g)	Gravimetric water content during study (θ_{act})[2] (g / 100 g)	Gravimetric water content at pF2 (θ_{ref})[3] (g / 100 g)	Correction factor (f_{moist}) (-)	(-)
[redacted] (2010, CA 7.1.1.1/001): Gartenacker	Loam	20	1.00	50[4]	21.4[5]	10.7	25	0.55	0.55
[redacted] (2010, CA 7.1.2.1.1/002) : Drusenheim	Loam	20	1.00	50[4]	17.6[5]	8.8	25	0.48	0.48
[redacted] (2010, CA 7.1.2.1.1/002) : Pappelacker	Loamy sand	20	1.00	50[4]	12.4[5]	6.2	14	0.57	0.57

Table 8.1.1.2-34: Temperature and moisture correction factors for normalisation of modelling endpoints (grey rows correspond to soils that were discarded by RMS)

Study	Soil type	Temperature		Moisture					Overall correction factor ($f_{overall}$) (-)
		During study[1] (°C)	Correction factor (f_{temp}) (-)	During study[1] (% of MWHC)	Gravimetric water content at MWHC[1] (g / 100 g)	Gravimetric water content during study (θ_{act})[2] (g / 100 g)	Gravimetric water content at pF2 (θ_{ref})[3] (g / 100 g)	Correction factor (f_{moist}) (-)	
[redacted] (2010, CA 7.1.2.1.1/002): 18-Acres	Sandy clay loam	20	1.00	50[4]	19.7[5]	9.9	22	0.57	0.57
[redacted] (1996, CA 7.1.1.1/003): Soil B	Sandy loam	25	1.61	75[4]	14.2[5]	10.7	19	0.67	1.07
[redacted] (1995, CA 7.1.1.1/005): Arrow	Sandy loam	20	1.00	40	27[3]	10.8	19	0.67	0.67
[redacted] (1993, CA 7.1.1.1/006): Les Evouettes	Silt loam	20	1.00	40	22.1	8.8	26	0.47	0.47
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.2	Sand	20	1.00	40	17.7	7.1	12	0.69	0.69
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.3	Loamy sand	20	1.00	40	14	5.6	14	0.53	0.53
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group A	Sand	20	1.00	40	32.95	13.2	12	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.1	Sand	20	1.00	45	31	14.0	12	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.2	Loamy sand	20	1.00	45	48	21.6	14	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	20	1.00	45	39	17.6	14	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	10	0.39	45	39	17.6	14	1.00	0.39
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.1	Sand	20	1.00	40	12.8	5.1	12	0.55	0.55
[redacted] (1992, CA 7.1.2.1.1/005)	Sand	20	1.00	20	32.95	6.6	12	0.66	0.66

Table 8.1.1.2-34: Temperature and moisture correction factors for normalisation of modelling endpoints (grey rows correspond to soils that were discarded by RMS)

Study	Soil type	Temperature		Moisture					Overall correction factor ($f_{overall}$) (-)
		During study[1] (°C)	Correction factor (f_{temp}) (-)	During study[1] (% of MWHC)	Gravimetric water content at MWHC[1] (g / 100 g)	Gravimetric water content during study (θ_{act})[2] (g / 100 g)	Gravimetric water content at pF2 (θ_{ref})[3] (g / 100 g)	Correction factor (f_{moist}) (-)	
: Speyer 2.1, dose group B									
[redacted] (1992, CA 7.1.2.1.1/005) : Speyer 2.1, dose group C	Sand	8	0.32	40	32.95	13.2	12	1.00	0.32
[redacted] (1992, CA 7.1.2.1.1/005) : Speyer 2.1, dose group D (sterile)	Sand	20	1.00	40	31.31	12.5	12	1.00	1.00
[redacted] (1992, CA 7.1.2.1.1/005) : Speyer 2.1, dose group E (lower rate)	Sand	20	1.00	40	32.95	13.2	12	1.00	1.00
[redacted] (1992, CA 7.1.2.1.1/005) : Beedon manor, dose group F	Clay loam	20	1.00	40	57.94	23.2	28	0.88	0.88

[1] Measured values taken from study reports
[2] Calculated: moisture during study (% MWHC) / 100 × gravimetric water content at MWHC
[3] FOCUS default value
[4] Percent of gravimetric water content at 1/3 bar
[5] Gravimetric water content at 1/3 bar, reported values

Overview of trigger and modelling endpoints

Table 8.1.1.2-35: Laboratory trigger and modelling endpoints of glyphosate (grey rows correspond to soils that were discarded by RMS)

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (2010, CA 7.1.1.1/001): Gartenacker	Loam	7.1	20 / 50	8.1 / 55.4	3.1	DFOP	9.2[5]	3.1	DFOP
[redacted] (2010, CA 7.1.2.1.1/002): Drusenheim	Loam	7.4	20 / 50	2.2 / 14.4	5.0	FOMC	2.1[5]	5.0	FOMC
[redacted] (2010, CA 7.1.2.1.1/002): Pappelacker	Loamy sand	7.0	20 / 50	3.6 / 37.6	5.5	DFOP	6.4[5]	5.5	DFOP
[redacted] (2010, CA 7.1.2.1.1/002): 18-Acres	Sandy clay loam	5.7	20 / 50	60.2 / >1000	2.0	FOMC	98.7[6]	2.9	DFOP

Table 8.1.1.2-35: Laboratory trigger and modelling endpoints of glyphosate (grey rows correspond to soils that were discarded by RMS)

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1996, CA 7.1.1.1/003): Soil B	Sandy loam	6.7	25 / 75[4]	1.1 / 21.3	7.0	FOMC	6.9[5]	7.0	FOMC
[redacted] (1995, CA 7.1.1.1/005): Arrow	Sandy loam	5.9[3]	20 / 40	37.8 / >1000	2.3	FOMC	126.2[6]	3.6	DFOP
[redacted] (1993, CA 7.1.1.1/006): Les Evouettes	Silt loam	6.1[2]	20 / 40	9.7 / 184	6.5	DFOP	26.0[5]	6.5	DFOP
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.2	Sand	6.0[2]	20 / 40	6.3 / 157	7.8	HS	47.0[6]	7.8	HS
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.3	Loamy sand	6.9[2]	20 / 40	5.8 / 22.2	2.5	DFOP	3.5[5]	2.5	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group A	Sand	6.9	20 / 40	4.5 / 68.9	5.6	DFOP	20.8[5]	5.6	DFOP
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.1	Sand	6.1[2]	20 / 40	10.8 / 84.0	3.3	DFOP	13.9[5]	3.3	DFOP
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.1	Sand	5.9[3]	20 / 45	8.3 / 51.3	2.5	DFOP	15.5[5]	2.5	DFOP
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.2	Loamy sand	5.6[3]	20 / 45	18.1 / 162	5.9	DFOP	64.2[6]	5.9	DFOP
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	20 / 45	2.7 / 13.0	7.5	DFOP	2.8	8.9	SFO
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	10 / 45	7.9 / 50.9	2.4	DFOP	5.9[5]	2.4	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group B	Sand	6.9	20 / 20	3.2 / 76.7	2.8	DFOP	15.2[5]	2.8	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group C	Sand	6.9	8 / 40	41.6 / 201	2.3	HS	22.0[6]	2.3	HS

Table 8.1.1.2-35: Laboratory trigger and modelling endpoints of glyphosate (grey rows correspond to soils that were discarded by RMS)

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group D (sterile)	Sand	6.3	20 / 40	15.8 / 134	4.4	DFOP	50.6[6]	4.4	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group E	Sand	6.9	20 / 40	7.0 / 73.2	6.2	DFOP	22.0[5]	6.2	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Beedon manor, dose group F	Clay loam	7.8	20 / 40	0.6 / 9.7	2.6	DFOP	2.6[5]	2.6	DFOP

[1] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[2] Buffer solution unknown
[3] Measured in $CaCl_2$ solution
[4] % moisture at 1/3 bar
[5] Calculated as DT_{90}/3.32 as 10 % of initially measured concentration reached within experimental period
[6] Calculated as ln(2)/k_2 as 10 % of initially measured concentration not reached within experimental period

Table 8.1.1.2-36: Laboratory trigger and modelling endpoints of AMPA (grey rows correspond to soils that were discarded by RMS)

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff (from parent)	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (2010, CA 7.1.1.1/001): Gartenacker	Loam	7.1	20 / 50	119 / 396	8.2	DFOP-SFO	0.183	65.7	8.2	DFOP-SFO
[redacted] (2010, CA 7.1.2.1.1/002): Drusenheim	Loam	7.4	20 / 50	29.4 / 97.7	3.8	FOMC-SFO	0.285	14.2	3.8	FOMC-SFO
[redacted] (2010, CA 7.1.2.1.1/002): Pappelacker	Loamy sand	7.0	20 / 50	90.9 / 302	6.2	DFOP-SFO	0.192	51.4	6.2	DFOP-SFO
[redacted] (2010, CA 7.1.2.1.1/002): 18-Acres	Sandy clay loam	5.7	20 / 50	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1996, CA 7.1.1.1/003): Soil B	Sandy loam	6.7	25 / 75[4]	99.4 / 330	8.9	FOMC-SFO	0.264	106	8.9	FOMC-SFO
[redacted] (1995, CA 7.1.1.1/005): Arrow	Sandy loam	5.9[3]	20 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1993, CA 7.1.1.1/006): Les Evouettes	Silt loam	6.1[2]	20 / 40	424 / >1000	15.4	DFOP-SFO	0.346	199	15.4	DFOP-SFO
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.2	Sand	6.0[2]	20 / 40	110 / 365	8.9	HS-SFO	0.683	76.0	8.9	HS-SFO

Table 8.1.1.2-36: Laboratory trigger and modelling endpoints of AMPA (grey rows correspond to soils that were discarded by RMS)

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff (from parent)	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.3	Loamy sand	6.9[2]	20 / 40	85.0 / 282	8.8	DFOP-SFO	0.336	44.8	8.8	DFOP-SFO
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group A	Sand	6.9	20 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.1	Sand	5.9[3]	20 / 45	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.2	Loamy sand	5.6[3]	20 / 45	497[6] / >1000[6]	8.8	DFOP-SFO	0.548[6]	497[6]	8.8	DFOP-SFO
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	20 / 45	41.4[6] / 137[6]	15.8	DFOP-SFO	0.424	43.1	18.2	SFO-SFO
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	10 / 45	129 / 429	8.2	DFOP-SFO	0.454	50.0	8.2	DFOP-SFO
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group B	Sand	6.9	20 / 20	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group C	Sand	6.9	8 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group D (sterile)	Sand	6.3	20 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group E	Sand	6.9	20 / 40	283 / 940	6.4	DFOP-SFO	0.393	283	6.4	DFOP-SFO
[redacted] (1992, CA 7.1.2.1.1/005): Beedon manor, dose group F	Clay loam	7.8	20 / 40	67.3 / 224	16.4	DFOP-SFO	0.149	59.0	16.4	DFOP-SFO
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.1	Sand	6.1[2]	20 / 40	86.5 / 288	13.7	DFOP-SFO	0.687	47.7	13.7	DFOP-SFO

Table 8.1.1.2-36: Laboratory trigger and modelling endpoints of AMPA (grey rows correspond to soils that were discarded by RMS)

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff (from parent)	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.

[1] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[2] Buffer solution unknown
[3] Measured in $CaCl_2$ solution
[4] % moisture at 1/3 bar
[5] No reliable endpoints were derived as no real decline phase was observed
[6] Endpoint derived from modified pathway fit with fixed parameters

Assessment and conclusion by applicant:

The kinetic evaluation was conducted according to current guidance. Therefore, the study and the endpoints derived are considered valid.

Assessment and conclusion by RMS:

Processing of the data:

As already indicated in the study summary, the initial concentration at t0 was not corrected for radiochemical purity as recommended in FOCUS guidance. Additional fits were performed by the RMS.

Kinetic evaluation

For easier reading, the RMS opinion on the kinetic model to be selected for each soil is reported in the study summary.

For the following soil, RMS considers that there is no need to derive modelling endpoints:

- Soil Speyer 2.1 from [redacted],1992, dose group B, C, D, E, performed with alternative conditions, but for which the dose A is already available with recommended 20°C, 40% MWHC and relevant dose.

Normalisation

The correction factors used by the study author are correct, except for the following cases:

- For Pappelacker ([redacted] 2010b): default FOCUS water content at pF2 for loamy sand (14 g/100 g) instead of sandy loam (19 g/100 g) was used;
- For Les Evouettes ([redacted] 1993), study was conducted at 40% MWHC, equivalent to 55% field capacity. RMS considers that correction factor for moisture should be calculated directly from $0.55^{0.7}$
- For Arrow ([redacted] 1995), the study author used a FOCUS default value for MWHC, whereas measured value is available from the study report.

Table 8.1.1.2-37: Temperature and moisture correction factors for normalisation of modelling endpoints

Study	Soil type	Temperature		Moisture (g / 100 g)				
		T° C	Correction factor (f_{temp})	During study	Gravimetric water content at MWHC or pF 2.5[1]	Gravimetric water content during study (θ_{act})[2]	Gravimetric water content at pF2 (θ_{ref})[3]	Correction factor (f_{moist})
[redacted] (2010a) Gartenacker	Loam	20	1.00	50% pF2.5	21.4[5]	10.7	25	0.55
[redacted] (2010b) Drusenheim	Loam	20	1.00	50% pF2.5	17.6[5]	8.8	25	0.48
[redacted] (2010b) Pappelacker	Sandy loam	20	1.00	50% pF2.5	12.4[5]	6.2	19	0.46
[redacted] (2010b) 18-Acres	Sandy clay loam	20	1.00	50% pF2.5	19.7[5]	9.9	22	0.57

[redacted] (1996) Soil B	Sandy loam	25	1.61	75% pF2.5	14.2[5]	10.7	19	0.67
[redacted] (1995) Arrow	Sandy loam	20	1.00	40% MWHC	37.95[4]	15.2	19	0.86
[redacted] (1993): Les Evouettes	Silt loam	20	1.00	55%FC	not needed	55%FC	26	0.66
[redacted] (1993) Speyer 2.2	Sand	20	1.00	40% MWHC	17.7[4]	7.1	12	0.69
[redacted] (1993) Speyer 2.3	Loamy sand	20	1.00	40% MWHC	14[4]	5.6	14	0.53
[redacted] (1992) Speyer 2.1, dose group A	Sand	20	1.00	40% MWHC	32.95[4]	13.2	12	1.00

[1] Measured values taken from study reports
[2] Calculated: moisture during study (% MWHC or pF2.5) / 100 × gravimetric water content at MWHC or pF 2.5
[3] FOCUS default value
[4] Gravimetric water content at MWHC, measured values
[5] Gravimetric water content at 1/3 bar, measured values

Summary on the trigger and modelling endpoints of glyphosate considered reliable by RMS

Laboratory trigger endpoints for glyphosate

Parent	Dark aerobic conditions – Persistence endpoints					
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50} /DT_{90} (d)	Kinetic parameters	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	8.8/57.3	k_1: 0.2138 k_2: 0.03023 g: 0.4345	2.9	DFOP
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	2.3 / 14.9	α: 1.414 β: 3.635	4.2	FOMC
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	3.9 / 38.7	k_1: 0.3125 k_2: 0.03172 g: 0.6584	5.0	DFOP
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	78.9 / 588	k_1: 0.05856 k_2: 0.003146 g: 0.3644	3.4	DFOP
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	0.7 / 16.2	k_1: 2.306 k_2: 0.08875 g: 0.58	8.2	DFOP
[redacted] (1995): Arrow Sandy loam	6.4[a]	20 / 40	37.8 / 1660	α: 0.4539 β: 10.47	2.3	FOMC
[redacted] (1993): Les Evouettes Silt loam	6.1[b]	20 / 40	11.5 / 358	α: 0.51 β: 3.96	5.9	FOMC
[redacted] (1993): Speyer 2.2 Sand	6.0[b]	20 / 40	2.0 / 151	k_1: 8.104 k_2: 0.01078 g: 0.4893	8.6	DFOP
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[b]	20 / 40	6.2 / 20.4	k: 0.1127	8.0	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	9.0/ 63.7	k1:0.3685 k2: 0.02889 g: 0.3702	9.7	DFOP

[a] Calculated with equation reported in EFSA guidance 2017[4]: $pH_{H2O}=0.982pH_{CaCl2} + 0.648$.
[b] Medium not reported, H_2O assumed

For modelling endpoints of glyphosate, the choice of kinetic model is sometimes different when considering the parent-only degradation and the parent-metabolite pathway (as explained in introduction of point B.8.1.1.2.1). A separate modelling endpoint table for parent alone is therefore also presented below, that could be used for parent-only modelling if needed.

Laboratory modelling endpoints for glyphosate based on parent-only fit

Parent	Dark aerobic conditions – Modelling endpoints						
Soil	pH (H_2O)	t. °C / % MWHC	Actual DT_{50} /DT_{90} (d)	Modelling DT_{50} (not normalized)[a]	DT_{50} (d) 20 °C pF2/10kPa[b]	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	9.0/60	18.1	9.9	4.0	FOMC
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	2.3/15	4.5	2.2	4.2	FOMC
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	4.0/37	11.1	5.1	4.5	FOMC
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	76.3/523	192.6	109.8	2.6	DFOP
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	1.0/20.1	6.1	6.5	8.6	FOMC
[redacted] (1995): Arrow Sandy loam	6.4[c]	20 / 40	37.4/440	187.3	161.1	3.6	DFOP
[redacted] (1993): Les Evouettes Silt loam	6.1[d]	20 / 40	11.5/358	107.8	71.2	5.9	FOMC
[redacted] (1993): Speyer 2.2 Sand	6.0[d]	20 / 40	2.0/151	64.3	44.4	8.6	DFOP
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[d]	20 / 40	6.1/20.3	6.1	3.2	8.0	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	6.0/165	49.7	49.7	6.8	FOMC

[a] DT_{90}/3.32 for FOMC kinetics; ln(2)/k2 value for DFOP kinetics
[b] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[c] Calculated with equation reported in EFSA guidance 2017[4]: $pH_{H2O}=0.982pH_{CaCl2} + 0.648$.
[d] Medium not reported, H_2O assumed

Laboratory modelling endpoints for glyphosate based on pathway fit (Glyphosate → AMPA)

Parent	Dark aerobic conditions – Modelling endpoints

4 EFSA (European Food Safety Authority), 2017. EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2017;15(10):4982, 115 pp. https://doi.org/10.2903/j.efsa.2017.4982

Soil	pH (H_2O)	t. °C / % MWHC	DT_{50} /DT_{90} (d)	Kinetic parameters	Fast | Slow DT_{50} (d) 20 °C pF2/10kPa[a]	DT $_{90}$[d] (d) 20 °C pF2/10kPa[a]	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	8.8 / 57.3	k_1: 0.2138 k_2: 0.03023 g: 0.4345	1.8 | 12.6	31.5	2.9	DFOP
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	2.3 / 13.4	k_1: 0.9889 k_2: 0.1375 g: 0.3704	0.3 | 2.4	6.4	4.8	DFOP
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	3.9 / 38.7	k_1: 0.3125 k_2: 0.03172 g: 0.6584	1.0 | 10.1	17.8	5.0	DFOP
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	78.6 / 588	k_1: 0.05856 k_2: 0.003146 g: 0.3644	6.7 | 125.6	335.2	3.4	DFOP
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	0.7 / 16.2	k_1: 2.306 k_2: 0.08875 g: 0.58	0.3 | 8.4	17.5	8.2	DFOP
[redacted] (1995): Arrow Sandy loam	6.4[b]	20 / 40	37.4 / 440	k_1: 0.0595 k_2: 0.0037 g: 0.4852	10.0 | 161.1	378.4	4.7	DFOP
[redacted] (1993): Les Evouettes Silt loam	6.1[c]	20 / 40	9.8 / 192	k_1: 0.2084 k_2: 0.008013 g: 0.5339	2.2 | 57.1	126.7	6.3	DFOP
[redacted] (1993): Speyer 2.2 Sand	6.0[c]	20 / 40	2.0 / 151	k_1: 8.104 k_2: 0.01078 g: 0.4893	0.1 | 44.4	104.2	8.6	DFOP
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[c]	20 / 40	6.2 / 20.4	k: 0.1127	3.3	10.8	8.0	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	9.0 / 63.7	k_1:0.3685 k_2: 0.02889 g: 0.3702	1.9 | 24.0	63.7	9.7	DFOP

[a] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[b] Calculated with equation reported in EFSA guidance 2017: pH_{H2O}=0.982pHc_{aCl2} + 0.648.
[c] Medium not reported, H_2O assumed
[d] Modelling DT_{90} also reported since it is used to assess pH-dependency

Laboratory trigger endpoints for AMPA – parent applied studies

AMPA	Persistence endpoints Dark aerobic conditions The precursor from which the f.f. was derived was glyphosate						
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50}/ DT_{90} (d)	f. f. k_f / k_{dp}	Kinetic parameters	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	112 / 373	0.1955	k: 0.006181	7.6	SFO
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	28.6 / 95.1	0.3000	k: 0.02421	3.5	SFO
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	88.2 / 293	0.2004	k: 0.007863	6.2	SFO

[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	1000 / 3320	0.2618	k: 0.00069	9.2	SFO
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	96.4 / 320	0.2793	k: 0.007187	10.1	SFO
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[a]	20 / 40	79.2 / 263	0.3406	k: 0.008753	8.2	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	200 / 666	0.4796	k: 0.003459	3.2	SFO

[a] Medium not reported, H_2O assumed

Laboratory modelling endpoints for AMPA – parent applied studies

AMPA	Modelling endpoints Dark aerobic conditions The precursor from which the f f. was derived was glyphosate						
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50}/ DT_{90} (d)	f. f. k_f / k_{dp}	DT_{50} (d) 20 °C pF2/10kPa[b)]	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	112 / 373	0.1955	61.6	7.6	SFO
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	28.6 / 95.1	0.3000	13.43	3.9	SFO
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	88.2 / 293	0.2004	40.6	6.2	SFO
Ponte (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	1000 / 3320	0.2618	570	9.2	SFO
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	96.4 / 320	0.2793	104	10.1	SFO
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[a]	20 / 40	79.2 / 263	0.3406	42	8.2	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	200 / 666	0.4796	200	3.2	SFO

[a] Medium not reported, H_2O assumed

A summary of the reliable endpoints is also presented in B.8.1.1.3.

Metabolites, breakdown and reaction products

In addition to the information available from parent applied studies, the rate of degradation of AMPA was investigated in 2 new studies with AMPA applied.

Table 8.1.1.2-38: List of new studies on aerobic degradation of AMPA

Annex point	Study	Study type	Substance(s)	Soil name	Status in RAR (2021)

CA 7.1.2.1.2/003	[redacted], 2017	Aerobic rate	AMPA	Warsop, UK	Acceptable
CA 7.1.2.1.2/002 & 004	[redacted], 2020	Aerobic rate	AMPA	18 Acres, UK Brierlow, UK	Acceptable

[redacted], 2017

Data point:	CA 7.1.2.1.2/003
Report author	[redacted]
Report year	2017
Report title	Aminomethylphosphonic Acid (AMPA): Rate of Degradation of AMPA in one Acidic Soil Incubated under Aerobic Conditions
Report No	S16-04460
Guidelines followed in study	OECD 307; SANCO/3029/99 rev.4
Deviations from current test guideline	From OECD 307: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Acceptable

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Aminomethylphosphonic acid (AMPA)

Lot No.: GLP-1508-24086-A

CAS number: 1066-51-9

Chemical purity: 98.8 %

2. Soil:

The soil was collected freshly in United Kingdom, no fertilizers or pesticides have been applied to the soil for 5 years. Following arrival at the testing facility the soil was sieved to ≤ 2 mm and stored at 4°C for 9 days. Before use, the soil was adjusted to 45 % WHC and stored at 20 °C Characteristics of the test soil are presented in the table below.

Table 8.1.1.2-39: Characteristics of test soil

Parameter	Results
Soil	Warsop
Country	United Kingdom
Textural Class (USDA)	Loamy sand
Sand (50 µm – 2 mm) (%)	84.2
Silt (2 µm – 50 µm) (%)	11.2
Clay (< 2 µm) (%)	4.6
pH (water)	4.71
pH ($CaCl_2$)	3.90
Organic carbon (%)	1.76
Organic matter (Organic carbon x 1.72) (%)	3.03
Cation exchange capacity (meq/ 100 g soil)	7.1
Maximum Water Holding Capacity (%)	37.25
Bulk Density (disturbed) (g/L)	1400
Microbial biomass (mg C/ 100 g soil)	
After arrival	20.5 (1.16% OC)
At the start (1 DAT)	21.1 treated (1.20% OC)/ 21.3 untreated (1.21% OC)
59 DAT	20.4 treated (1.16% OC)/ 17.6 untreated (1.00% OC)

Study end (120 DAT)	17.6 treated (1.00% OC)/ 18.6 untreated (1.06% OC)

B. STUDY DESIGN

1. Experimental conditions

Static test systems were used, consisting of Erlenmeyer flasks filled with soil closed by polyurethane plug.

100 g of sieved soil (dry weight equivalents) were weighed into each test vessel, soil moisture was adjusted to 45 % of the maximum water holding capacity, and the test systems were acclimated for 11 days at test conditions.

The application rate of AMPA was 280 µg/100 g soil (dry weight). AMPA was dissolved in water and 560 µL of this solution were applied to each test system. The verification of application concentration was performed by determination of recoveries at levels of 110 % of the applied concentration and the LOQ of the method at each sampling date. Determined recoveries were in the range from 92.7 and 108.6 %, demonstrating the validity of the extraction and analysis.

Test systems were incubated under aerobic conditions in the dark for 120 days at 20 ± 2°C and 45 % of the maximum water holding capacity.

2. Sampling

Duplicate samples from each system were processed and analysed at 0, 2, 8, 13, 30, 62, 90, and 120 days after treatment (DAT). All soil samples were processed on the designated sampling day. At every sampling time point both flasks were extracted on the same day of collection, extracts were stored in a freezer at ≤ -18 °C and analysed by LC-MS/MS within 10 days of collection.

3. Analytical procedures

At each sampling interval, soil samples were extracted with 1000 mL of 1 N NaOH and agitated for 30 seconds by hand followed by agitation on a flatbed shaker for 20 minutes at ambient temperature. Extracts and soil were separated by centrifugation and decantation. 10 mL of the extract was filtrated through a single use syringe filter. 0.2 mL of a 500 ng/mL internal standard solution (^{13}C and ^{15}N isotope enriched AMPA) was mixed with 0.1 mL formic acid and 1.7 mL of the filtrated extract. About 1 mL of the mixed solution was cleaned-up through a SPE cartridge and transferred into a glass vial for LC-MS/MS analysis.

AMPA was identified by HPLC-MS/MS with multiple reaction monitoring (MRM) mode using AMPA standards in solvent for calibration.

4. Kinetics

The analytical results were evaluated using CAKE 3.3 (2016) software according to FOCUS Guideline using four kinetic models (single first order (SFO), double first order in parallel (DFOP), first order multi compartment (FOMC) and hockey-stick (HS)) using replicate values. The data were directly fitted un-weighted with the complete data set and unconstrained initial concentration (M_0). IRLS was used as solver as implemented in CAKE 3.3.

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration *vs.* time plots and residual plots) and statistically (χ^2, t-test, confidence interval).

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.2-40: Degradation of AMPA in Warsop soil under aerobic conditions (expressed as percent of applied analyte)

Compound		Replicate	DAT							
			0	2	8	13	30	62	90	120
AMPA	Soil	A	108.2	105.0	108.6	105.7	102.5	97.9	91.4	83.6
		B	108.6	107.5	108.6	106.1	97.5	97.9	88.2	81.8
		Mean[1]	108.6	106.4	108.6	106.1	100.0	97.9	89.8	82.9

DAT: days after treatment
[1] Mean was calculated from two replicates

B. DEGRADATION OF TEST ITEM

The mean residues of AMPA in soil extracts slowly decreased from 0 DAT to 120 DAT from 108.6 to 82.9 % of applied concentration.

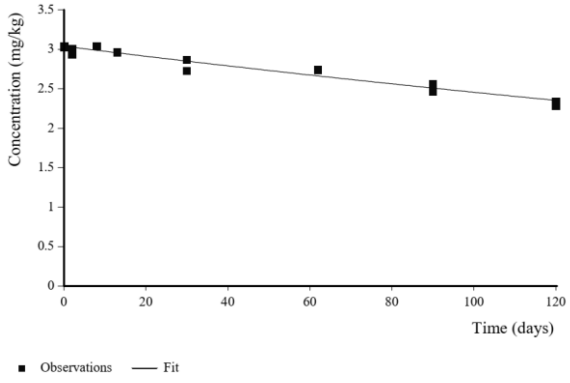
C. KINETICS

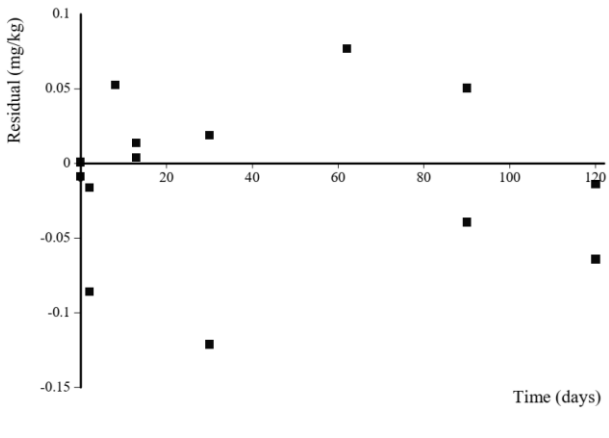
The degradation of AMPA was best described using Single First-Order kinetics (SFO) where the time for a decrease in the concentration of the test item is constant throughout the experiment and independent of its initial concentration. SFO results were selected since this model yielded a low percent chi^2 error (1.25 %), acceptable statistical parameters and visually acceptable goodness-of-fit and hence the best fit. SFO will also be chosen as modelling endpoint. The DT_{50} value determined was 326 days and the DT_{90} value was 1080 days.

Table 8.1.1.2-41: Kinetic models and goodness-of-fit statistics

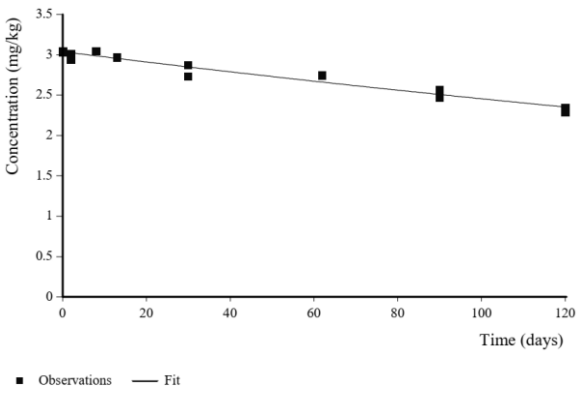
Kinetic model	Visual assessment	M_0 (mg/kg)	Kinetic parameters	χ^2 error [%]	Prob > t (5 % level)	Lower CI (95%)	Upper CI (95%)	DT_{50} [d]	DT_{90} [d]
SFO	good	3.039	0.002128	1.25	k: <0.001	0.00184	0.002	326	1080
FOMC	good	3.039	α: 25.97 β: 0.000122	1.34	N/A	β: -84340	β: 109000	329	1130
DFOP	good	3.039	k_1: 0.002143 k_2: 0.00027 g: 0.9929	1.44	k_1: 0.3601 k_2: 0.4998	k_1: -0.01059 k_2: -1.148	k_1: 0.015 k_2: 1.149	326	1100
HS	good	3.023	k_1: 0.001739 k_2: 0.002763 tb: 63.22	1.19	k_1: <0.001 k_2: 0.002	k_1: 0.001017 k_2: 0.001101	k_1: 0.002 k_2: 0.004	274	857

SFO





FMOC



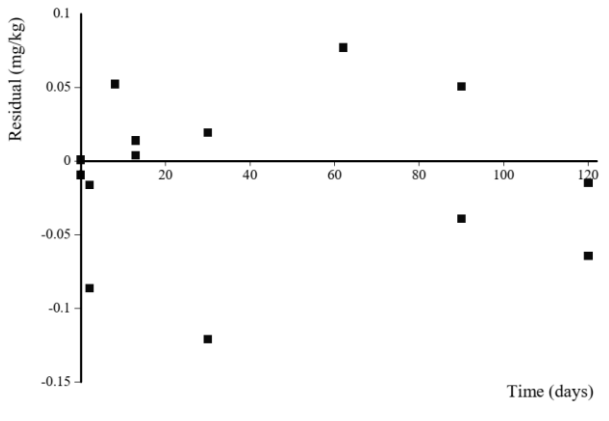
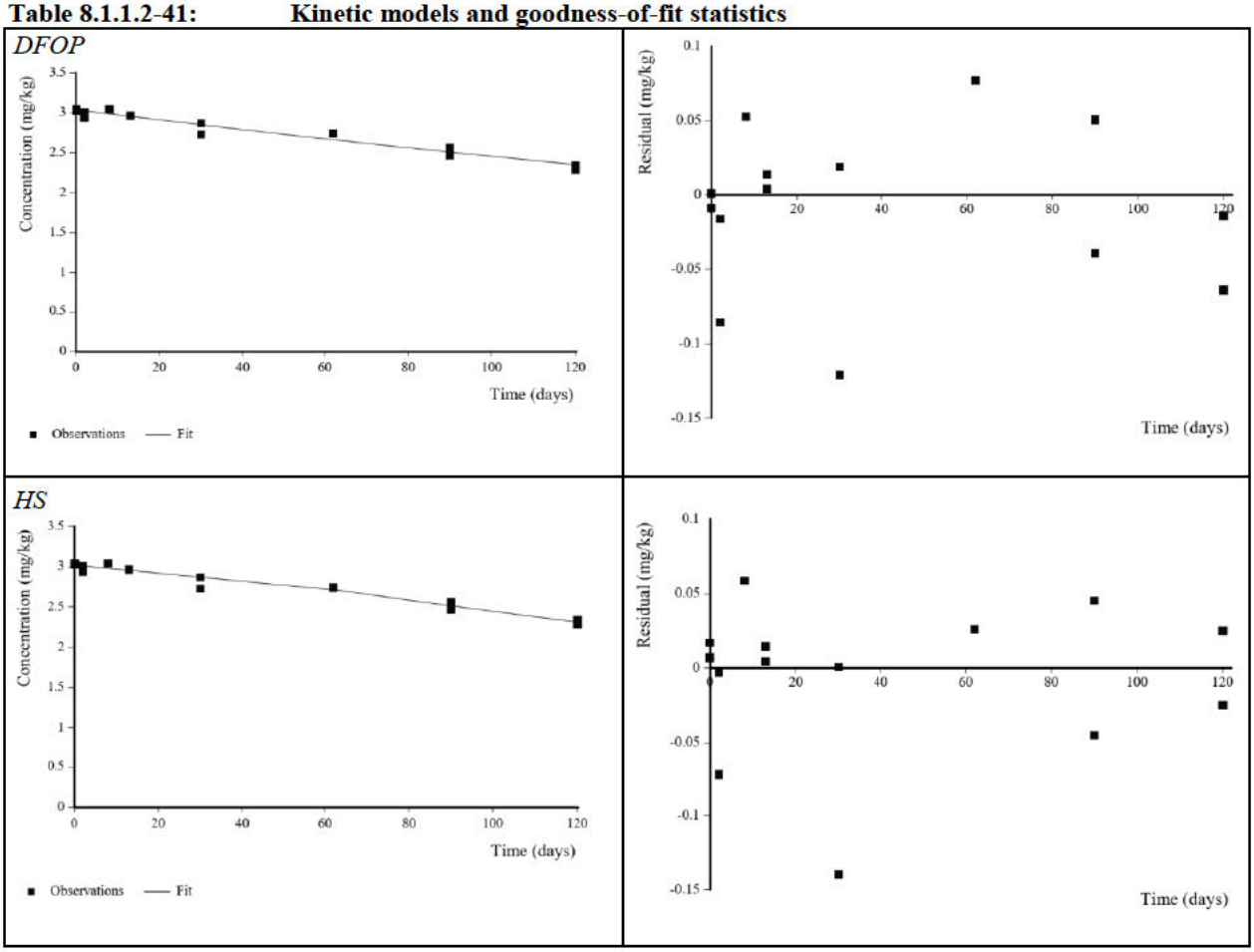


Table 8.1.1.2-41: Kinetic models and goodness-of-fit statistics



III. CONCLUSIONS

The aim of this study was to determine the degradation rate of the non-labelled test item AMPA in one microbially active highly acidic soil. The study was performed in the dark at 20 °C under aerobic conditions over an incubation period of 120 days.

The test item degraded slowly under aerobic laboratory conditions with a half-life of 326 days and a DT_{90} value of 1080 days. The slow rate of degradation of AMPA in this soil is consistent in general with the strong ability of AMPA to tightly bind to most soils, which limits its availability to microorganisms for degradation.

The study suggests that degradation of AMPA follows a Single First-Order kinetic as best fit and hence also modelling endpoint indicating that the time for the decrease in the concentration of AMPA will be constant while present in soil and is independent of its initial concentration.

Assessment and conclusion by applicant:

The study is conducted according to the current guidelines and is therefore considered valid.

Assessment and conclusion by RMS:

This study was performed in order to assess the degradation rate of AMPA in an acidic soil, to address a data gap identified in EFSA Journal 2015. The study is well performed and documented.

The study duration was set to 120 days but less than 20% of the compound had degraded at this time. A longer study duration would have been relevant.

The kinetic fitting was performed according to FOCUS guidance. The initial concentration was not corrected for chemical purity, but no significant impact is expected considering the high chemical purity (98.8%) and the slow degradation. RMS agrees with the selection of SFO for trigger and modelling endpoint.

The study is acceptable.

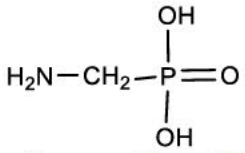
[redacted], *2020*

Data point:	CA 7.1.2.1.2/002
Report author	[redacted]
Report year	2020
Report title	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil
Report No	3202599
Guidelines followed in study	OECD 307 EPA 835.4100 Commission Regulation (EU) No. 283/2013 Regulation (EC) No. 1107/2009 (2009)
Deviations from current test guideline	From OECD 307: High organic carbon content for Brierlow soil
GLP/Officially recognised testing facilities	Yes
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

Data point:	CA 7.1.2.1.2/004
Report author	[redacted]
Report year	2020
Report title	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil Final Report Addendum
Report No	3202599
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Aminomethylphosphonic acid
	
Description:	White crystalline solid
Lot/Batch #:	107785
Purity:	≥ 99 2%
Stability of test compound:	Stable, >99 days in 0.1% formic acid (aq) (refrigerated) ≥23 months in water (refrigerated)
Application vehicle:	RO (reverse osmosis) Water

Two soils were collected freshly in United Kingdom, no fertilizers or pesticides have been applied to the soils for 5 years. Following arrival at the testing facility the soils were stored at 4°C for 48 days in loosely tied plastic bags. Before use, the soils were thoroughly mixed and passed through a 2 mm mesh sieve, with the minimum of air drying. Soils were adjusted to just below the water holding capacity at pF 2.0 by the addition of reverse osmosis water, for 7 days.

Table 8.1.1.2-42: Soil Characteristics

Name	Soil 1	Soil 2
Sampling location	[redacted], [redacted] Berks, UK	[redacted] Derbyshire, UK
GPS co-ordinates	[redacted]	[redacted]
Date of collection	14 November 2019	18 September 2019 (source site) 21 November 2019 (soil nursery)
Batch reference	S19/18A/042	S19/BRI/058
Pesticide History	No pesticide use in last 5 years	No pesticide use in last 5 years
Sampling depth (cm)	5 cm to 15-20 cm	10 cm to 20 cm
Collection procedures	Excavator	In accordance with ISO 18400-206
Particle size (% w/w):	-	-
Sand (2000-50 µm)	48	30
Silt (50-2 µm)	27	60
Clay (<2 µm)	25	10
Texture (USDA)	Sandy clay loam	Silt loam
pH (1:1 w/v soil:water)	5.5	5.7
pH (1:2 w/v soil:0.01M $CaCl_2$)	5.3	5.4
Organic matter[1] (%)	3.3	7.3
Organic carbon[1] (%)	1.9	4.3
CEC[2] (meq/100 g soil)	15.7	12.9
Moisture content at pF 2.0[3] (0.1bar, % w/w)	23.2	34.7
Moisture content at pF 2.5[4] (0.33 bar, % w/w)	17.5	24.4
Moisture content at 15 bar (% w/w)	11.1	16.0
Moisture content on arrival (% w/w)	20.47	27.94
Moisture content used in study (% w/w)	23.03	34.47
Initial biomass (start of study)	574 µg C/g dry soil 3 % OC	633 µg C/g dry soil 1.5 % OC
Final biomass (end of study)	615 µg C/g dry soil 3.2 % OC	551 µg C/g dry soil 1.3 % OC

[1] Organic carbon (OC) % = organic matter (OM) %/1.724
[2] CEC = cation exchange capacity
[3] Measured in an undisturbed (non-sieved) soil core
[4] Measured in 2 mm sieved soil

B. STUDY DESIGN

1. Experimental design

Parameter		Description
Duration of the test		120 days
Soil condition		Fresh soil, passed through 2 mm sieve prior to use
Soil sample weight		100 g (dry weight) per replicate
Test concentration		(Nominal) 2.80 (mg/kg soil dry weight) (Achieved) 2.99 (mg/kg soil dry weight)
Number of replicates	Treated soil samples	2
	Control soil samples	1
	Recovery soil samples	1
Test apparatus		250 mL Duran borosilicate glass incubation vessels (*ca.* 7 cm diameter) plugged with polyurethane bungs to allow continuous air exchange
	Identity of solvent	Treated in RO water

Test material application	Volume of test solution used/treatment	0.56 mL
	Application method	Positive displacement pipette
	Evaporation of application solvent	No
Experimental conditions	Temperature (°C)	20±2
	Moisture content	At 0 DAT, soil adjusted to just below pF2 moisture tension (to allow for water to be added in the treatment solution)
	Moisture maintenance method	The polyurethane bungs were moistened with RO water, incubation vessels weighed periodically and any weight loss relative to 0 DAT attributed to water loss. Water added to restore original system
	Continuous darkness	Yes

2. Sampling

Parameter		Description
Sampling intervals	Treated soil samples	Duplicate samples from 18 Acres and Brierlow: 0, 2, 8, 13, 29, 43, 62, 92 and 120 DAT.
	Control soil samples	Single samples from 18 Acres and Brierlow: 0, 2, 8, 13, 29, 43, 62 92 and 120 DAT
	Recovery soil samples	Single samples from 18 Acres and Brierlow: 2, 8, 13, 29, 43, 62, 92 and 120 DAT
	Untreated soils for biomass	At 0 and 120 DAT
Soil sampling procedures		Treated test, blank control and recovery vessels were removed at each sampling interval. At the time of sampling, recovery soil samples were fortified with AMPA and subjected to the same extraction procedures as the test samples and blank controls. Soil samples were extracted in 1M NaOH (aq), (manually and mechanically shaken), centrifuged and a portion of the supernatant filtered. A portion of the filtrate was spiked with internal reference standard and acidified with formic acid (aq) (≥ 98 %) before undergoing SPE. The sample was then quantified by LC-MS/MS.
Sample storage before analysis		All samples were extracted on the day of sampling and analysed by LC-MS/MS within 8 days of refrigerated storage. 8 DAT and 13 DAT samples were re-analysed 22 and 17 days after extraction respectively. Samples were stored refrigerated between repeat analysis.

120 DAT sample analysis started immediately after the incubation vessels were removed for analysis.

3. Description of analytical procedures

20 g (or 100 g for incubation vessels) dry weight equivalent of soil sample was transferred to plastic pots (recovery vessels fortified with known amounts of AMPA) and extracted with 200 mL (1000 mL for incubation vessels) 1M NaOH(aq) (minus the volume of water already present in the soil) for 20 minutes via mechanical agitation. A portion of extract was transferred into a centrifuge tube centrifuged at 1455 g for 5 minutes.

A portion of the resulting supernatant (3 mL) was cleaned-up via filtration (passed through a Macherery-Nagel™ Chromafil™ MV Cellulose Mixed Esters syringe filter; 2.5 mm diameter, 0.45 µm pore). The filtrate (1.7 mL) was acidified with ≥ 98 % formic acid (0.1 mL) and spiked with 0.5 µg/mL internal reference standard (0.2 mL). An aliquot (1 mL) was cleaned-up further by solid phase extraction, SPE (Strata-X 33u Polymeric RP 3 mL; 60 mg) prior to LC-MS/MS analysis.

Injected samples were quantified by peak area ratio with reference to a calibration curve. The latter was obtained by correlation of the peak area ratio of the calibration standards (made up in 0.1 % formic acid (v/v), non-matrix matched) with the corresponding concentrations of the test item.

At each sampling interval, control (untreated) samples and recovery samples (fortified after sampling with a known amount of AMPA) were processed in the same way as the treated soil samples to determine the specificity and efficiency of the analytical method.

4. Kinetics

The analytical results were evaluated using CAKE 3.3 (2016) software according to FOCUS Guideline using only the single first order (SFO) model, using replicate values. The data were directly fitted un-weighted with the complete data set and unconstrained initial concentration (M_0). IRLS was used as solver as implemented in CAKE 3.3.

The goodness of fit of the estimated to the measured residue data was evaluated statistically (χ^2, t-test, confidence interval).

II. RESULTS AND DISCUSSION

Microbial Biomass

The microbial biomass was ≥ 1.0 % OC at the start and end of the study in all soils. Hence, the soils were deemed to be suitably microbially active during the course of the study.

Specificity of the Analytical Method

Control (blank) soil extracts were free from components that interfered with the analysis of AMPA. Therefore, the analytical procedure was considered specific for AMPA.

Recovery of AMPA in Fortified Samples

Recoveries ranged from 76.8 to 105.0 % and 77.8 to 116.0 % in 18 Acres and Brierlow soils, respectively. The mean recoveries were 89.3 and 101.6% for 18 Acres soil and 93.1 and 108.7% for Brierlow soil for low and high fortification levels. Since all the mean recoveries were within acceptable limits (70-110 %), no correction was made for procedural recoveries in the test samples. There were no interferences at the retention time of AMPA > 30 % of the LOQ (> 0.042 mg/kg).

Degradation of AMPA in Soils

The AMPA concentration in treated samples is shown below.

Table 8.1.1.2-43: Concentration of AMPA in soil (values as % of applied)

Soil	Replicate	% of applied at Time (DAT)[1]								
		0	2	8	13	29	43	62	92	120
18 Acres	A	98.4	91.2	92.3	91.6	90.1	85.4	83.1	92.6	87.4
	B	97.2	95.2	93.5	91.2	89.1	86.0	83.3	91.2	87.1
	Mean	97.8	93.2	92.9	91.4	89.6	85.7	83.2	91.9	87.3
Brierlow	A	106.0	100.0	98.2	99.6	93.4	98.4	87.5	102.0	101.0
	B	103.0	104.0	97.0	97.4	106.0	95.5	94.2	99.2	115.0
	Mean	104.5	102.0	97.6	98.5	99.7	97.0	90.9	100.6	108.0

[1] Based on a nominal application rate of 2.80 mg/kg

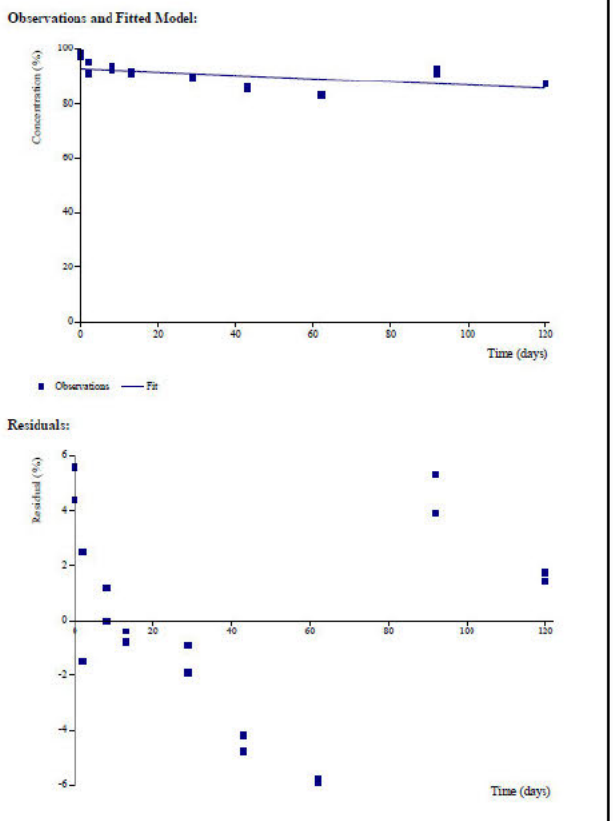
Kinetic fittings

The kinetic fitting with SFO model gives the following results.

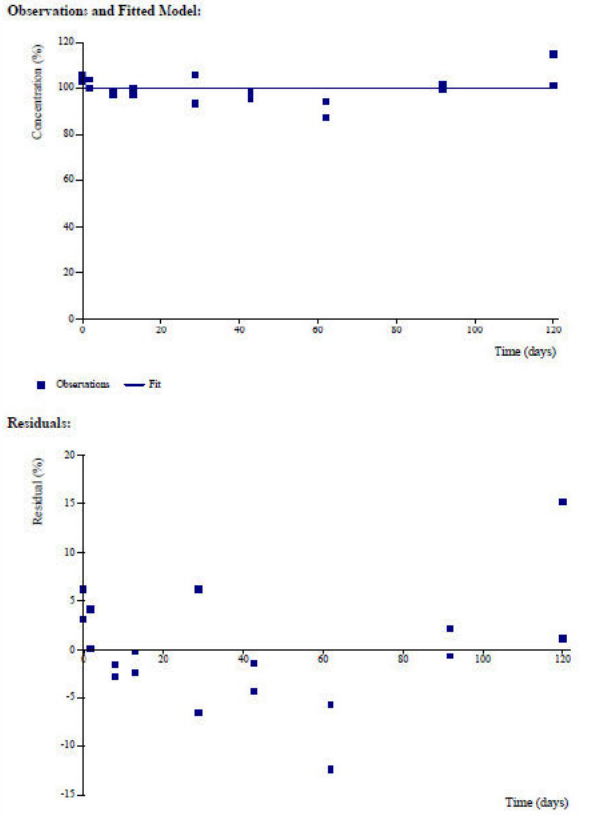
Table 8.1.1.2-44: Kinetic models and goodness-of-fit statistics

Soil	M_0 (mg/kg)	Kinetic parameters	χ^2 error [%]	Prob t<0.05	CI contains 0	DT_{50} [d]	DT_{90} [d]
18 Acres	92.8	0.000667	3.04	Yes	-	1040	3450
Briarlow	99.86	$8.78x10^{-13}$	3.68	No (0.5)	-	$7.9 x10^{11}$	$2.62x10^{12}$

SFO – 18 Acres



SFO – Briarlow



Assessment and conclusion by applicant:

The study was performed according to the guidelines that were in force at time of submission of this dossier. The rate of degradation of AMPA was investigated in two different aerobic soils incubated at a temperature of 20°C and a moisture content of *ca.* pF 2. AMPA was applied at a nominal rate of 2.80 mg/kg dry weight soil (based on the proportions of AMPA found from parent (glyphosate) degradation in previous studies).

The rate of degradation of AMPA was investigated in two soils. The DT_{50} value was calculated to be 1040 days for 18 Acres soil using SFO kinetic modelling, therefore, the modelling endpoint (study conducted at 20±2°C and *ca.* pF 2) is a DT_{50} of 1040 days for 18 Acres.
The SFO kinetic model does not properly estimate the degradation (no statistically reliable fit) of AMPA in Brierlow soil, therefore, endpoints could not be derived using this model.

The study is considered valid.

Assessment and conclusion by RMS

This study was performed in order to assess the degradation rate of AMPA in acidic soils, to address a data gap identified in EFSA Journal 2015. The study is well performed and documented.

RMS notes that the organic content of Brierlow soil is very high, way above the recommended 2.5 %OC.

The study duration was set to 120 days but less than 20% of the compound had degraded at this time. A longer study duration would have been relevant.

Only SFO fits were provided by the applicant. Considering that no significant degradation is observed, no better visual fits would be obtained with biphasic models. Therefore no further kinetic analysis is deemed necessary.

In RMS opinion, the DT_{50} of 1040 d calculated by the model for 18 Acres soil can be used. Indeed, the fit describes accurately the very low degradation of AMPA in this soil, visually and statistically.

Regarding Brierlow soil, RMS notes that no degradation is observed during the 120 days of the study and residues even increase at the end of the study. No accurate DT_{50} can be calculated for this soil. RMS proposes considering a default DT_{50} of 1000 days. Although it may not be an absolute worst-case value, it is the commonly agreed default value. This DT_{50} may underestimate the degradation rate of AMPA in this soil, however it is important to account for its persistence and it is therefore a better option than completely excluding this soil.

It is also underlined that setting this default value is consistent with the approach proposed for glyphosate-applied soils, for which endpoints were derived for AMPA when persistence was observed and acceptable visual fits could be obtained.

The study is acceptable.

Summary of the endpoints for AMPA – AMPA based studies:

No normalization of endpoints from AMPA-applied studies is required since both [redacted] (2017) and [redacted] (2020) have been performed at 20°C and *ca* pF2.

Laboratory trigger endpoints for AMPA – AMPA applied studies

AMPA	Trigger endpoints Dark aerobic conditions Metabolite dosed						
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50}/ DT_{90} (d)	f. f. k_f / k_{dp}	Kinetic parameters	St. (χ^2)	Method of calculation
[redacted] (2017): Warsop Loamy sand	4.71	20 / pF 2	326 / 1080	-	k: 0.002128	1.3	SFO
[redacted], 2020: 18-Acres Sandy clay loam	5.5	20 / pF 2	1040 / 3450	-	k: 0.000666	3.0	SFO
[redacted], 2020: Brierlow, Silt loam	5.7	20 / pF 2	1000 / 3320	-	k: 0.000693	3.2	SFO

Laboratory modelling endpoints for AMPA – AMPA applied studies

AMPA	Modelling endpoints Dark aerobic conditions Metabolite dosed						
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50}/ DT_{90} (d)	f. f. k_f / k_{dp}	DT_{50} (d) 20 °C pF2/10kPa[b)]	St. (χ^2)	Method of calculation
[redacted] (2017): Warsop Loamy sand	4.71	20 / pF 2	326 / 1080	-	326	1.6	SFO
[redacted], 2020: 18-Acres Sandy clay loam	5.5	20 / pF 2	1040 / 3450	-	1040	3.0	SFO
[redacted], 2020: Brierlow, Silt loam	5.7	20 / pF 2	1000 / 3320	-	1000	3.2	SFO

Relevant articles from literature search

In the scientific literature review for glyphosate (2010-2020), twelve articles were identified to provide further information relevant to the data point.

Table 8.1.1.2-45: Aerobic rate of degradation - relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status
CA 7.1.2.1.1/010	Zhelezova *et al*., 2017	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/011	Cassigneul *et al*., 2016	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/012	Norgaard *et al*., 2015	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/013	Kanissery *et al*., 2015	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/014	Rampoldi *et al*., 2014	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/015	Al-Rajab & Hakami, 2014	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/016	Nguyen *et al*., 2013	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/017	Bergstrom *et al*., 2011	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/018	Ghafoor *et al*., 2011	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/019	Alexa *et al*., 2010	Aerobic rate	Glyphosate	Reliable with restrictions
CA 7.1.2.1.1/020	Al-Rajab & Schiavon, 2010	Aerobic rate	Glyphosate, AMPA	Reliable with restrictions
CA 7.1.2.1.1/021	Bento C. P. M. *et al.* 2016	Aerobic rate	Glyphosate, AMPA	Reliable with restrictions

Zhelezova, A. et al

Data point:	CA 7.1.2.1.1/010
Report author	Zhelezova, A. et al.
Report year	2017
Report title	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides
Document No	DOI 10.1007/s11270-017-3392-7 ISSN 0049-6979
Guidelines followed in study	Degradation experiment: none Adsorption experiment: OECD 106 (2000)
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Biochar amendment can alter soil properties, for instance, the ability to adsorb and degrade different chemicals. However, ageing of the biochar, due to processes occurring in the soil over time, can influence such biochar-mediated effects. This study examined how biochar affected adsorption and degradation of two herbicides, glyphosate (N-(phosphonomethyl)-glycine) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in soil and how these effects were modulated by ageing of the biochar. One sandy and one clayey soil that had been freshly amended with a wood-based biochar (0, 1, 10, 20 and 30% w/w) were studied. An ageing experiment, in which the soil-biochar mixtures were aged for 3.5 months in the laboratory, was also performed. Adsorption and degradation were studied in these soil and soil-biochar mixtures, and compared to results from a soil historically enriched with charcoal. Biochar amendment increased the pH in both soils and increased the water-holding capacity of the sandy soil. Adsorption of diuron was enhanced by biochar amendment in both soils, while glyphosate adsorption was decreased in the sandy soil. Ageing of soil-biochar mixtures decreased adsorption of both herbicides in comparison with freshly biochar-amended soil. Herbicide degradation rates were not consistently affected by biochar amendment or ageing in any of the soils. However, glyphosate half-lives correlated with the Freundlich Kf values in the clayey soil, indicating that degradation was limited by availability there.

Materials & Methods

Soil Sampling and Processing

The soil samples were collected in September 2015 from arable fields at two locations: Länna (L) (59° 52′ N, 17° 58′ E) and Ulleråker (U) (59° 49′ N, 17° 39′ E). Soil sampling at L was performed in two parts of the arable field: an untreated part (L) and a historically charcoal-enriched part (LB). Because of the long-term charcoal amendment, the latter soil was characterised by lower bulk density and higher loss on ignition and water-holding capacity (WHC) than the unamended soil from the same field, which leads to higher

yields in dry years. In each soil, about 10 samples were taken from the upper layer (5–15 cm below surface) and pooled. After sieving, the Ø < 2 mm fraction was homogenised and stored at −20 °C in plastic bags until the start of the experiment. Moisture content and WHC were measured for all soil samples. Moisture content was determined by drying at 110 °C for 10 h, while WHC was defined as the moisture content after saturation of 30 g soil with distilled water for 10 h followed by 4 h of free drainage. Chemical and physical properties of the three soils studied (L, LB, U) were determined by a commercial laboratory and are presented below.

Preparation and Ageing of Soil-Biochar Mixtures

The biochar used was the commercial product Skogens kol, which is produced from a mixture of about 80% hardwood, mainly birchwood (Betula sp.) and 20% wood from Norway spruce (Picea abies), by slow pyrolysis with a maximum process temperature of 380– 430 °C (Cederlund et al. 2016). Soil-biochar mixtures were prepared by mixing soil (L and U) with sieved biochar (Ø < 2 mm) at a rate of 1, 10, 20 and 30% biochar per unit soil dry weight (designated L1, L10, L20 and L30 and U1, U10, U20 and U30). WHC was determined as described above and pH for all mixtures was measured in a 1:2 slurry of soil and distilled water (w/v) after shaking and stabilisation for 10 h. Biochar ageing was performed with soil-biochar mixtures made from U soil. These mixtures were incubated in darkness at 20 °C for 3.5 months. The moisture content was adjusted to 55% of WHC and monitored and adjusted weekly by addition of deionised water.

Chemicals Used in Herbicide Adsorption and Degradation Experiments

Glyphosate (N-(phosphonomethyl)-glycine, CAS [1071-83-6], 98%) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), CAS [330-54-1], 99.0%) were provided by Dr. Ehrenstorfer GmbH, Augsburg, Germany. 14C-labelled diuron ([ring-U-14C], 96.4%, 5.71 MBq/mg) and glyphosate ([P- methylene-14C], 4.87 MBq/mg) were provided by the Institute of Isotopes Co. Ltd., Budapest, Hungary.

Measurement of Herbicide Adsorption in Soils and Soil-Biochar Mixtures

Adsorption was determined in a batch-equilibrium system according to OECD guideline 106 (OECD 2000). A pre-study was performed to estimate the time when the equilibrium between adsorbed herbicide and herbicide in solution was reached (8, 24 and 32 h). In all cases, equilibrium was reached within 24 h. For high-percentage soil-biochar mixtures with U soil, an additional pre-study was performed to estimate an appropriate soil to solution ratio as defined in the OECD guideline. Soil and soil-biochar mixtures, corresponding to 1 g of soil or mixture dry weight, were weighed into tubes (15-mL glass tubes for diuron and 50-mL polypropylene tubes for glyphosate) and adjusted with 0.01 M CaCl2 to reach the appropriate soil-solution ratio. This was 1:40 for all samples with glyphosate and for U20, U30, U20a and U30a with diuron and 1:4 for all other samples with diuron. The samples were shaken for 24 h (20 °C, 200 revolutions/min). After that, herbicides were added to reach concentrations of 1, 5, 10, 50 and 100 μg/g dry weight (dw) soil for glyphosate and 0.1, 0.5, 1, 5 and 10 μg/g dw soil for diuron, due to its lower water solubility. In addition, a fixed amount (10 μL for glyphosate and 20 μL for diuron) of 14C- labelled herbicide was added to each tube to reach an activity of 2000 DPM (3.333×10^{-5} MBq) per sample. There were two replicate tubes of each concentration. After 24 h, the tubes were centrifuged (3000 revolutions/min for 30 min), samples of supernatant were transferred to scintillation vials (4 mL for diuron and 10 mL for glyphosate samples) and Quicksafe A (Scintvaruhuset, LAB-service, Uppsala, Sweden) was added directly before measurement of scintillation. 14C activity was measured on a Beckman LS 6000TA liquid scintillation counter (Beckman Counter Inc., Fullerton, CA). Controls without herbicides were measured for all samples to exclude the level of background radioactivity. The data obtained were fitted using the linear form of the Freundlich isotherm.

Herbicide Degradation Experiment

The herbicides were dissolved in water (glyphosate) or methanol (diuron) and added dropwise to a fraction (10%) of the soils and soil-biochar mixtures. Water and methanol were allowed to evaporate from the samples for 10 h. The herbicide-treated part was then mixed with the rest of each sample to give an initial nominal concentration of 10 mg/kg soil dry weight. Portions of soil corresponding to 5 g of dry weight were weighed into 50-mL plastic tubes and the water content was adjusted to 60% of WHC and kept at this level for the duration of the experiment. The tubes were sealed with caps and were incubated at 20 °C in

the dark. After 1, 2, 5, 8, 16, 23 (only for U samples) and 31 days of incubation, two replicate tubes from each treatment were placed in the freezer (−20 °C) for future extraction and analysis.

Table 8.1.1.2-46: Chemical properties of the soils studied

Soil	Code	HCl extracted K (mg 100 g^{-1})	HCl extracted P (mg 100 g^{-1})	Al-K[a] (mg 100 g^{-1})	Al-P[a] (mg 100 g^{-1})	Total C (%)	Total N (%)	pH
Charcoal-amended soil from Länna	LB	68.35	85.02	3.82	16.48	17.57	0.37	5.57
Untreated soil from Länna	L	229.36	78.77	37.28	16.67	4.86	0.34	5.27
Soil from Ulleråker	U	287.59	68.16	34.95	4.87	1.36	0.1	6.41

[a] Al–K/Al-P = ammonium lactate-extractable K and P—Swedish standard method for estimation of plant available K and P fractions (Otabbong et al. 2009)

Table 8.1.1.2-47: Physical properties of the soils studied

Soil code	Clay Ø < 0.002 mm	Fine silt 0.002–0.006 mm	Medium silt 0.006–0.02 mm	Coarse silt 0.02–0.06 mm	Fine sand 0.06–0.2 mm	Medium sand 0.2–0.6 mm	Coarse sand 0.6–2 mm	Loss on ignition %
LB	n.d.	n.d.	n.d.	n.d.	5.9	3.3	4.1	39.4
L	66.5	14.8	9.1	6.5	2.1	0.7	0.3	13.7
U	7.5	3.2	2.4	3.2	12.1	63.8	7.8	3.3

n.d. not determined

Data from the degradation experiment after recovery correction were used to estimate herbicide half-life. Recovery was calculated as:

$$\text{Recovery} = \left(\frac{C_0}{C_{\text{nominal}}}\right) \times 100$$

where C0 is the herbicide concentration determined at day 0. Natural logarithms of remaining concentrations for days 0–31 were plotted against time, giving the first- order rate constant k as the slope of the linear regression line. Half-life (T½) was calculated as:

$$T^{1/2} = \frac{\ln 2}{k}$$

Analysis of Diuron

For diuron extraction from soil and soil-biochar mixtures, the following protocol was used: 10 mL methanol were added using a Vogel pipette to the tubes with sample. The tubes were shaken at 200 revolutions/min for 60 min, centrifuged at 4000 revolutions/min for 10 min and the supernatant was filtered (OOH Whatman; 11 cm). Portions (1 mL) of filtrate were transferred to sample vials and HPLC analysis was performed according to the protocol in Cederlund et al. (2007). Standard solutions with concentration range 0.05–50 µg/mL were analysed with extracts from samples. The HPLC was equipped with a G1314A UV detector, a G1311A pump, a G1329A auto injector (Agilent Technologies AB; 1100 Series; Sweden) and a Zorbax SB-C18 column (12.5 × 4.6 mm, 5 mm; ChromTech AB, Sundbyberg, Sweden).

Analysis of Glyphosate

Extraction of glyphosate, derivatisation and measurement on GC-MS were performed using the same reagents for analytical standards, glyphosate extraction and internal standards as previously described (Bergström, Börjesson, and Stenström, 2011).

Results & Discussion

Effect of Biochar on Soil Water-Holding Capacity and pH

The studied soils had different physical texture: the dominant particle fractions in the L soil were clay and fine silt, while the U soil was dominated by medium and fine sand. The texture of the LB soil could not be fully determined due to its high organic matter content, as traces of organic C remained in the sample after digestion (oxidation by H2O2). Coming from the same field as L, it is likely that the LB soil was also dominated by clay. However, the proportion of sand was higher. This agrees with Kihlberg et al. (unpublished), who also reported a coarser particle size distribution in LB compared with L soil, but also did not subdivide particles with Ø < 0.06 mm. The WHC of the clayey L soil (53%) was higher than in the sandy U soil, where it was only 27%, and was not affected by biochar addition. However, the LB soil, which was historically amended by charcoal, had a higher WHC (57%) than the L soil with or without fresh biochar amendment. In the sandy soil, the WHC increased from 27 to 42% with biochar addition and was correlated positively (r = 0.98) with the biochar percentage (Figure 8.1.1.2-1). Biochar addition increased the pH from 5.27 to 6.07 in the L soil and from 6.41 to 7.69 in the U soil (Figure 8.1.1.2-2). Ageing of the biochar led to a further pH increase in most of the soil-biochar mixtures (U10a- U30a). In the LB soil, the pH was higher (5.77) than in the L soil. The pH of soil-biochar mixtures was correlated with the percentage of biochar added in all cases (r = 0.99 for L soil-biochar mixtures; r = 0.99 for fresh U soil-biochar mixtures; r = 0.98 for aged U soil- biochar mixtures).

Figure 8.1.1.2-1: Water-holding capacity (WHC) of the soil samples ± standard deviations plotted against biochar percentage added

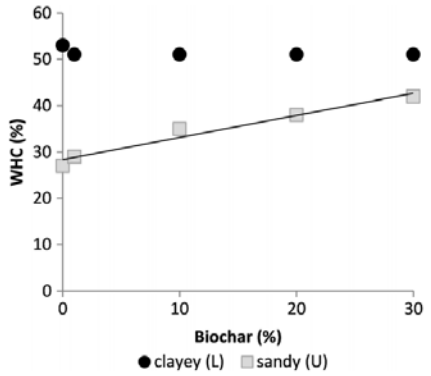
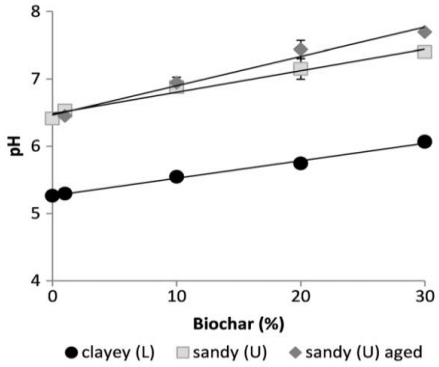


Figure 8.1.1.2-2: pH of the soil samples (N = 2) ± standard deviations plotted against biochar percentage added

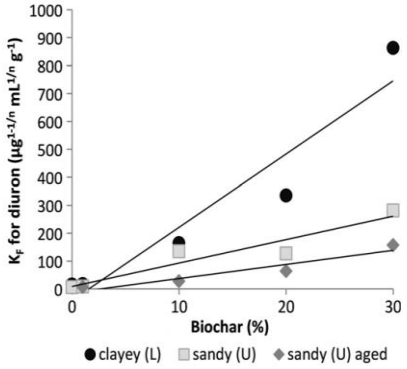


Adsorption of Diuron

Biochar amendment increased diuron adsorption in both the L and U soils. In the LB soil, KF was 364 µg1–1/n(mL) 1/n g−1, which is quite close to the KF value of the L20 soil-biochar mixture. KF values in the aged soil-biochar mixtures were lower than in mixtures with fresh biochar addition. There were positive

correlations between the diuron KF values and biochar percentage for L, U and aged U soils (r = 0.96, r = 0.95, and r = 0.95, respectively).

Figure 8.1.1.2-3: Freundlich KF values for diuron plotted against biochar percentage added in samples from Länna (L) and Ulleråker (U)



Adsorption of Glyphosate

Glyphosate was more strongly adsorbed in the L soil (KF = 1218 µg1–1/n mL1/n g−1) than in the U soil (KF = 146 µg1–1/n mL1/n g−1). No consistent effect of biochar amendment on glyphosate adsorption in L soil was observed (Figure 8.1.1.2-4). A very high KF value was observed for the sample with 1% biochar addition (KF = 1892 µg1–1/n mL1/n g−1), while the KF values for the unamended L soil and the other soil-biochar mixtures varied between 1099 and 1294 µg1–1/n mL1/n g−1. The LB soil had a much lower KF value (539 µg1–1/ n mL1/n g−1) than the L soil and soil-biochar mixtures. However, in the U soil, glyphosate adsorption was correlated negatively (r = −0.99) with the biochar percent- age (Figure 8.1.1.2-4). Ageing of the biochar decreased adsorption further.

Figure 8.1.1.2-4: Freundlich KF values for glyphosate plotted against biochar percentage added in samples from Länna (L) and Ulleråker (U)

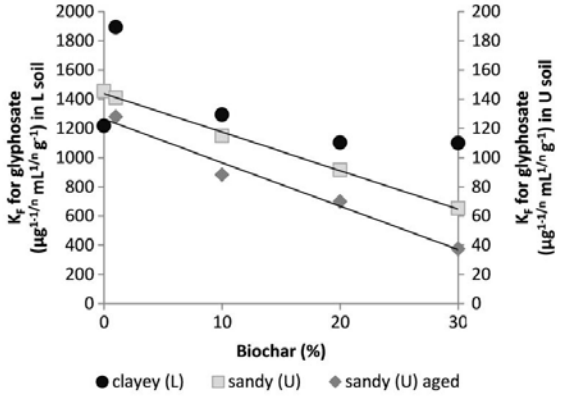


Table 8.1.1.2-48: Freundlich parameters (KF, 1/n and R2 value) for adsorption and half-life of diuron and glyphosate

Sampling site		Adsorption						Degradation			
		Diuron			Glyphosate			Diuron		Glyphosate	
		K_F[a]	1/*n*	R^2	K_F[a]	1/*n*	R^2	$T½$[b]	R^2	$T½$[b]	R^2
Länna	LB	364	0.859	0.99	539	0.890	0.99	36	0.965	17	0.97
	L	15.21	0.863	0.99	1218	0.842	0.99	40	0.963	87	0.606
	L1	17.10	0.807	0.99	1892	0.872	0.99	47	0.708	187	0.333
	L10	164	0.859	0.99	1294	0.806	0.99	42	0.853	151	0.385
	L20	335	0.822	0.99	1102	0.796	0.99	56	0.918	131	0.402
	L30	863	0.978	0.96	1099	0.780	0.98	45	0.86	51	0.945
Ulleråker	U	5.73	0.798	0.99	145.5	0.783	0.99	112	0.663	182	0.482
	U1	8.60	0.586	0.95	140.9	0.765	0.99	58	0.718	83	0.767
	U10	135	0.789	0.99	114.8	0.754	0.99	33	0.866	66	0.674
	U20	127	0.727	0.85	91.6	0.780	0.99	35	0.868	78	0.621
	U30	281	0.753	0.97	65.2	0.750	0.99	40	0.888	53	0.861
	U1a	6.44	0.760	0.99	127.9	0.761	0.99	37	0.71	51	0.716
	U10a	27.4	0.824	0.99	88.3	0.776	0.99	27	0.785	81	0.683
	U20a	64	0.547	0.94	70.0	0.788	0.99	29	0.849	49	0.917
	U30a	157	0.686	0.97	37.4	0.751	0.99	35	0.871	68	0.885

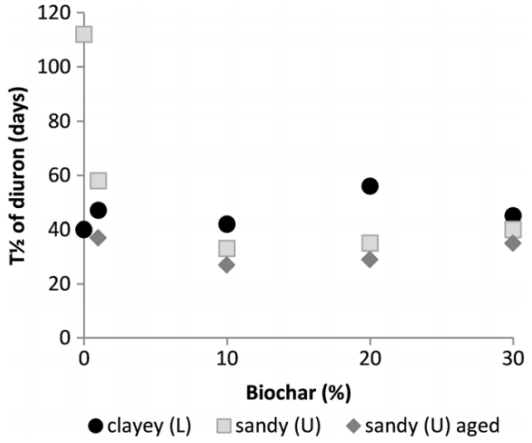
[a] The unit of K_F is $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}

[b] The unit of $T½$ is days

Degradation of Diuron

In the L and U soils and soil-biochar mixtures, from 20 to 50% of the added diuron was degraded during the experimental period. Diuron half-life varied between 40 to 56 days in the L soil, was 36 days in the LB soil and varied between 26 to 112 days in the U soil (Figure 8.1.1.2-5). No correlation was seen between the biochar percentage and diuron half-life in any of the soils. However, in the U soil, the half-life was shorter in all samples with biochar addition compared with the unamended soil. Here, it should be noted that the half-life of 112 days found for the U soil without biochar may be a less accurate estimation, since the dynamics of diuron degradation did not fit well with a first-order kinetic model in this sample. The degradation kinetics of all other samples followed first-order kinetics reasonably well, with R2 values of 0.7–0.96. Ageing of the biochar consistently decreased diuron half-life in the U soil.

Figure 8.1.1.2-5: Diuron half-life in the Länna (L) and Ulleråker (U) soils and soil-biochar mixtures



Degradation of Glyphosate

In the L and U soils and soil-biochar mixtures, 10–70% of the added glyphosate was degraded during the experimental period. Glyphosate half-life in the L soil varied between 51 and 187 days. However, in the L, L1, L10 and L20 samples, the data fitted poorly to the first-order kinetic model (R2 = 0.33–0.61), mostly due to great variation in glyphosate concentrations during the first week of degradation. This fact can explain the some-what inconsistent pattern of half-life variation for the soil-biochar mixes. However, degradation in the LB and L30 samples followed first-order kinetics well (R2 = 0.97 and 0.94). The shortest glyphosate half-life (19 days) was observed in the LB soil. Degradation of glyphosate was relatively slow in the unamended U soil, but was faster in all samples with biochar amendment. In the unamended U soil, the half- life of glyphosate was 182 days, while in the U soil- biochar mixtures, it varied between 49 and 83 days. However, as in the case of diuron, data from the un-amended U soil were a poor fit to the first-order model (R2 = 0.48) and the degradation rate in the biochar-amended samples did not appear to be related to the biochar percentage added. The fastest degradation was observed in the U1a and U20a soil-biochar mixtures, but ageing of the biochar did not consistently affect degradation rates (Figure 8.1.1.2-6). No correlations between glyphosate half-life and amount of added biochar were found for any of the L and U soils. However, the half-life was correlated with the KF value for glyphosate (r = 0.88) in samples of the L soil when the LB sample was included. In the U soil and soil-biochar mixtures, the adsorption coefficient of glyphosate was generally lower and its half-life was not correlated with the KF value.

Figure 8.1.1.2-6: Glyphosate half-life in the Länna (L) and Ulleråker (U) soils and soil-biochar mixtures

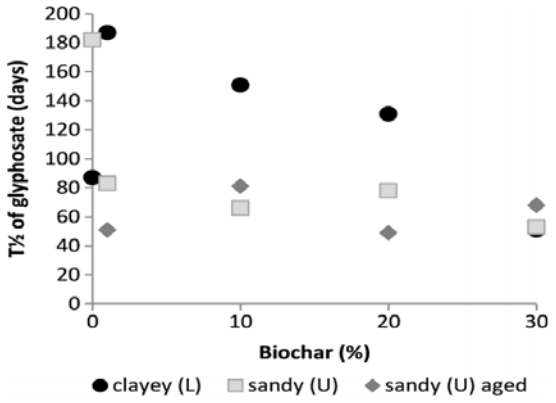
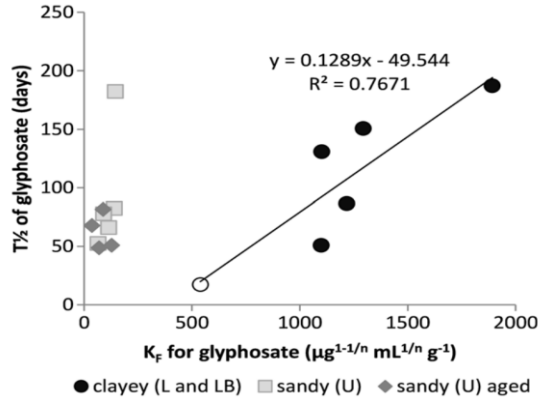


Figure 8.1.1.2-7: Correlation between glyphosate half-life and adsorption coefficient (KF). The open circle is for the LB soil



Effects of Biochar on Herbicide Adsorption

Diuron adsorption increased after biochar amendment in both the L and U soils. This effect of biochar addition has been observed in previous studies for silty loam and sandy soil. Biochar contains many

adsorption sites that can bind non-polar herbicides, so diuron adsorption increased with amount of biochar added, and the risk of it leaching is lower. The increased pH obtained with biochar addition is not likely to have contributed to the increased sorption since diuron is uncharged at relevant soil pH-levels. In a previous study, we also found that pH has no effect on diuron adsorption when studying this particular biochar without soil (Cederlund et al. 2016).

Biochar addition decreased glyphosate adsorption in the sandy U soil, but not in the clayey L soil. The difference in effects of biochar on glyphosate adsorption between the L and U soils may be explained by the different soil texture and physical properties of these soils. The decreased glyphosate adsorption in the U soil is likely to be related to the induced pH changes. According to several studies, soil pH is negatively correlated with glyphosate adsorption (Gimsing et al. 2004b; Mamy and Barriuso 2005; Vereecken 2005). Increased soil pH can increase the negative charge of both soil surfaces and glyphosate itself, which leads to enhanced repulsion. Glyphosate has a pH-dependent OH− group with a pKa value of 5.7, so its charge is likely to have been affected in the pH range studied here. The same relationship with pH has been observed for glyphosate adsorption on pure biochar: Herath et al. (2016) studied the effect of pH on adsorption of glyphosate on a rice husk biochar and found that the adsorption percentage varied from 75 to 85% at pH 3–5, decreased to 75–65% at pH 6–8 and then significantly dropped to 55% at pH 9. However, in a previous study, we showed that glyphosate adsorption by the studied biochar was low at both low and high pH (Cederlund et al. 2016). In the L soil, there was no linear relationship between glyphosate adsorption coefficient and biochar amendment. The overall strong adsorption in this soil possibly contributed to masking the relatively minor effects of the biochar. It is known that inorganic components of soil, such as Al- and Fe-oxides, adsorb glyphosate effectively (Gimsing et al. 2004a) and that this herbicide is less available in soils with a high clay content. The induced pH changes in this soil also occurred over a different pH interval, which may have contributed to the less clear outcome.

Effects of Biochar Ageing on Adsorption

Short-term ageing of the biochar mixtures in the laboratory decreased adsorption of both herbicides. This suggests that processes that have the potential to reduce sorption, such as organo-mineral interactions with the biochar surface (Pignatello et al. 2006; Singh and Kookana 2009; Lin et al. 2012), were the dominant forces affecting the biochar during our ageing experiment. For diuron, our results are consistent with findings in a field study on biochar amendment of Australian ferrosols, in which diuron and atrazine adsorption to soils amended by poultry litter and paper mill biochar was significantly reduced after 32 months of ageing. For glyphosate, it is possible that the further increase in pH during the 3 months of ageing contributed to the additional decrease observed in sorption. Although we cannot know the original properties of the charcoal applied to the historically charcoal-enriched LB soil, it may be informative to compare the adsorption results from this soil. In LB, the KF value for diuron was comparable to that determined in the 20% soil-biochar mixture (L20) and, considering that the total carbon content of the LB soil is about 18%, this suggests limited effects of ageing. However, for glyphosate, the KF value of the LB soil was only 539 μg1−1/n mL1/n g−1, which is only about half the KF value found for any of the fresh biochar mixtures or the unamended L soil. Since the adsorption of glyphosate on the biochar itself is very weak, this low adsorption is difficult to explain in terms of reduced adsorptive affinity of the charcoal. It is more likely to reflect a reduced affinity for glyphosate of the soil itself. Kihlberg et al. (unpublished) suggest that the heat from the charcoal kilns in LB may have contributed to sintering the clay particles in the soil, causing a shift towards a coarser particle size distribution. Heating clay soils to 500 °C has been shown to change soil physical texture and increase the amount of silt and sand particles. Such a reduction in the proportion of clay would consequently reduce the amount of surfaces available for glyphosate adsorption. Heating may also cause other mineralogical changes in soil that affect adsorption, for instance de Santana et al. (2006) reported reduced interaction between glyphosate and Al2O3 and Fe2O3 in soil after burning. Our results for glyphosate differ somewhat from those of Kumari et al. (2016), who found that glyphosate sorption was increased in a silty loam soil amended with the same wood-based biochar that we used (Skogens kol) after 7–10 months of ageing under field conditions. The application rates used in their study varied from 10 to 100 Mg biochar/ha added to the topsoil layer (0– 10 cm), which corresponds to about 0.8–8% of biochar per gramme dry weight assuming a bulk density of the soil of 1.3 g/cm³. Increases in glyphosate sorption occurred in plots amended with 10, 20 and 40 Mg/ha of biochar (i.e. corresponding to 0.8, 1.6 and 3.2% w/w), while the plot amended with 100 Mg/ha, where the glyphosate adsorption was the same as in the unamended plots, was considered to be an outlier (Kumari et al. 2016). In the present study,

the clayey L soil with the lowest application rate was the outlier: the adsorption coefficient in the L1 soil-biochar mixture was much higher than in L soil without amendment, while the adsorption coefficient in the L10, L20 and L30 soil-biochar mixtures was the same or lower than in the unamended clayey L soil. However, we cannot offer an explanation for this pattern. In the sandy U soil, the adsorption of glyphosate was reduced after the ageing process, which can be explained by a further pH increase and low affinity to sorb glyphosate in both sandy soil and biochar itself.

Herbicide Degradation before and after Biochar Amendment

Microbial degradation of chemicals in soil has often been reported to be limited by strong sorption (Bergström et al. 2011; Gimsing et al. 2004a; Wu et al. 2011). Moreover, pesticide degradation is often inhibited after fresh biochar addition (Kookana 2010), which can be explained by a decrease in their bioavailability. In the present case, it seems that despite the fact that adsorption of diuron increased in both soils and that adsorption of glyphosate decreased in the sandy soil, biochar amendment had no clear effect on either diuron or glyphosate degradation. However, even though neither the KF value nor the half-life of glyphosate was clearly correlated with the added biochar percentage in the clayey L soil, the half-life was correlated with the KF value (Figure 8.1.1.2-7). This indicates that in the case of glyphosate in the clayey L soil, which had KF values >1000 µg1−1/n mL1/n g−1, availability of glyphosate may have been a rate-limiting factor for its degradation, while in the other cases adsorption was too weak to have an effect.

Conclusion

As hypothesised, fresh biochar addition increased diuron adsorption in both clayey (L) and sandy (U) soils. However, glyphosate adsorption decreased only in the sandy U soil. These effects are most likely due to adsorption of diuron on the biochar itself, while in the case of glyphosate the decreased sorption may be explained by an increase in soil pH after biochar addition. No consistent effect of biochar amendment on herbicide degradation was observed in the studied soils, which contradicts our initial hypothesis. However, there was a positive relationship between adsorption and glyphosate half-life in the clayey soil-biochar mixtures, indicating that availability may be the rate-limiting step, but only where adsorption is strong. The consequences of biochar ageing under laboratory conditions were further increases in soil pH and a reduction in adsorption of both herbicides. Changes in biochar adsorptive properties during ageing in soil should be taken into consideration when planning its use in agriculture and for soil remediation purposes.

Assessment and conclusion by applicant:

The article describes the adsorption and degradation behavior of two agrochemicals in two agricultural soils from Northern Europe following amendment of biochar.

The tests resulted in data on adsorption and degradation of glyphosate in the presence and absence of biochar amended to soil samples. The tests designs are described and the adsorption parameters are sufficiently reported. For adsorption experiments, conduct according to OECD guideline 106 is claimed for. However, validity criteria in terms of OECD Guideline 106 and the EU Evaluators Checklist could not be checked due to a lack of such detail in reporting.

For the evaluation of the degradation tests, no information was reported in the publication whether a specific guideline was followed including details in design, conduct and analysis. The results were kinetically evaluated against Single First Order kinetics only to partly result in poor fits. No detailed information on findings at the different time points is reported thus preventing kinetic re-evaluation based on the presented data.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

This article was mainly performed to assess the influence of biochar on the degradation and adsorption of glyphosate and diuron. However the available information does not allow to check the validity against current guidelines (*e.g.* the characteristics of the soils are incomplete, no sufficient details regarding study design, analytical method, *etc*.).

The article provides supportive information on the degradation and adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Cassigneul, A. et al

Data point:	CA 7.1.2.1.1/011
Report author	Cassigneul, A. et al.
Report year	2016
Report title	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study
Document No	DOI 10.1016/j.scitotenv.2015.12.052 E-ISSN: 1879-1026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The increasing use of cover crops (CC) may lead to an increase in glyphosate application for their destruction. Sorption and degradation of 14C–glyphosate on and within 4 decaying CC–amended soils were compared to its fate in a bare soil. 14C–Glyphosate and its metabolites distribution between mineralized, water–soluble, NH4OH–soluble and non–extractable fractions was determined at 5 dates during a 20°C/84-d period. The presence of CC extends 14C–glyphosate degradation half–life from 7 to 28 days depending on the CC. 14C–Glyphosate dissipation occurred mainly through mineralization in soils and through mineralization and bound residue formation in decaying CC. Differences in sorption and degradation levels were attributed to differences in composition and availability to microorganisms. CC– and soil–specific dissipation patterns were established with the help of explicit relationships between extractability and microbial activity.

Materials and Methods

Soil and mulch sampling

Common vetch (Vicia sativa), white mustard (Sinapis alba), hybrid ryegrass (Lolium hybridum) and a mixture of common vetch + oat (Avena sativa) were grown as cover crops (CC) on the Lamothe INP–EI Purpan experimental station (near Toulouse, SW France) on a clay loam soil from June to September 2012. Prior to this cover crop, the whole field had grown a durum wheat–sunflower rotation without glyphosate application for more than 10 years. Aerial parts of the 4 cover crops were collected, dried at 40°C and cut into 1 cm square pieces. The underlying 0–5 cm topsoil was collected, sieved (5 mm) and stored at 4°C. CC–associated soils were sampled on each CC plot to record any possible plant–specific soil–borne microbial populations.

Herbicides

Both experiments were conducted with a mixture of technical–grade and [phosphonomethyl–14C] glyphosate (Sigma–Aldrich), prepared in 0.01 M CaCl2. Specific radioactivity and radiochemical purities of GLY were 81.4 MBq/mmol and 98.8 %, respectively.

Experiment 1: glyphosate adsorption on decaying cover crop residues

Incubations and CC characterization/description

CC– were subjected to accelerated decomposition in the dark for 6, 28 or 56 days at 28°C and in non–limiting moisture conditions. Each CC–was moistened and placed on top of its associated soil in a plastic tray (24 ∗ 37 ∗ 7 cm). Soils had been previously brought to field capacity (pF 2.5). At days 0, 6, 28 and 56 of the incubation, CC were dried, ground and analyzed (i) in duplicate for their carbon and nitrogen content

and (ii) on a single aliquot for their biochemical composition as assessed by Van Soest fractionation (Van Soest and Robertson, 1979). CC and soils characteristics are described below

Table 8.1.1.2-49: Cover crops (a) and associated soils (b) characteristics at different incubation times. OM: organic matter, SOL: water–soluble, NDF: neutral detergent fiber soluble, HEM: hemicellulose–like, CEL: cellulose–like, LIC: lignin–like, C: carbon, N: nitrogen

(a)

CC	Incubation Time (days)	OM	SOL	NDF	HEM	CEL	LIC	C ($mg \cdot g^{-1}$)	N ($mg \cdot g^{-1}$)	C/N
Vetch + oat	0	89.1	31.3	7.1	21.1	33.7	6.8	427.6	34.7	12.3
	6	81.0	29.4	12.0	12.7	38.7	7.1	427.5	34.8	12.3
	28	73.8	27.8	18.7	16.2	23.7	13.6	370.6	37.7	9.8
	56	75.8	18.8	24.3	18.2	23.5	15.2	374.0	36.5	10.3
Vetch	0	88.6	27.4	18.5	13.9	31.1	9.1	432.0	44.3	9.8
	6	66.8	29.6	13.0	11.6	33.9	11.9	352.7	27.9	12.6
	28	62.8	19.0	20.1	14.7	23.8	22.3	321.2	33.5	9.6
	56	54.2	7.4	30.3	11.5	27.1	23.6	290.3	29.9	9.7
White mustard	0	64.0	14.4	23.0	24.3	31.4	6.9	391.9	40.4	9.7
	6	62.8	35.9	24.7	7.8	22.1	9.6	323.4	29.5	11.0
	28	63.9	16.1	19.8	14.8	26.8	22.5	317.6	32.8	9.7
	56	69.9	23.1	20.8	15.2	25.5	15.4	336.1	30.5	11.0
Ryegrass	0	87.8	37.7	3.1	28.8	26.8	3.6	423.9	34.9	12.1
	6	72.1	24.4	26.1	15.1	18.2	16.1	377.6	44.6	8.5
	28	74.9	39.5	9.6	14.2	28.1	8.6	382.3	33.9	11.3
	56	72.6	23.5	27.3	15.7	19.2	14.3	380.9	43.2	8.8

(b)

CC	pH	OM (%)	C/N	N (%)	Organic C (%)	CEC (méq/100 g)	$CaCO_3$ (%)	CaO exangeable ($mg \cdot kg^{-1}$)	K_2O exangeable ($mg \cdot kg^{-1}$)	MgO exangeable ($mg \cdot kg^{-1}$)	P_2O_5 Olsen ($mg \cdot kg^{-1}$)
Vetch + oat	7.4	2.1	8.2	0.148	1.21	19.5	<0.1	4671	158	734	29.4
Vetch	7.5	1.8	7.4	0.138	1.02	16.2	<0.1	4255	153	582	20.3
White mustard	7.4	1.8	7.5	0.138	1.04	17.5	<0.1	4721	147	631	33.3
Ryegrass	7.4	2.2	9.2	0.140	1.28	17.0	<0.1	4387	184	645	24.0

Sorption characterization

Sorption of glyphosate onto CC residues and soil was determined using a batch equilibration technique, as detailed in Cassigneul et al. (2015). The sorbent:glyphosate solution ratio was 1:9 (g/mL) for soil and 1:5.8 for CC residues. Amounts of sorbed glyphosate were described using the partition coefficient Kd (L/kg) and the normalised organic carbon content Kd i.e. Koc (L/kg OC)

Experiment 2: glyphosate degradation in microcosms of soil and cover crop residues

Microcosm setup/construction/description – Microcosms, i.e. cylinders containing soil (118 g dw) covered by CC mulch (2.5 g dw), were set up as detailed in Aslam et al. (2014). The amount of mulch corresponds to 8 t/ha of biomass in the field, soil and mulch densities being 1.2 g/cm3 and 0.05 g/cm3 respectively. This amount of biomass was chosen to ensure a sufficient soil coverage given our objectives. After determination of their retention curve using pressure plates, water content of both soil and mulch was brought to field capacity (pF 2.5) in order to ensure water availability to microorganisms. Microcosms were placed in a 2 L hermetically sealed jar and incubated in the dark (20 ± 1°C). To maintain constant soil moisture, a 10 mL vial filled with deionized water was placed in each jar and water content was adjusted weekly by weighing and adding water as necessary. Two 84–days incubations were performed, both with treatments including a bare soil (control) and 4 studied CC amended soils, but with and without 14C–glyphosate application. The aim was to characterize separately (i) glyphosate fate in 'soil + mulch' and (ii) carbon mineralization from mulch. Each treatment was repeated thrice.

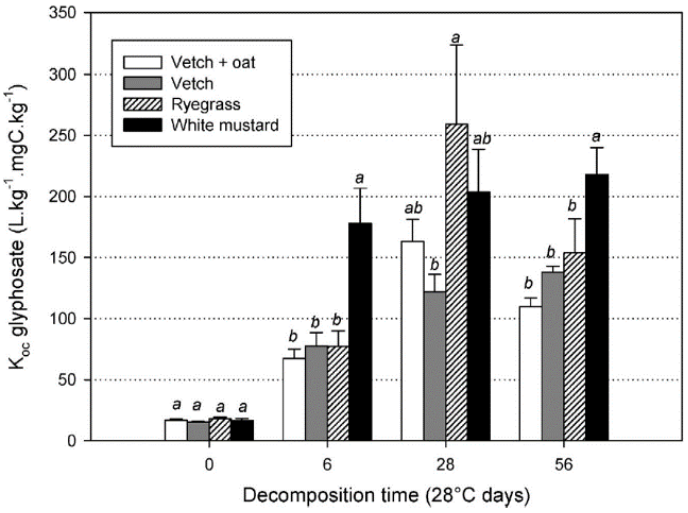
Organic C mineralization – CO2–C produced by soil respiration and mulch decomposition was trapped in a vial containing 20 mL of 0.1 M NaOH, which was replaced weekly throughout the incubation. From a 1 mL aliquot, CO2–C was analyzed by colorimetry on a continuous flow–analyzer. Net mineralization of CC carbon was calculated by subtracting the mineralization measured in the control soil treatment from that of the CC–amended treatment, and expressing the difference as a percentage of the initially–introduced organic carbon content.

Degradation study: pesticide monitoring in soil and mulch samples – At day 0, the recommended rate of glyphosate (2 L/ha) was applied at the microcosm surface (soil or mulch) in 2 mL of aqueous solution with

a micropipette. The water volume thus added had been subtracted from the total amount of water that had to be added to reach the targeted water content.

Mineralized fraction – 14CO2–C originating from glyphosate mineralization in the mulch and/or underlying soil was trapped by the same procedure as total CO2–C. The vials containing 20 mL of 0.1 M NaOH were replaced weekly throughout the incubation.

Figure 8.1.1.2-8: Sorption of glyphosate on cover crop residues. Letters correspond to LSD grouping within a single incubation time



Extractable fractions – At 0, 7, 22, 49 and 84 DAT (days after treatment), microcosms were destructively sampled. Soils (top 1 cm) and mulches were separately submitted to 4 sequential extractions. Substrates were placed in polypropylene tubes containing solvent and shaken in a rotary shaker for 24 h in the dark at room temperature. The substrate:solvent ratio was 1:20 (g/g) and 1:3 (g/g) for mulches and soils respectively. Extractions were performed first with CaCl2 (0.01 M) and then 3 times with NH4OH (0.1 M), providing access to the weakly–sorbed and to the strongly–sorbed 14C–glyphosate. Between each extraction, tubes were centrifuged for 10 min at 10,000 g and 6000 g for the mulch and the soil respectively. Supernatants were sampled for radioactivity counting, and the remaining volumes were stored at 4°C until HPLC analysis.

Non–extractable fraction – CC or soil material pellets remaining after the last extraction were oven–dried for 72 h (40°C) and ground for 10 min (Retsch GmbH, Germany). Duplicate aliquots of 500 mg were burnt in a Sample Oxidizer 307 where evolved 14CO2 was trapped in a scintillation vial containing Oxysolve T. The vial was immediately subjected to scintillation counting.

Analytical determinations – Radioactivity content in the liquid samples was measured by scintillation counting from a 1 mL aliquot mixed with 10 mL of scintillation liquid (Ultima Gold™ XR, Perkin Elmer, USA), using a Packard Tri–Card counter (GMI, Inc., USA). To prevent a chemiluminescence reaction, NaOH and NH4OH scintillation vials were submitted to a 24 h period in the dark prior to counting. A blank sample containing solvent or NaOH solution was inserted in each counting series. To determine the amount of glyphosate and metabolites, soil and mulch extracts containing sufficient radioactivity (83.3 Bq/mL) were previously filtered, concentrated by evaporation under vacuum at 50°C (Rotavapor ®, Büchi, Switzerland), and centrifuged to ensure maximum particle removal. Samples of NH4OH extracts included the extracts of the 3 successive extractions. HPLC analysis was performed coupled with a Flexar (PerkinElmer, USA) coupled with a radioactive flow detector (Radiomatic Flow Scintillation Analyzer 150TR, PerkinElmer, USA). Samples (200–500 µL) were injected into an Allsep ™ A–2 anion exchanger column (100 mm × 4.6 mm, 7 µm, Grace Davison Discovery Science, USA) preceded by a GA–1 Anion guard column (7.5 × 4.6 mm, Grace Davison Discovery Science, USA) to ensure an efficient separation, eluted with a KH2PO4 solution (0.34 g/L) adjusted to pH 2 with a 85 % H3PO4 solution. The mobile phase flow was 10−3 L/min. Under these conditions, the retention time was 3–5 min for GLY and 1–3 min for its main metabolite.

Glyphosate in the extracts was identified by comparison with the standard solution on the basis of retention time. Other detected peaks were considered as "main metabolite" (MM) or "unidentified" (UI) peaks. The main metabolite was suspected to be AMPA from previous experience with the same analytical method but, in the absence of a radiolabeled standard, this could not be verified in this particular experiment. The area of each peak was integrated (Chromera ® chromatography Data System, PerkinElmer) and expressed as a percentage of initial radioactivity applied in the microcosm.

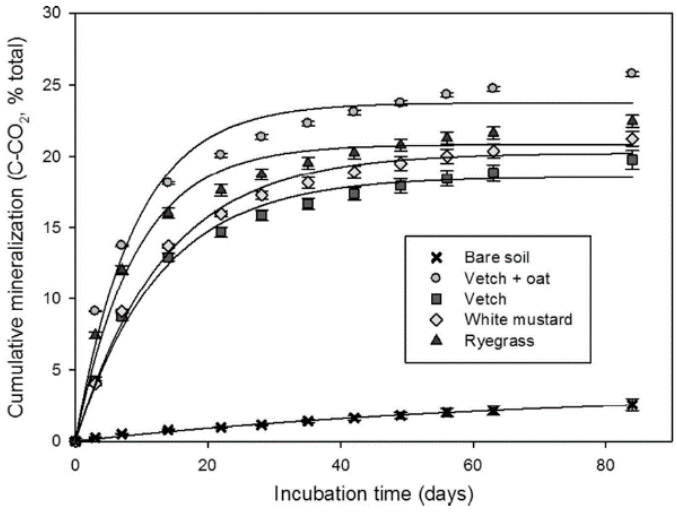
Degradation half–life modeling.

The percentage of glyphosate in the extractable fraction in the microcosm (corresponding to the extractable fraction in the mulch + the extractable fraction in the underlying soil) was fitted to a single first–order kinetic model with untransformed data, C(t) = C0e–kt where C(t) is the measured concentration in glyphosate at time t, C0 is the initial concentration measured immediately after application, and k is the first–order rate constant (day−1). Following this model, the degradation half–life (DT50), time (days) for 50 % disappearance of the initial amount of glyphosate, was calculated for C = C0/2 and corresponds to the ln 2/k ratio.

Data analysis

Data handling and modeling – The radioactivity measured in the different glyphosate fractions was expressed as a percentage of the initially applied radioactivity. Cumulative net CO2–C and 14CO2–C mineralization were fitted to an exponential model that describes mineralization at incubation time t as aMIN ∗ (1–exp.(–kMIN ∗ t)). The parameters αMIN and kMIN describe the maximum % C mineralized, and the rate at which it is reached, respectively. The kinetics data for extractable and non–extractable fractions proportions were fitted to an exponential model with 2 (y = aEXT ∗ exp.(–kEXT ∗ t)) and 3 (y = y0 + aNER ∗ (1–exp.(–kNER ∗ t))) parameters respectively. In the former case, αEXT is the initial extractable proportion and kEXT the rate of decrease, and in the latter case y0 is the initial NER proportion, aNER the direction of variation, increase or decrease in NER and kNER the rate of NER variation.

Figure 8.1.1.2-9: Organic carbon mineralization. Error bars represent the standard error of the mean of 3 replicates



Statistical analysis

Analyses of variance were performed to ascertain whether each glyphosate fraction proportion was influenced by the incubation time, the treatment or the compartment (soil or mulch) at/on which it was measured. Then, for each fraction, a Fisher's LSD test was used to rank the treatments or compartments.

Additionally, an analysis of the correlations between the different glyphosate fractions was carried out at the column and compartment level, with treatments considered together and alone. Parameters of the different kinetic models were also subjected to analysis of variance and post–hoc LSD Fisher test to rank the different treatments, with a level of significance set at 0.05.

Results

Adsorption

Sorption was significantly higher on soil than on cover crop residues (Figure 8.1.1.2-8). Kd was 53 and 8 times higher on soil than on fresh and decomposed (56–d) CC residues, respectively. Furthermore, the statistical analysis performed within the CC revealed a significant effect of both decomposition degree and CC type. Sorption increased with the decomposition degree of cover crops ($p < 0.0001$), Kd and Koc for decomposed CC (56 d) being on average 8 or 9 times higher than those measured on fresh CC (0 d). Kd was significantly higher on white mustard than on other CC for 6– and 56–d old CC, being 57 L/kg and 75 L/kg while other CC averaged 28 and 50 L/kg, respectively. For 28–d old CC, Kd was significantly higher on ryegrass (99 L/kg) than on vetch (39 L/kg), other CC being intermediate (66 L/kg). In CC, the analysis of correlations between sorption coefficients and organic matter descriptors did not show any significant relations for Kd. Koc was inversely correlated with the hemicellulose–like fraction ($r = -0.55$, $p < 0.05$).

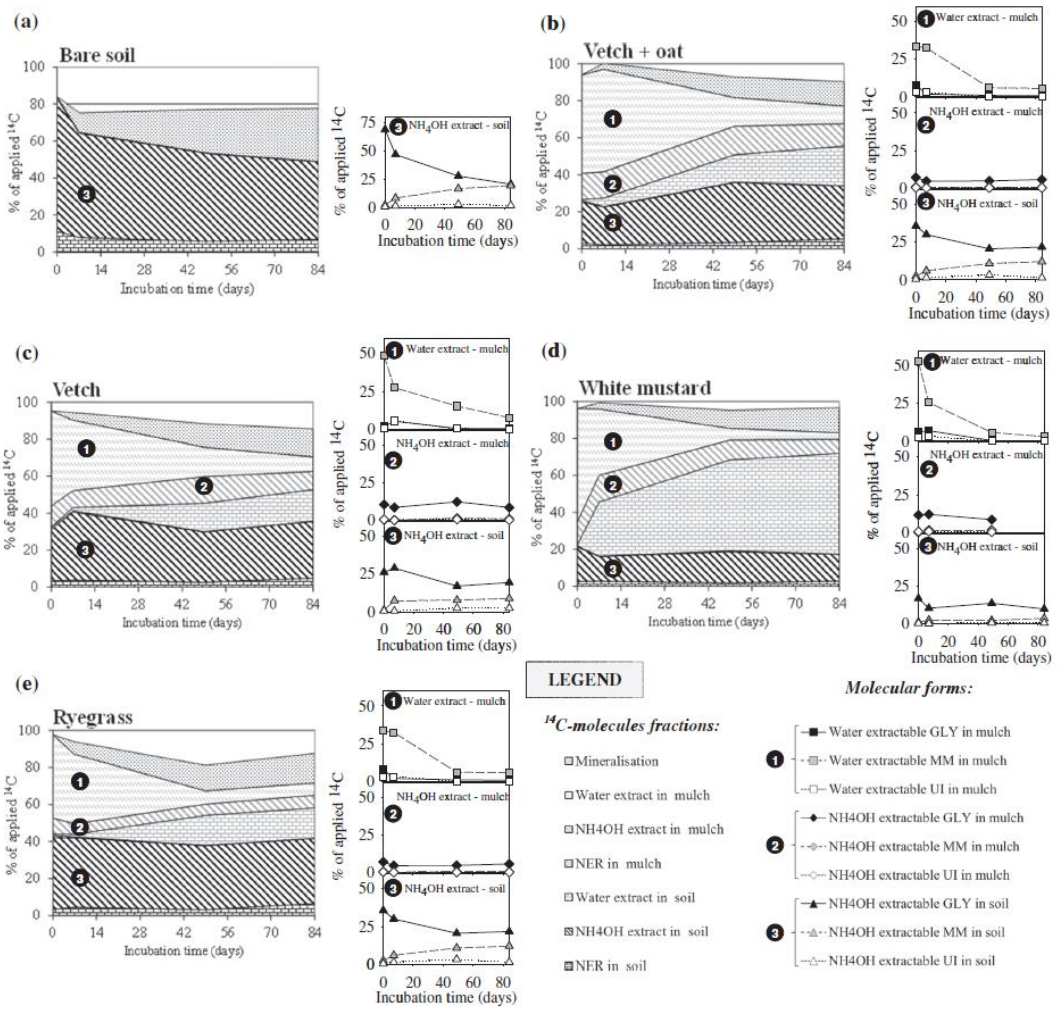
Degradation study

Mulch characteristics during incubation – During the whole incubation period, 2.6 % of the microcosms' carbon content was mineralized from the bare soil. In CC–amended soils, carbon mineralization ranged from 19 % (vetch) to 25 % (vetch + oat) (Figure 8.1.1.2-9), corresponding to a weight loss of approximately 35 %. Water content remained constant, being 17 % (w:w) in the soil and 60–72 % according to the mulch (data not shown).

Variability across intercepting material, plant type and time – Glyphosate recovery in the microcosms averaged 90 % of the initially applied dose (Figure 8.1.1.2-10). 14C glyphosate fractions were significantly influenced by time, intercepting material (i.e. decaying residue or soil) and plant type (i.e. cover crop species).

Mineralized fraction – Glyphosate mineralization started immediately after application, without any lag phase. It fitted the chosen exponential model well ($R2 > 0.95$), with parameter kMIN, the speed at which the maximum is reached, and a, the maximum value reached. Treatments differed significantly from each other for the parameter aMIN, with a higher cumulative glyphosate mineralization in the bare soil microcosms, compared to the mineralisation in the CC–amended microcosms, with values ranging from 13.0 to 15.8 %. Analysis of the plant type effect showed that the maximum mineralized glyphosate was reached significantly faster in ryegrass.

Figure 8.1.1.2-10: Fate of glyphosate in the microcosms. Letters indicate the treatment (a: bare soil, b: vetch + oat, c: vetch, d: white mustard, e: ryegrass) and numbers 1, 2 or 3 indicate the fraction within which molecular forms were analyzed (water- or NH4OH–extractable in mulch and/or soil). Results are expressed as % of applied ^{14}C



Extractable fraction – Total extractable fraction (corresponding to the water and NH4OH extracts) was well fitted by the chosen exponential model, with R2 >0.8 and R2 = 0.65 for CC–amended and bare soil treatments respectively. The extractable fraction decreased over time for all treatments. Differences were observed between the treatments, extractability falling faster in white mustard than in other treatments (param bEXT). At the end of the experiment, significantly less glyphosate was extractable in the white mustard treatment. The extractable fraction is separated into a water–extractable and an ammonia–extractable fraction, for which more details of molecular forms are given below. The water–extractable fraction decreased rapidly from 52.7 ± 3.4 to 7.0 ± 1.3 % of the applied 14C between 0 DAT and 84 DAT in the mulch compartment while it remained low in the soil compartment (<1 % of applied 14C). A larger proportion of 14C was extracted with water in the vetch + oat microcosms, until 49 days of incubation. In mulches, more metabolites than glyphosate (GLY) were found in the water extracts. Both GLY and its main metabolite decreased during incubation, averaging 7.9 ± 2.7 % to 0.9 ± 0.3 % and 45.0 ± 4.7 % to 6.7 ± 1.2 % of the applied 14C between 0 DAT and 84 DAT, respectively. The ammonia–extractable fraction remained stable with time in the mulch compartment and varied with no clear trend in the soil compartment while it decreased during incubation in the bare soil treatment (Figure 8.1.1.2-10, fractions ❷ and ❸). In the bare soil treatment, GLY proportions decreased from 70 to 20 % of the applied dose between 0 DAT and 84 DAT, whereas MM proportions increased from 2 to 19 % in the same period (Figure 8.1.1.2-10a). In all CC–amended treatments, the GLY proportion decreased from an average of 26.5 ± 4.7 % to 14.4 ± 2.1 % and from 11.9 ± 0.1 % to 8.8 ± 1.3 % in soil and in mulch compartments between 0 DAT and 84 DAT, respectively. Meanwhile, MM proportion (i) increased from 1.5 ± 0.3 % to 8.5 ± 1.9 % in soils and (ii) averaged 1.01 ± 0.15 % in mulches.

Table 8.1.1.2-50: Fraction–dynamics model parameters. Letters correspond to LSD groups

Fraction	Treatment	y_0			a_i (i = MIN, EXT, NER)			k_i (i = MIN, EXT, NER)		
		Value	Effect of mulch vs. bare soil	Effect of plant type	Value	Effect of mulch vs. bare soil	Effect of plant type	Value	Effect of mulch vs. bare soil	Effect of plant type
Mineralized fraction	Bare soil	–	–	–	27.1 ± 0.9	a	–	0.06 ± 0.01	ab	–
$y = a * (1\text{-exp.}(-kt))$	Vetch + oat	–	–	–	15.0 ± 2.5	b	ns	0.04 ± 0.01	b	b
	Vetch	–	–	–	15.8 ± 1.4	b	ns	0.04 ± 0.01	b	b
	White mustard	–	–	–	13.0 ± 0.4	b	ns	0.04 ± 0.01	b	b
	Ryegrass	–	–	–	15.1 ± 0.2	b	ns	0.08 ± 0.01	a	a
Extractable fraction	Bare soil	–	–	–	66.5 ± 0.6	b	–	0.01 ± 0.00	b	–
$y = a * \exp.(-kt)$	Vetch + oat	–	–	–	92.2 ± 0.5	a	ns	0.00 ± 0.00	b	b
	Vetch	–	–	–	89.6 ± 0.8	ab	ns	0.01 ± 0.00	b	b
	White mustard	–	–	–	84.9 ± 7.8	ab	ns	0.02 ± 0.00	a	a
	Ryegrass	–	–	–	89.0 ± 0.8	ab	ns	0.01 ± 0.00	b	b
Non-extractable fraction	Bare soil	8.9 ± 1.0	a	–	−2.7 ± 0.9	c	–	0.12 ± 0.04	a	–
$y = y_0 + a * (1\text{-exp.}(-kt))$	Vetch + oat	4.1 ± 1.0	b	ns	59.8 ± 27.7	a	ns	0.01 ± 0.01	b	b
	Vetch	3.1 ± 0.7	b	ns	28.2 ± 6.3	b	ns	0.02 ± 0.01	b	b
	White mustard	3.4 ± 1.0	b	ns	53.2 ± 2.3	ab	ns	0.05 ± 0.01	b	a
	Ryegrass	3.6 ± 0.9	b	ns	31.1 ± 5.9	ab	ns	0.02 ± 0.01	b	b

Non–extractable fraction – The NER fraction increased with time for CC–amended treatments, especially in the mulch compartment. On the contrary, NER decreased in the bare soil from 11 to 7 % of the applied dose between 0 and 84 DAT (a <0). By comparison, in the soil compartment below the mulch NER increased from 3 to 5 % or remained constant (white mustard). At the end of the experiment, three statistical groups differing in their NER proportions were distinguished: (i) white mustard with 59.7 ± 2.8 %, (ii) the 3 other mulches with 27.2 ± 0.8 % and (iii) bare soil with 9.0 ± 1.1 % of the initially applied 14C. The modeling of NER formation showed that NER formation rate was significantly greater in white mustard (kNER parameter) than in other mulches.

Glyphosate degradation half–life – In presence of a cover crop mulch, glyphosate degradation half–life was longer than in bare soil, being respectively 28–47 days and 20 days. DT50 values showed that glyphosate persistence was increased in the presence of a mulch layer at the soil surface, whatever the type of mulch.

Table 8.1.1.2-51: Glyphosate half–life (DT50) calculated from fitting of experimental data to C = C0.e–kt model. Data are mean ± standard–error

Treatment	DT_{50} (days)	LSD group	R^2
Bare soil	21 ± 1	*b*	0.91
Vetch + oat	28 ± 10	*ab*	0.80
Vetch	47 ± 4	*a*	0.74
White mustard	43	*ab*	0.77
Ryegrass	38 ± 5	*ab*	0.73

Correlation between processes – Considering all treatments, glyphosate mineralization was (i) positively correlated with carbon mineralization (r = 0.80 for CC– amended and r = 0.99 for bare soil) and non–extractable fraction (r = 0.54 for CC–amended and r = 0.99 for bare soil, p <0.05); (ii) negatively correlated with water–extractable fraction (r = −0.69 for CC–amended and r = −0.97 for bare soil, p <0.01). Furthermore, NER fraction was (i) positively correlated with mineralized glyphosate fraction (r >0.96, p <0.01) and carbon mineralization (r = 0.55 for CC–amended and r = 0.99 for bare soil, p <0.05) and (ii) negatively correlated with water–extractable fraction (r <−0.96, p <0.05) in bare soil and all CC–treatments except vetch. Vetch specific correlations were found between the ammonia–extractable fraction and carbon (r = −0.98) and glyphosate (r = −0.99) mineralization. At the compartment level, the analysis revealed a correlation of NER formation either with water–extractable fraction in mulch (r = −0.98) or with ammonia extractable fraction in soil (r = −0.94).

Discussion

Glyphosate fate depends on the intercepting material

After application, glyphosate fate presented specificities according to the intercepting material, i.e. soil or CC mulch. It was much strongly retained by soil than by mulch, being mainly extractable with ammonia and with water, respectively. These results are in agreement with the sorption measurements (Figure 8.1.1.2-8) and are mainly explained by the sorption affinity of glyphosate to soil mineral constituents (clays, oxides). Furthermore, despite a high microbial activity in the mulches, reflected by the

carbon mineralization (Figure 8.1.1.2-9), glyphosate mineralization in the presence of mulch was lowered as compared to the bare soil treatment. These observations partly suggest a difference in glyphosate accessibility to microorganisms in the two compartments. In soil, although glyphosate is strongly retained, as stated above, the herbicide remains accessible for a complete biological degradation. These results are in agreement with those of Schnurer et al. (2006) who observed biodegradation of soil–sorbed glyphosate. In mulches, the absence of change in the molecular forms with time in the ammonia extracts (Figure 8.1.1.2-10, fraction ❷) suggests that mulch–sorbed molecules were not available for microorganisms. In contrast, soluble glyphosate and its degradates are available in the mulch–water extracts, as shown by the decrease in their respective proportion (Figure 8.1.1.2-10, fraction ❶). However, microbial populations that colonize the decaying mulch are not as efficient as soil microbial population in mineralizing glyphosate. NER formation is generally considered to be the result of either microbial incorporation of pesticide, physical entrapment in the nanoporosity, chemical stabilization by bounding, or diffusion to less accessible sites during a long period of contact. In this study, NER formation was positively correlated to glyphosate mineralization by microorganisms in both soil and mulch compartments. As the mulch compartment is prone to a higher microbial activity (Figure 8.1.1.2-9), NER formation is clearly one of the main dissipation pathways of glyphosate in mulches, while it is a minor pathway for soils. In our study, NER proportion was negatively correlated either to sorbed (ammonia extracted) glyphosate fraction in soil or to soluble (water extracted) glyphosate fraction in mulches. In CC mulches, the decrease with time in soluble glyphosate is combined with a weak mineralization and its nearly constant sorbed proportion (recovery of glyphosate in the ammonia fraction). This supports the hypothesis of a direct transfer from the 'soluble' to the 'NER' fraction.

Glyphosate fate as influenced by the nature of the intercepting plant material

Glyphosate fate in the mulch compartment is similar whatever the mulch, i.e. the time evolutions of different fractions are generally similar. However, two of the four cover crop species stand out from the others. Glyphosate was less mineralized in ryegrass than in other cover crops, which we cannot explain, and NER formation is maximal in white mustard. This latter result was not expected but can be explained in view of the results of the sorption study where white mustard was the mulch which maximized sorption at day 6 and 56.

Glyphosate fate in cover crop residues and environmental risk assessment

In this study, glyphosate fate was studied at a fine scale by considering several fractions. The results can be interpreted at a broader scale by considering only two fractions: (i) the dissipated glyphosate i.e. the glyphosate mineralized as CO2 and immobilized as NER; and (ii) the available glyphosate and metabolites i.e. the molecules which remain available and could be leached in field conditions. At this scale, except for the white mustard treatment, both dissipated and available glyphosate were statistically the same in all treatments. This does not lead to the conclusion that glyphosate fate is not influenced by the presence of a cover crop since (i) dissipation pathways are treatment–specific, i.e. mineralization and metabolites (AMPA) formation are greater in bare soil and more non–extractable residues are formed in CC–amended treatments; and (ii) the NER formation pathway in mulch is time–dependent, leading to a potential decrease in availability of glyphosate in CC–amended treatment. According to the mechanisms potentially involved in NER formation routes we have proposed for mulch, such release cannot be excluded. The extent to which these results can be extrapolated to field conditions will be determined by (i) weather conditions, especially during the time between application and the first rain and the temperature; (ii) agricultural practices, especially cover crop incorporation and fertilization; and (iii) mulch biomass, coverage, and contact with soil as well as soil type.

Conclusions

This study aimed at evaluating the effects of a mulch of cover crop residues located at the soil surface on the environmental behavior of glyphosate. In the presence of a cover crop mulch, glyphosate and its metabolite remained mainly water–soluble, but with time, a higher proportion of the herbicide became non–extractable. Unlike in soil conditions, bound residue formation was the main process involved in glyphosate dissipation in cover crop mulches. Variations in the intensity of each process were observed among the four cover crop residues studied, but remained unexplained by the biochemical composition of the residues. Finally, degradation half–life of glyphosate was increased with all type of mulches.

Assessment and conclusion by applicant:

The article describes investigations on the degradation and adsorption of Glyphosate to soil under the potential influence by cover crops. The article is well described and provides potential endpoints for degradation and sorption. However, the available information does not allow to check the validity against current guidelines, and not enough parameters are provided to evaluate the kinetic behavior.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

This experimental study seems to be well performed. It focuses on the impact of cover crop on the degradation and adsorption of glyphosate but presents also bare soil conditions for comparison. However the available information does not allow to check the validity against current guidelines (*e.g.* raw residues, mass balances and stability of glyphosate are not reported).

The article provides supportive information on the degradation and adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Norgaard, T. et al

Data point:	CA 7.1.2.1.1/012
Report author	Norgaard, T. et al.
Report year	2015
Report title	Can Simple Soil Parameters Explain Field-Scale Variations in Glyphosate-, Bromoxyniloctanoate-, Diflufenican-, and Bentazone Mineralization?
Document No	DOI 10.1007/s11270-015-2518-z ISSN 0049-6979
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The large spatial heterogeneity in soil physico-chemical and microbial parameters challenges our ability to predict and model pesticide leaching from agricultural land. Microbial mineralization of pesticides is an important process with respect to pesticide leaching since mineralization is the major process for the complete degradation of pesticides without generation of metabolites. The aim of our study was to determine field-scale variation in the potential for mineralization of the herbicides glyphosate, bromoxyniloctanoate, diflufenican, and bentazone and to investigate whether this variation can be predicted by variations in basic soil parameters. Sixty-five soil samples were sampled from an agricultural, loamy field in Silstrup, Denmark, from a 60×165 m rectangular grid. The mineralization potential of the four pesticides was determined using a 96-well microplate 14C-radiorespirometric method. Initial mineralization rates were determined using first-order kinetics for glyphosate and bromoxyniloctanoate and zero-order kinetics for diflufenican and bentazone. The mineralization rates of the four pesticides varied between the different pesticides and the different soil samples, but we could not establish correlations between the pesticide mineralization rates and the measured soil parameters. Only the glyphosate mineralization rates showed slightly increasing mineralization potentials towards the northern area of the field, with increasing clay and decreasing OC contents. The mineralization potentials for glyphosate and bentazone were compared with 9-years leaching data from two horizontal wells 3.5 m below the field. The field-scale leaching patterns, however, could not be explained by the pesticide mineralization data. Instead, field-scale pesticide leaching may have been governed by soil structure and preferential flow events.

Materials and Methods

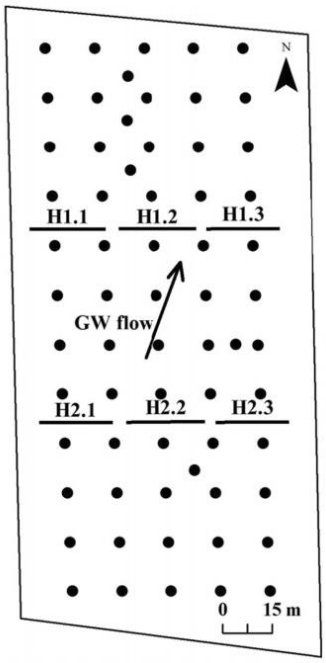
Field Site

The agricultural test field (Silstrup, northwestern Jutland, Denmark) was a conventionally cultivated, loamy field with a cultivated area of 1.69 ha. The climate is coastal, cold temperate. The field has been cultivated as part of a routine agricultural practice with management and pesticide records dating back to 1983. Glyphosate was sprayed on the field five times since 1983, bentazone was sprayed four times, bromoxyniloctanoate only once, and diflufenican not at all. Application dates for the pesticides and their commercial formulations are shown in the tables. Two horizontal wells, H1 and H2 (Figure 8.1.1.2-11), are located 3.5 m below the surface, and each consists of three screen sections of 18 m. Water samples from the middle screen section of each well (H1.2 and H2.2) have been analyzed for pesticides every month, and the samples from the outer screen sections (H1.1, H1.3, H2.1, and H2.3) have been analyzed twice a year (Rosenbom et al. 2010). During 9 years screening (2000–2009), pesticides were detected in 44 % of the water samples from the middle section of the northern horizontal well (H1) whereas only 5 % of the water samples from the middle screen section of the southern horizontal well (H2) contained detectable pesticide concentrations. In the outer screen sections of the northern well (H1.1 and H1.3), pesticides were detected in 30 and 27 % of the water samples whereas there were no pesticide detections in the outer screen sections of H2 (Norgaard et al. 2012). Consequently, pesticides seem to leach only from the northern part of the field.

Table 8.1.1.2-52: Pesticide application history. There is no record of which Roundup formulation was applied in 1988 and 1999

	Application date	Formulation
Glyphosate	25 October 1988	Roundup (unknown)
	10 October 1994	Touchdown
	5 August 1999	Roundup (unknown)
	25 October 2001	Roundup Bio
	15 September 2003	Roundup Bio
Bromoxyniloctanoate	20 April 1999	Oxitril
Bentazone	24 May 1994	Basagran 480
	17 June 1994	Basagran 480
	17 May 2003	Basagran 480
	19 May 2009	Fighter 480

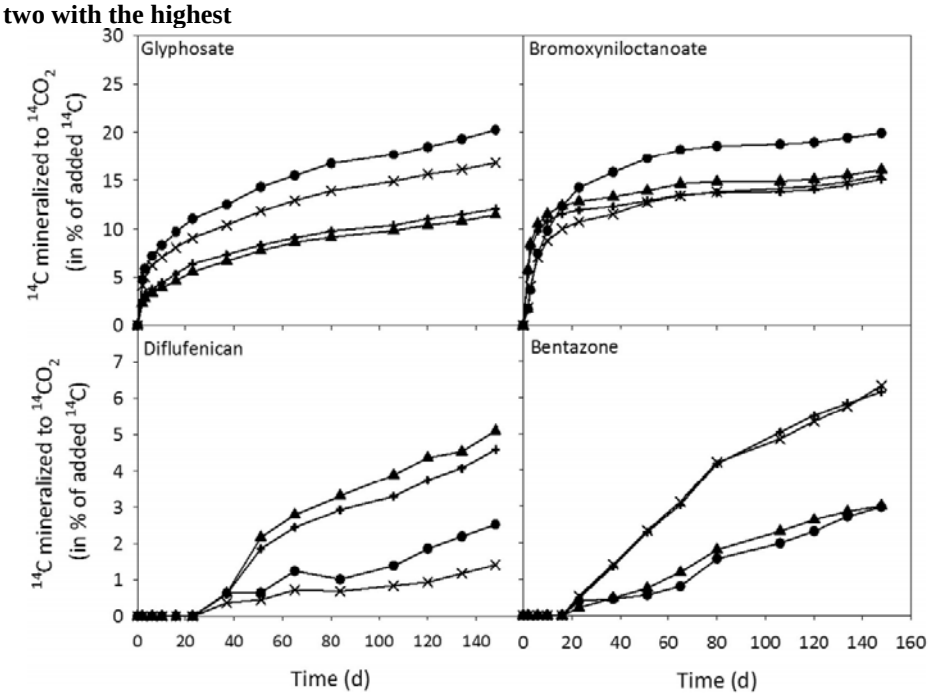
Figure 8.1.1.2-11: Schematic presentation of the Silstrup field. Sample positions are indicated by the black dots. The horizontal wells (H1 and H2) and the screen sections in each well are indicated by the lines. The arrow indicates the groundwater flow direction



Soil Sampling

Sixty-five samples were sampled from a 60 × 165 m rectangular field with a distance of 15 m between sampling points (Figure 8.1.1.2-11) on 6 December 2011. Soil was sampled from the plough layer at a depth of approximately 8–16 cm. First, the upper 8-cm top soil was removed and then a sample was taken by pounding a sterile 50-ml centrifuge tube (upside down) into the ground until the tube was almost full and then the tube was sealed. In the lab, each sample was homogenized by sieving twice through a sterile 4-mm mesh. The soil was further mixed thoroughly and stored at 2 °C for 1 week.

Figure 8.1.1.2-12: Mineralization curves depicting the variation in mineralization within the field. The curves represent for each herbicide the two soil samples with the lowest initial mineralization rate and the two with the highest



Physical and Chemical Soil Analyses

Soil texture was determined according to Gee and Or (2002) using a combined sieve/hydrometer method. Organic carbon was determined on a LECO analyzer coupled with an infrared CO2 detector. Bulk density was determined from weights of 20×20 cm intact soil columns after drying at 105 °C for 2 weeks. The soil pH was measured in a soil/water suspension of 1:4 (v/v), and the soil electrical conductivity (EC) was measured in a soil/water suspension of 1:9 (v/v). Oxalate-extractable iron, aluminum, and phosphorus were determined at AGROLAB GmbH, Germany, using the procedure described by Schoumans (2000). The Dexter index (Dexter n) for each soil sample was calculated as the ratio (w/w) between clay and organic carbon.

Table 8.1.1.2-53: Basic soil parameters. Minimum, maximum, mean, and coefficient of variation (CV) of soil texture, organic carbon (OC), Dexter n, bulk density, oxalate-extractable aluminum (Al), oxalate-extractable iron (Fe), and oxalate-extractable phosphorus (P), pH, and electrical conductivity (EC)

	Clay (<2 µm)	Silt (2–50 µm)	Sand (0.05–2 mm)	OC	Dexter *n*	Bulk density	Al	Fe	P	pH	EC
	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	–	g cm^{-3}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	–	mS cm^{-1}
Min. value	0.14	0.23	0.45	0.017	6.75	1.39	24	33	7.60	6.39	0.40
Max. value	0.19	0.33	0.59	0.022	10.43	1.60	36	53	15	7.45	0.71
Mean	0.16	0.30	0.51	0.02	8.11	1.50	28.3	43.3	10.6	6.75	0.47
CV	8.4	5.5	4.2	6.9	11.7	3.4	7.7	10.4	14.2	2.2	11.0

Mineralization Potentials

The mineralization potential of the four pesticides was tested using a modified version of a radiorespirometric microplate method. [P-methylene-14C]glyphosate (>99 % radiochemical purity) was purchased from IZOTOP, Institute of Isotopes (Budapest, Hungary). Radioactive pesticide solutions (10 mg/mL, approximately 870 Bq/mL) were prepared by dissolving appropriate amounts of radioactive pesticide and the corresponding non-labeled pesticide in sterile water. For each of the 65 homogenized soil samples, subsamples of 0.5 g were transferred to microplate wells, one microplate for each of the four pesticides and one subsample per pesticide. The microplates were 96-well polypropylene microplates (Nunc 278752) with a well volume of 2.0 mL to minimize oxygen depletion. Fifty microliters of 14C-labeled pesticide solution was added to all wells, corresponding to an initial pesticide concentration of 1 mg/kg soil. The microplates were sealed with PCR sealing tapes on which 96 14CO2 traps (Ca(OH)-amended filter paper discs) were placed in a pattern corresponding to the microplate wells. Polyurethane foam sheets (the size of a microplate lid) were placed on top of the sealing tapes, microplate lids were added, and the plates and lids were held tightly together with strong rubber bands. The sealing tapes were changed after approximately 2, 3, 6, 10 16, 23, 37, 51, 65, 80, 106, 120, 134, and 148 days of incubation at 10 °C. The trapped 14CO2 from each well, captured on the Ca(OH)2-impregnated filters, was quantified from a standard series of NaH14CO3 using digital autoradiography and subsequent digital image analysis as described by Hybholt et al. (2011).

Mineralization Kinetics

A two-parameter exponential model (first-order kinetics, Eq. 1) was used to fit the mineralization curves for glyphosate and bromoxyniloctanoate.

$y = \alpha \cdot (1 - e^{-bt})$ (1)

where y is the accumulated 14CO2 (% of added 14C) released at time t (day), α is the maximum 14C mineralized (% of added 14C), and b is the mineralization rate constant (day−1).

Since we were interested in estimating the in-situ mineralization potentials, we fitted only the first 23 days of mineralization, where the mineralization followed first-order kinetics. The initial rate at time zero was then calculated from the first derivative function (Eq. 2).

$dy/dt0 = ba$ (2)

A linear regression model (Eq. 3) was used to fit the mineralization curves for diflufenican and bentazone.

$y = a + bt$ (3)

For diflufenican and bentazone, the models are based on the mineralization data from days 23–84 and 16–65, respectively. This was done in order to capture the initial, linear part of the mineralization curves from the first detection of mineralization in each of the two cases. The slope of the linear models was used as an estimate of the initial mineralization rate.

Two-Dimensional Interpolation and Statistical Analysis

The spatial, field-scale variation in soil texture, organic carbon content and the mineralization rates were mapped using minimum curvature interpolation with regularized spline interpolation in ArcMap 10.1. The number of points used in the calculation of each interpolated cell was set to 12 and the weight parameter to 0.1. The mineralization rates were correlated to soil physical and chemical parameters using the linear correlation coefficient (R2), as it shows the fraction of the variation in the mineralization potentials that can be explained by the variation in the physical or chemical soil parameters. Coefficients of variation (CVs) for the pesticide mineralization rates and the soil parameters were calculated as the standard deviation divided by the mean and are given as percentage.

Most Probable Number of Pesticide Degraders

The most probable numbers (MPNs) of cultivable glyphosate-, bromoxyniloctanoate-, diflufenican-, and bentazone degraders were estimated by a modification of the above microplate radiotracer method. To represent the gradients in clay and organic carbon across the field, selected samples were pooled into groups with high clay and low organic carbon content, low clay and high organic carbon content, and intermediate clay and organic carbon content (five to seven subsamples for each group: group A with 17.6–18.9 % clay and 1.8– 1.9 % organic carbon, group B with 14.2–14.3 % clay and 2.0–2.1 % organic carbon, and group C with 16.1– 6.2 % clay and 1.9–2.0 % organic carbon).

A well was considered mineralization-positive if the accumulated amount of 14CO2 at the end of the experiment (148 days at 10 °C) exceeded 5 % of the initially added 14C-labeled pesticide. The MPNs were calculated according to Hurley and Roscoe (1983) from the distributions of positive and negative microplate wells. The lower detection limit was calculated by assuming only one mineralization-positive well at the lowest dilution (10 - fold), and the upper limit was calculated from only one mineralization-negative well at the highest dilution (21,870-fold).

Results

Pesticide Mineralization

The MPNs of the microbial degrader populations were for glyphosate and bromoxyniloctanoate above the detection limit of 6.6×104 cells/g soil, which indicates a large potential for microbial degradation of these pesticides. This was reflected in the rapid mineralization without any-lag phase of both glyphosate and bromoxyniloctanoate (Figure 8.1.1.2-12). After a fast, immediate phase, the mineralization of glyphosate leveled off at 10–20 % and bromoxyniloctanoate at 13–26 %. Diflufenican and bentazone both showed slow linear mineralization with a lag-phase, and both pesticides reached very low mineralization levels (diflufenican 1–5 %, bentazone 3–7 %) within the 148 days of the experiment (Figure 8.1.1.2-12). The first mineralization was detected on day 23 for bentazone and on day 37 for diflufenican. We did not detect any microorganisms that could utilize diflufenican or bentazone as a source of carbon and energy (MPN <4 cells/g soil), which probably explains the long lag-phases in the mineralization of these two herbicides. The mineralization of bromoxyniloctanoate showed the best model fits (R2 = 0.980–0.996, average 0.992), whereas the glyphosate mineralization was slightly underestimated within the first 3 days and slightly overestimated the following 13 days (R2 = 0.933–0.987, average 0.968). The model fits for diflufenican mineralization (R2 = 0.734–0.995, average 0.963) and bentazone mineralization (R2 = 0.850–1.00, average 0.992) were more variable.

Field-Scale Variation in Pesticide Mineralization Rates

The spatial variability of the initial mineralization rates, derived either from the initial rate at time zero for glyphosate and bromoxyniloctanoate or as the slope of the linear regression models for diflufenican and bentazone, is depicted in Figure 8.1.1.2-14. Throughout the field, the initial glyphosate mineralization rates varied from 12.1 to 26.0 µg/(kg day) (average 17.1 µg/(kg day), CV=16.7 %), with a slight indication of lower mineralization in the southern part of the field. Bromoxyniloctanoate had the largest initial mineralization rates varying from 14.9 to 42.0 µg/(kg day) (average 29.6 µg/(kg day), CV=16.5 %).

Diflufenican and bentazone showed very limited mineralization of only 0.11– 0.58 µg/(kg day) (average 0.32 µg/(kg day), CV= 24.7 %) and 0.13–0.64 µg/(kg day) (average 0.47 µg/(kg day), CV= 22.4 %).

Figure 8.1.1.2-13: Examples of herbicide mineralizations and the corresponding model fits. The data represent for each herbicide the two soil samples with the lowest initial mineralization rate and the two with the highest initial mineralization rate within the fitted time period

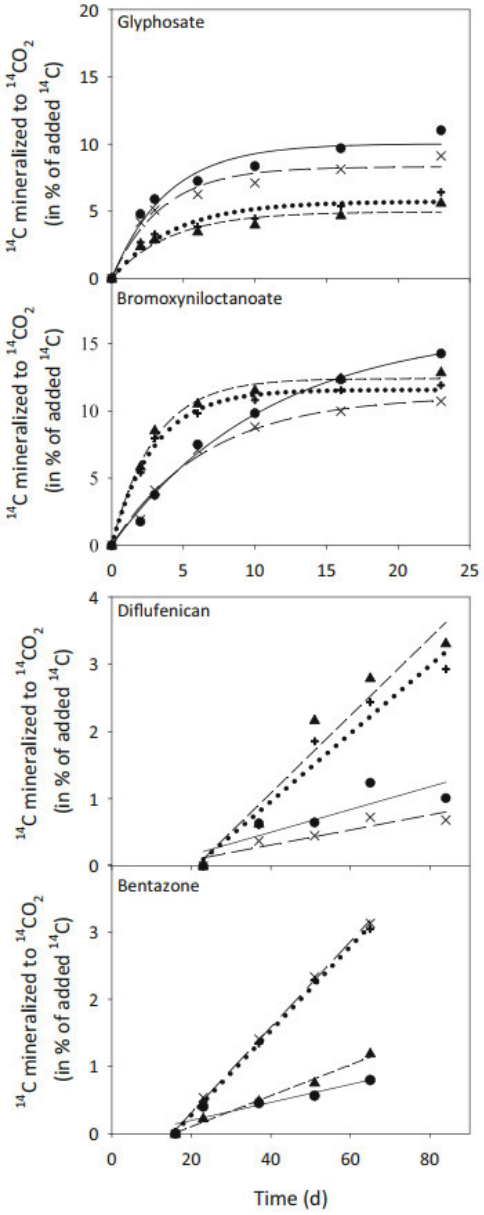


Figure 8.1.1.2-14: Spatial distributions of herbicide mineralization rate, clay-, silt-, sand-, and organic carbon (OC) content, and Dexter n (clay/OC ratio). The dots denote the sampling points (n=65)

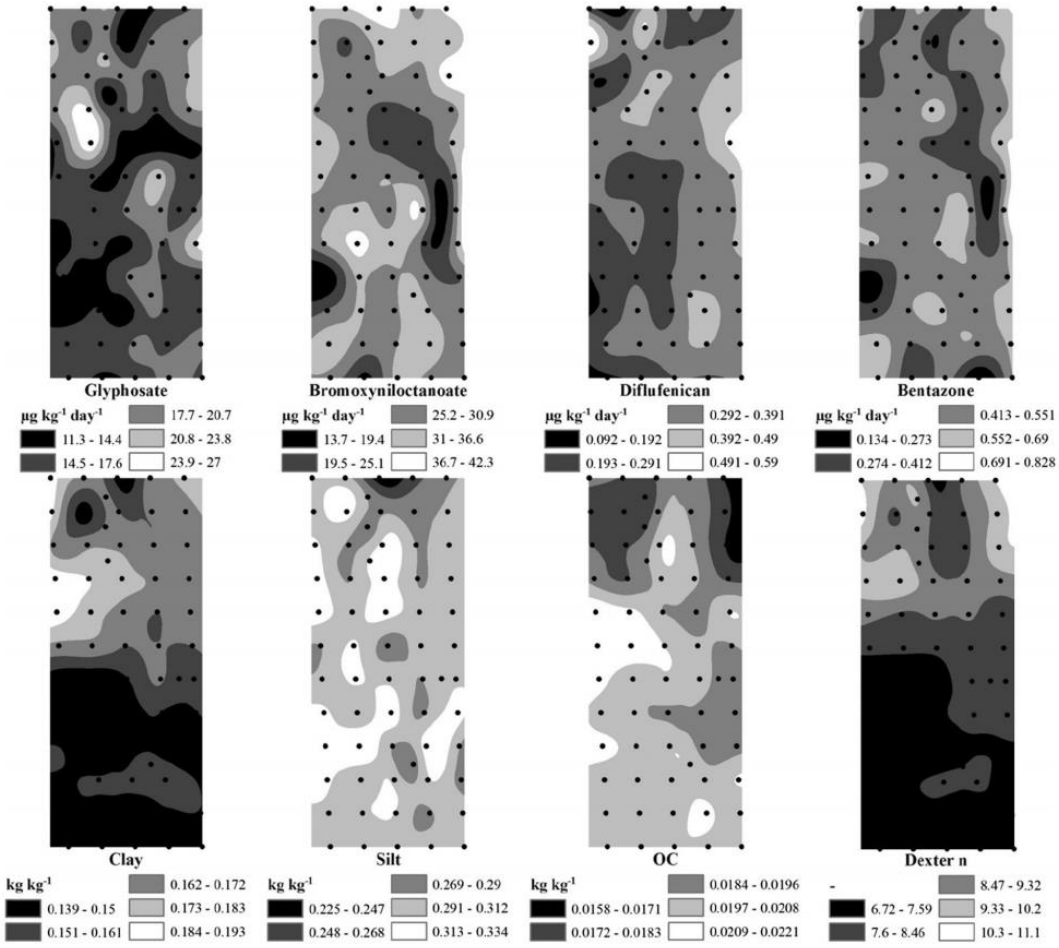


Table 8.1.1.2-54: The linear correlation coefficients (R2) between the pesticide mineralization rates and the basic soil parameters

	Clay (<2 µm)	Silt (2–50 µm)	Sand (0.05–2 mm)	OC	Dexter *n*	Bulk density	Al	Fe	P	pH	EC
Glyphosate	0.09	0.01	0.00	0.11	0.17	0.06	0.06	0.03	0.10	0.00	0.00
Bromoxyniloctanoate	0.01	0.10	0.04	0.04	0.04	0.01	0.08	0.04	0.05	0.05	0.01
Diflufenican	0.06	0.02	0.00	0.06	0.10	0.03	0.01	0.01	0.07	0.00	0.00
Bentazone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

Mineralization Rates and Soil Characteristics

The range, mean, and CV for the measured soil parameters are reported in the tables. Gradients in clay and organic carbon content run in opposite directions within the field. Thus, highest clay contents and lowest organic carbon contents were found in the northern part of the field and lowest clay and highest organic carbon contents were found in the southern part of the field (Figure 8.1.1.2-14). The ratio between clay and organic carbon, Dexter n, therefore increased from south to north (Figure 8.1.1.2-14). The mineralization rates for each of the four pesticides generally showed no correlation or very little correlation to the soil parameters. The highest correlation was between the glyphosate mineralization and the Dexter n, but this correlation was also weak (R2=0.17). Linear correlations between the mineralization rates of the four pesticides are reported below. The correlation coefficients are weak and the strongest correlation was between the mineralization rates of bromoxyniloctanoate and bentazone (R2=0.16).

Table 8.1.1.2-55: The linear correlation coefficients (R2) between the pesticide mineralization rates

	Glyphosate	Bromoxyniloctanoate	Diflufenican	Bentazone
Glyphosate	1.00	0.07	0.08	0.01
Bromoxyniloctanoate		1.00	0.02	0.16
Diflufenican			1.00	0.03
Bentazone				1.00

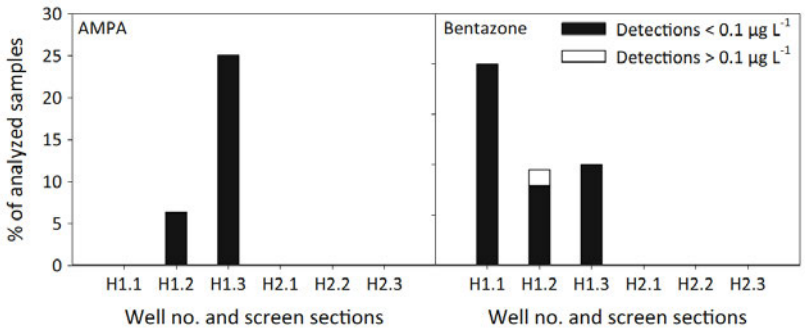
Table 8.1.1.2-56: Number of groundwater samples from H1 and H2 analyzed for glyphosate, aminomethylphosphonic acid (AMPA), bentazone, and 2-amino-N-isopropylbenzamide

	Number of samples, *n*					
	H1.1	H1.2	H1.3	H2.1	H2.2	H2.3
Glyphosate	8	48	8	10	46	8
AMPA	8	48	8	10	46	8
Bentazone	10	63	10	7	46	6
2-Amino-*N*-isopropylbenzamide	4	29	4	4	29	4

Field-Scale Leaching

Water from the two horizontal wells (H1 and H2, Figure 8.1.1.2-11) was analyzed for glyphosate and bentazone and their main metabolites aminomethylphosphonic acid (AMPA) and 2-amino-N-isopropylbenzamide. Glyphosate was applied five times on the field during the period from 1988 to soil sampling in 2011 with two applications within the monitoring period (2001 and 2003). The glyphosate and AMPA contents in water from different subsections of H1 and H2 were analyzed from 2001 to 2005. Glyphosate was not detected in any of the samples. The glyphosate degradation product AMPA, however, was detected in 6.3 % of the analyzed samples from H1.2 and 25 % of the analyzed samples from H1.3 (Figure 8.1.1.2-15). None of the AMPA concentrations exceeded the drinking water quality criterion of 0.1 µg/L. Bentazone was applied four times on the field from 1994 to 2011, and two of these applications were within the monitoring program (2003 and 2009). Bentazone was analyzed for in the periods from 2003 to 2006 and from 2009 to 2011. The bentazone metabolite was analyzed for only in the period from 2003 to 2006. In total, bentazone was detected in 20 % of the samples from H1, 19.5 % of the H1.2 samples, and 10 % of the H1.3 samples (Figure 8.1.1.2-15). One of the detections in H1.2 was above the criterion of 0.1 µg/L. The bentazone degradation product, 2-amino-N-isopropylbenzamide, was not detected in any of the analyzed samples. The metabolite, however, was analyzed for only in the period from 2003 to 2006, whereas bentazone was analyzed for in the periods from 2003 to 2006 and from 2009 to 2011. The horizontal monitoring well, H2, was suspended from 2009.

Figure 8.1.1.2-15: Percentage of samples AMPA Bentazone from the two horizontal wells, H1 and H2 (Figure 8.1.1.2-11), containing detectable levels of the glyphosate degradation product AMPA or bentazone. Water was collected monthly (H1.2 and H2.2, n=29–63) or half yearly (remaining filter sections, n=4–10)



Discussion

In this study, we have investigated the potential mineralization of four herbicides commonly used in agriculture (Miljøstyrelsen 2014). These herbicides represent different physico-chemical properties with very different literature reports on hydrophobicity and sorption. Glyphosate was an easily mineralized, hydrophilic compound with strong sorption to clay loam. Bromoxyniloctanoate was also easily mineralized and strongly sorbing, but hydrophobic. Diflufenican was difficult to mineralize, hydrophobic, and strongly sorbing, and bentazone was also difficult to mineralize, in spite of being hydrophilic with low sorption.

It is clear from the above that bioavailability, expressed as the soil/water distribution coefficient Kd, did not determine the different mineralization patterns between the four herbicides. One reason could be that we added the Tween-80 detergent to the solutions of bromoxyniloctanoate and diflufenican to be able to handle these compounds in aqueous solution. Also, bentazone was not mineralized to any great extent in spite of high bioavailability, which suggest a microbiological limitation rather than a physico-chemical limitation. Glyphosate, in contrast, was easily mineralized in spite of a high distribution coefficient and thus low bioavailability, indicating that sorption may be less important when degraders are very numerous in the soil.

We used first-order kinetics to quantify the mineralization of glyphosate and bromoxyniloctanoate for the first 23 days. Linear regression was used to quantify the mineralization of diflufenican and bentazone covering the time periods 23–84 and 16–65 days, respectively. The linear mineralization patterns indicate that these pesticides were probably mineralized by slow co-metabolic metabolism without growth of the degrader organisms, which is consistent with the absence of bacteria that could utilize them for growth. The 2–3-week delay in mineralization may imply that the degrader organisms were fungi.

The mineralization potentials of bromoxyniloctanoate, diflufenican, and bentazone did not correlate with the gradients in clay and organic carbon across the field or any other of the measured soil parameters. Only the glyphosate mineralization rates tended to increase towards the northern part of the field, correlating slightly with increasing clay and decreasing organic carbon contents . The highest correlation was, however, between the glyphosate mineralization and Dexter n, so that it was the ratio between clay and organic carbon more than the total contents that influenced the glyphosate mineralization.

Our results indicate that the development of generally valid models for predicting pesticide mineralization across field sites, based on simple soil characteristics and in-vitro mineralization rates, may be unrealistic. Furthermore, if the mineralization of two or more of the herbicides were determined by the same soil parameters, we would have seen correlations between these herbicides, which were not the case. It seems difficult to connect pesticide mineralization (or degradation) and specific topsoil parameters, but what about pesticide mineralization and leaching? Though included in the analyses, we did not detect glyphosate in the samples from the horizontal monitoring wells, but the glyphosate degradation product, AMPA, was detected. In contrast, only bentazone was detected, and not the degradation product, 2-amino-Nisopropylbenzamide. All detections of AMPA and bentazone were from the H1 well that collected water from the northern part of the field. Neither AMPA nor bentazone was detected in the samples from H2 which collected water from the southern part of the field. This pattern does not correspond well with the rather random distribution of mineralization potentials of the two herbicides (Figure 8.1.1.2-14).

Conclusion

Glyphosate was an easily mineralized, hydrophilic compound with strong sorption to clay loam. Bromoxyniloctanoate was also easily mineralized and strongly sorbing, but hydrophobic. Diflufenican was difficult to mineralize, hydrophobic, and strongly sorbing, and bentazone was also difficult to mineralize, in spite of being hydrophilic with low sorption. It is clear from the above that bioavailability, expressed as the soil/water distribution coefficient Kd, did not determine the different mineralization patterns between the four herbicides. The linear mineralization patterns indicate that these pesticides were probably mineralized by slow co-metabolic metabolism without growth of the degrader organisms, which is consistent with the absence of bacteria that could utilize them for growth.

The mineralization potentials of bromoxyniloctanoate, diflufenican, and bentazone did not correlate with the gradients in clay and organic carbon across the field or any other of the measured soil parameters. Only the glyphosate mineralization rates tended to increase towards the northern part of the field, correlating slightly with increasing clay and decreasing organic carbon contents. Our results indicate that the development of generally valid models for predicting pesticide mineralization across field sites, based on simple soil characteristics and in-vitro mineralization rates, may be unrealistic. Furthermore, if the

mineralization of two or more of the herbicides were determined by the same soil parameters, we would have seen correlations between these herbicides, which were not the case. It seems difficult to connect pesticide mineralization (or degradation) and specific topsoil parameters.

Assessment and conclusion by applicant:

The article deals with investigations on mineralization in aerobic soil under laboratory and field conditions. Amongst other active substances, laboratory tests were performed with glyphosate. In parallel the leaching behavior was investigated under field conditions for the soils used in laboratory tests on mineralization.

The study did not follow guidelines in design and conduct. Moreover, the level of detail of provided data does not allow for a check of validity of the study against current guidelines. Furthermore, nor data on glyphosate content per sampling point, neither half-lives were provided.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, the available information does not allow to check the validity against current guidelines. The soils have also been treated repeatedly with glyphosate, which may influence the behaviour of the microbial biomass of the soil.

The article provides supportive information on the degradation and mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Kanissery, R. G. et al

Data point:	CA 7.1.2.1.1/013
Report author	Kanissery, R. G. et al.
Report year	2015
Report title	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate
Document No	DOI 10.2134/jeq2014.08.0331 E-ISSN 1537-2537
Guidelines followed in study	Adsorption experiment: USEPA guidelines for adsorption studies (USEPA, 2008) Degradation experiment: None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The adsorption, desorption, degradation, and mineralization of 14C-glyphosate [N-(phosphonomethyl)glycine] were examined in Catlin (a fine-silty, mixed, superactive, mesic Oxyaquic Argiudoll), Flanagan (a fine, smectitic, mesic Aquic Argiudoll), and Drummer (a fine-silty, mixed, superactive, mesic Typic Endoaquoll) soils under oxic and anoxic soil conditions. With the exception of the Drummer soil, soil aeration did not significantly alter the adsorption pattern of 14C-glyphosate to soils. Herbicide desorption was generally enhanced with anaerobiosis in all the soil types. Anoxic soils demonstrated slower microbial degradation and mineralization kinetics of 14C-glyphosate than oxic soils in all the soil types studied. Phosphate additions significantly reduced the adsorption of 14C-glyphosate to soils irrespective of soil aeration and confirmed the well-established competitive adsorption theory. The addition of soil phosphate stimulated degradation only in anoxic soils. The results from this research

highlight the importance of soil redox conditions as an important factor affecting the bioavailability and mobility of glyphosate in soils.

Materials and Methods

Chemicals

Carbon-14-glyphosate (phosphonomethyl-14C) (specifc activity: 1.85 × 109 Bq/mmol) was obtained from American Radiolabeled Chemicals. Unlabeled glyphosate (chemical purity: 99 %) was procured from Sigma Chemical Company. Organic solvents and water were of Optima grade from Fisher Scientifc and used without further purification.

Soils

The soils used were a Drummer silty clay loam, a Flanagan silt loam, and a Catlin silt loam. The moderately well drained Catlin, the somewhat poorly drained Flanagan, and the poorly drained Drummer soils occur in proximity in landscapes and form a soil catena; they thus have similar parent materials but vary in organic matter content, landscape position, and soil drainage class. The soils were collected (to a depth of 15 cm) from a field with no previous glyphosate application history at the University of Illinois Crop Sciences Research and Education Center in Urbana. All soil samples were sieved through a 2-mm screen and stored at 4 °C for 4 weeks. Relevant physical and chemical properties of the three soil types used in this study were analyzed at the A&L Great Lake Laboratories and are listed in Table 8.1.1.2-57. Each soil type had three replicates for each treatment in the subsequent experiments.

Table 8.1.1.2-57: Selected properties of the soils used in the experiment (analysis by A&L Great Lakes Laboratories, Inc.)

Soil	pH	Texture†			WHC‡	CEC§	Organic matter¶	P
		Sand	Silt	Clay				
		%				$cmol_c\ kg^{-1}$	%	$mg\ kg^{-1}$
Catlin	7.6	10	58	32	31.4	13.9	3.3	69
Flanagan	6.5	12	56	32	32.0	17.5	3.7	154
Drummer	7.1	14	46	40	33.0	23.8	4.9	81

† Determined by hydrometer method.

‡ Water holding capacity at 33 1/3 kPa (determined by porous plate/pressure apparatus).

§ Cation exchange capacity.

¶ Determined by loss-on-ignition.

Adsorption-Desorption Study

Adsorption isotherms of ^{14}C-glyphosate were determined using the batch equilibrium method for the three soil types. The initial concentrations of ^{14}C-glyphosate (0.1, 1, 5, and 10 mg/L) were prepared in 0.1 mol/L KCl solution and the adsorption experiment followed USEPA guidelines for adsorption studies. Two grams of air-dried soil was equilibrated with 10 mL of ^{14}C-glyphosate in 20-mL Teflon centrifuge tubes in a horizontal shaker (150 rpm) for 24 h (sufficient for apparent equilibrium in a preliminary study) at room temperature (25 ± 1°C). For the anaerobic treatments, 2-g portions of the soil samples contained in the Teflon centrifuge tubes were flooded with sterile, deoxygenated water. Preliminary studies revealed that <5 % of added glyphosate was degraded during 24 h of contact with any of the soils used (data not shown). The tubes were flushed with N_2 gas, sealed, and incubated in an anaerobic chamber from Coy Laboratory Products containing a primary headspace of $N_{2(g)}$ with 5 % $CO_{2(g)}$ and <2 % $H_{2(g)}$ at room temperature (25 °C) for 2 wk to allow reduction. To study anaerobic adsorption, ^{14}C-glyphosate was added, using a concentrated O_2-free stock solution, to the reduced (anoxic) soil to attain final concentrations of 0.1, 1, 5, and 10 mg/L. Sealed tubes were equilibrated on a shaker inside the anaerobic chamber for 24 h, where the O_2 was maintained at zero concentration. The effect of phosphate addition on glyphosate adsorption in oxic and anoxic soils was examined by incorporating $CaHPO_4$ into the Catlin, Flanagan, and Drummer soils at an overwhelming concentration (500 mg/kg soil), followed by thorough mixing of the soils before the addition of the herbicide.

At the end of the equilibration period, the soil suspension was centrifuged (15 min, 12,000 × g) and aliquots removed from each tube for a radioactivity assay using a Packard Tri-Carb (1900TR) scintillation counter. Controls (treatment without herbicide) were included for calibration and background correction purposes.

The amount of ^{14}C-glyphosate adsorbed to the soil was calculated based on the difference between the initial and final concentrations of herbicide in the solution.

Following equilibration and removal of 5 mL of the initial 10 mL of supernatant, herbicide desorption from the soil was estimated by adding equal amounts of fresh 0.1 mol/L KCl solution to the centrifuge tubes, dispersing the soil aggregates by vibration, and shaking for 24 h. Sampling from the anaerobic soil treatments was handled inside the anaerobic chamber. Soil samples were centrifuged (15 min, 12,000 × g), and an aliquot of the supernatant was removed and analyzed utilizing the radioactivity assay. The desorption process was repeated four times. Desorption was estimated by determining the amount of herbicide (described below) in the soil solution following equilibration and calculated by subtracting the amount of herbicide remaining on the soil surface.

Degradation Study

Microcosm Preparation

Soil incubations were performed for 56 d under reduced (anoxic) or oxidized (oxic) conditions using serum bottle microcosms to determine the degradation kinetics of ^{14}C-glyphosate. No degradation was detected in aqueous or organic stock solutions of glyphosate during the experiment.

Anaerobic incubations: Microcosms consisting of serum bottles (60 mL) were amended with soil (10 g) and were spiked with phosphonomethyl-C-labeled ^{14}C-glyphosate (specifc activity of 3.33 x 103 Bq/mmol, diluted with unlabeled glyphosate) in 50 mL of methanol to produce a final concentration of 2 mg/kg of soil that corresponded to the recommended agricultural application rate. The glyphosate-spiked soils were agitated on a reciprocating shaker for 24 h at room temperature to ensure thorough mixing and to evaporate the solvent. To determine the effect of soil phosphate on glyphosate degradation, $CaHPO_4$ was uniformly mixed into the soils at a concentration of 500 mg/kg soil before the addition of the herbicide. The soil was then flooded with 20 mL of sterile (autoclaved), deoxygenated water to mimic soil saturation by rainfall. The microcosm headspace was flushed with N_2 gas and immediately crimp sealed with a butyl stopper fitted with a vial containing 1 mL of 0.5 mol/L NaOH to trap the mineralized $^{14}CO_2$. These microcosms were incubated in a dark, temperature-controlled chamber at 25°C. Sterilized soil microcosms were included as controls for each soil type. Sterilization was achieved by autoclaving the soils twice at 121°C for 1 h on successive days.

Aerobic incubations: Soil microcosms were built from serum bottles as described above. Sterile, distilled water was added to the glyphosate-spiked soils to adjust the moisture content to about 60 % of the field water-holding capacity. The serum bottles were lightly capped (no crimp seal) with a butyl stopper fitted with a NaOH trap and stored in the dark at 25°C. At 1-wk intervals, the microcosms were aerated by equilibrating the headspace with the atmosphere, and the soil moisture content was adjusted by returning each vessel to its initial weight with sterile, distilled water.

Sample Extraction and Analysis

Anaerobic and aerobic microcosms were destructively sampled at consecutive intervals (0.5, 3, 7, 14, 28, 42, and 56 d) by removing the NaOH trap, followed by agitating the microcosm for 1 min and transferring the contents to a 50-mL Teflon centrifuge tube. Quantification of $^{14}CO_2$ in the NaOH traps was accomplished by direct liquid scintillation spectrometry (LSS) using a Packard Tri-Carb (1900TR) scintillation counter. The solid and liquid phases of the soil slurry were then separated by centrifugation (15 min, 12,000 × g). Aqueous samples were removed and filtered (0.2 µm), and the total aqueous radioactivity was estimated using LSS. The soil was extracted with 20 mL of NaOH (0.1 mol/L) in a Teflon centrifuge tube with horizontal shaking following the method described by Druart *et al.* (2011). Extracts were centrifuged at 12,000 rpm for 15 min, an aliquot was removed for LSS (to quantify extractable radioactivity), and the supernatant was retained for analysis of the herbicide. The recovery values of glyphosate from oxic soils were 73 to 78 % and 74 to 76 % from anoxic soils. The recovery efficiencies obtained were taken into consideration in the calculations of the results. Soil extract samples containing ^{14}C-glyphosate were analyzed using high-performance liquid chromatography with a Packard Radiomatic Flo-one Beta scintillation detector. Separation was achieved with an isocratic elution of the mobile phase

composed of acetonitrile/water (10:90 v/v) through a 4.6 × 150 mm, 5-µm particle size, C_{18} column from Prontosil. Glyphosate had a reproducible retention time of 4.1 min at a flow rate of 1 mL/min.

Data Analysis

The adsorption and desorption parameters of glyphosate under oxic and anoxic conditions for each soil type were calculated using the transformed Freundlich equation; equation: $\log C_s = \log K + 1/n \log C_e$, where C_s is the amount of glyphosate adsorbed to the soil (mg/kg), C_e is the equilibrium concentration in the soil solution (mg/L), and K and $1/n$ are empirical constants that reflect the affinity of the soil for the herbicide and the degree of linearity between the amount adsorbed and the solution concentration, respectively. Regression analysis was performed on adsorption and desorption isotherms to calculate K (intercept) and $1/n$ (slope) values of glyphosate in oxic and anoxic soils. Hereafter, K_{ads} and $1/n_{ads}$ will indicate Freundlich parameters for adsorption, and K_{des} and $1/n_{des}$ will refer to desorption parameters. The data on the degradation of glyphosate in soils were fitted into the first-order kinetics model $C_t = C_0 \exp(-kt)$, where C_0 is the initial concentration (mg/kg soil) of the herbicide in the soil, C_t is the herbicide concentration (mg/kg soil) detected in the soil at time t, and k is the first-order rate constant. Degradation rate constants were calculated by linear regression of the natural logarithm of the percentage of herbicide remaining against the time. The aerobic and anaerobic degradation half-lives ($T_{1/2}$) for each soil type were calculated using the equation $T_{1/2} = \ln 2/k$. The statistical program SAS Version 9.3 from SAS Institute was used to calculate the treatment means and standard errors (n = 3). The experiments were set up as a completely randomized design, and the differences between treatments were evaluated using one-way analysis of variance followed by a least significant difference test at p <0.05.

Results

Adsorption-Desorption

Adsorption data from the experiment were very well fitted by the Freundlich equation (R^2 = 1) for the range of herbicide concentrations (0.1-10 mg/L) and soils tested regardless of the soil redox conditions (Table 8.1.1.2-58). Among the different soils and treatments, the slope ($1/n_{ads}$) values ranged from 0.76 to 0.93 and the Freundlich adsorption coefficient (K_{ads}) from 62.21 to 103.46. Soil redox conditions did not alter glyphosate adsorption to the Catlin and Flanagan soils, as evident from their nearly equal K_{ads} values. However, the herbicide exhibited a noticeably lower K_{ads} value in the anaerobically treated Drummer soil vs. the aerobic Drummer soil incubations. Further, K_{ads} was observed to be lowest for Catlin and highest for Drummer regardless of the soil redox conditions. A higher K_{ads} indicates a higher adsorption affinity of the herbicide to the soils. Desorption isotherms for glyphosate in all the soils fit well into the Freundlich model (R^2 >0.92). The calculated desorption parameters of glyphosate in the oxic and anoxic soils are presented in Table 8.1.1.2-59. Freundlich desorption coefficient (K_{des}) values of glyphosate were considerably lower in the anoxic soils than the oxic soils. Among the three soils tested, the highest K_{des} was observed in the Catlin soil irrespective of the soil redox conditions. A higher K_{des} indicates a greater retention of glyphosate on the soil surface.

Table 8.1.1.2-58: Adsorption (Freundlich model) of ^{14}C-glyphosate in different soil types under oxic and anoxic environmental conditions

Soil	K_{ads}†		$1/n_{ads}$‡		R^2§	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
Catlin	62.21 (±1.71)¶	72.38 (±5.80)	0.92 (±0.02)	0.76 (±0.05)	0.999	0.999
Flanagan	78.14 (±2.05)	69.64 (±4.02)	0.90 (±0.02)	0.78 (±0.04)	0.999	1.000
Drummer	103.46 (±5.11)	84.82 (±4.36)	0.93 (±0.03)	0.88 (±0.03)	0.998	0.998

† Freundlich adsorption coefficient.

‡ Adsorption isotherm slope.

§ Goodness of fit for Freundlich model.

¶ 95% confidence intervals in parentheses.

Table 8.1.1.2-59: Desorption (Freundlich model) of ^{14}C-glyphosate in different soil types under oxic and anoxic environmental conditions

Soil	K_{des}†		$1/n_{des}$‡		R^2§	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
Catlin	46.52 (±0.03)¶	42.85 (±0.20)	0.02 (±0.002)	0.28 (±0.003)	0.94	0.94
Flanagan	17.08 (±0.03)	5.81 (±0.02)	0.09 (±0.002)	0.25 (±0.005)	0.92	0.92
Drummer	17.95 (±0.04)	7.75 (±0.05)	0.02 (±0.003)	0.25 (±0.005)	0.92	0.92

† Freundlich desorption coefficient.

‡ Desorption isotherm slope.

§ Goodness of fit for Freundlich model.

¶ 95% confidence intervals in parentheses.

Degradation and Mineralization

Figure 8.1.1.2-16 a to c depict the degradation pattern of ^{14}C-glyphosate in the Catlin, Flanagan, and Drummer soils incubated under oxic and anoxic conditions. The first-order parameters including the rate constant (k) and degradation half-life ($T_{1/2}$) of the ^{14}C-glyphosate in the different soil types and redox conditions are presented in the tables. The ^{14}C-glyphosate degradation followed first-order kinetics in all the nonsterile oxic and anoxic soils, as obvious from their R^2 values (0.83 -1.00). The loss of herbicide from the sterile soil control microcosms was not substantial in either aerobic or anaerobic incubations (Figure 8.1.1.2-16 a to c). In all three soil types studied, the aerobic $T_{1/2}$ values (15 – 18 d) calculated for glyphosate were significantly lower than the corresponding anaerobic values (42 -51 d). The $T_{1/2}$ of the herbicide in the Catlin, Flanagan, and Drummer soils were comparable in the aerobic incubations. On the other hand, compared with the other soils, glyphosate degradation was relatively slow in the Flanagan soil in the anaerobic incubations. Figure 8.1.1.2-16 d to f illustrate the comparative microbial mineralization trends of glyphosate amendments observed as the amount of $^{14}CO_2$ measured from the alkali trap from aerobic and anaerobic soil microcosms. More than half (53 – 63 %) of the radioactivity in the applied ^{14}C-glyphosate was mineralized as $^{14}CO_2$ from the oxic soils, and only 38 to 41 % of the applied ^{14}C-glyphosate was mineralized in the anaerobic microcosms by the end of incubation. Conversely, aerobically or anaerobically incubated sterilized microcosms had little or no mineralization of the herbicide in all the soil types considered. Another interesting observation from the study is the absence of a lag phase before the evolution of $^{14}CO_2$ from the soils. The evolution of $^{14}CO_2$ from soils was evident immediately after Day Zero of the incubation in both oxic and anoxic soils. Glyphosate mineralization in oxic soils was initially rapid, followed by a gradually decreasing rate. However, in anoxic soils, mineralization of the glyphosate started out slowly and steadily increased toward the end of incubation.

Figure 8.1.1.2-16: (a,b,c) Degradation kinetics and (d,e,f) mineralization patterns of ^{14}C-glyphosate under oxic (Ox) and anoxic (An) soil conditions in Catlin, Flanagan, and Drummer soils. Data from oxic (Ox Ster) and anoxic (An Ster) sterilized control soils are also shown

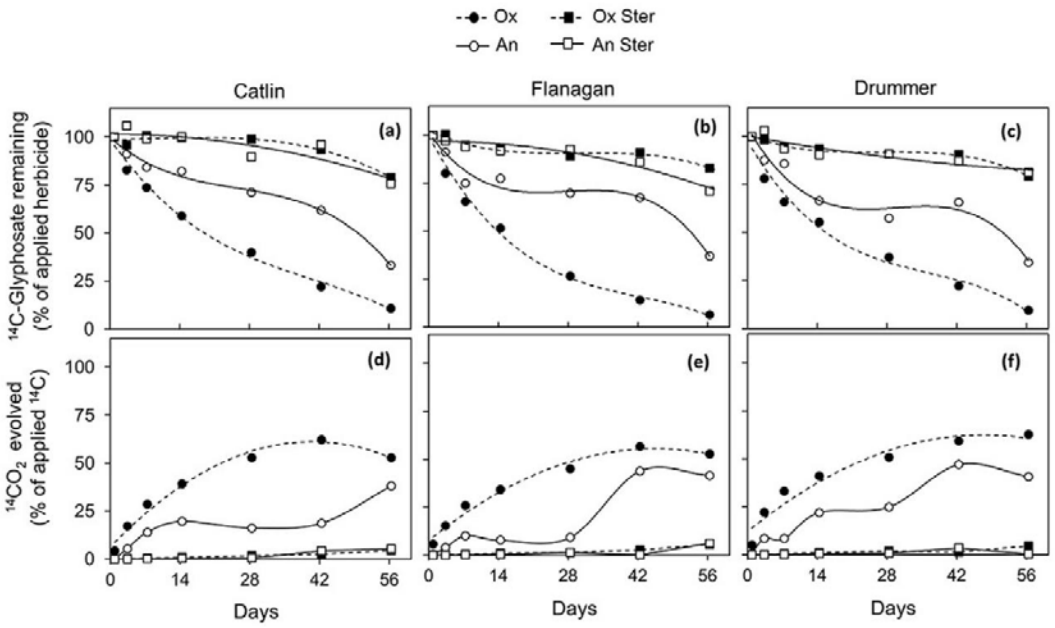


Table 8.1.1.2-60: Degradation (first-order kinetics) parameters of ^{14}C-glyphosate in different soil types under oxic and anoxic environmental conditions

Soil	k†		$T_{1/2}$‡		R^2§	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
	d^{-1}		d			
Catlin	0.038 (0.003)¶	0.016 (0.005)	18 c# (209)	42 b (154)	0.99 (0.73)	0.88 (0.68)
Flanagan	0.048 (0.003)	0.014 (0.005)	15 c (228)	51 a (140)	1.00 (0.84)	0.81 (0.85)
Drummer	0.038 (0.003)	0.015 (0.004)	18 c (210)	45 b (200)	1.00 (0.85)	0.83 (0.86)

† Rate constant.

‡ Degradation half-life.

§ Goodness of fit for first-order degradation model.

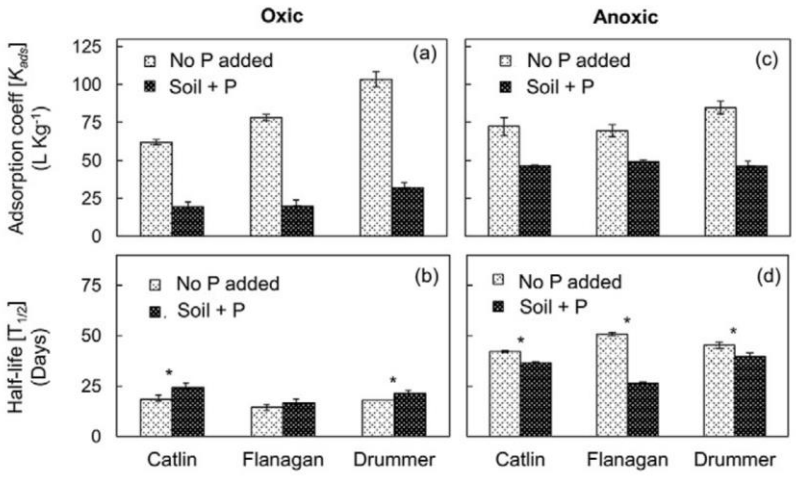
¶ Corresponding values for the sterilized soil control in parentheses.

Means followed by the same letter are not significantly different ($p < 0.05$).

Effect of Phosphate Addition on Adsorption and Degradation

The addition of phosphate to the Catlin, Drummer, and Flanagan soils significantly reduced the ^{14}C-glyphosate adsorption to oxic and anoxic soils (Figure 8.1.1.2-17 a - c). Moreover, the extent of the reduction in herbicide adsorption was more pronounced in the oxic soils. Phosphate additions did not improve or had no effect on the degradation of ^{14}C-glyphosate in the oxic soils, as observed from the degradation half-life values ($T_{1/2}$) of the herbicide in the respective soils (Figure 8.1.1.2-17 b). Conversely, the presence of soil phosphate significantly enhanced the anaerobic degradation of ^{14}C-glyphosate in all three soil types studied (Figure 8.1.1.2-17 d).

Figure 8.1.1.2-17: Effect of phosphate addition (500 mg/kg soil) on the adsorption and degradation of ^{14}C-glyphosate in oxic and anoxic soils: comparison of (a,c) the adsorption coefficient and (b,d) the degradation half-life of glyphosate in oxic and anoxic soils without (No P added) and with (Soil + P) phosphate amendment *Signifcantly different at p <0.05. Error bars represent standard errors (n = 3)



Discussion

Adsorption-Desorption

High K_{ads} values of ^{14}C-glyphosate from the present study (62.21-103.46) clearly indicate a strong adsorption affinity of the herbicide to the soils (Table 8.1.1.2-58). The results obtained from this study were comparable to reported K_{ads} values (33-152.9) for glyphosate. The greatest extent of ^{14}C-glyphosate desorption was observed in the soils having the least adsorption (Table 8.1.1.2-59). Relatively lower K_{des} values in the anaerobic treatments than the corresponding aerobic treatments in all the tested soils indicate that desorption of the herbicide was enhanced in the anoxic, reduced soils. Increased desorption of the herbicide under anoxic conditions may result in an enhanced bioavailability of glyphosate, increasing the risk of movement or crop damage and possibly enhancing degradation of the herbicide under anoxic soil conditions.

Degradation and Mineralization

Degradation of ^{14}C-glyphosate occurred more rapidly in the aerobically incubated Catlin, Flanagan, and Drummer soils than in the corresponding anaerobic incubations, as evident from the significantly lower aerobic $T_{1/2}$ values (Table 8.1.1.2-60). This concurs with previous studies. Glyphosate degradation could be inferred to be a purely microbially mediated process because practically no degradation or mineralization occurred in the sterile control soils in any soil type or redox condition. The slow start in the anaerobic mineralization may be ascribed to the acclimation of specialized herbicide degrading microbial populations in the anoxic soil.

Impact of Soil Phosphate

Suppression of glyphosate adsorption in both oxic and anoxic soils with phosphate addition explicitly demonstrated the competition for adsorption sites between glyphosate and phosphate despite differences in redox conditions (Figure 8.1.1.2-17). Several studies have confirmed similar competitive adsorption of glyphosate and phosphate on Al^{3+} and Fe^{3+} surface sites in soil. The effect of phosphate addition on the enhanced microbial bioavailability of glyphosate was found only in the anoxic soils, where the $T_{1/2}$ of glyphosate was noticeably reduced in all the soil types treated anaerobically with phosphate (Figure 8.1.1.2-17). Phosphate addition did not stimulate glyphosate degradation in oxic soils.

Implications

This study examined the significance of oxic and anoxic soil conditions on the microbial bioavailability of glyphosate in soils. Although 14C-glyphosate was highly adsorbed to the soils regardless of the soil type and redox conditions, desorption or release of the adsorbed herbicide was enhanced in anoxic soils. The degradation and mineralization of 14C-glyphosate exhibited slower kinetics in anoxic soils than oxic soils in all the soil types investigated. The addition of phosphate to the soil suppressed the adsorption of glyphosate in both oxic and anoxic soils and improved the degradation rate in anoxic soils. The effects of anaerobiosis on the observed Kads and Kdes suggest greater glyphosate bioavailability in saturated soils.

Significant decreases in degradation kinetics observed under anaerobiosis across soils could confer a greater potential for transport in water and subsequent environmental impacts. These findings are based on soils in corn (Zea mays L.) - soybean [Glycine max (L.) Merr.] rotations from the Upper Midwest and may not reflect outcomes in soils in warmer climates or situations involving frequent flooding cycles, such as in wetland rice (Oryza sativa L.) production or crop areas in river floodplains. The conflicting observations between oxic and anoxic soil conditions on the environmental fate of glyphosate in the presence of soil phosphate requires additional research attention.

Assessment and conclusion by applicant:

The article describes the sorption and degradation behavior of glyphosate in three different US soils under consideration of aerobic and anaerobic conditions and the addition of phosphates. The sorption experiment is well described stating that USEPA guidelines was followed. However, design, conduct and results are missing details in reporting (ads/des results at each concentration not available numerically) to allow for a check of validity.

A degradation test was conducted – being non-standard compared to Guideline OECD 307 - in a microcosm while again lacking of details in description of results to allow for the calculation of degradation or dissipation rates according to current EU guidance.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, while the article provides a well described sorption experiment and degradation test, detailed results of the former are not provided and the later was performed with specific conditions (microcosm). The available information does not allow to check the validity against current guidelines.

The article provides supportive information on the degradation and adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Rampoldi, E. et al

Data point:	CA 7.1.2.1.1/014
Report author	Rampoldi, E. et al.
Report year	2014
Report title	Carbon-14-Glyphosate Behavior in Relationship to Pedoclimatic Conditions and Crop Sequence
Document No	DOI 10.3844/ajessp.2014.94.101 E-ISSN 1558-3910
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The recognition of glyphosate [(N-phosphonomethyl) glycine] behavioral patterns can be readily examined using a pedoclimatic gradient. In the present study, glyphosate adsorption–desorption and degradation were examined under different scenarios in relationship to soil properties and soil use applications. Three sites with varied pedoclimatic conditions and two crop sequences were selected. Adsorption–desorption and

glyphosate distribution in mineralized, extractable, and non-extractable fractions were assessed under laboratory conditions. Glyphosate sorption was characterized by isotherms and glyphosate degradation using the distribution of 14C-glyphosate radioactivity among mineralized fractions, two extractable fractions (in water, ER1; in NH4OH, ER2), and non-extractable fractions. Results showed sorption indices (distribution coefficient Kd and Freundlich sorption coefficient Kf: 13.4 ± 0.3–64.1 ± 0.9 L/kg and 16.2–60.6, respectively), and hysteresis increased among soil sites associated with decreasing soil particle size <2 µm, soil organic matter, and other soil properties associated with soil granulometry. A multiple stepwise regression analysis was applied to estimate the relationship between Kd values and soil properties. Cation exchange capacity, water field capacity, and Bray-1 P were the soil properties retained in the equation. Soils under continuous soybean [Glycine max (L.) Merr.] (monoculture) treatment exhibited reduced glyphosate adsorption and decreased hysteresis desorption relative to soils under rotation. To our knowledge, these results are the first to demonstrate that soils with identical properties exhibited different glyphosate retention capacities based on crop sequence. We propose possible explanations for this observation. Our results suggested that characterization of the variability in soil property gradients can serve to determine glyphosate behavioral patterns, which can establish a criterion for use in reducing potential environmental risks.

Materials and Methods

Soils

The province of Cordoba, Argentina, is characterized by broken relief to the west and plains in the central and eastern parts. The dominant parent materials are sediments transported by wind, called loess, from the mountain range of Los Andes. Three sites were selected: Pampa de Pocho (PP), Manfredi (M), and Marcos Juarez (MJ). At each site, two crop sequences were investigated, a monoculture of soybean with four glyphosate applications of 6 L/ha (2880 g a.i./ha) during the year and a soybean–maize rotation with only one glyphosate application of 2 L/ha (960 g a.i./ha). The soil was sampled at 0-5 cm. All samples were characterized by particle size determined by sedimentation, water-holding capacity (WHC) by membrane pressure plate, and the permanent wilting point (PWP) by ceramic pressure plate. Soil pH in water (soil/water, 1:1), and total organic C content (TOC) by wet combustion, extractable P by Bray 1, cation-exchange capacity (CEC) by NH4OAc saturation, exchangeable Ca2+ and Mg2+ by complexometric titration with ethylenediaminetetraacetic acid, and exchangeable Na+ and K+ by flame photometer.

Table 8.1.1.2-61: Main characteristics of the three soils under two cropping sequences, a soybean monoculture and a soybean-maize rotation

Property	Marcos Juárez		Manfredi		Pampa de Pocho	
Altitude, m asl	110		292		1026	
Annual avg. temperature, °C	17.9		16.8		16.6	
Mean annual precipitation, mm	931		787		523	
Soil type	Typic Argiudoll		Typic Haplustoll		Entic Haplustoll	
Main textural class†	clay loam		loam		sandy loam	
	Monoculture	Rotation	Monoculture	Rotation	Monoculture	Rotation
pH	5.2 ± 0.1	5.5 ± 0.2	6.1 ± 0.1	6.3 ± 0.1	6.7 ± 0.1	6.2 ± 0.1
Water holding capacity, g kg^{-1}	300	280	220	250	80	150
Permanent wilting point, g kg^{-1}	130	130	100	100	40	70
Clay, g kg^{-1}	278 ± 2	242 ± 18	200 ± 4	216 ± 18	146 ± 15	170 ± 8
Silt, g kg^{-1}	580 ± 9	602 ± 15	558 ± 20	520 ± 20	88 ± 11	268 ± 21
Sand, g kg^{-1}	142 ± 7	156 ± 3	242 ± 5	264 ± 5	766 ± 22	562 ± 15
Total organic C, g kg^{-1}	17.2 ± 0.9	17.0 ± 0.1	14.3 ± 0.27	15.8 ± 0.5	7.3 ± 0.9	9.9 ± 0.3
Bray-1 P, mg kg^{-1}	58.0 ± 1.0	57.0 ± 1.0	53.0 ± 1.0	55.0 ± 1.0	12.0 ± 0.3	45.0 ± 1.0
Cation exchange capacity, cmol kg^{-1}	19.6 ± 0.1	18.8 ± 0.2	18.9 ± 0.1	19.0 ± 0.1	15.0 ± 0.1	9.0 ± 0.1
Ca, cmol kg^{-1}	11.8 ± 0.1	10.9 ± 0.1	12.1 ± 0.1	12.1 ± 0.1	9.4 ± 0.1	6.3 ± 0.1
Mg, cmol kg^{-1}	3.2 ± 0.1	3.4 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.0 ± 0.1	1.5 ± 0.1
K, cmol kg^{-1}	2.3 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.2 ± 0.1	0.7 ± 0.1
Na, cmol kg^{-1}	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Soil size fractionation was done by dispersion of soils in water. The fractions 2000 to 200, 200 to 50, and <50 µm were recovered from the dispersed suspension by sieving and dried at 50°C. The soil weight and organic C concentration in each fraction were quantified.

Carbon-14-Glyphosate Retention

A solution of [methyl-14C]glyphosate was purchased from Sigma Chemical Co. (81 MBq/mmol, 99.2% radiopurity) and was prepared in Milli-Q water by isotopic dilution with unlabeled glyphosate (>99% purity) at six different concentrations (0.2, 0.5, 1, 2, 5, and 10 mg/L). Each solution contained 0.166 MBq/L. Two-gram subsamples of air-dried soil were placed in 25-mL Corex glass centrifuge tubes and 10 mL of 14C-glyphosate solution at one of the six different concentrations was added. Blanks without soil were included and each soil and glyphosate concentration combination was prepared in triplicate. The tubes were shaken by rotation for 24 h at 20 ± 2°C in the darkness. After shaking, the tubes were centrifuged for 15 min at 1800 x g and supernatant removed. The 14C-glyphosate concentrations in the supernatant solution were calculated with a Packard Tri-Carb 2100 TR liquid scintillation counter (Packard Instruments) from the supernatant radioactivity measurements. The amount of sorbed glyphosate per mass of soil was calculated from the difference in herbicide concentration before and after sorption. Desorption of 14C-glyphosate was studied in all samples initially treated with 10 mg glyphosate/L during the adsorption study. After sorption equilibration, most of the supernatant was removed and replaced by an equivalent volume of Milli-Q water. The tubes were vortexed to disperse the soil pellets, and the suspensions were mechanically shaken for 24 h at 20 ± 2°C.The suspensions were then centrifuged for 15 min at 1899 x g, and the supernatant was again replaced with Milli-Q water. Five successive desorption treatments were done for each sample. The supernatant radioactivity was determined after each desorption to quantify the amount of desorbed herbicide.

Table 8.1.1.2-62: Freundlich sorption-desorption isotherm parameters (adsorption $K_{f,ads}$ and n_{ads} , desorption $K_{f,des}$ and n_{des} and hysteretic index H) and distribution coefficients (K_d) of glyphosate in three soils under two cropping sequences

Location	Cropping sequence	Sorption					Desorption			H
		$K_{f,ads}$	n_{ads}	R^2	K_d	R^2	K_{fdes}	n_{des}	R^2	
		L kg^{-1}								
Marcos Juárez	monoculture	48.8 ± 0.3	0.91 ± 0.01	0.99	50.1 ± 0.5	0.98	44.9 ± 0.6	0.17 ± 0.01	0.90	0.19 ± 0.02
	rotation	60.6 ± 0.8	0.90 ± 0.02	0.99	64.1 ± 0.9	0.95	45.2 ± 0.5	0.11 ± 0.01	0.93	0.12 ± 0.03

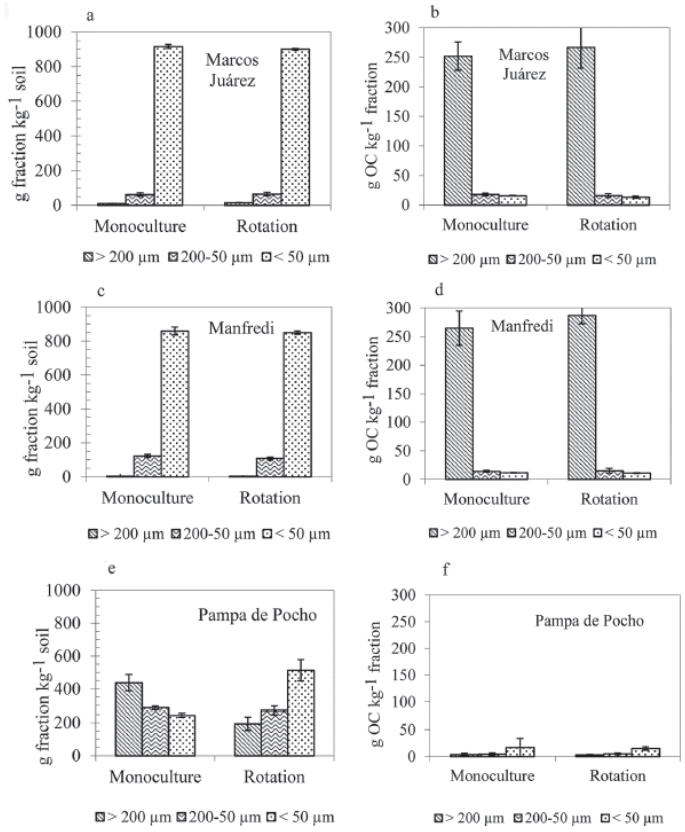
Carbon-14-Glyphosate Behavior

The mineralization of 14C-glyphosate was followed during laboratory incubations (in triplicate) of 49 d at 28 ± 1°C in the dark. One milliliter of the 14C-glyphosate solution was added to 10 g of each soil. The soil water content was adjusted to 85% of WHC of each soil with Milli-Q water, taking into account the glyphosate solution. The 14C-CO2 evolved during the incubation was trapped in NaOH. The vials containing NaOH were sampled and replaced after 3, 7, 14, 21, 28, 35, 42, and 49 d. The total radioactivity content was measured by liquid scintillation counting using a Tri-Carb 2100 TR counter and with external standardization and Ultima Gold XR as a scintillation cocktail.

Extractable and Non-extractable Residues

At the 49th d of the incubation period, four sequential extractions were done for the corresponding soil samples. The extractable fraction of 14C-glyphosate was obtained in two steps. The first extraction was done using 50 mL of Milli-Q water during 24 h, the supernatant was recovered, and the radioactivity was measured by scintillation counting (ER1). After that, three successive extractions were performed, each 24, 24, and 4 h, respectively, with 50 mL of 0.5 mol/L NH4 OH in glass centrifuge tubes. The three successive extracts were pooled for each soil sample and the radioactivity was measured by scintillation counting (ER2). Radioactivity in the solid soil samples containing non-extractable 14C-glyphosate residues (NER) were recovered and dried at 40°C. The radioactivity was measured on three subsamples (100–200 mg) by scintillation counting after combustion at 800°C under O2 flow in a sampler oxidizer (Packard) followed by 14C-CO2 trapping in 8 mL of Carbosorb E (Packard) mixed with 12 mL of Permafluor E+ (Packard).

Figure 8.1.1.2-18: Distribution of soil mass and organic C (OC) content in three soil size fractions (2000-200, 200-50, and <50 µm) in three soils and two cropping sequences



Mathematical Adjustment and Statistical Analysis

Sorption Isotherms

The amounts of 14C-glyphosate adsorbed on the soil (x/m, mg glyphosate/kg solid) were calculated as the difference between the initial 14C-glyphosate concentration and the supernatant concentration (C, mg glyphosate/L supernatant solution). Glyphosate sorption isotherms were described by the Freundlich model and the linear model.

Kinetics of Degradation

Cumulative 14C-CO2 glyphosate and C-CO2 evolved were adjusted to a first-order model:

Statistical Analysis

An ANOVA procedure was performed using the soil type (location) as the main factor, with six replicates per soil. Fisher's test of comparison of means was used. Multiple regression analysis was also performed between glyphosate Kd values and soil properties: sand, clay, silt, pH, TOC, CEC, Ca2+, Mg2+, Na+, K+, WHC, PWP, Bray-1 P, and three organic C fractions (>200, 50–200, and <50 µm). The criteria for the selection of the variables were $p < 0.05$. For each crop sequence (monoculture or rotation), a simple ANOVA by each soil with three replicates was used. The statistics software used was Infostat (Di Rienzo et al., 2009).

Table 8.1.1.2-63: Stepwise linear regression of glyphosate sorption index K_d

Variable	Coefficient	SE	*P*	R^2	Adjusted R^2
	–48.04	13.22	0.0027		
Water field capacity	130.68	29.6	0.0006		
Bray-1 P	–0.54	0.14	0.0018		
Cation exchange capacity	3.53	1.4	0.0243		
				0.97	0.96

Results and Discussion

Soil Characterization

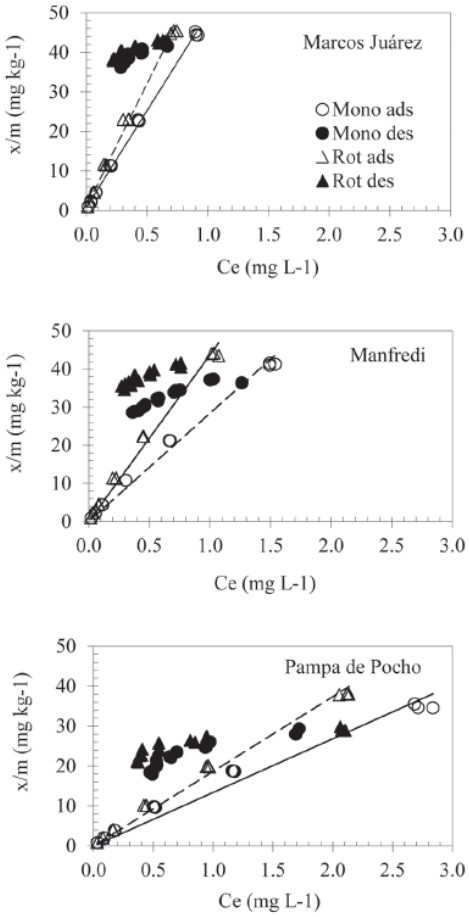
Edaphoclimatic characteristics from each of the three scenarios studied are shown in Table 8.1.1.2-61. The PP soils positioned at the northwestern sampling site exhibited a sandy texture, the highest pH and the lowest TOC, CEC, PWP, and WHC. At the extreme southeastern sampling site, the MJ soils showed the lowest pH and the highest TOC, CEC, PWP, WHC, and clay and silt contents. The M soils located in the geographically intermediate sampling site also exhibited intermediate edaphic properties in relationship to the other two sampling sites.

The soil size distribution among fractions ranged from 2000 to 200, 200 to 50, and <50 µm (Figure 8.1.1.2-18 a, c, e) and showed granulometric differences among the three sample sites. The M and MJ sites primarily differed in the fraction proportion in micrometers (i.e., 200-50), corresponding to the categories of fine sand and very fine sand. The PP soils differed from the other two sampling sites in the proportion and distribution of the three soil size categories evaluated. Rotation and monoculture treatments revealed identical soil size distributions for M and MJ. However, the two cropping sequence treatments from the PP site were not congruent with M and MJ, and significant differences in soil particle size distribution were observed ($P < 0.05$). Results showed that the coarsest soil size fraction (2000 – 200 µm) containing fresh soil organic matter (SOM) represented the largest organic C concentration in MJ and M soils and both treatments (monoculture and rotation) Figure 8.1.1.2-18 b and d). Nevertheless, the highest TOC proportion corresponded to humified organic matter associated with a soil size fraction <50 µm, i.e., the highest proportion of this fraction was present in these soils (between 75 and 85 %). Carbon enrichment in some of the three soil size fractions, which was associated with soil texture and granulometry, was not found in the PP soils (Figure 8.1.1.2-18 f).

Carbon-14-Glyphosate Sorption–Desorption

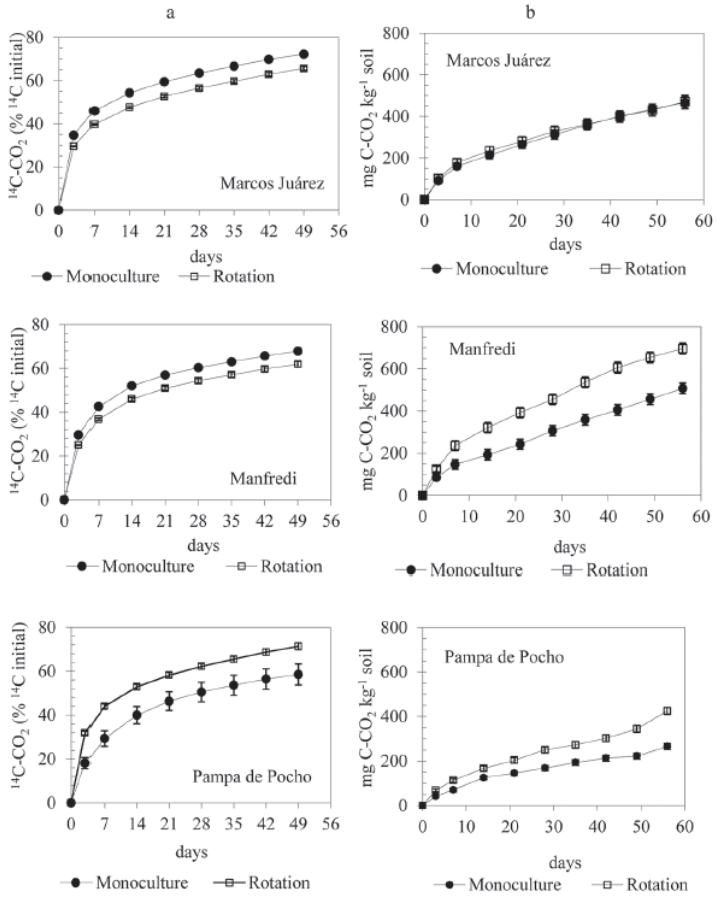
Carbon-14-glyphosate sorption-desorption isotherms obtained from the three scenarios evaluated are shown in Figure 8.1.1.2-19. The experimental data were fitted with two mathematical models: the Freundlich adsorption isotherm and the linear model. Empirical evidence indicated that as the value of n_{ads} decreased, the linear approximation became less satisfactory, especially at high and low concentrations, and discrepancies between K_f and K_d values occurred. Our results showed that the equilibrium concentration range was 0.1 to 1.0 mg/L (MJ soils), 0.1 to 1.5 mg/L (M soils), and 0.1 to 3 mg/L (PP soils), and $K_{f,ads}/K_d$ ranged between 0.9 and 1.1.

Figure 8.1.1.2-19: Glyphosate adsorption (open symbols) and desorption (filled symbols) isotherms in three soils and two cropping sequences: monoculture (circles) and rotation (triangels)



The three scenarios examined clearly differed in glyphosate adsorption. The $K_{f,ads}$ and K_d indices showed a threefold increase from PP soils (northwest position) to MJ soils (southeast position). Our results from the three sampling sites showed a geographic gradation in soil characteristics that were associated with glyphosate adsorption, i.e., we detected a relation among geographic position, edaphic characteristics, and glyphosate adsorption capacity. That behavioral pattern was confirmed by adsorption studies. The PP site collective soil characteristics were associated with low glyphosate adsorption capacity, i.e., high pH, low clay content and SOM, while the MJ site exhibited inverse soil attributes and an overall high adsorption capacity. A multiple linear regression analysis was performed to estimate the relationship between glyphosate K_d values and soil properties (Table 8.1.1.2-63). The PWP, CEC, and Bray-1 P were the regressed variables retained in the analysis, which explained 93 % (R^2 = 0.97) of the total variation. Desorption isotherms were fitted for the Freundlich model. An irreversible desorption process was shown by the lack of overlap in the sorption - desorption isotherms (Figure 8.1.1.2-19). Glyphosate desorption indices were higher than corresponding adsorption indices. Our study revealed that glyphosate desorption hysteresis increased from the northwest toward the southeast sampling sites. The *H* indices, which ranged from 0.12 to 0.39, were used to compare the hysteresis degree among soils. Between 15 and 57 % of the glyphosate initially applied was desorbed. The PP soils exhibited the highest ^{14}C-glyphosate desorption, with >50 % recovered in the first desorption step. On the contrary, for the M and MJ soils in the first desorption step, only about 30 % of the total ^{14}C-glyphosate was desorbed. Crop sequence effects were evaluated only for the M and MJ soils. These two sample sites had similar soil characteristics, while the PP site differed and was therefore excluded. The extent of glyphosate adsorption and desorption hysteresis was higher in rotation than monoculture soils (Figure 8.1.1.2-20; Table 8.1.1.2-62). To our knowledge, our results are the first to document that soils with identical properties exhibited different glyphosate retention capacities due to cropping sequence.

Figure 8.1.1.2-20: Kinetics of (a) 14C-glyphosate mineralisation and (b) total organic carbon mineralisation interpreted as indicator of total microbial activity during laboratory incubations of three soils and the two cropping sequences monoculture and rotation; standard deviations (error bars) are shown when larger than the symbol size



Carbon-14-Glyphosate Mineralization

Carbon-14-glyphosate mineralization kinetics together with C-CO2 evolution are shown in the figures. In addition, cumulative glyphosate mineralization and oxidizable C after 49 d of incubation are shown in the tables. At the end of the incubation period, the 14C-CO2 released ranged from 61.7 to 72.8% of the 14C applied, and the time needed to reduce the 14C initially applied to 50% was 5.0 ± 0.7 d (calculated from ln2/k).

Decreased 14C-glyphosate mineralization detected in the MJ and M sites relative to the PP site might be associated with increased glyphosate adsorption in the MJ and M soils. We found that the TMA (total microbial activity) was significantly different ($P < 0.01$) among sampling site soils: M > PP > MJ. The M soils, with the highest TMA, did not have the highest 14C-glyphosate mineralization. Glyphosate mineralization was affected by cropping sequence. At the end of incubation, the 14C-CO2 evolved was monoculture MJ = 69% vs. rotation MJ = 63.6% and monoculture Mm = 68% vs. rotation M = 61.7% ($P < 0.05$). These results provide additional support to our interpretations regarding glyphosate mineralization differences detected among study sites, given that monoculture soils showed reduced glyphosate adsorption and a history of glyphosate use.

Table 8.1.1.2-64: Carbon-^{14}C-glyphosate mineralised, C-CO_2 expressed as a percentage of the total organic C, and setting parameters for three soils and two cropping sequences using the equation $C_t=C_0[1-\exp(-kt)]$, where C_t is the percentage of ^{14}C-CO_2 or C-CO_2 mineralized at time t, C_0 is the percentage of C potentially mineralizable, k is the daily mineralization rate, and t is time in days

Soils	Cropping sequence	Mineralization of glyphosate			Mineralization of soil organic C		
		$^{14}C\text{-}CO_2$	C_o	k	$C\text{-}CO_2$	C_o	k
		%		d^{-1}	%		d^{-1}
Marcos Juarez	monoculture	69.1 ± 0.3	62.8 ± 1.3	0.178 ± 0.018	2.8 ± 0.03	3.0 ± 0.11	0.044 ± 0.003
	rotation	63.8 ± 0.2	57.7 ± 1.2	0.170 ± 0.017	2.4 ± 0.02	2.5 ± 0.09	0.053 ± 0.005
Manfredi	monoculture	68.3 ± 0.3	63.1 ± 1.0	0.163 ± 0.012	3.3 ± 0.03	4.1 ± 0.31	0.031 ± 0.004
	rotation	61.6 ± 0.1	57.4 ± 1.0	0.141 ± 0.011	4.3 ± 0.02	4.7 ± 0.21	0.044 ± 0.004
Pampa de Pocho	monoculture	65.3 ± 0.5	60.9 ± 1.2	0.117 ± 0.009	3.2 ± 0.03	3.0 ± 0.11	0.063 ± 0.006
	rotation	74.3 ± 2.8	66.9 ± 1.5	0.159 ± 0.017	3.2 ± 0.03	3.3 ± 0.11	0.054 ± 0.005

Carbon-14-Glyphosate Distribution among Mineralized, Extractable, and Nonextractable Residues

The three study sites differed in the distribution of initial radioactivity applied and in the proportion remaining in ER and NER forms. The lower proportion of ER2 and NER in the PP soils corresponded with soil properties involving low sorbent surfaces. Sequential extraction of ER and NER was conducted following 49 d of incubation; consequently equilibrium between soluble and sorbed forms of glyphosate should have occurred. The PP soil contained 19% ER (ER1 + ER2), which clearly contrasted with 30% ER obtained from the M and MJ soils. The ER1 fraction, extracted with water, represented the weakly adsorbed herbicide and on average was <5% of the total ER for the three sample sites. The ER1 from the PP soils was slightly higher than that from the other two sample sites (MJ and M), indicating weak glyphosate adsorption properties and high sorption reversibility. Nonextractable residues constituted a small fraction (4-6%) of the 14C-glyphosate initially applied. Small differences among soils were observed, such as decreasing order of NER proportions: M > MJ > PP ($P < 0.05$). The M soils showed the highest TMA and NER proportion.

Conclusions

The study of glyphosate retention and degradation processes through a pedoclimatic gradient turned out to be a useful tool to recognize and establish some behavioral patterns. Identification of soil indicators that allow inference of glyphosate behavior is one of the goals in studies of sustainable soil use. We found that along a distance of approximately 280 km, gradual changes in glyphosate behavior were associated with pedoclimatic characteristics. Soil properties associated with soil surface reactivity, such as CEC, WHC, and PWP, increased in from northwest to southeast together with the increase in glyphosate adsorption and the increase in hysteresis of desorption. Changes in the glyphosate distribution between adsorbed and soluble forms establish, in part, a behavior pattern of extractable (ER) and mineralized forms. The extent of glyphosate adsorption and also the hysteresis of desorption were higher in rotation soils than monoculture soils; that is, soils with identical properties exhibited a different glyphosate retention capacity due to the cropping sequence. The results of this study contribute to our understanding of glyphosate behavioral patterns in relation to different edaphoclimatic scenarios and establish criteria for use in reducing potential environmental risks.

Assessment and conclusion by applicant:

The article investigates 14C-glyphosate adsorption–desorption and degradation under different scenarios in relationship to soil properties and soil use applications. Three Argentinian sites/soils with varied pedoclimatic conditions and two crop sequences were selected. Sorption parameters and degradation in terms of mineralization are reported. Essential details to assess the quality of data, for example, in terms of the EU Evaluators Checklist, are not available, described, and there are some deviations from current guidelines. In addition, the pedo-climatic conditions do not correspond to EU conditions.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

The article is well documented. The study followed degradation and adsorption of glyphosate under mono culture or rotations in Argentinian soils. The soils in monoculture were treated multiple times,

which could influence the degradation rate and adsorption. Therefore it is not considered as reliable for deriving endpoints for the risk assessment. The rotational conditions were investigated with crops on the field, which is a major deviation from current guidance for validity of field dissipation studies.

The article provides supportive information on the degradation and adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Al-Rajab, A. et al

Data point:	CA 7.1.2.1.1/015
Report author	Al-Rajab, A., Hakami, O.M.
Report year	2014
Report title	Behavior of the non-selective herbicide glyphosate in agricultural soil
Document No	DOI 10.3844/ajessp.2014.94.101 E-ISSN 1558-3910
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate [N-phosphonomethyl]glycine is a systematic, non-selective, organophosphorus herbicide used worldwide in agriculture and industrial zones. In this study, the authors followed the degradation, stabilisation, remobilisation and leaching of 14C-glyphosate in three agricultural soils in laboratory incubations and in lysimeters under field conditions. Glyphosate degradation was relatively rapid with a half-life of 14.5 days in the silt clay loam soil incubated at 20°C. Glyphosate's degradation product, aminomethylphosphonic acid (AMPA), represented more than 85 % of residues after 80 days of laboratory incubation. Leaching of glyphosate in lysimeters of three different investigated soils under outdoor conditions was very slow, less than 1 % of the initial applied amount has been detected in the leachates after 100 days of experimentation. Glyphosate rapidly formed non-extractable residues after treatment. In summary, glyphosate was removed from soil very rapidly and its leaching seems to be very slow regardless the type of treated soil. On the other hand, the contamination risk of groundwater with its metabolite AMPA at long term is probably due to the release of the non-extractable residues.

Materials & Methods

Chemicals

[Phosphonomethyl-14C]-glyphosate diluted, purity 99 % was purchased from ARC-ISOBIO (Belgium). Glyphosate [N-phosphonomethyl]glycine, purity 99 % was purchased from Cluzeau (CIL, Paris). Aminomethylphosphonic Acid (AMPA), 10 ng µ/L in water, was purchased from Dr. Ehrenstorfer GmbH (Germany). Sarcosine [N-methylglycine], purity 99 % was purchased from Fluka (Germany). H2PO4, FMOC-chloride, Potassium hydroxide and Sodium tetraborate decahydrate were purchased from Fluka (Germany). Methanol and acetonitrile (HPLC grade) were purchased from SDS (France).

Sampling

Soils used in this study were obtained from three different agricultural lands in Lorraine region (France). Therefore, based on information provided by the landowners, these soils were never exposed to direct agricultural application of glyphosate and their properties were as following: Sandy loam soil (Sand:Silt:Clay (59:30:11), pH 5.1; % organic matter 0.82); silt clay loam soil (Sand:Silt:Clay (16:53:31),

pH 6.3; % organic matter 1.45); and clay loam soil (Sand:Silt:Clay (35:30:35), pH 7.9; % organic matter 1.91).

In the laboratory studies, soils were air dried then sieved at 2 mm and stored in fridge at 4°C until treatment. Otherwise, in the outdoor leaching study, lysimeters were prepared in site using an undisturbed soil for each type of soil separately, a total of 7 columns of each soil were used in this study. Laboratory lysimeters were polyvinyl chloride pipes of 10 cm wide and 35 cm long. Therefore, the 21 lysimeters of the three selected soils were placed in the experimental field of ENSAIA (54500 Vandoeuvre-lès-Nancy, France) for 100 days.

Extraction of Glyphosate

The efficacy of different solvents for extraction of glyphosate from soil was evaluated as follows. A 5 g portion of each soil (in triplicate) was treated with a 0.5 mL solution of H2O (concentration of 19.4 Bq/g) of [Phosphonomethyl-^{14}C]-glyphosate and 0.1 µg/g of unlabelled glyphosate. Treated soil was placed into a 250 mL PPCO (Nalgene®, VWR, USA) centrifuge bottle and 25 mL of selected solvent were added. Five different solvents were tested separately for the glyphosate extraction efficacy: Ammonium oxalate monohydrate 0.1 M; potassium dihydrogen phosphate (KH2PO4) 0.1 M; a mixture of (NH4OH 0.5 M + KH2PO4 0.1 M + H3PO4 0.5 %); $CaCl_2$ 0.1 M and distilled water. Bottles were rotary shaken for 2 h, then centrifuged at 5000 g for 20 min, the supernatant of each sample was recovered. Extraction of each sample has been repeated twice, the supernatants of the same sample were combined and a portion of 1 mL counted by Liquid Scintillation Counter (LSC). Thereafter, extraction of glyphosate from soil samples was effectuated with (KH2PO4) 0.1 M.

Laboratory Degradation Study

About 25-g soil samples were placed in glass jars (60 mm diameter, 40 mm high). Samples of silt clay loam soil were prepared in triplicates for each sampling time. Each sample was amended by 0.51 mg of glyphosate and 45.1 kBq in water. Final soil moisture was 80 % of the soil retention capacity. After treatment, each sample was added to a Mason jars (1.5 L). At the same time, a plastic vial of 10 mL H2O was added to each jar in order to maintain the humidity of soil (Al-Rajab et al., 2009). Another plastic scintillation vial with 10 mL of 0.5 N NaOH was placed into each jar for trapping 14CO2. Jars were incubated at 20°C in the dark for 80 days. The radioactivity trapped in NaOH was counted at each sampling time using a Liquid Scintillation Counter LSC Packard Tri-Carb 1900 CA (Packard, USA). 1 mL of NaOH was added to 10 mL of scintillation cocktail in a plastic scintillation vial to measure the radioactivity in the LSC during 10 min. At each sampling date, the 25-g soil samples were extracted separately using KH2PO4 as described previously. Then, after the 3rd and last extraction, soil samples were air-dried at the lab ambient temperature for 3 days. The remaining 14C-radioactivity in the samples after extraction was referred as (non-extractable residues) which was determined by combustion at 900°C using a 307 Packard Oxidiser (Packard, USA).

Leaching Study

Laboratory lysimeters were prepared and placed in the experimental field of Lorraine University (France) 3 months before the treatment. During the experimentation of 100 days, the average temperature was 10°C; total precipitation was 235 mm; in total 8 leachates samples were collected. Leached radioactivity from each lysimeter was determined directly after collection. Therefore, water samples were stored at -18°C until analysis.

Analytical Methods

14C-Radioactivity has been determined using a Liquid Scintillation Counter LSC. Glyphosate residues were determined using a Varian HPLC (USA) equipped with two detectors: A fluorescence detector and a β-radioactivity detector. A Lichrosorb (NH2) column (4×250 mm, 5 µm) purchased from (CIL-Cluzeau, France) was used and thermostated at 30°C. Fluorescence detector was set at (λ 260 and 310 nm), while the flow rate of 1.2 mL/min was adopted in the β-radioactivity detector with a counting cell of 500 µL. The mobile phase was a mixture of (KH2PO4 0.05 mol-1, pH 5.7)/acetonitrile (70/30: V/V) at flow rate of 0.8 mL/min. The injected volume was 50 µL. Within these conditions, the retention times were 4.2, 6.6 and 13.3 for sarcosine, AMPA and glyphosate respectively. Determination of the non-extractable residues in soil has been effectuated by combustion of 0.5 g portions at 900°C using an oxidizer (Packard, USA). Statistical analyses were conducted using Stat Box (Version 6.4, Grimmer Software, France).

Results

Extraction of Glyphosate

Extraction recovery of glyphosate varied from 4 to 74 % of the initial applied amount (Table 8.1.1.2-65). $CaCl_2$ (0.1 M) and water were the less effective solvents in glyphosate extraction in the three investigated soils. However, ammonium oxalate (0.1 M) was the most efficient solvent with a recovery rate ranged from 60 to 74 %. The only issue with the extraction with ammonium oxalate was that the extracts were very dark and need an intensive clean up. On the other hand, potassium dihydrogen phosphate (KH_2PO_4 0.1 M) was adopted as a suitable solvent since the extracts were clear and it showed an acceptable recovery rate varied from 45 to 49 % in investigated soils (Table 8.1.1.2-65). Recovery rate with citric acid (20 %) was not high enough (less than 37 %) for the three investigated soils.

Dissipation of Glyphosate

Results showed an immediate and high degradation rate of glyphosate after its application on the soil (Figure 8.1.1.2-21). Mineralization of glyphosate after 17 days of incubation reached 39.7 % of the initial amount applied. Thereafter, the mineralization of glyphosate declined gradually. The half-life of glyphosate derived from the mineralization rates was 31 days for silt clay loam soil. However, the extraction curves are opposite to those of the mineralization (Figure 8.1.1.2-22). The percentage of extracted residues from the silt clay loam soil at T0 was only 56.9±0.7 %. This availability to extraction decreased overtime, it reached 6.9 % of the initial amount for silt clay loam soil. HPLC analysis showed the appearance of two degradation products of glyphosate AMPA and sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co-eluted and unlabeled organic compounds. The half-life of glyphosate extractable was 14.5.

Leaching of Glyphosate

Our study showed that the residues of glyphosate were detected in the first leachates samples of three soils, the cumulated precipitation was 85 mm. In the case of silt clay loam soil, the maximum residues concentration of 9.5±7 µg L-1 has been reached after 2 months of application. Concentration of leached residues decreased dramatically after 2 months until the end of experiment (Figure 8.1.1.2-23).

Table 8.1.1.2-65: Extraction efficiency of glyphosate from the selected soils using different solvents

Extraction efficiency (%: Mean±standard deviation: n = 3)

Solvent	Sandy loam	Silt clay loam	Clay loam
KH_2PO_4 (0.1M)	44.9 (±0.3)	48.8 (±0.7)	48 (±0.5)
Ammonium oxalate (0.1 M)	59.9 (±0.7)	73.5 (±0.2)	61.1 (±0.1)
Citric acid (20%)	34.2 (±0.1)	36.4 (±0.2)	28.9 (±0.2)
$CaCl_2$ (0.1 M)	5.7 (±0.5)	3.6 (±0.9)	10.3 (±0.6)
H_2O	14.3 (±0.2)	23.5 (±0.1)	31.7 (±0.1)

Figure 8.1.1.2-21: Residues evolution of glyphosate and AMPA in the extractable residues in silt clay loam soil during incubation at 20 °C

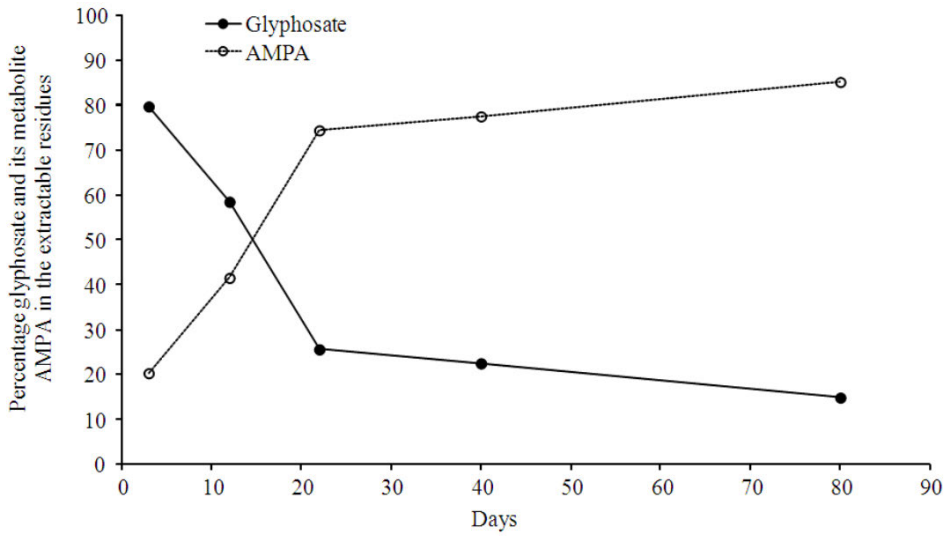


Figure 8.1.1.2-22: Evolution of different portions of 14C-glyphosate residues (extractable, mineralization as 14CO2 and Non-extractable) in silt clay loam soil during incubation at 20 °C

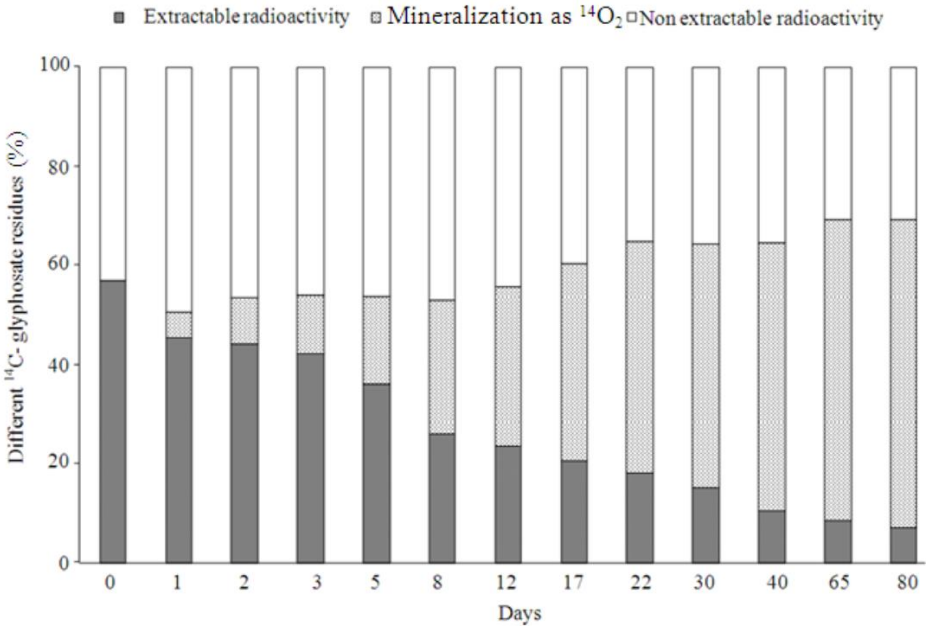
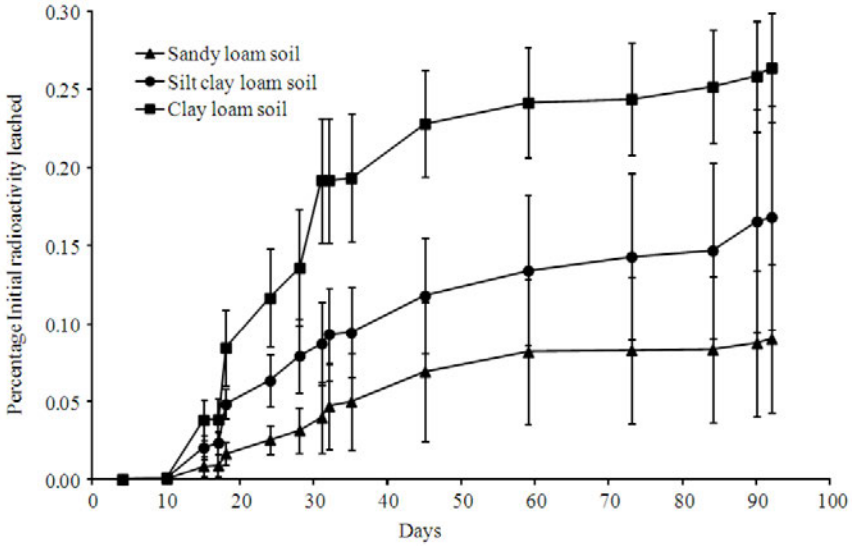


Figure 8.1.1.2-23: Radioactivity leached from lysimeters of the investigated soils treated with 14C-glyphosate under outdoor conditions



Discussion

Extraction of Glyphosate

Extraction and determination of glyphosate in agricultural soil is problematic due to its high solubility and its physic-chemical properties (Botero-Coy *et al.*, 2013). In the present study, extraction recovery of glyphosate varied from 4 to 74 % of the initial applied amount (Table 8.1.1.2-65). $CaCl_2$ (0.1 M) and water were the less effective solvents in glyphosate extraction in the three investigated soils. However, ammonium oxalate (0.1 M) was the most efficient solvent with a recovery rate ranged from 60 to 74 %. The only issue with the extraction with ammonium oxalate was that the extracts were very dark and need an intensive clean up.

On the other hand, potassium dihydrogen phosphate (KH_2PO_4 0.1 M) was adopted as a suitable solvent since the extracts were clear and it showed an acceptable recovery rate varied from 45 to 49 % in investigated soils (Table 8.1.1.2-65), this rate was similar to that one reported by other studies (Cheah and Lum, 1998; Landry *et al.*, 2005). Recovery rate with citric acid (20 %) was not high enough (less than 37 %) for the three investigated soils. Non-extractable residues of glyphosate in soil increase with the time; consequently, glyphosate will be less available for extraction or degradation.

Dissipation of Glyphosate

Monitoring of mineralization of glyphosate labelled on the phosphonomethyl group allows assessing both the loss of glyphosate and AMPA. We observed an immediate and high degradation rate of glyphosate after its application on the soil (Figure 8.1.1.2-21). The absence of lag phase indicates that the microflora of soil already had an enzymatic system capable of degrading glyphosate and as such did not need an adaptation period. Mineralization of glyphosate after 17 days of incubation reached 39.7 % of the initial amount applied. Thereafter, the mineralization of glyphosate declined gradually. The fast mineralization of glyphosate in the soil appears due to its bioavailability. The half-life of glyphosate derived from the mineralization rates was 31 days for silt clay loam soil. On the other hand, the effect of organic matter content in the soil on mineralization of glyphosate was not clear under the conditions of this study. The extraction rate of glyphosate is an indication of the accessibility of the residues for microbial degradation and/or their transfer to groundwater under natural conditions. The extraction curves are opposite to those of the mineralization (Figure 8.1.1.2-22). The percentage of extracted residues from the silt clay loam soil at T0 was only 56.9±0.7 %. We can assume that the treatment in a dry soil may cause an entry of glyphosate into the microporisity of aggregates during the capillary invasion by the aqueous solution of treatment (Guimont *et al.*, 2005; Al-Rajab *et al.*, 2010b). The size of this compartment would be defined at the time of treatment and may depend on the physicochemical and physical properties and the moisture rate of soil at the application moment. This availability to extraction decreased overtime, it reached 6.9 % of the initial amount for silt clay loam soil. The evolution of extraction rate with KH_2PO_4 over time in the soil is related to the mineralization of residues and the availability of non-extractable residues for mineralization or

extraction. A similar behaviour of extractable residues of glyphosate over time was reported by (Getenga, 2004; Miles, 1998). HPLC analysis showed the appearance of two degradation products of glyphosate AMPA and sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co-eluted and unlabelled organic compounds.

The appearance of AMPA during the first days of incubation is due the fast mineralization of glyphosate in soil, reaching about 85.1 % of residues after 80 days of treatment (Figure 8.1.1.2-21). The half-life of glyphosate extractable was 14.5 days. The fraction of non-extractable residues represent the residues which cannot be extracted from the soil by the series of KH_2PO_4 extractions (exhaustive extraction) (Figure 8.1.1.2-22). The formation of the non-extractable residues NER in the silt clay loam soil reached 43 % of the initial applied amount at T0 and 49.4 % at T1. The rate stayed stable until T2 after which it decreased to 30.9 % by the end of experiment. The rate of non-extractable residues decreased over time unlike other pesticides such as atrazine where the rate of non-extractable residues increases gradually over dozens of days (Winkelmann, 1991). The rate of non-extractable residues is probably dependent on the properties and physical aspects of the soils including the size of the microporal compartment. This rapid formation of non-extractable residues immediately after treatment is very specific for glyphosate. The treatment of herbicide on a dry soil promotes the capillary invasion and the rapid transport of the solution of treatment in the microporisity intra aggregate, subsequently making the herbicide inaccessible for extraction (Guimont *et al.*, 2005). We also reported that the initiation of the degradation of glyphosate did not affect the evolution of extractable residues rate. The very slow decrease of non-extractable residues showed that these residues can return by diffusion and under the effect of a concentration gradient, to areas accessible to microorganisms to subsequently undergo mineralization.

Leaching of Glyphosate

This study showed that water circulation in the soil might has an important role in contamination of groundwater with glyphosate. The diminution of soil macroporosity on the surface layer (where most residues usually present) with the time slows the water infiltration and might encourage the desorption of glyphosate residues. The circulation of glyphosate residues in soil could be due to a preferential water flow regarding the presence of its residues in the 1st collected leachates (Figure 8.1.1.2-23). In disaccording with results reported by (Dousset *et al.*, 2004), our study showed that the residues of glyphosate were detected in the first leachates samples of three soils, the cumulated precipitation was 85 mm. Detection of glyphosate residues in the 1st leachates was due to the preferential flow (Laitinen *et al.*, 2006). In the case of silt clay loam soil, the maximum residues concentration of 9.5±7 µg/L has been reached after 2 months of application. However, (De Jonge and Jacobsen, 2000) have reported residues concentration of glyphosate much higher than what was obtained from the current study. Concentration of leached residues decreased dramatically after 2 months until the end of experiment (Figure 8.1.1.2-23). Our findings were in accord with results reported by (De Jonge and Jacobsen, 2000; Landry *et al.*, 2005) who detected the glyphosate residues in the soil leachates after 3 months of application. Overall, the total residues (extractable and non-extractable) of glyphosate in the soil should be considered to evaluate its persistence in the soil, not only the extractable residues.

Conclusion

The present study monitored the residue dynamics of glyphosate in agricultural soil in controlled and outdoor conditions. Results obtained for the fate study suggest that the water pollution with this herbicide is closely related to the adsorption and the formation of non-extractable residues, which are themselves dependent on soil texture and its moisture condition at the time of treatment. In case of rain following treatment, the risk of groundwater pollution by glyphosate will be low but may continue to be present for long time since the mineralization is slow. The silt clay loam soil could be less favourable for water pollution since it showed a formation of large amount of non-extractable residues. In the semi-field lysimeters study, leaching of 14C-glyphosate was limited, but its metabolite AMPA seems to be the main potential pollutant of the groundwater. The water circulation mode in the soil was preferential flow which facilitate a fast leaching of residues to reach the groundwater.

In summary, these results suggest that the organophosphorus herbicide glyphosate is rapidly degradable in the agricultural soil. Leaching of glyphosate seems to be very slow regardless the type of the soil. Release

of the non-extractable residues of glyphosate probably increases the risk of groundwater pollution with its metabolite AMPA at long term. More investigations are requested for a better understanding of the effect of soil content of organic carbon and soil microflora on environmental behavior of glyphosate.

Assessment and conclusion by applicant:

The article investigates the degradation/dissipation and the potential for mobility of glyphosate and its metabolite AMPA in three French soils. The soil degradation tests perfomed with 14C-labelled glyphosate cannot be assesses fully for their quality and deviations from current guideline due to a lack of detail in reporting. This includes, for example, that no detailed values per sampling interval are reported for all soils.

The semi-field leaching experiments were small-scale soil columns consisting of 35-cm with undisturbed soil with low diameter. It is a common observation that this design can cause preferential flow as some artefact thus having potential to result in false-positive findings in percolates of such type of 'lysimeter'. Being indicative in the best case, the results cannot be compared to those of 'full lysimeter studies' that are typically run for more than a year under outdoor conditions.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, the lack of details – especially results- for the degradation tests prevent the data to be used to derive reliable endpoints. Additionally, at DAT0 up to 43% of non extractable radioactivity was found, which is very high. Extraction recovery of glyphosate varied from 4 to 74% of the initial applied amount in the study.

Regarding the leaching experiments, the size of the lysimeters is very limited (35 cm long, 10 cm wide), compared to recommendations from OECD 22 (1 m depth, minimum surface area 0.5 m2). No sufficient details are given in the article to assess the reliability of the experiment against OECD 22 criteria.

The article provides supportive information on the degradation and mobility of glyphosate and AMPA, but no reliable endpoints can be derived for use in risk assessment.

Nghia, N.K. et al

Data point:	CA 7.1.2.1.1/016
Report author	Nghia, N.K. et al.
Report year	2013
Report title	Soil properties governing biodegradation of the herbicide glyphosate in agricultural soils
Document No	ISBN 978-602-96519-2-8
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The relationships between soil properties and glyphosate biodegradation in different agricultural soils was investigated in this study. Soils differ hugely in soil texture, soil organic matter content. pH, oxalate extractable A13+ and Fe3+. The biodegradation experiments were conducted under test conditions: water

tension of -15 kPa as soil moisture, a soil density of 1.3 g/cm3 and at 20 °C in the dark. The biodegradation experiments showed that the mineralization of glyphosate in 21 agricultural soils greatly varied. Between 7.6 to 68.7 % of the applied 14C-glyphosate was mineralized to 14CO2 in the 21 different soils within 32 days of incubation. The highest and lowest mineralized glyphosates were observed in Feldkirchen (68.7 %) and Brejze soil sample (7.6 %), respectively. Glyphosate was mineralized rapidly by the microorganisms in the soil solution and the highest mineralization rate was reached shortly after application. The mineralization of glyphosate in soils was individually regulated by exchangeable H+, soil pH-CaCl2, oxalate extractable AI 3+ and bacterial cell numbers at the end of the experiments, but it was collectively controlled by exchangeable H+, Ca2+ ions and plant available K. Moreover, soil textures, soil organic content, P2O5, Cu2+, oxalate extractable Fe3+ and CEC were found not to have any correlation with mineralization of glyphosate. The NaOH extractable residues were bioavailable for degradation whereas the bound residues of glyphosate in soils were mostly formed by microbial activity.

Materials and Methods

Soil

The experiment was conducted using 21 agricultural soils typical of Germany and Slovenia. There was a big variation in the different soil characteristics (soil textures, organic matter content, total N, C/N, plant available P, oxalate extractable Al3+, Fe3+, Cu2+, CEC, pH-CaCl2, water content at water potential of -15 kPa and heterotrophic bacteria). All soils were taken from the upper Ap layer of arable fields (0-30 cm), sieved (2 mm) after sampling, homogenized and stored at 4°C in the dark before use. At the beginning of the experiments all soils were conditioned and moistened to a water potential close to -15 kPa at room temperature (20 ± 2 °C) for 2 weeks and compacted to the soil density of 1.3 g/cm3 to equilibrate the microbial processes and to make sure that all soils have the comparable conditions at the start of the experiments.

Table 8.1.1.2-66: Some characteristics of soil samples

Name of soil (site of origin)	Sand [%]	Silt [%]	Clay [%]	Water content -15 kPa (%)	pH ($CaCl_2$)	Organic mattter [%]	C [%]	N [%]	P_2O_5 (mg $100g^{-1}$)	K_2O (mg $100g^{-1}$)
1 (Ada-A02)	62.5	27.4	10.1	21.9	5.7	2.9	1.7	0.2	17	4.1
2 (Apace-njiva)	66.4	31.2	2.4	20.7	7.0	2.6	1.5	0.2	4	22.6
3 (Berta-A02)	46.4	39.4	14.2	28.1	5.7	2.5	1.5	0.2	8	12.5
4 (Brezje)	8.3	73.2	18.5	32.7	5.2	2.8	1.6	0.2	5	21.1
5 (Dunja - A06)	62.4	25.9	11.7	17.4	5.4	2.2	1.3	0.2	11	13.2
6 (Feldkirchen)	34.8	47.0	18.2	28.2	7.0	3.4	2.0	0.3	39	9.4
7 (Grace - A13)	50.3	41.3	8.4	21.0	5.4	2.6	1.5	0.2	12	9.6
8 (Hanna - A15)	62.3	24.2	13.5	18.4	5.2	1.7	1.0	0.1	7	8.2
9 (Hohenwart)	67.2	20.5	12.3	22.4	6.2	1.7	1.0	0.1	21	21.1
10 (Joy -A19)	31.6	45.6	22.8	31.9	5.9	2.7	1.6	0.2	34	43.2
11 (Kelheim)	76.2	15.5	8.3	12.5	6.5	1.2	0.7	0.1	23	17.0
12 (Konjise)	33.8	60.2	6.0	34.6	6.9	4.5	2.6	0.2	4	7.9
13 (Lamanose)	10.3	69.6	20.1	35.8	5.8	4.3	2.5	0.3	5	18.7
14 (Lea -A18)	18.9	66.8	14.3	28.9	5.2	1.9	1.1	0.2	6	23.8
15 (Lomanose)	21.9	60.2	17.9	25.8	5.8	1.7	1.0	0.2	11	16.8
16 (Neumark)	85.5	8.8	5.7	12.6	5.2	1.6	0.9	0.1	11	12.2
17 (Pearl - A20)	29.3	51.8	18.9	28.3	5.0	2.3	1.3	0.2	12	31.7
18 (Scheyern Lysi)	17.2	62.6	20.2	30.1	5.5	2.7	1.6	0.2	20	5.3
19 (Skrinjar)	67.5	27.0	5.5	19.2	7.1	1.6	0.9	0.1	21	24.7
20 (Zepovci)	41.3	43.1	15.6	24.0	5.7	2.9	1.7	0.2	11	24.7
21 (Zepovci (Plitv.))	11.8	72.2	16.0	27.4	5.2	1.9	1.1	0.2	8	20.2

Name of soil (site of origin)	Al_{ox} (mg $100g^{-1}$)	Fe_{ox} (mg $100g^{-1}$)	Cu^{2+} (mg kg^{-1})	Ca	Mg	K	Na	H	CEC	Heterotrophic bacteria (x10^7 CFU g^{-1})*
				[$mmol_c$ $100g^{-1}$]						
1	63	198	4	8.5	0.8	1.0	0.04	5.7	16.0	0.3
2	62	248	4	11.1	2.3	0.1	0.04	1.5	15.0	0.5
3	76	265	3	9.0	1.0	0.6	0.04	5.3	15.9	0.4
4	187	518	2	7.2	0.9	0.6	0.07	11.1	19.8	0.1
5	80	211	62	7.0	0.6	0.6	0.04	5.3	13.6	0.3
6	139	310	12	26.4	2.5	0.5	0.05	3.5	32.9	1.1
7	106	259	3	8.7	0.7	0.6	0.04	7.4	17.5	0.6
8	83	215	2	7.2	0.5	0.2	0.04	5.7	13.6	0.5
9	75	206	4	5.5	1.2	0.4	0.05	3.9	11.1	1.6
10	101	320	39	13.1	1.8	0.7	0.06	6.7	22.4	0.9
11	44	132	8	5.5	1.2	0.3	0.05	2.0	9.1	0.8
12	88	381	7	10.8	4.6	0.1	0.06	3.2	18.8	0.1
13	134	456	4	16.4	3.6	0.3	0.06	9.2	29.6	0.4
14	107	345	3	6.4	0.8	0.4	0.07	6.9	14.6	0.3
15	72	252	3	9.5	1.8	0.3	0.09	5.4	17.0	0.7
16	88	110	1	2.6	0.4	0.2	0.05	4.3	7.5	0.7
17	125	319	4	6.9	0.8	0.5	0.05	8.8	17.0	0.8
18	102	349	10	9.1	1.6	0.6	0.06	7.1	18.4	0.9
19	57	257	4	10.8	0.5	0.1	0.06	1.5	13.0	0.5
20	165	476	4	7.8	0.7	0.9	0.05	10.6	20.0	0.3
21	147	430	2	4.4	0.5	0.7	0.10	9.4	15.1	0.1

* CFU = colony-forming unit at the start of degradation experiments

Chemicals

14C-labelled glyphosate [N-(phosphonomethyl)glycine purity >97.0%.] was labelled on the phosphonomethyl group. 14C-glyphosate was mixed with non-labelled glyphosate (purity 98%) resulting in a final specific radioactivity of 1.6 Bq/mg (for degradation experiments). Aminomethylphosphonic acid (AMPA) had the purity of 98 %. Sodium hydroxide (NaOH), monopotassium phosphate (KH2PO4), sodium chloride (NaCI), calcium chloride dihydrate (CaCl2x2H20), NH4CI, NH4NO3, methanol (CH40), diatomaceous earth, water for chromatography were purchased commercially.

Glyphosate mineralization experiments

Application - All biodegradation experiments were performed in 4 replicates with 50 g soil (dry mass) for each replicate. 14C-glyphosate was dissolved in autoclaved and distilled water and mixed with non-labeled glyphosate which was also dissolved in sterilized distilled water. This was the application standard solution with a concentration of a.i of 5.42 µg/L and a specific radioactivity of 166.70 Bq/µg. The application standard (0 .089 mL) was applied to an oven dried, pulverized soil and carefully stirred for 1 minute with a spatula. The spiked aliquot was transferred to another glass beaker containing the rest of equilibrated soils and mixed for another 2 min. The total concentration of glyphosate was 10 µg/g in each set corresponding to a total radioactivity of 83,000 Bq.

Test system, experimental conditions and samplings - The spiked soils were compacted to a soil density of 1.3 g/cm3 and soil water was adjusted to a water potential of -15 kPa. The f1asks were covered with special rubber caps, and incubated at 20 ± 1°C in the dark for a maximum period of 32 days. The soil humidity was controlled weekly. The rubber caps were equipped with an air inlet and outlet system as well as a facility to trap the evolved CO2. The air exchange system should prevent unaerobiosis in the incubation flasks and consisted of a canal which was made of a stainless needle with a diameter of 1 mm. To eliminate CO2 from the ambient air entering the flasks, a 12 mL plastic syringe filled with granular CO2 absorber (soda lime) was connected to the canal at the top of the cap. Below the cap a small plastic beaker was placed containing 0.1 M NaOH solution to capture 14CO2 released from glyphosate mineralization from the soil samples. The NaOH solution was exchanged three times per week and from the collected solution an aliquot of 2 mL was mixed with 3 mL of scintillation cocktail Ultim a Flo AF to determine 14CO2 in a liquid scintillation counter. At the end of the experiment, 30 g of each soil sample (dry mass) were used for pore water extraction, 7 g of each soil sample were extracted with NaOH to determine the quantity and quality of the extractable residues as well as to quantify the non-extractable residues, while 1 g of each soil sample was used for cell counts.

NaOH extraction, clean up and HPLC analysis- For NaOH extraction, the method used by Gimsing et al. (2004a) was applied. At the end of the experiment, soil was extracted with 0.1 M NaOH by shaking on overhead shaker for 17 hours. The supernatant was collected after centrifuging for 10 min at 3020 rcf. Radioactivity of the filtered supernatant was measured by scintillation counting using 100 µl of supernatant aliquot and 5mL of scintillation cocktail Ultima Gold XR to quantify the NaOH extractable pesticide residues. Subsequently, extracts were concentrated and cleaned up before injecting to HPLC. Twenty µl of each sample (NaOH extract) were injected via an Auto Sampler AS50 to a HPLC system that was connected with a Radioflow detector LB 509. 14C-glyphosate and its metabolites (AMPA, sarcorsine, glycine, methylamine) were identified by comparison of their retention times with standard substances. After each analysis the column was regenerated with Regenerant-RG019 at a flow velocity (isocratic) of 0.5 mL/min for 30 min.

Quantification of non-extractable 14C-labelled residues - After extraction with 0.1 M NaOH, the rest of radioactivity remaining in the soil was considered as non-extractable residues. Soil material was intensively mixed and homogenized with diatomaceous earth for 2 min in a mortar. Four aliquots of each soil sample were weighed in combustion cups and mixed with 8 drops of saturated aqueous sugar solution to accelerate and ensure a complete oxidation of the 14C. The oxidation step was done with an automatic sample-oxidizer 306. 14CO2 from the combustion was trapped in Carbo-Sorb E and mixed with Permaflour E before scintillation counting. The extractable and non-extractable glyphosate residues were calculated after the combustion.

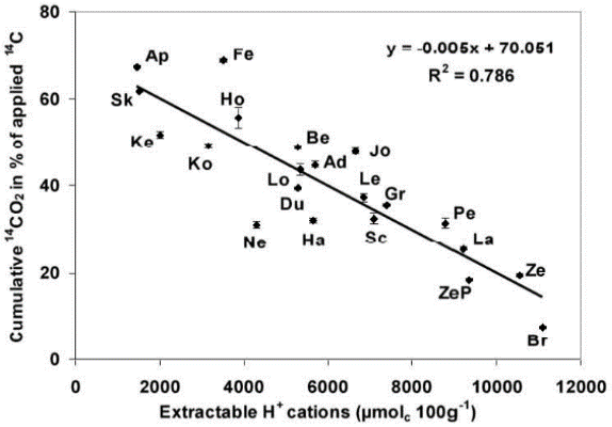
Bacterial cell counts- Bacterial cell counts were performed to count the cultivable and heterotrophic bacteria in the different soils. The method for bacterial cell counts was adapted from Ngigi et al. (2011). Soil bacteria were extracted from the soil by mixing soil with a buffer solution. Before use the buffer solution was autoclaved and shaken vigorously for 1 hour on a shaker at 150 rpm. The soil particles were allowed to sediment for 10 min. Then 0.1 mL of the supernatant was transferred to sterilized buffer solution for further dilution steps. A total of 4 dilutions (10-1 to 10-4) were established. Finally, 0.1 mL of each dilution was spread in triplicates on Lysogeny broth (LB) agar media. This medium was also autoclaved before use. The number of CFU was determined after three days of incubation at 25 0C by counting.

Table 8.1.1.2-67: Behavior of ^{14}C-glyphosate in different soils

Soil	Cum. Min (%)[a] (1)	NaOH extract. residues (%)[a]* (2)	Non-extract. residues (%)[a] (3)	Total recovery** (%)[a] (4)	Quality of NaOH extract. residues		
					Glyphosate (%) (5)	AMPA[d] (%) (6)	Unknown (%) (7)
1	44.7	48.3	4.8	97.8	37.7	2.3	8.3
2	67.3	24.5	9.6	101.4	18.9	2.2	3.4
3	48.9	42.7	6.3	97.9	34.4	0.0	8.3
4	7.6	91.0	2.5	101.1	88.0	0.0	3.0
5	39.5	53.9	3.9	97.3	45.2	0.0	8.7
6	68.7	23.3	9.0	101.0	12.2	7.6	3.6
7	35.5	57.7	3.7	96.9	44.1	2.8	10.8
8	32.2	59.8	4.1	96.1	54.0	0.0	5.8
9	55.8	37.7	6.3	99.8	24.8	5.7	7.3
10	47.9	46.9	8.2	103.0	31.3	7.1	8.5
11	51.8	37.3	6.2	95.3	25.1	4.1	8.1
12	49.1	35.7	9.5	94.3	23.3	4.5	7.9
13	25.5	64.4	6.7	96.6	45.0	8.3	11.1
14	37.3	55.7	5.4	98.4	30.0	0.0	25.7
15	43.7	46.8	6.4	96.9	30.4	8.0	8.4
16	31.2	63.0	3.1	97.3	48.8	2.5	11.7
17	31.5	63.6	3.7	98.8	29.5	0.0	34.1
18	32.5	59.8	5.0	97.3	40.9	5.5	13.4
19	61.6	28.8	11.4	101.8	16.9	4.8	7.1
20	19.5	73.3	4.1	96.9	65.8	0.0	7.5
21	18.4	78.5	2.7	99.6	55.9	11.3	11.3

a) % of applied ^{14}C-glyphosate after 32 days; the mean value is presented
b) Aminomethylphosphonic acid
* Total NaOH extractable residues (2) = (5) + (6) + (7)
** Total recovery (4) = (1) + (2) + (3)

Figure 8.1.1.2-24: Correlation between cumulative mineralization of glyphosate and extractable H+ cations in soils (bars indicate standard deviation of 4 samples)



Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA) and multiple regression analysis.

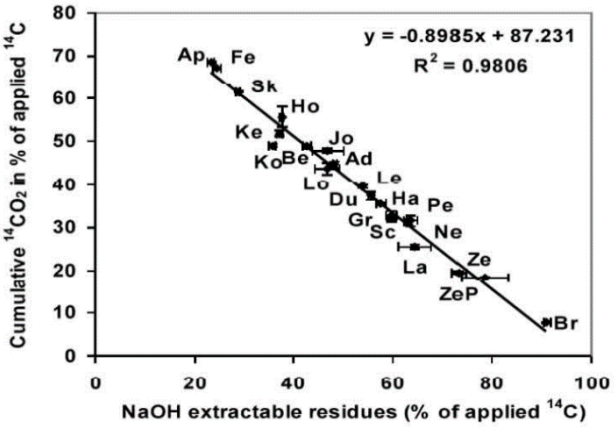
Results and Discussion

The main aim in this part of the study is to check the correlation between soil parameters and glyphosate mineralization. The selected soil parameters for correlation were exchangeable [H+], silt, clay, soil organic matter, C, N, C/N, P2O5, Cu2+, oxalate extractable Al3+, oxalate extractable Fe3+, K2O, CFUbeginning and CFUend, Ca2+ , Mg2+ , K+ , Na+ , CEC, and Ph.

Mineralization of glyphosate

After 33 days of incubation a big variance of cumulative mineralization can be observed. Between 7.6 to 68.7 % of the applied ^{14}C -glyphosate was mineralized to $^{14}CO_2$ in the 21 different soil types (Table 8.1.1.2-67). Shortly after application, a high amount of glyphosate was mineralized. The lowest mineralization of ^{14}C-glyphosate was identified in Brezje soil while the highest mineralization of ^{14}C-glyphosate was obtained in Feldkirchen and Apace-njiva soils. Low mineralization of glyphosate was also observed in Zepovci, Zepovci(Plitv.) and Lamanose soils. In these 3 soils less than 30 % of the initial glyphosate was mineralized after 32 days. In contrast, other soils had a higher mineralization activity and $^{14}CO_2$ production after 32 days reached 31.2-68.7 % of the initial glyphosate. A big difference in biomineralization of glyphosate among 21 soils indicates that agricultural soils have difference in ability to degrade glyphosate. The firstly rapid mineralization of glyphosate was observed for most soils during the first 4 days without a lag phase, but mineralization rates subsequently decreased over time, as found in other earlier studies (von Wiren-Lehr *et al.*, 1997; Gimsing *et al.*, 2004a). At the end of the biodegradation experiments, mass balances were established. Mass balances of ^{14}C-glyphosate are presented in Table 8.1.1.2-67. In all soils, the ^{14}C mass balances were quite good: over 94 % of the totally applied ^{14}C-glyphosate was recovered at the end of the biodegradation experiments.

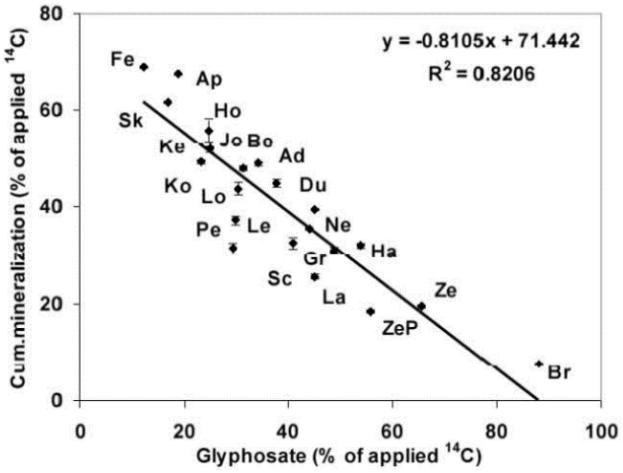
Figure 8.1.1.2-25: Correlation between cumulative mineralization of glyphosate and NaOH extractable residues (bars indicate standard deviation of 4 samples)



Identification of the parameters governing mineralization of glyphosate

In order to identify the factors which govern glyphosate mineralization in the 21 soils, soil parameters, NaOH extractable residues, 14C-glyphosate residues, non-extractable residues and the mineralized glyphosate were compared at the end of the biodegradation experiments and several significant correlations could be discovered.

Figure 8.1.1.2-26 Correlation between cumulative mineralization of glyphosate and glyphosate residues (from extractable residues) (bars indicate standard deviation of 4 samples).



Relationship between mineralized glyphosate and extractable acidity (extractable H+ cations)

According to univariate correlation analysis there was highly significant and negative correlation between the cumulative mineralization glyphosate and extractable H+ cations (p = 0.000). This illustrates that the extractable H+ cations interfered the mineralization process in soils. Therefore, the assessment of extractable H+ cations in soils appears suitable for ranking of soil according to the mineralization of the compound.

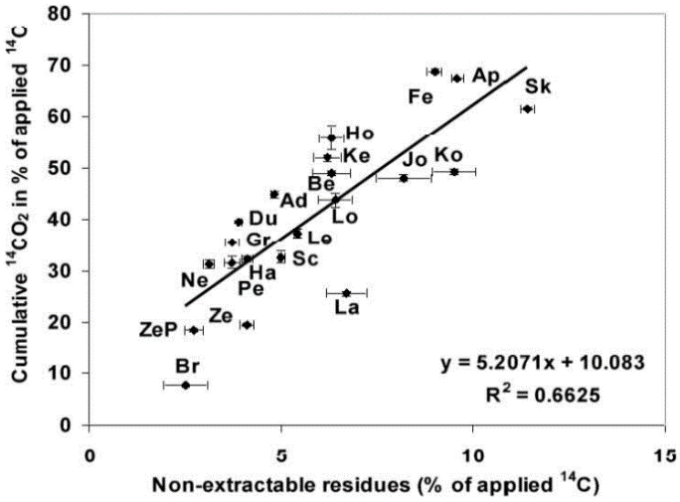
Relationship between mineralized glyphosate and NaOH extractable residues

NaOH extractable residues of the 21 investigated soils were performed after 32 days. The results show that the NaOH extractable fraction in all soils was relatively high and very various. Approximately between 23 and 91 % of initial glyphosate after 32 days incubation was extracted with NaOH 0.1 M. Soils with higher mineralization had lower NaOH extractable fraction. A correlation was performed to check the relationship between mineralized glyphosate and NaOH extractable residues. There was a negative correlation between mineralized glyphosate within 32 days and NaOH extractable residues (p = 0.0000). This shows that NaOH extractable residues were non-available for microorganisms to be degraded.

Relationship between mineralized glyphosate and 14C-glyphosate residues from extractable pool

14C-glyphosate is the major component in the NaOH extract as compared to AMPA and unknown metabolites. To test whether there is any relationship between the mineralized glyphosate and NaOH extractable residues, we calculated correlation between both values. There is exist significantly negative correlation between 14C-glyphosate residues from extractable pool and mineralized glyphosate (p = 0.0000). This indicates that in soils with low mineralization glyphosate is present in a high amount and that this glyphosate could not be degraded/mineralized because it was adsorbed to Al- or Fe-oxides.

Figure 8.1.1.2-27: Correlation between cumulative mineralization and non-extractable residues (bars indicate standard deviation of 4 samples)



Relationship between mineralized glyphosate and non-extractable residues

The amount of non-extractable residues was relatively low. It varied between 2.5 % and 11.4 % of the initial glyphosate. The non-extractable residues and mineralized glyphosate were compared together to see whether there is any relationship between both parameters. A significant and positive correlation between mineralized glyphosate and non-extractable residues (p = 0.0000) was found. The high mineralization of glyphosate in soils coincided with non-extractable residues at the end of the experiment.

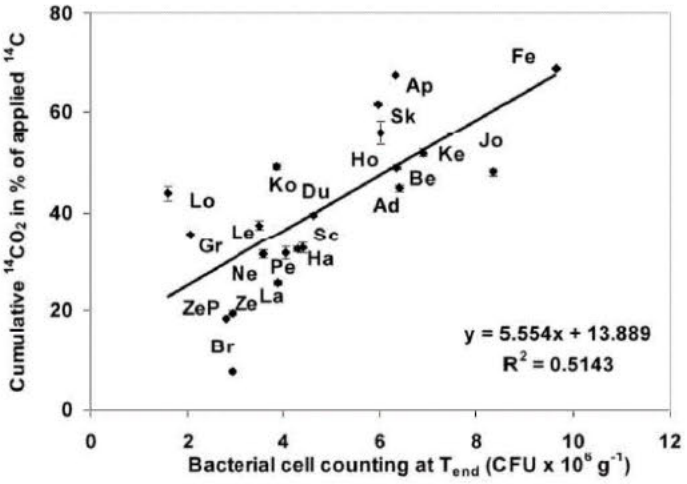
Relationship between mineralized glyphosate and bacterial cell counts

There was a significantly positive correlation between mineralization of glyphosate and bacterial cell counts (p = 0.003). This shows that the mineralization of glyphosate in soils is limited not only by availability of glyphosate and its degradation products, but also by the bacterial activity. Therefore, it can be assumed that the bacterial cell numbers at the end of the experiment seemed to be the degrading microorganisms for glyphosate in soils and it was likely that microbes capable of degrading glyphosate aerobically exist in soils.

The interacting junctions of the different soil parameters on mineralized glyphosate

In order to investigate the interacting functions of the different soil parameters on cumulative glyphosate mineralization, a multiple regression analysis was used. The input parameters were extractable H+ cations, silt, clay, soil organic matter, C, N, C/N, plant available P, Cu2+, oxalate extractable AI3+, oxalate extractable Fe3+, plant available K, Ca2+, Mg2+, K+, Na+, CEC, pH , CFUs at beginning and CFUs at the end of the experiments. The result of multiple regression analysis reveals extractable H+ cations, Ca2+ and plant available K as key parameters governing glyphosate mineralization in the 21 tested soils and Ca2+ and plant available K contributes additionally to extractable H+ cations to the mineralization of glyphosate. In this multiple regress ion, extractable H+ cations has a negative correlation with mineralization of glyphosate, whereas exchangeable Ca2+ and plant available K have a positive correlation with cumulative mineralization of glyphosate. Once again, this result indicates that extractable H+ cations is an important factor which reduces the bioavailability of glyphosate in soils, and as a consequence the mineralization of glyphosate is reduced. Regarding Ca2+ and plant available K, cumulative mineralization was found to be positively correlated with exchangeable Ca2+ and plant available K, respectively. Therefore, it is proposed in this study that a complexation between glyphosate with exchangeable Ca2+/plant available K will not reduce the bioavailability and mineralization of glyphosate. In the contrary, Ca2+-glyphosate complexes may be transported more efficiently across microbial cell walls than sole glyphosate compound as it has already been argued for Cu2+ complexes in literature (Kools et al., 2005). However, these mechanisms have not been documented and should be clarified.

Figure 8.1.1.2-28: Correlation between cumulative mineralization of glyphosate and bacterial cell counting (bars indicate standard deviation of 4 sample)



Conclusions

Degradation of glyphosate in soils greatly varies depending on soil properties. Mineralized glyphosate is affected by extractable acidity (H+ cations) and bacterial cell counts. Sorption behavior and bioavailability of glyphosate in soil are important to regulate mineralization. Extractable H+ cations. Ca2+ ions and plant available K have been identified as important soil parameters that collectively control the mineralization of glyphosate in soil. Glyphosate that is absorbed by AI/Fe-oxides and extractable H+ cations can be extractable with NaOH 0.1 M, but it is not available for degradation by soil microorganisms. Non-extractable residues of glyphosate which have been identified as a result of microbial activity.

Assessment and conclusion by applicant:

The article describes the dissipation of glyphosate in agricultural soils in Europe. While a lot of experimental details are reported, the data are insufficient for kinetic evaluation since tests were run for 32 days in maximum only and determination of mineralization only, i.e. no detailed analysis for active substance and metabolites.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, the article lacks data on residues of glyphosate and AMPA to allow determination of endpoints. In addition the study duration was very short.

The article provides supportive information on the degradation of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Bergström, L. et al

Data point:	CA 7.1.2.1.1/017
Report author	Bergström, L. et al.
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Document No	DOI 10.2134/jeq2010.0179 E-ISSN 1537-2537
Guidelines followed in study	Degradation experiment: none Adsorption experiment: OECD 106 Guideline Lysimeter experiment: none

Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Due to the increasing concern about the appearance of glyphosate [N– (phosphonomethyl) glycine] and its major metabolite aminomethylphosphonic acid (AMPA) in natural waters, batch laboratory and lysimeter transport studies were performed to assess the potential for leaching of the compounds in two agricultural soils. Unlabelled and ^{14}C–labelled glyphosate were added at a rate corresponding to 1.54 kg a.s./ha on undisturbed sand and clay columns. Leachate was sampled weekly during a period of 748 d for analyses of glyphosate, AMPA, total ^{14}C, and particle–bound residues. Topsoil and subsoil samples were used for determination of glyphosate adsorption, glyphosate degradation, and formation of AMPA and its degradation. The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation rate in the clay soil (half–life 110–151 d). The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation rate can be faster than that of glyphosate. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. Despite large amounts of precipitation in the autumn and winter after application, however, these residues were mainly located in the topsoil, and only 0.009 and 0.019 % of the initial amount of glyphosate added leached during the whole study period in the sand and clay, respectively. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil.

Materials and methods

Lysimeter Experiment

Soil Characteristics, Lysimeter Collection, and Management

Three undisturbed soil columns of a sandy soil and four of a clay soil were used. The smaller number of sand columns was based on the fact that sandy soils are usually more homogeneous and therefore show less variability in flow processes (Bergström & Shirmohammadi, 1999). Some physical and chemical properties of the two soils used are listed in Table 8.1.1.2-68. The soil columns were collected using coring equipment in which a polyvinyl chloride pipe (1.18–m long and 0.295–m inner diam.) is gently pushed into the soil by a steel cylinder with cutting teeth, which rotates around the pipe as it penetrates the soil (Persson and Bergström, 1991). After collection at the two field sites, Lanna in southwest Sweden (58°21′ N, 13°08′ E) and Nåntuna close to Uppsala (59°49′ N, 17°39′ E), the columns were prepared for gravity drainage by removing about 0.07 m of soil at the base, which was replaced by gravel, two stainless steel meshes, and a fiberglass lid, giving a final length of the soil columns of ∼1.05 m. The lysimeters were then placed in vertical pipes permanently installed below ground at a lysimeter station located at the Swedish University of Agricultural Sciences in Uppsala, Sweden (Bergström, 1992).

Table 8.1.1.2-68: Selected soil characteristics of the Lanna clay and the Nåntuna sand. Standard laboratory methods were used throughout (Bergström *et al.*, 1994)

Layer	Soil texture (Gr/Sa/Si/Cl)†	Organic matter	Cation exchange capacity	Bulk density	pH‡	Water content at tensions (cm)		
						0§	100	15,000
cm	%		$cmol_c\ kg^{-1}$	$g\ cm^{-3}$		$m^3\ m^{-3}$		
Clay								
0–30	1.3/6.0/46.2/46.5	4.4	28.4	1.24	7.2	0.524	0.359	0.193
30–60	0.6/2.7/40.6/56.1	0	33.6	1.43	7.4	0.477	–	0.249
60–90	0.2/1.8/37.4/60.6	0	–	1.46	7.4	0.468	–	0.297
Sand								
0–30	0/87.8/4.5/7.7	2.0	4.7	1.43	7.4	0.448	0.180	0.034
30–60	0/95.4/4.6/0	1.0	1.8	1.47	6.4	0.427	0.165	0.019
60–90	–	1.0	1.4	1.46	7.0	0.450	0.065	0.016

† Gr = gravel, >2 mm; Sa = sand, 0.06–2 mm; Si = silt, 0.002–0.06 mm; Cl = clay, <0.002 mm.

‡ Determined in water.

§ Equivalent to porosity.

All management practices performed on the lysimeters were intended to reproduce field conditions as closely as possible. Just before sowing in each year, the soil in each lysimeter was hand–tilled to simulate light harrowing. Spring barley (Hordeum distichum L.) was sown at a rate of 2 g per lysimeter on 21 May 2006, 26 May 2007, and 30 May 2008. On each occasion, mineral fertilizers were applied at rates of 100 kg N/ha, 22 kg P/ha, and 56 kg K/ha. The barley was harvested on 1 Sept. 2006, 28 Sept. 2007, and 16 Sept. 2008 by cutting the aboveground plant parts at ground level.

In addition to natural precipitation, all lysimeters received supplemental irrigation on two occasions during the 2–yr experimental period (in total, 22 mm). On each occasion, water was added with spray bottles over a few hours at rates typical of heavy rain storms, but not exceeding the infiltration capacity of the soil.

Chemical Application

Glyphosate was applied to two lysimeters of the sand soil and to three lysimeters of the clay soil on 18 Sept. 2006 at a rate corresponding to 1.54 kg a.s./ha, which represents a normal dose in Swedish cereal production systems. Radiolabeled [^{14}C] glyphosate (ARC 1313 glyphosate-[phosphonomethyl–^{14}C], 50 mCi/mmol, American Radiolabeled Chemicals, Inc., St. Louis, MO) was used to obtain fast screening of the leachate samples using scintillation counting analysis. The radiolabeled portion (5.32 MBq) was mixed with formulated (Roundup BIO, contains 486 g glyphosate/L as isopropylamin salt, Monsanto Crop Sciences), unlabeled glyphosate (in total 10.5 mg/lysimeter), which was dissolved in 11 mL (0.16 mm) of water. This solution was applied to the lysimeters by dripping it on the soil surface using a syringe. After the solution had been applied, 5 mL (0.07 mm) of water was drawn up into the syringe and also applied to each lysimeter. In addition to glyphosate, KBr at a rate of 0.268 g Br^- per lysimeter (~40 kg Br^-/ha) was applied to provide information on the movement of water through the soil columns. The KBr was dissolved in water (0.4 g KBr in 5 mL), which was applied separately to the lysimeters, also using a syringe.

Soil and Water Sampling

On 17 Oct. 2007, samples of the topsoil (0–30 cm) and subsoil (30–80 cm) of each soil were collected for determination of adsorption and degradation characteristics. These samples were taken from the lysimeter of each soil used as control (i.e., no glyphosate added). Three soil cores from each lysimeter were collected with a tube drill. The individual samples were then mixed by layers into a topsoil and a subsoil sample for each lysimeter. On 5 Oct. 2008, after leaching measurements were terminated, soil samples were collected from the lysimeters to which glyphosate had been applied to determine the residual amounts of glyphosate and AMPA about 2 yr after application. Three cores from each lysimeter were taken with a tube drill and divided into three layers (0–30, 30–60, and 60–90 cm), which were pooled to one sample for each lysimeter and layer. After collection, all soil samples were stored in a freezer (–20 °C) until analyzed.

Leachate from the lysimeters was collected and weighed each week during the 2–yr period when drainage water was available. After collection, all leachate samples were stored in a freezer (–20 °C) until analyzed. The amount of ^{14}C was measured in 10 mL of the leachate using a Beckman LS 6000TA liquid scintillation counter (Beckman Coulter Inc, Fullerton, CA) after addition of 10 mL of Insta–Gel Plus (PerkinElmer, Waltham, MA).

Adsorption Study

The adsorption study was performed according to the OECD 106 guideline (OECD, 2001). Adsorption data were obtained at five different concentrations in two replicate samples. Four grams dry weight (DW) of field–moist soil were shaken at 200 rpm on a shaker for pre–equilibration with 39 mL of 0.01 M $CaCl_2$ for 24 h at 20°C in 50–mL plastic tubes. Thereafter, the soil slurry was spiked with 1 mL of a mixture of labeled (1.98 kBq) and unlabeled glyphosate in 0.01 M $CaCl_2$ to give five initial concentrations in the range 0.1 to 10 µg/g dw of soil. After shaking for 24 h, the tubes were centrifuged for 20 min at 4000 rpm and then the radioactivity was measured in 10 mL of the supernatant. Tubes without soil and ^{14}C–labeled glyphosate were included for subtraction of background radiation, and tubes without soil were used to give the initial amount of ^{14}C activity added. No significant adsorption of glyphosate occurred on the plastic tubes. A pre–study showed that adsorption equilibrium was obtained after 24 h of contact time between soil and solution, which also indicates that negligible amounts of AMPA had been formed.

Adsorption data were fitted by nonlinear regression to the Freundlich adsorption isotherm:

[1]

$$c_{soil} = K_f c_{aq}^{1/n}$$

where c_{soil} (µg/g) is the adsorbed amount, c_{aq} (µg/mL) is the concentration in the aqueous phase, K_f [$\mu g^{1-1/n}$ $(mL)^{1/n}$/g] is the Freundlich adsorption coefficient, and $1/n$ (–) the measure of nonlinearity.

Degradation Study

Glyphosate dissolved in water (1.4 mg/mL) was applied dropwise (1.0 mL) to 15 g of fresh soil. The soil was dried and mixed, after which an additional amount of fresh soil (to give 140 g DW in total) was thoroughly mixed into the spiked soil to give an initial concentration of 10 µg glyphosate per g DW of soil. Portions corresponding to 10 g of dry soil were transferred to 50–mL plastic tubes. The water content was adjusted to 60 % of the water–holding capacity. The tubes were sealed with plastic caps that allow gas exchange and incubated at 20°C in the dark. After 2, 4, 8, 16, 32, and 64 d, two tubes were put in the freezer (−20 °C) until analysis for residual concentrations of glyphosate and the metabolite AMPA. The weight of the tubes was measured once a week during the incubation, and when necessary, the moisture content was adjusted to 60 % of the water–holding capacity.

Residual values of glyphosate were used for a least squares fitting procedure to determine values of the parameters of the function for first order exponential decay:

[2]

$$c_G(t) = c_{G0} e^{-k\,t}$$

where c_G (mg/kg) is the residual concentration of glyphosate at time t days after application, c_{G0} (mg/kg) is the initial concentration of glyphosate, and k (d^{-1}) is the first–order rate coefficient for degradation.

A branched reaction scheme was applied to describe the degradation of glyphosate to AMPA and sarcosine (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008) and the degradation of AMPA (Figure 8.1.1.2-29). According to this scheme and assuming first–order kinetics, the rate of AMPA formation and degradation is then

[3]

$$\frac{dc_A}{dt} = 0.66 k_1 c_G - k_2 c_A$$

where c_A (mg/kg) is the concentration of AMPA at the time t. Because the concentrations of glyphosate and AMPA were expressed in units mg/kg, the value of c_{G0} obtained from Eq. [2] was multiplied by the stoichiometric factor 0.66 (i.e., the ratio of the molecular weights of the dominant species of AMPA and glyphosate at pH 7) in these calculations. The equation describing the concentration of AMPA was obtained by combining Eq. [2] and [3], and integrating:

[4]

$$c_A = \frac{0.66 k_1 c_{G0}}{k_2 - k}(e^{-kt} - e^{-k_2 t})$$

In this branched pathway, k for glyphosate degradation in Eq. [2] equals the sum of k_1 for AMPA formation and k_3 for sarcosine formation. Then $k_3 = k - k_1$ and the fractions of glyphosate transformed into AMPA and sarcosine are k_1/k and k_3/k, respectively. Since no more than 100 % of the glyphosate can be transformed into AMPA, the upper limit for k_1 is k, in which case $k_3 = 0$. The maximum concentration of AMPA, c_{Amax}, occurs at time t_{Amax} when $dC_A/dt = 0$. Inserting this value into Eq. [3], replacing c_G and c_A in Eq. [3] by their expressions in Eq. [2] and [4], respectively, and rearranging gives the following:

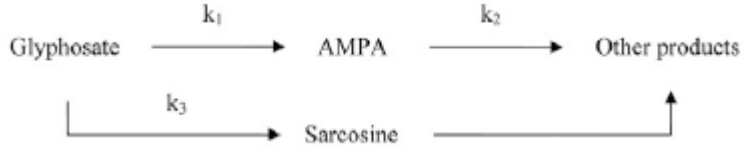
[5]

$$t_{A\,max} = \frac{\ln(k) - \ln(k_2)}{k - k_2}$$

Nonlinear Regression

Least squares fits of data on adsorption and on residual values of glyphosate and AMPA were fitted to their respective equations by nonlinear regression. Residual values of AMPA were fitted using the values of c_{GO} and k for glyphosate degradation as obtained from Eq. [2]. The calculations were performed on a PC with the application SigmaPlot for Windows version 10.0 (Systat Software, Inc., San Jose, CA); the nonlinear regression method is based on the Levenberg and Marquardt method.

Figure 8.1.1.2-29: Branched reaction scheme with the first-order rate coefficients k_1 and k_3 for the degradation of glyphosate to aminomethylphosphonic acid (AMPA) and sarcosine, respectively, and k_2 for the degradation of AMPA



Glyphosate and AMPA Analyses

Reagents

Analytical standards used for calibration were (trivial names in italics): N−(phosphonomethyl) glycine, *glyphosate*, (Riedel−de−Haën, Sigma−Aldrich, Sweden AB) and (aminomethyl) phosphonic acid, *AMPA*, (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Internal standards were ^{13}C; ^{15}N; ^{2}D−labeled AMPA and ^{13}C; and ^{15}N−labeled glyphosate (LCG Standards AB, Borås, Sweden). Concentrated HCl, ethyl acetate, and NaOH (analytical reagent grade from VWR, Stockholm, Sweden), were used for extraction and solvation. The AG1−X8, 100−200, formate form (Bio Rad Laboratories, Sundbyberg, Sweden) and Isolute C18 EC 200 mg (Sorbent AB, Gothenburg, Sweden) were used for ion exchange and clean−up. Trifl uoroacetic anhydride (TFAA) and trifluoroethanol (TFE), both analytical reagent grade from Sigma Aldrich Sweden AB (Stockholm, Sweden), were used for the derivatization. The 0.22−µm glass fiber filters # GSWP04700 were from Millipore VWR (Stockholm, Sweden).

Calibration

Stock solutions of glyphosate and AMPA were diluted in water to concentrations of 100 µg/mL and stored at +4°C. A solution containing 1 µg/mL of glyphosate and AMPA was prepared daily as a working standard. The labeled glyphosate and AMPA were diluted in deionized water to a concentration of 1 µg/mL and stored at −20 °C in 2−mL portions.

Clean−Up and Derivatization: Water Samples

A 50−mL volume of a water sample and 0.1 µg each of glyphosate and AMPA internal standard were adjusted to pH 2 with 6 M HCl in a plastic tube. The sample was left to precipitate for 1 h and centrifuged at 5000 rpm for 10 min. The upper, clear phase was adjusted to pH 7 to 8. Ag1−X8 (2.3 g) was weighed into an empty 6 mL−plastic column equipped with a piece of cotton at the bottom, and the column was wetted with deionized water. A 3−mL (200 mg) C18 SPE column was activated with 3 mL of methanol

and 3 mL of water and connected on top of the AG1−X8 column. An empty 75−mL plastic column was connected on top of the C18 and Ag1−X8 columns, and the sample was applied at a rate of 2 mL/min. The two upper columns were removed and the analytes were eluted with 3×4 mL of 0.6 M HCl at a rate of 1 mL/min and collected in a 100−mL pear−shaped fl ask. The sample was evaporated to approximately 2 mL under vacuum, quantitatively transferred to an 8−mL glass tube and evaporated to dryness under an air stream at 50°C. The derivatization was performed by adding 1 mL of trifluoroethanol and 2 mL of trifluoroacetic anhydride, and the sample was held at 100°C for 1 h. After being cooled to room temperature, the sample was evaporated under nitrogen and redissolved in 1.00 mL of ethyl acetate before analysis.

Clean−Up and Derivatization: Particle−Bound Glyphosate and AMPA in Leachate

Leachate samples from three lysimeters of each soil were analyzed for particle−bound glyphosate and AMPA. These samples comprised two samples from the untreated lysimeters and four samples from the glyphosate−treated lysimeters on sampling occasions when the highest concentrations of glyphosate and AMPA were detected in the leachate. A 300−mL portion of each sample was filtered through a 0.22−µm glass fiber filter. The filter was weighed before and after filtration, dried at 105°C, and the dry weight of the particles was calculated. The dry filter and the particles were analyzed for glyphosate and AMPA by extraction with 7 mL of 0.1 M NaOH following the same procedure as for soil samples (see below).

Clean−Up and Derivatization: Soil Sample

Ten grams of soil were extracted with 40 mL (for the degradation study) or 75 mL (for the lysimeter soil residue analysis) of 0.1 M NaOH by shaking for 30 min at 200 rpm, sonicated for 10 min and centrifuged for 10 min at 5000 rpm. The internal standards (0.1 µg each of glyphosate and AMPA) were added to a portion (40 µL and 4 mL for the degradation and the lysimeter studies, respectively) of the clear upper part of the sample, which was then analyzed according to the procedures described for the water samples. The portion from the degradation study was evaporated and derivatized directly after precipitation of the extract, since no column clean−up was needed due to the high residual concentrations in these samples.

Instrumentation

The gas chomatography−mass spectrometry (GC−MS) analyses were performed with a Hewlett−Packard 6890 GC (Agilent Technologies Sweden AB), equipped with a 30 m by 0.25 mm i.d. (0.25−µm fi lm thickness) fused silica capillary column (HP−5 for GC–MS), a mass spectrometer 5973, a split/splitless injector, and the software Chemstation, all from Agilent Technologies (Kista, Sweden). One microliter of the samples was injected (in the splitless mode at 270°C, oven temperature 70°C). After 2 min, the oven temperature was raised to 170°C at 30°C/min and then from 170 to 250°C at 120°C/min. Helium (N47 grade, 99.997 %) was used as the carrier gas and the flow rate was 1.2 mL/min. The mass spectrometer was operated in the electron impact (EI) mode; the transfer line and manifold temperatures were 270 and 230 °C, respectively. Fragment ions were detected by selected ion monitoring (SIM) and used for identification of the AMPA and glyphosate derivates as shown in Table 8.1.1.2-69.

Table 8.1.1.2-69: Molecular weights, retention times (RT) and specific selected ions for compound derivatives.

Molecule	Molecular weight	RT (min)	Quantification ion (m/z, % relative abundance)	Qualification ion (m/z, % relative abundance)
AMPA†	371	4.49	126 (100)	302 (23)
AMPA‡	375	4.49	130 (100)	306 (22)
Glyphosate	511	5.35	411 (100)	384 (50)
Glyphosate‡	513	5.35	413 (100)	386 (48)

† AMPA = aminomethylphosphonic acid.

‡ Internal standard.

Verification of compound identification was based on comparison of the areas of the selected ions in the samples with those of the standards. For quantification, the response areas for AMPA and glyphosate target ions were calculated in relation to those of the internal standards. The response was found to be linear in the practical concentration range (2.5−100 pg) of individual components injected.

The quantification levels for glyphosate and AMPA were 0.1 µg/L in water and 0.01 µg/g in soil. In some samples, however, the quantification level was higher due to the specific background.

Results

Adsorption of Glyphosate

The high correlation coefficients (R^2 ≥0.997; Table 8.1.1.2-70) obtained when sorption data for both soils and soil layers were fitted to the Freundlich adsorption isotherm show that they could be accurately described by this model. The values of the K_f parameter obtained were considerably higher in the clay soil than in the sand and are similar to values previously reported for glyphosate sorption to soils of similar textures (Vereecken, 2005). In the sand, K_f was higher in the topsoil than in the sub−soil, whereas the opposite was true for the clay. The correlation between K_f and the amount of clay in the different soils was 0.987. Although based on only four soils (topsoil and subsoil in the respective soils), this result supports the generally held view that glyphosate is primarily sorbed to clay particles and their associated iron oxides (Vereecken, 2005). Normalisation of the distribution coefficients for glyphosate should therefore also account for the amount of clay and oxides present in soil and not organic carbon only, which is used to calculate K_{oc}. The 1/*n* parameter, which expresses the degree of linear relationship between c_{soil} and c_{aq}, was close to 1 for both layers of the clay soil and the sand topsoil, showing an almost constant distribution coefficient between sorbed and dissolved glyphosate in these soil layers in the range of concentrations studied. In the subsoil of the sand, the parameter 1/*n* was 0.82, indicating that the availability of sites for sorption in this layer becomes limiting at high glyphosate concentrations.

Table 8.1.1.2-70: Freundlich coefficients (K_f) (± SE, *n* = 10) for adsorption of glyphosate obtained by nonlinear regression according to Eq. [1]

Soil	K_f	1/*n*	R^2
	$\mu g^{1-n}(mL)^n g^{-1}$	–	–
Sand topsoil	40.0 ± 2.9	0.92 ± 0.04	0.997
Sand subsoil	28.7 ± 1.2	0.82 ± 0.02	0.998
Clay topsoil	118 ± 4.4	0.95 ± 0.01	1.000
Clay subsoil	165 ± 10.7	1.03 ± 0.02	0.999

Degradation of Glyphosate and AMPA

Best fits of glyphosate and AMPA residue data to Eq. [2] and [4], respectively, are shown in Figure 8.1.1.2-30 and the parameter values obtained in Table 8.1.1.2-71 and Table 8.1.1.2-72 5, respectively. Initial extraction efficiencies of glyphosate were 112 to 123 % as shown by comparing the initial concentrations obtained (Table 8.1.1.2-71) with the nominal value of 10 µg/g DW. All parameter values were significantly different from zero ($p < 0.05$, $n = 12$). The models gave good fits of the data for all soils ($R^2 \geq 0.90$), except for glyphosate in the clay subsoil ($R^2 = 0.56$). This poor fit could be due to difficulties in getting glyphosate homogeneously distributed in this clay−rich (56.1 %) subsoil with no organic matter (0 %). Another explanation could be that the R^2 values obtained by nonlinear and linear regression are not comparable. In nonlinear regression, R^2 refers to the fraction of the variance explained and is the model efficiency (EF). A disadvantage of EF is its dependency on the slope of the curve, as it is always relatively small for relatively flat decline patterns, or can even be negative for curves describing for instance formation and degradation of metabolites, irrespective of the scatter of measured data around the calculated curve (FOCUS, 2005). Therefore, from visual inspection of the fits to the data (Figure 8.1.1.2-30) and from the generally small standard errors in the parameters determined (Table 8.1.1.2-71 and Table 8.1.1.2-72), we concluded that the equations provide relevant quantitative information.

Figure 8.1.1.2-30: Best fits (A) to Eq. [2] of data on glyphosate and (B) to Eq. [4] of data on aminomethylphosphonic acid (AMPA) concentrations for sand topsoil (•), sand subsoil (○), clay topsoil (▼), and clay subsoil (^) (mean ± SE, n = 2). dw = dry weight

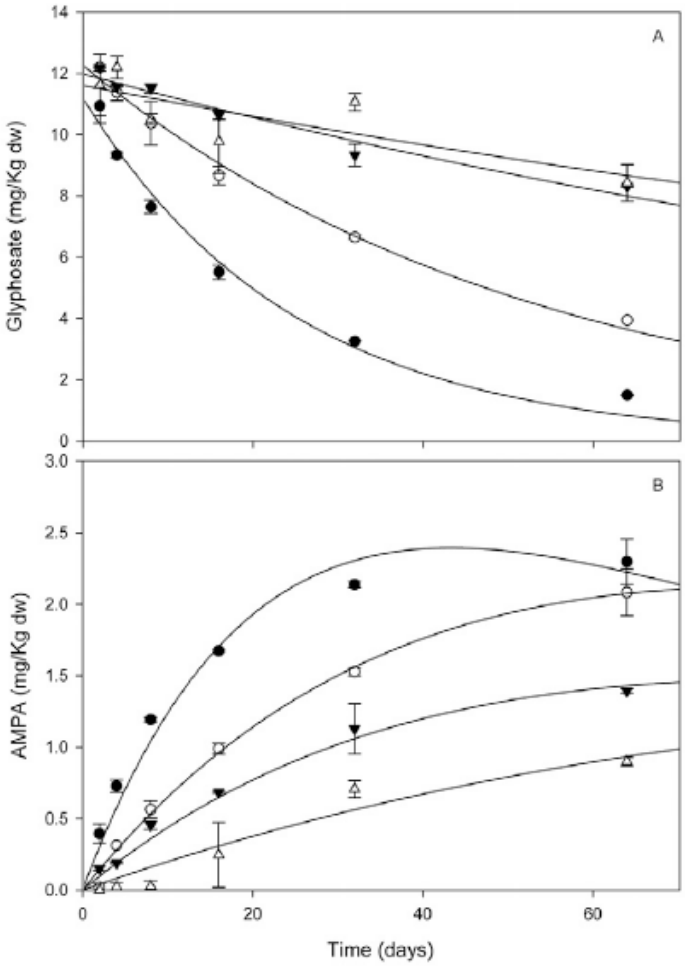


Table 8.1.1.2-71: Coefficients (± SE, n = 12) obtained by nonlinear regression for degradation of glyphosate according to first−order kinetics (Eq. [2]).

Soil	c_{G0}†	k	R^2	$t_{1/2}$‡	DT_{90}§
	mg kg^{-1}	d^{-1}	–	d	d
Sand topsoil	11.21 ± 0.33	0.041 ± 0.003	0.978	16.9	56.2
Sand subsoil	12.27 ± 0.19	0.019 ± 0.001	0.985	36.5	121
Clay topsoil	11.99 ± 0.15	0.0063 ± 0.0005	0.948	110	365
Clay subsoil	11.61 ± 0.41	0.0046 ± 0.0014	0.562	151	501

† c_{G0} = initial concentration of glyphosate; k = first-order rate coefficient for degradation; t1/2, = half-life; DT_{90} = time for 90% degradation.

‡ Calculated as ln(2)/k.

§ Calculated as ln(10)/k.

Table 8.1.1.2-72: Coefficients (± SE, n = 12) obtained by nonlinear regression for formation and degradation of aminomethylphosphonic acid (AMPA) according to Eq. [4].

Soil	k_1†	k_2	$t_{1/2}$‡	R^2
	d^{-1}		d	–
Sand topsoil	0.0216 ± 0.0011	0.0115 ± 0.0019	60.4	0.965
Sand subsoil	0.0092 ± 0.0005	0.0076 ± 0.0018	91.3	0.988
Clay topsoil	0.0063§ ± 0.0005	0.0199 ± 0.0013	34.9	0.973
Clay subsoil	0.0028 ± 0.0006	0.0071 ± 0.0087	97.6	0.901

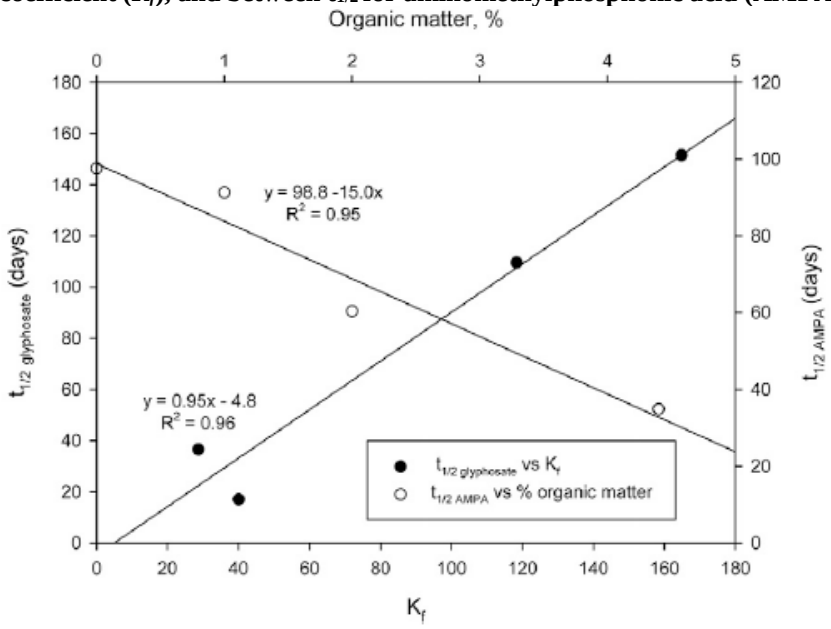
† k_1 = first-order rate coefficient for degradation of glyphosate to AMPA; k_2 = first-order rate coefficient for degradation of AMPA ; $t_{1/2}$ = half-life.

‡ Calculated as ln(2)/k_2.

§ In the clay topsoil, k_1 became 0.0073, i.e., larger than k (first-order rate coefficient for degradation; Table 4), leading to a formation fraction > 1, and was therefore set equal to k.

Glyphosate degraded relatively rapidly in the sand, with a half−life of 16.9 and 36.5 d in the topsoil and subsoil, respectively (Table 8.1.1.2-71). In the clay, very long half−life values of 110 and 151 d were obtained, and remarkable values of 365 and 500 d for 90 % degradation (DT_{90}). These half−life values are within the range previously reported for glyphosate degradation in agricultural soils (Giesy *et al.*, 2000). There was a high correlation between half−life and K_f (Figure 8.1.1.2-31), suggesting that adsorption is important for the amount of glyphosate available in the soil water for degradation.

Figure 8.1.1.2-31: Relationship between half−life ($t_{1/2}$) for glyphosate and Freundlich adsorption coefficient (K_f), and between $t_{1/2}$ for aminomethylphosphonic acid (AMPA) and % organic matter



The concentration of AMPA steadily increased during the incubation period of 64 d in all soils except the sand topsoil (Figure 8.1.1.2-30 B), where it peaked after 43.4 d at 2.4 mg of AMPA/kg, representing 32.4 % of the initial amount of glyphosate added (Table 8.1.1.2-73). The degradation rate of AMPA, as quantified by k_2, gave a half−life of 35 to 98 d, with slower rates in the subsoil (Table 8.1.1.2-72). The correlation between these half−life values and the amount of organic matter was −0.973 (Figure 8.1.1.2-31), suggesting that increasing amounts of organic matter, or perhaps AMPA−degrading microorganisms dwelling there, increase degradation rates.

Table 8.1.1.2-73: Derived parameter values on fraction of glyphosate degraded to aminomethylphosphonic acid (AMPA) ($k1/k$), rate constant for formation of sarcosine (k_3), incubation time (t_{Amax}) at which the AMPA−concentration peaks (c_{Amax}), and c_{Amax} as fraction of initially added glyphosate

Soil	k_1/k†	k_3‡	t_{Amax}§	c_{Amax}¶	c_{Amax} fraction#
	–	d^{-1}	d	mg kg^{-1}	%
Sand topsoil	0.53	0.0190	43.4	2.40	32.4
Sand subsoil	0.48	0.0098	80.4	2.12	26.2
Clay topsoil	1	0	84.7	1.47	18.6
Clay subsoil	0.61	0.0018	174	1.34	17.5

† Values of k (first-order rate coefficient for glyphosate degradation) from Eq. [2] (Table 4) and of k_1 (first-order rate coefficient for degradation of glyphosate to AMPA) from Eq. [4] (Table 5).

‡ $k_2 = k - k_1$.

§ Calculated according to Eq. [5].

¶ Obtained by inserting derived values of k, k_1, k_2, c_{G0}, and t_{Amax} into Eq. [4].

Calculated as $(c_{Amax} \times 1.52 \times 100)/c_{G0}$, where 1.52 is the stoichiometric factor for conversion of AMPA to glyphosate concentration.

The degradation of AMPA is reported to be slower than that of glyphosate (Giesy *et al.*, 2000). In the Footprint database, AMPA is classified as persistent, with a typical half−life of 151 d, compared with 12 d for glyphosate (Footprint, 2009). The fact that AMPA is formed when glyphosate is degraded clearly means

that the persistence of AMPA has to be equal to or longer than that of glyphosate. However, we did not find any previous study in which the degradation of AMPA was studied and compared with that of glyphosate in the same soil. In a study where glyphosate degraded with a half−life of 9 d, Simonsen *et al.* (2008) estimated a half−life of 32 d for AMPA from the descending part of data on AMPA residues. However, this is a worst−case scenario as these data represent the sum of AMPA formation from degradation of the glyphosate still present and AMPA degradation. This does not reveal how fast the AMPA molecule per se is degraded. Our data suggest that the AMPA degradation rate can be faster than that for glyphosate, for instance in soils with high clay content, which slows down glyphosate degradation, and high organic matter content, which stimulates AMPA degradation (Figure 8.1.1.2-31).

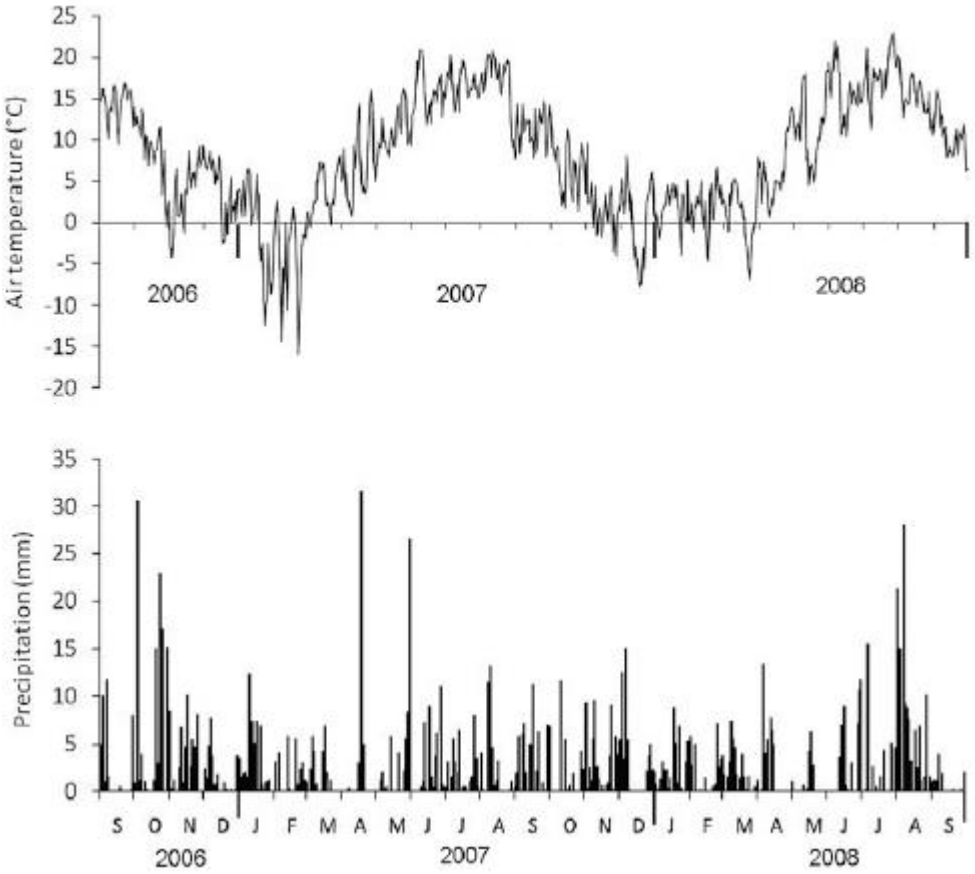
Microbial degradation is the main process controlling the disappearance of glyphosate in soil, and there are two well−described biological pathways for such degradation that give AMPA and sarcosine as the respective metabolites (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008). It has recently been shown that ligninolytic enzymes can also transform glyphosate into AMPA (Pizzul *et al.*, 2009). Because AMPA is the only significant soil metabolite found in soil degradation studies, it is frequently suggested that metabolism of glyphosate in soil usually proceeds via the AMPA pathway (Giesy *et al.*, 2000; Karpouzas and Singh, 2006). However, the fractions of AMPA formed in our study (48−100 %, Table 8.1.1.2-73) suggest that both pathways can be active in soil, with up to 52 % not following the AMPA pathway. Reasons for the sarcosine pathway not being considered significant in soil could be that soil residues of sarcosine are not determined in most studies and that sarcosine rapidly degrades to glycine (Karpouzas and Singh, 2006) in biologically active soil.

Precipitation and Drainage Conditions

Daily precipitation and average air temperatures at the lysimeter station are shown in Figure 8.1.1.2-32. Over the 2−yr study period (15 Sept. 2006−15 Sept. 2008), cumulative precipitation was 1192 mm, which, in combination with supplemental irrigation, resulted in a total water input to the lysimeters of 1214 mm. This total water input is slightly higher (10 %) than the long−term average precipitation for the Uppsala region (554 mm/yr). Average air temperature during the experimental period (7.8°C) was also higher than the long−term average at Uppsala (5.3°C).

A few weeks after glyphosate was applied, from 30 September onward, rain events were quite frequent (Figure 8.1.1.2-32), and precipitation totaled 232 mm by the end of 2006. This clearly created worst−case conditions for leaching of the herbicide, and the average amounts of leachate were 169 and 156 mm from the sand and clay soil, respectively, during this period. Peak weekly amounts of leachate, reaching 42 (sand) and 33 (clay) mm, occurred 8 wk after herbicide application. During 2007, precipitation was close to the normal for the area, although quite unevenly distributed. During periods with low evaporation (November, December, and January), monthly precipitation was about 60 mm, which was clearly above the average and increased the risk of leaching. In 2008, precipitation was again above normal, causing large amounts of leachate.

Figure 8.1.1.2-32: Average daily temperature (upper graph) and daily precipitation (lower graph) at the lysimeter site during the experimental period



The cumulative amounts of leachate from the lysimeters each year are shown in Table 8.1.1.2-74. In total over the 2−yr period, the amount of leachate was 572 (± 17) mm from the sand and 461 (± 15) from the clay soil. In relation to water input, these amounts constituted 47 and 38 % of precipitation plus irrigation, which is considerably higher than in other similar leaching studies performed in Sweden (Bergström & Jokela, 2001).

Table 8.1.1.2-74: Water inputs to the lysimeters and mean annual amounts of leachate from the lysimeters to which glyphosate was applied (± SD; n = 2 for sand, n = 3 for clay)

Year	No. of days	Precipitation + irrigation	Leachate	
			Sand lysimeters	Clay lysimeters
		mm		
2006†	104	232	169 (± 1.4)	156 (± 4.7)
2007	365	550	229 (± 3.2)	158 (± 9.1)
2008‡	261	432§	174 (± 13)	148 (± 4.0)
Total	730	1214	572 (± 17)	461 (± 15)

† Measurements made during the period 18 September to 31 December.

‡ Measurements made during the period 1 January to 18 September.

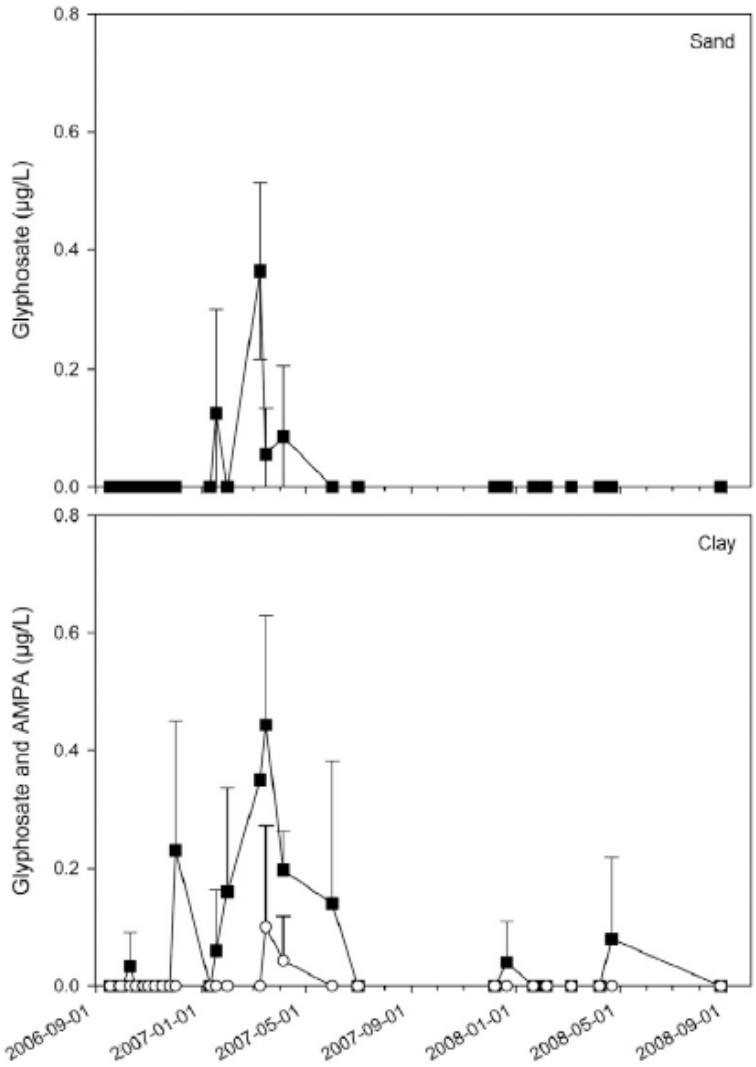
§ Includes 22 mm irrigation.

Leaching of Glyphosate and AMPA

Average concentrations of glyphosate and AMPA in leachate are shown in Figure 8.1.1.2-33. In the sand, the average peak concentration of glyphosate reached 0.36 µg/L in the beginning of March 2007, when temperatures were consistently above freezing, about 25 wk after pesticide application. During this period, the amount of leachate was about 250 mm (i.e., equivalent to 1.5 effective pore volumes). Thereafter the glyphosate concentration decreased and the average concentration was below 0.1 µg/L from 16 March 2007 onward. This leaching pattern indicates limited preferential transport of the herbicide through the sand profile, although some preferential transport must have occurred considering the strong sorption of glyphosate (Table 8.1.1.2-69) and thereby expected large retardation. This is a flow behavior reported in

several other leaching studies in sandy soils (e.g., Bergström & Shirmohammadi, 1999). The fact that the glyphosate peak occurred about 15 wk later than the corresponding bromide peak is a reflection of bromide being a nonreactive tracer. In the clay soil, the initial glyphosate peak occurred in the beginning of December and reached 0.23 µg/L after about 150 mm of water (i.e., equivalent to 0.8 effective pore volumes) had leached out of the soil columns. This considerably smaller amount of leachate suggests that glyphosate was partly transported through preferential flow paths in the clay profile, as was the case for bromide. This flow pattern has been documented earlier in this clay soil for reactive solutes (Djodjic *et al.*, 1999; Bergström, 1995) and for nonreactive tracers (Bergström & Jarvis, 1993; Bergström & Shirmohammadi, 1999). However, the highest glyphosate peak (0.44 µg/L) in leachate from the clay soil coincided with that in the sand, i.e., in the beginning of March 2007. This glyphosate peak was washed out of the columns slightly earlier than the corresponding bromide peak, which was rather unexpected. Apart from preferential flow, another explanation could be that the highly water−soluble bromide diffused into micropores in the clay soil relatively soon after application and once in these pores it was largely protected from percolating water (Bergström & Stenström, 1998). From July 2007 onward, the average glyphosate concentration in clay soil leachate was <0.1 µg/L, although single samples had concentrations slightly exceeding the detection limit (0.1 µg/L). Average concentrations of AMPA in leachate were at or below 0.1 µg/L in both soils (Figure 8.1.1.2-33), with the highest concentration (0.30 µg/L) in a sample from one of the clay lysimeters. The average total amount of glyphosate that leached from the sand was 0.13 (± 0.03) g/ha and from the clay soil 0.28 (± 0.08) g/ha. These amounts correspond to 0.009 and 0.019 % of the amount of glyphosate applied to the soils. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil. Total leaching of the ^{14}C applied in September 2006 was on average 0.31 % from the sand soil and 0.25 % from the clay. This shows that constituents other than glyphosate and AMPA that were not positively identified formed the major proportion of the total radioactivity in leachate. The leaching rates determined in this study are quite small compared with those in many other studies. For example, in a study performed by Al−Rajab *et al.* (2008), which included microlysimeters of three soils (clay loam, silty clay loam, and sandy loam), the amounts of glyphosate leached during 11 mo ranged between 0.11 and 0.28 % of the amount applied. However, there are also studies showing similar results to those obtained in the present experiment. In a study in France performed using lysimeters filled with calcareous soil (Landry *et al.*, 2005), leaching of glyphosate was between 0.02 and 0.06 % of that applied after 680 mm of rainfall. Similarly, Cheah *et al.* (1997) recovered 0.04 to 0.07 % of applied glyphosate in lysimeter leachate after 200 mm of simulated rainfall. However, the conditions in all the above−mentioned studies were quite different from those in this study; the lysimeters were only 9.8 to 25 cm long, the experimental periods were considerably shorter (a few days to 1 yr), and the amounts of rainfall were much smaller (200 to 869 mm). These differences certainly have to be taken into account in a comparison of results.

Figure 8.1.1.2-33: Average concentrations of glyphosate (■) and aminomethylphosphonic acid (AMPA) (○) in the leachate (mean + SD, n = 2 for sand and n = 3 for clay). No AMPA was found in the leachate from sand



No glyphosate or AMPA was determined to be particle−bound, even though large quantities of particles were present in leachate from the clay soil. It is noteworthy that the particles were operationally defined as those being retained on a 0.22−µm glass−fiber filter. Some studies have shown that colloid−facilitated transport of glyphosate can occur. For example, de Jonge *et al.* (2000) showed in a study on lysimeters filled with undisturbed topsoil of a sandy loam that 1 to 27 % of leached glyphosate was particle−bound. Considering the overall low total concentrations of glyphosate in the present study (Figure 8.1.1.2-33), the particle−bound proportion would be below the detection limit (0.1 µg/L) if it constituted less than 25 % of what was leached. It is also important to bear in mind that topsoil lysimeters only include about 30 % of the profiles used in this study and may in fact, as indicated above, generate results that are quite atypical of results obtained in full−length lysimeters, such as those used here. The underlying subsoil can act as a sink or source for particles leaching through the soil profile.

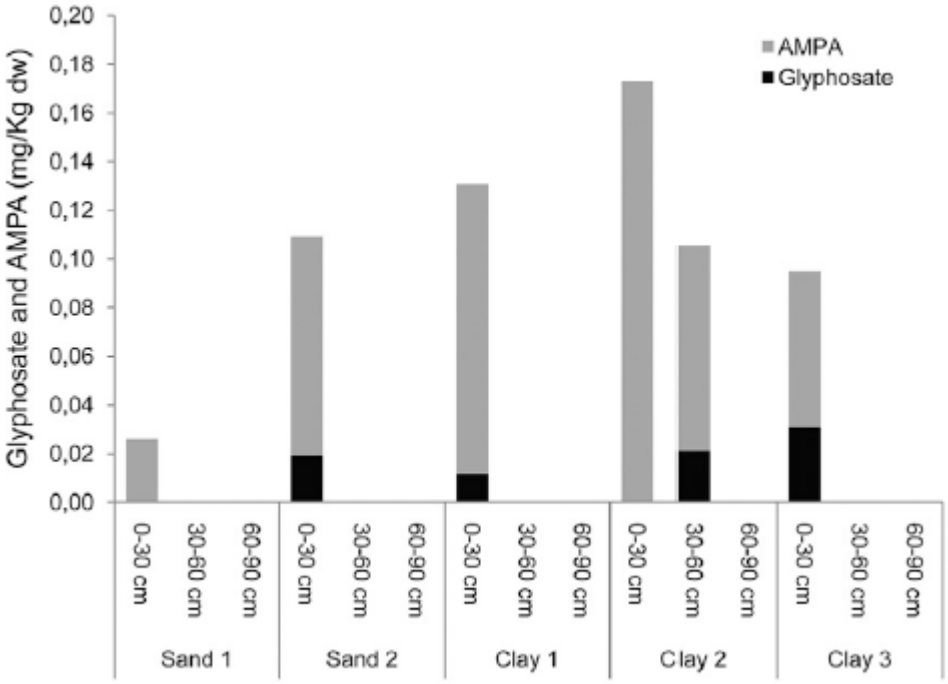
The trend for glyphosate to leach in larger amounts from clay soils than from sandy soils is relatively well documented. In a Danish study, this was attributed to periods of high intensity rainfall shortly after application, when glyphosate was located on the soil surface and thereby exposed to rapid water transport in clay macropores extending up to the surface (Kjaer *et al.*, 2003).

Residues of Glyphosate and AMPA in Soil

Residues of glyphosate and AMPA in the 0− to 30−, 30− to 60−, and 60− to 90−cm soil layers 748 d after application are shown in Figure 8.1.1.2-34. Residues were found in the 0− to 30−cm layer in all lysimeters and also in the 30− to 60−cm layer in one of the lysimeters with clay soil, possibly due to preferential flow in clay macropores and translocation in plant roots (Laitinen *et al.*, 2007). No residues were found in the

60− to 90−cm layer in any of the lysimeters. Considering the worst−case conditions prevailing for leaching after application of glyphosate in the autumn of 2006, these results confirm the generally low mobility found for these compounds (Giesy *et al.*, 2000; Vereecken, 2005).

Figure 8.1.1.2-34: Residues of glyphosate and aminomethylphosphonic acid (AMPA) in the 0− to 30−, 30− to 60−, and 60− to 90−cm soil layers 748 d after application. dw = dry weight



No glyphosate was detected in one of the sand lysimeters, and 0.019 mg/kg remained at 0 to 30 cm in the other one. Low concentrations could be expected from the fast degradation in the sand topsoil (laboratory half−life 16.9 d). The concentrations of AMPA (0.026 and 0.090 mg/kg) remaining can be due to a combination of slow degradation (laboratory half-life 60.4 d) and continuous supply from degradation of remaining glyphosate. Related to the initial amount of glyphosate added, the remaining glyphosate residues represented 2.7 % and total residues of glyphosate + AMPA, calculated as glyphosate equivalents, represented 27 %.

In the clay soil, glyphosate and AMPA were found in all three lysimeters, probably due to very slow degradation of glyphosate in the topsoil and subsoil (Table 8.1.1.2-71), and thereby a long−term supply of AMPA, slow degradation of AMPA in the clay subsoil, and 100 % formation of AMPA from glyphosate degradation in the topsoil. Glyphosate residues represented 5.1 % and total residues 59 % of the initial amount of glyphosate added. Similar field persistence of glyphosate and AMPA residues was found in a sandy soil in Finland, where total residues in the 0− to 60−cm layer accounted for 72 % of the amount applied 20 mo after application (Laitinen *et al.*, 2009).

Conclusion

The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation in the clay soil. The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation can be faster, for instance in soils with a high clay content, which slows down glyphosate degradation, and a high organic matter content, which stimulates AMPA degradation. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. However, despite quite frequent rain events and large amounts of precipitation in the autumn and winter after application, these residues were mainly located in the topsoil, confirming the generally low mobility reported for these compounds. This conclusion is also supported by the small amounts of glyphosate and AMPA leached during the whole study period. Possible residues of glyphosate and AMPA due to transport on particles >0.22 µm were below the limit of

detection (0.1 µg/L), and this does not appear to be an important transport mechanism in the soils included in this study.

Assessment and conclusion by applicant:

The article investigates the behaviour of glyphosate and AMPA under conditions of outdoor lysimeters including the determination of sorption parameters and degradation data for two Swedish soils. The invesigations were performed with non-labelled test substances for which further information such as purity was not reported. The use of non-labelled test material does not allow for determination of mass balances. No detailed tabulated results per sample point are provided.

Lysimeter experiment: Not all required information is reported to allow for a check of the overall quality of the study.

Degradation and sorption tests: The tests for glyphosate were claimed to follow OECD 106 guideline while being unclear for soil degradation. Due to a lack of detail in reporting, information is insufficient to check the quality of data. In addition and for example, the LOD of the analytical methods used seem inappropriate to fulfill the requirements for EU data generation methods.

The article is therefore classified as reliable with restrictions for the three experiments.

Assessment and conclusion by RMS:

This article lacks details to allow assessment of its validity against current guidelines. Specifically the mass balances and stability of the substance are not available, a mixture of labelled and unlabelled glyphosate has been applied to the soil for the adsorption experiment, unlabelled glyphosate on the degradation test. The lack of information on the efficacy of the extraction method also is a major deviation that prevents validating the degradation test and the soil analysis in the lysimeter experiment.

The lysimeter experiment shows limited contamination of the leachates by either glyphosate or AMPA over the two years study. However, some of the analysis were >0.1 µg/L for both AMPA and glyphosate, especially around May. The results presented in this study do not confirm the preferential flow to be the cause of contamination of leachates. Very low concentration (below LOQ) of glyphosate or AMPA was due to their bound to particles >0.22µm. The peak concentrations in leachate did not match the heavy rainfall events. the highest glyphosate peak (0.44 µg L−1) in leachate from the clay soil coincided with that in the sand, i.e., in the beginning of March 2007. Without more information, further interpretation of the results is difficult.

The article provides supportive information on the degradation, adsorption and of glyphosate and AMPA, but no reliable endpoints can be derived for use in risk assessment.

Ghafoor, A. et al

Data point:	CA 7.1.2.1.1/018
Report author	Ghafoor, A. et al.
Report year	2011
Report title	Measurements and modeling of pesticide persistence in soil at the catchment scale
Document No	DOI 10.1016/j.scitotenv.2011.01.049 E-ISSN 1879-1026
Guidelines followed in study	Degradation experiment: None Adsorption experiment: OECD 106 Guidance
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No

Acceptability/Reliability: Reliable with restrictions

Full summary

Degradation of pesticides in soils is both spatially variable and also one of the most sensitive factors determining losses to surface water and groundwater. To date, no general guidance is available on suitable approaches for dealing with spatial variation in pesticide degradation in catchment or regional scale modeling applications. The purpose of the study was therefore to study the influence of various soil physical, chemical and microbiological characteristics on pesticide persistence in the contrasting cultivated soils found in a small (13 km2) agricultural catchment in Sweden and to develop and test a simple model approach that could support catchment scale modeling. Persistence of bentazone, glyphosate and isoproturon was investigated in laboratory incubation experiments. Degradation rate constants were highly variable with coefficients of variation ranging between 42 and 64 % for the three herbicides. Multiple linear regression analysis and Mallows Cp statistic were employed to select the best set of independent parameters accounting for the variation in degradation. Soil pH and the proportion of active microorganisms (r) together explained 69 % of the variation in the bentazone degradation rate constant; the Freundlich sorption co–efficient (Kf) and soil laccase activity together explained 88 % of the variation in degradation rate of glyphosate, while soil pH was a significant predictor ($p < 0.05$) for isoproturon persistence. However, correlations between many potential predictor variables made clear interpretations of the statistical analysis difficult. Multiplicative models based on two predictors chosen 'a priori', one accounting for microbial activity (e.g. microbial respiration, laccase activity or the surrogate variable soil organic carbon, SOC) and one accounting for the effects of sorption on bioavailability, showed promise to support predictions of degradation for large–scale modeling applications, explaining up to 50 % of the variation in herbicide persistence.

Materials and methods

Study site and soils

The study was carried out in the E21 monitoring catchment in Östergötland, southern Sweden. The total catchment area of 13 km2 consists of 95 % agricultural land, with main crops of winter and spring sown cereals, rape, potatoes and peas. The soils, which are derived from glacial and post–glacial fluvial sediments and glacial till (moraine), have a wide range of texture, from loamy sand to clay. Soil samples were collected from 60 locations in the catchment (1 location every 20 ha) on a grid pattern. Five soil samples from each location were taken in the surface 20 cm, bulked, homogenized by passing through a 2 mm sieve, put into plastic bags and stored at 4°C until use (within 48 days). Sixteen of these sampled locations were selected for further study to cover the range of measured textures, organic matter contents and pH values.

Soil pH was measured on fresh samples after shaking the samples in de–ionised water (1:2.5) at room temperature (Swedish Standard Institute, 1994). Particle size distributions were evaluated using the standard pipette method (Day, 1965). The contents of clay, sand, and silt are usually correlated with one another (Iqbal et al., 2005). Thus the geometric mean particle diameter, dg, was derived from the fundamental particle size classes as (Shirazi & Boersma, 1984):

[1]

$$d_g = exp(\sum_i m_i ln(X_i))$$

where m is the mass fraction of particle size class i and X is the mean diameter of that class. For the Swedish system, x–values are 0.001, 0.03 and 1.03 mm for clay, silt, and sand, respectively. Total organic C and N were measured using a Leco CN 2000 (LECO Corp., St. Joseph, MI, USA). Water contents at a pressure potential of –100 cm (pF 2) were measured on a sand table (Jamison, 1958). Ammonium–lactate extractable phosphorus and potassium were measured according to the method described by Egner et al. (1960).

The physical and chemical characteristics of the 16 soils are given below. There was a relatively wide range of SOC contents, ranging from 0.9 to 10.2 %. Soil pH ranged from 6.0 to 7.6. Soil texture is very variable for such a small catchment: clay, sand and silt contents ranged from 4–45 %, 12–87 %, and 8–54 % respectively, and 8 of the 11 USDA texture classes are represented. Ammonium–lactate extractable phosphorus and potassium ranged from 56–148 mg/kg and 54–209 mg/kg, respectively.

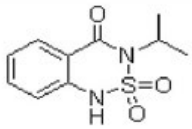
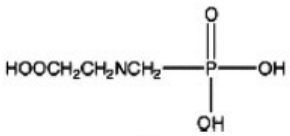
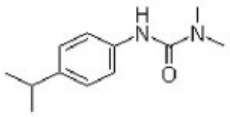
Table 8.1.1.2-75: Physico–chemical properties of soils

Soils	pH	Texture			Textural class	SOC	$CaCO_3$	Total N	Water content at pF_2 (−100 cm)	Geometric mean particle diameter (d_g)	Available P	Available K
		Sand	Silt	Clay								
		%	%	%	International	%	%	%	g/g	mm	mg kg^{-1}	mg kg^{-1}
1	7.6	49	32	18	Loam	1.6	9.2	0.16	0.253	0.095	58	68
2	6.2	87	8	4	Sand	1.2	0.1	0.08	0.115	0.588	63	69
3	7.0	43	27	30	Clay Loam	2.3	0.1	0.22	0.363	0.049	99	116
4	7.1	58	25	17	Sandy Loam	2.1	0.4	0.21	0.3	0.131	89	84
5	6.9	68	17	15	Sandy Loam	2.1	0.2	0.21	0.285	0.199	111	114
6	6.5	70	21	9	Sandy Loam	1.1	0.1	0.09	0.175	0.263	90	120
7	6.5	85	9	6	Loamy Sand	1.6	0.1	0.15	0.169	0.494	159	70
8	7.6	55	28	17	Sandy Loam	2.6	0.2	0.25	0.265	0.118	56	68
9	6.4	12	45	44	Silty Clay	6.7	0.3	0.54	0.643	0.010	57	205
10	6.9	17	54	29	Silty Clay Loam	10.2	0.5	0.87	0.540	0.020	73	170
11	6.9	22	33	45	Clay	2.5	0.9	0.25	0.383	0.014	134	162
12	7.3	56	24	20	Sandy Loam	5.4	0.4	0.53	0.477	0.110	142	209
13	6.0	63	27	10	Sandy Loam	0.9	0.1	0.08	0.240	0.198	132	126
14	6.1	83	11	6	Loamy Sand	1.3	0.1	0.13	0.227	0.460	148	54
15	7.5	35	39	25	Clay Loam	3	0.1	0.28	0.410	0.046	101	97
16	7.1	31	40	29	Clay Loam	1.9	0.2	0.19	0.347	0.033	89	164

Chemicals

Unlabelled isoproturon (N,N–dimethyl–N′–[4–(1–methylethyl) phenyl]urea; 99 % purity), bentazone (3–(1–methylethyl)–1 H–2,1,3–benzothiadiazin–4(3 H)–one 2,2–dioxide; 97 % purity) and glyphosate (N–(phosphonomethyl)glycine, 98 % purity) were purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Ring (14C) isoproturon (4.044 MBq/mg; purity >95 %) and [P–methylene–14C]glyphosate (5.155 MBq/mg, purity >99 %) were purchased from Izotop, Institute of Isotopes, Budapest, Hungary. 14C–labelled bentazone (3–(1–methylethyl–1 H–2,1,3–benzothiadiazin–4(3 H)–one 2,2–dioxide–[phenyl–U–14C]; 5.211 MBq/mg; 100 % purity) was a gift from BASF, Limburgerhof, Germany. The table below gives the structural formulae and some physical and chemical properties of the three compounds. The 3–methyl–2–benzothiazolinone hydrazone (MBTH), 3–(dimethylamino) benzoic acid (DMAB) and 2,2′–azinobis(3–ethyl–benzthiazoline–6–sulfonic acid) (ABTS) were supplied by Sigma–Aldrich Sweden AB.

Table 8.1.1.2-76: *Selected* pesticides and their properties (data from the e–Pesticide Manual (3.0), British Crop Protection Council, 2003)

Herbicides	Structural formulae	pKa	mol wt	Solubility in water (mg L^{-1})
Bentazone		3.3	240.3	570
Glyphosate		2.3, 5.7,10.2	169.1	10,500
Isoproturon		n.a	206.3	65

Degradation

Incubation experiments for each soil/pesticide combination were carried out on two replicate samples. A sub–sample of each soil (7 g) was spiked with glyphosate dissolved in water (0.7 mg herbicide/mL water) or isoproturon or bentazone dissolved in methanol (0.7 mg herbicide/mL methanol). The soil was dried, after which an additional amount of fresh soil (63 g) was thoroughly mixed into the spiked soil to give an initial concentration of 10 μg/g dry weight (d.w.) of soil (procedure adopted from Brinch et al. (2002)). Water contents were adjusted to and maintained at pF 2 throughout the experiment by the addition of de–ionized water as necessary. The samples were incubated in aerated glass tubes in the dark at 20°C for

64 days. Duplicate samples (5 g) were taken after 0, 2, 4, 8, 16, 32 and 64 days of incubation for measurement of the residual concentrations of glyphosate, isoproturon, and bentazone.

Analyses of bentazone and isoproturon in soil samples were carried out by HPLC as described by Larsbo et al. (2009), while for glyphosate, the GC–MS method developed by Börjesson & Torstensson (2000) was employed. The data from the incubation study were fitted to first–order degradation kinetics using non–linear regression:

[2]

$$c = c_0 e^{-kt}$$

where c is the mass of compound in the soil (µg/g) at a given time t (days), c0 is the original mass of compound added to the soil (µg/g), and k (day−1) is the first–order degradation rate coefficient. Degradation half–lives (DT50, days) were calculated as ln(2)/k.

Adsorption

The adsorption experiments were carried out according to the OECD 106 guideline (Organization for Economic Cooperation and Development, 2000) on two replicates. Soil (four grams d.w. for glyphosate, two grams d.w. for isoproturon and bentazone) was shaken to pre–equilibrate with 0.01 M CaCl2 (39 mL for glyphosate, 1.5 mL for bentazone and isoproturon) for 24 h at 20°C in test tubes (50 mL plastic tubes (Sarstedt) for glyphosate and 10 mL glass tubes for bentazone and isoproturon). Thereafter, the soil slurry was spiked with a 1 mL mixture of labeled (ca 7000–11000 dpm) and unlabeled pesticides in 0.01 M CaCl2 to give 5 initial concentrations in the range of 0.1–10 µg/g soil. The tubes were shaken for 24 h and then centrifuged for 20 min at 4000 rpm. After mixing with 10 mL of Insta–Gel Plus (glyphosate) or 6 mL of Ultima Gold emulsifying cocktail (bentazone and isoproturon) (PerkinElmer, Waltham, MA, USA), the radioactivity was measured in the supernatant (10 mL for glyphosate, 1 mL for bentazone and isoproturon) using a LS 6000TA liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA). Tubes without soil and 14C–labelled substances were included for subtraction of background radiation and tubes without soil were used to give the initial amount of 14C activity. No significant adsorption of the tested substances occurred on the tubes.

The sorption measurements for each pesticide in the 16 soils were fitted to the Freundlich equation using non–linear regression:

[3]

$$S = K_f c_e^n$$

where S is the adsorbed amount (µg/g), ce is the equilibrium concentration (µg/mL), Kf is the Freundlich constant (µg1-n mLn/g), and n (–) is an exponent that expresses the degree of isotherm nonlinearity.

Manganese peroxidase and laccase enzyme activities

Manganese peroxidase (MnP, EC 1.11.1.13) activity

Ten g of soil was mixed with 20 mL of a 1:1 mixture of 500 mM lactic acid/sodium succinate buffer (pH 4.5) in a Waring blender and homogenized for 3×30 seconds at high speed. The aliquots were centrifuged in 50 mL centrifuge tubes at 4000 rpm for 15 min at 4°C. The supernatant was filtered through 0.45 µm filter paper. Manganese peroxidase (MnP) activity was measured according to the method described by Castillo et al. (1994). Briefly, the assay is based on the oxidative coupling of MBTH and DMAB in the presence of H2O2, Mn+2 and MnP. This reaction gives a deep purple–blue color with a broad absorption band with a peak at 590 nm. The reaction mixture contained 300 µL 6.6 mM DMAB, 100 µL 1.4 mM MBTH, 30 µL 30 mMMnSO4, 10 µL 10 mM H2O2 and 1.56 mL of sample extract in a total volume of 2 mL. A reagent blank without any sample extract was also run. Time zero was registered at the moment of addition of H2O2 and the increase in absorbance was then followed at 590 nm for 5 min by using a Shimadzu UV 1800–A spectrophotometer fitted with a time scan function. The initial rates were calculated by using linear regression. MnP activity (mU/min/g soil) in soil was calculated as:

[4]

$$\text{Unit of enzyme } g^{-1}\text{soil} = \frac{\text{Abs}/min \times 0.002}{E_m \times \text{mL of sample} \times \text{Dry weight of soil} \times \text{mL of buffer added}}$$

where Em is the molar extinction coefficient (0.053 μM−1/cm). One unit is defined as the amount of enzyme needed to form 1 μmol of product in 1 min.

Laccase (EC 1.10.3.2) activity

Laccase activity was measured by monitoring the oxidation of ABTS (Wolfenden & Willson, 1982) in a citrate/phosphate (100 mM citrate, 200 mM phosphate) buffer (pH 4.5) at 420 nm. Briefly, five g of soil was extracted with 20 mL 100 mM citrate/phosphate buffer (pH 4.5) for 1 h and then centrifuged for 15 min at 4000 rpm. The supernatant was filtered through a 0.45 μm filter. The reaction mixture contained 900 μL soil extract and 100 μL 30 m MABTS solution. The absorbance was measured at 420 nm at 25°C for 1 min with Shimadzu UV 1800–A spectrophotometer. Absorbance per minute (Abs/min) was calculated from the linear range of the curve and laccase activity was calculated as:

Unit of enzyme g−1soil

[5]

$$= \frac{\text{Abs}/min \times \text{mL of buffer added} \times 1000 \times \text{Volume}_{\text{reaction mixture}(\mu L)}}{E_m \times \text{Volume}_{\text{enzyme solution}(\mu L)} \times \text{Dry weight of soil}}$$

where Em is 0.036 μM–1/cm.

Respiration

Respiration was measured as described previously (Stenström et al., 2001) with some modifications. Two replicates of soil (10 g d.w.) were weighed into 250 mL respirometric jars. The jars were installed inside a respirometer, and the accumulation of CO2 trapped in KOH solution (0.2 M; 10 mL) was determined automatically twice every hour for each jar by measuring the electrical conductivity. The soil samples were incubated until a constant basal respiration rate (BR) was established (after about 3 days) at a constant temperature of 22°C and with a moisture content adjusted to pF 2. A substrate was prepared, consisting of glucose (7.5 g), (NH4)2SO4 (1.13 g), KH2PO4 (0.35 g) and talcum powder (10 g), and 0.19 g of this mixture was thoroughly mixed into each jar. Empty jars were incubated as controls. The BR was calculated by linear regression of accumulated CO2 produced versus time. The instantaneous rate of CO2 formation after addition of the substrate (substrate–induced respiration, SIR) was calculated using non–linear regression. The SIR was divided into the CO2 production rate of active, exponentiallly growing (r) and dormant, non–growing (K) microorganisms as described by Stenström et al. (2001).

14C–DHP mineralization

Synthetic 14C–ring–labeled dehydrogenated polymerizate (14C–DHP) of coniferyl alcohol (gift from Paul Ander, Department of Forest Products, SLU) with a molecular weight of 4–10 kDa and a specific activity of 0.16 MBq/mg was used to quantify lignin degrading activity in situ. The 14C–DHP was added as a DMF–water suspension to 10 g dw of soil in 20 mL plastic jars. The final radioactivity was approximately 13,000 dpm per sample. The water contents were adjusted to pF 2. The plastic jars were each installed into air–tight glass jars together with scintillation vials containing NaOH (0.2 M; 4 mL) to trap carbon dioxide. The glass jars were incubated in the dark at 20°C and the base traps were changed regularly. The amount of 14C in the base traps was measured on an LS 6000TA liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA) after mixing with 4 mL of Insta–gel Plus and incubated in the dark overnight. The 14C liberated was corrected for the background radiation in controls without soil. Kinetic parameters describing 14C–DHP mineralization were determined by non–linear regression according to first–order kinetics:

[6]

$$P = P_{max}\left(1 - e^{-kt}\right)$$

where P is the accumulated 14C–CO2 released (% of the added 14C) at time t, Pmax is the maximum 14C mineralized (% of applied) and k is the mineralization rate constant (day−1).

Statistical analysis

General regression models (GRM) for best–subset–regression were fitted to the data, where replicate 1 was cross–validated with replicate 2 under the assumption of homogeneous variance. Hence, the two replicates were pooled for variance estimation, and all possible combinations of regressors examined with respect to explanatory power of the response variable (k). When the best–subset–regression models were built, our objective was to identify the subset of explanatory variables that combine optimal orthogonality with maximum explanatory power, in order to explain the variance of the response variable across soil samples. Orthogonality is synonymous with independence across regressor variables, and many methods have been suggested for estimating the ideal subset. Mallow's Cp statistic (Mallows, 1973) is an effective way to punish a linear combination of potential regressors with respect to multi–collinearity and accumulated error (Ryan, 1997). Mallow's Cp is identical to Akaike's information criteria when the generic variance σ2 is known 'a priori'. Since we estimate σ2 from our data, Mallow's Cp is a better choice. Statistical analyses were performed with STATISTICA™ (StatSoft, 1995).

Results and discussion

Degradation

The results from the degradation experiments are presented below. Bentazone, glyphosate and isoproturon degradation in soils generally followed first–order kinetics (all R2>0.91 and statistically significant at p<0.001). The degradation rate constants listed below show considerable differences between soils with coefficients of variation ranging from 42 to 64 % for the three compounds. Degradation rate constants for bentazone were in the range 0.005–0.034/day which corresponds to half–lives of 20 to 139 days. Our data are consistent with those (8–133 days) reported by others (Rodriguez–Cruz et al., 2006; Thorstensen & Lode, 2001). Degradation rate constants for isoproturon were in the range 0.011–0.104/day which corresponds to half–lives of 7–63 days. Again, this degree of variation is similar to that reported in the literature for isoproturon, with values ranging from 1.4 to 40 days (Larsbo et al., 2009; Rodriguez–Cruz et al., 2006; Walker et al., 2001). The degradation rates of glyphosate (0.006–0.05/day, which correspond to half–lives of 14–116 days) are also consistent with other studies where the DT50 values in a variety of different soil types have been reported in the range of 1.7 to 197.3 days (Giesy et al., 2000; Sorensen et al., 2006).

Table 8.1.1.2-77: Degradation rate constant of bentazone, isoproturon, and glyphosate in different soils

Soils	Bentazone		Isoproturon		Glyphosate	
	k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2
1	0.024 ± 0.002[a]	0.963	0.045 ± 0.002	0.981	0.044 ± 0.006	0.93
2	0.013 ± 0.001	0.984	0.031 ± 0.000	0.977	0.018 ± 0.001	0.95
3	0.019 ± 0.001	0.980	0.024 ± 0.001	0.986	0.032 ± 0.002	0.86
4	0.021 ± 0.001	0.986	0.044 ± 0.003	0.940	0.046 ± 0.002	0.88
5	0.015 ± 0.001	0.959	0.016 ± 0.001	0.980	0.031 ± 0.001	0.87
6	0.015 ± 0.002	0.972	0.041 ± 0.001	0.968	0.033 ± 0.003	0.97
7	0.010 ± 0.000	0.983	0.032 ± 0.005	0.990	0.024 ± 0.000	0.95
8	0.018 ± 0.002	0.976	0.062 ± 0.006	0.968	0.050 ± 0.001	0.89
9	0.032 ± 0.001	0.984	0.027 ± 0.000	0.941	0.017 ± 0.004	0.98
10	0.034 ± 0.001	0.986	0.015 ± 0.001	0.960	0.006 ± 0.001	0.95
11	0.014 ± 0.000	0.936	0.027 ± 0.003	0.983	0.013 ± 0.001	0.96
12	0.015 ± 0.002	0.961	0.034 ± 0.001	0.967	0.029 ± 0.001	0.96
13	0.006 ± 0.000	0.987	0.023 ± 0.003	0.964	0.022 ± 0.000	0.95
14	0.005 ± 0.000	0.910	0.011 ± 0.001	0.933	0.027 ± 0.000	0.97
15	0.017 ± 0.002	0.985	0.104 ± 0.002	0.937	0.028 ± 0.001	0.96
16	0.023 ± 0.002	0.968	0.077 ± 0.007	0.939	0.032 ± 0.004	0.98
Mean	0.018		0.039		0.028	
CV %	46		64		42	

[a] indicates the ± standard deviation of two replicates.

Correlations between variables

Soil physical, chemical and microbial parameters

Correlations between basic soil properties, microbiological parameters, sorption strength and the degradation rate of pesticides are reported below. As is quite typical, the sandy soils in our catchment (large

dg values) generally had lower pH and SOC contents than the finer–textured loamy and clayey soils. Activities of ligninolytic enzymes (MnP and laccase) were highly variable in our soils. Sinsabaugh et al. (2008) found a coefficient of variation for phenol oxidase (e.g., laccase) and peroxidase (e.g., MnP) activities among ecosystems of nearly 300 %. This variability can be attributed to differences in both the enzymology of various enzyme–producing white–rot species and differences in growth and enzyme production responses of the fungi to different soil and environmental factors (Sinsabaugh, 2010). Correlation analysis suggested that significantly higher enzyme activities in our soils were associated with higher soil organic carbon and soil pH. MnP was positively correlated with SOC ($r = 0.78$; $p < 0.0001$). For peat soils, Sinsabaugh et al. (2008) also found that peroxidase activity increased with SOC. The activity of laccase was positively correlated with soil pH ($r = 0.55$; $p < 0.001$). This has also been found in other studies (Sinsabaugh, 2010; Sinsabaugh et al., 2008). Laccases deprotonate at high soil pH, which reduces their redox potential and increases their solubility, both of which may enhance their reaction potential (Sinsabaugh, 2010). Because laccases are widely produced for varied purposes, it is arguable that the diversity of the soil enzyme pool and potentially its range of action may also increase with soil pH (Sinsabaugh et al., 2008). Soil pH is also known to be an important predictor of microbial diversity (Sinsabaugh, 2010). Thus, SIR was positively correlated with soil pH, as well as SOC and available potassium, whereas it was negatively correlated with dg and available P. The proportion of active microorganisms (r) was positively correlated with SOC, available K, and MnP whereas it was negatively correlated with dg.

Table 8.1.1.2-78: Linear correlation coefficients

	pH	d_g	SOC	P	K (Potassium)	MnP
d_g	0.621**					
SOC	0.186	−0.479*				
P	−0.32	0.299	−0.254			
K (potassium)	0.057	−0.605**	0.645***	0.018		
MnP	−0.132	−0.172	0.777***	0.043	0.700**	
Laccase	0.546**	−0.305	−0.274	−0.432*	−0.26	−0.588**
SIR	0.458*	−0.699***	0.494*	−0.489*	0.516**	0.323
r (active)	−0.015	−0.455*	0.834***	−0.448*	0.579**	0.548**
DHP k	0.099	−0.032	0.509**	0.229	0.253	0.649**
Bentazone k	0.449*	−0.655**	0.736***	−0.706***	0.477*	0.361
Isoproturon k	0.592**	−0.295	−0.169	−0.268	−0.115	−0.308
Glyphosate k	0.516**	−0.053	−0.503**	−0.223	−0.492*	−0.503**
Bentazone K_f	−0.286	−0.178	0.551**	0.035	0.610**	0.803***
Isoproturon K_f	0.025	−0.496*	0.814***	−0.221	0.697***	0.719***
Glyphosate K_f	−0.523**	0.059	0.428*	0.054	0.444*	0.363

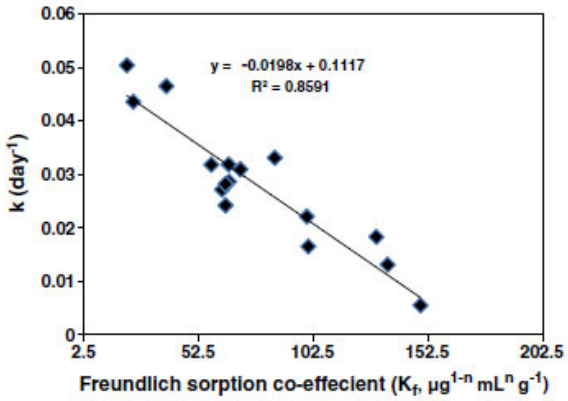
*, **, and *** indicate significance at $p < 0.1$, 0.05 and 0.01, respectively.

Laccase	SIR	r	DHP k	Bentazone k	Isoproturon k	Glyphosate k
0.324						
−0.084	0.646***					
−0.504**	−0.019	0.13				
0.204	0.761***	0.787***	0.077			
0.546**	0.429*	−0.054	−0.274	0.130		
0.588**	−0.026	−0.536**	−0.012	−0.10	0.413	
−0.423	0.525**	0.555**	0.347	0.274	−0.14	−0.538**
−0.225	0.629***	0.761***	0.284	0.694	−0.23	0.413
−0.404	−0.078	0.498**	−0.162	0.112	0.11	−0.924***

Pesticide degradation

Glyphosate degradation was significantly positively correlated to soil pH and laccase activity and negatively correlated with SOC and Kf. The strong relationship between glyphosate degradation and the Freundlich sorption coefficient is illustrated in Figure 8.1.1.2-35. A negative correlation between glyphosate adsorption and degradation in soil has also been reported by others (Zablotowicz et al., 2009).

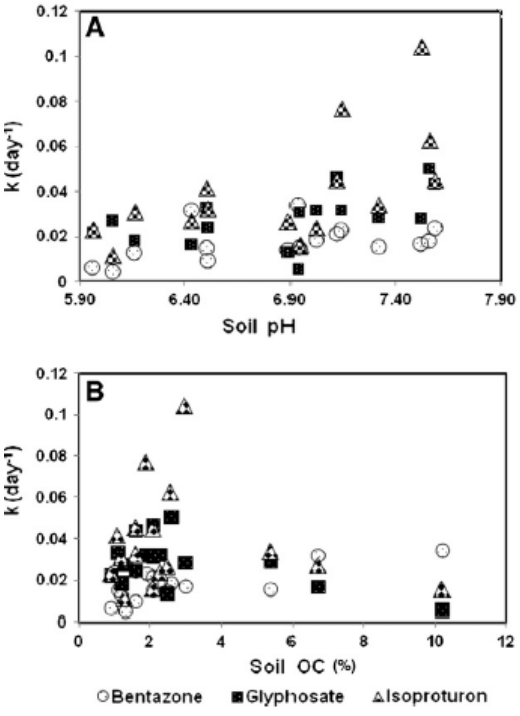
Figure 8.1.1.2-35: Relationship between the degradation rate constant k (day−1) for glyphosate and the Freundlich sorption coefficient (Kf, µg1− n mLn g−1).



In this catchment, the finer-textured loamy and clay soils of higher pH showed faster degradation than sandy soils of low pH (Figure 8.1.1.2-36 A). Soil bacterial diversity and richness decline as pH decreases (Sinsabaugh et al., 2008) and other studies have also found pesticide persistence to increase as soil pH decreases (Walker et al., 2001). However, caution should be exercised in interpreting our data, since pH and SOC are strongly (positively) correlated if the three locations with highly organic (peaty) topsoils are excluded.

Figure 8.1.1.2-36 B shows that the influence of SOC on pesticide degradation was rather complicated. An increase in soil organic matter increases biological activity and pesticide degradation rates in soil by providing conditions favorable to microbial growth (Thorstensen & Lode, 2001). On the other hand, pesticide sorption in soil, which is often positively related to SOC (Kah et al., 2007), may reduce the bioavailability of pesticides (Boivin et al., 2005; Kah et al., 2007; Sorensen et al., 2006; Thorstensen and Lode, 2001). Many studies have therefore demonstrated strong negative relationships between pesticide sorption and degradation in soils (Bolan & Baskaran, 1996; Dyson et al., 2002; Lehmann et al., 1992). In our study we observed a strong negative relationship between glyphosate degradation and its Freundlich sorption coefficient (Figure 8.1.1.2-35). The competing effects of organic matter content on microbial activity and sorption (bio–availability) mean that both positive and negative relationships between sorption and degradation have been reported, as well as non–monotonic relation–ships which display an optimum (Bolan & Baskaran, 1996), as in Figure 8.1.1.2-36 B for isoproturon.

Figure 8.1.1.2-36: Relationships between soil pH (A) and organic carbon content (B) and the degradation rate constants of all three pesticides



Regression analysis

Table 8.1.1.2-79 shows the results of best-subset regression analysis. The use of Mallows Cp led to the selection of five out of 12 potential regressor variables: soil pH, SOC, r, laccase, and Kf. Soil pH and r together explained 69 % of the total variation in the bentazone degradation rate constant. There was, however, a problem with this model, arising from the skewed distribution of r, which resulted in heteroscedasticity. After applying Box–Cox transformation to the original r variable (rBC), we obtained a regression equation for which the residual distributions were approximately normal homoscedastic:

[7]

$$k = 0.006(\mathrm{pH}) + 0.004(\mathrm{rBC}) - 0.012$$
$$R^2 = 0.57, \mathrm{Adj}\ R^2 = 0.51, F(2,14) = 8.75, p < 0.01$$

As an alternative model, pH and SOC explained 56 % of the variation in bentazone degradation, with an acceptable behaviour of the residuals. This is because SOC and r are strongly correlated. For glyphosate, 88 % of the variation in degradation rate coefficient could be explained by the Freundlich co–efficient Kf and soil laccase activity. Soil pH was the most significant predictor (pb 0.05) for isoproturon degradation and the inclusion of two more terms (SOC and r) significantly increased R2 from 0.29 to 0.42.

Table 8.1.1.2-79: Best subset regression models relating first–order degradation rate constants for bentazone, glyphosate and isoproturon to soil parameters (β is the unscaled regression coefficient)

Pesticide	Intercept	Soil pH	SOC	r	Laccase	K_f	Overall performance
Bentazone	−0.030	β = 0.006 p <0.05 F (1, 15) = 8.25	–	β = 0.007 p<0.001 F (1, 15) = 25.2	–	–	Adj R^2 = 0.69 F (2, 14) = 17.8 p<0.001
	−0.024	β = 0.005 p = 0.07 F (1, 15) = 3.85	β = 0.002 p<0.01 F (1, 15) = 13.9	–	–	–	Adj R^2 = 0.56 F (2, 14) = 10.6 p<0.01
Glyphosate	0.04	–	–	–	β = 0.000011 p <0.05 F (1, 15) = 7.5	β = −0.0002 p<0.0001 F (1, 15) = 60.7	Adj R^2 = 0.88 F (2, 14) = 56.5 p<0.000
Isoproturon	−0.14	β = 0.030 p<0.05 F (1, 15) = 7.08	–	–	–	–	Adj R^2 = 0.29 F (1, 15) = 7.08 p<0.05
	−0.17	β = 0.032 p<0.01 F (1, 15) = 11.6	β = −0.008 p<0.05 F (1, 15) = 4.9	β = 0.02 p = 0.13 F (1, 15) = 2.7	–	–	Adj R^2 = 0.42 F (3, 13) = 4.57 p<0.05

As discussed above, these predictor variables are more or less strongly correlated, both with each other and with other potential predictors. Furthermore, multiple linear regression models comprising linear additive terms (e.g. for SOC and Kf) cannot reproduce observed non–monotonic relationships between degradation rate coefficients and either sorption constants or soil organic carbon content (see Figure 8.1.1.2-36 B). It may therefore be more fruitful to develop models based on a mechanistic understanding of the processes controlling degradation. For microbial degradation, Allen and Walker (1987) suggested that degradation rates should be controlled by some measure of microbial activity multiplied by a factor related to the bio–availability of the compound. We can write:

[8]

$$k = k_{ref}.(B)^m.(A)^n$$

where k is the degradation rate constant, kref is a pesticide–specific reference rate coefficient which, in addition to the influence of variables not included in the model, should be related to the inherent degradability of the compound as determined by its molecular structure, m and n are constants, A is some measure of microbial activity and B is some measure of bioavailability. We tested six different forms of Eq. 8, combining three potential descriptors of microbial activity (laccase activity, SIR, and SOC) with two for bioavailability, Kf or the calculated fraction of pesticide in soil solution, Fs, given by:

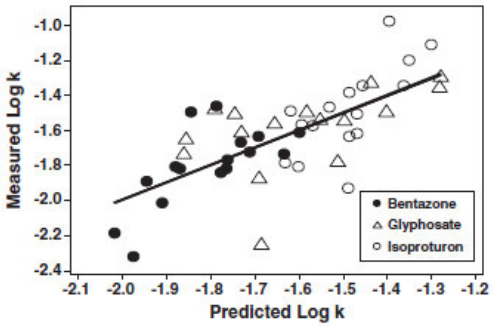
[9]

$$F_s = \frac{m_g}{m_g + K_f c_e^{n-1}}$$

where mg is the gravimetric water content at pF 2 and ce is the equilibrium concentration of the pesticide in solution at the start of the incubation experiment, which was iteratively calculated from the applied amount, the gravimetric moisture content and the parameters of the Freundlich equation. Although pH could also have been considered, we chose SOC as a surrogate variable for microbial activity, since it was strongly correlated to the microbial parameters MnP, SIR and r.

The data for all three pesticides were fitted to the logarithmic form of Eq. (8). The parameter values were estimated by introducing three 'class' variables into the data set (B, G, and I for bentazone, glyphosate and isoproturon, respectively, which take values of either 1 or zero) and kref values were obtained as regression coefficients for G, I and B. tables show that several of the models fitted the data well, especially models 1, 3 and 5, which had R2 values ranging from 39 to 50 % and regression co–efficients that were all significant ($p<0.1$). As an example, Figure 8.1.1.2-37 shows a comparison of measured k with predictions using model 1 (i.e. Eq. (8) based on Kf and SIR). In contrast, model 6 (using Fs with SOC as a surrogate measure of microbial activity) gave the poorest results, with no significant effect of SOC and clear bias in the residuals (not shown). However, after excluding glyphosate from the model, the overall regression became highly significant ($p<0.0001$) and the distribution of residuals was unbiased. It is interesting that bioavailability, as reflected in the parameter Fs, emerges here as a significant factor controlling degradation rates of bentazone and isoproturon, something which was not readily apparent from the classical correlation and regression analysis. Furthermore, although Kf is a very good predictor of glyphosate degradation (Figure 8.1.1.2-35), Fs is not. The reason for this is not clear.

Figure 8.1.1.2-37: Comparison of measured degradation rate constants with those predicted using model 1



No single model of pesticide degradation can be generally valid (Kah et al., 2007) as the mode of degradation varies considerably between compounds (e.g. chemical hydrolysis, co–metabolic or metabolic microbial degradation). However, although further testing is required, these results suggest that at least for some particular classes of pesticides, a multiplicative model based on soil organic carbon content and the sorption co–efficient (e.g. models 3 and 6) may be an effective and practical way to account for the effects of microbial activity and bio-availability on pesticide degradation in the context of modeling applications at catchment or regional scales.

Table 8.1.1.2-80: Parameter values and their significance for different models developed to predict degradation rate for all three pesticides together

With K_f in the models					With F_s in models				
Model	Parameters	Values	P value	Centered R^2	Model	Parameters	Values	P value	Centered R^2
1	Log ($k_{ref\ (B)}$)	−2.274	<.0001	0.50	4	Log ($k_{ref\ (B)}$)	−1.797	<.0001	0.42
	Log ($k_{ref\ (G)}$)	−0.893	0.0006			Log ($k_{ref\ (G)}$)	−0.659	0.1515	
	Log ($k_{ref\ (T)}$)	−1.472	<.0001			Log ($k_{ref\ (T)}$)	−1.182	<.0001	
	Log K_f	−0.500	0.0005			Log F_s	0.433	0.0179	
	Log SIR	0.366	0.0006			Log SIR	0.176	0.1134	
2	Log ($k_{ref\ (B)}$)	−2.825	<.0001	0.45	5	Log ($k_{ref\ (B)}$)	−2.586	<.0001	0.49
	Log ($k_{ref\ (G)}$)	−2.106	<.0001			Log ($k_{ref\ (G)}$)	−1.510	0.0076	
	Log ($k_{ref\ (T)}$)	−2.296	<.0001			Log ($k_{ref\ (T)}$)	−1.991	<.0001	
	Log K_f	−0.218	0.1408			Log F_s	0.404	0.0149	
	Log Laccase	0.353	0.0057			Log Laccase	0.344	0.0038	
3	Log ($k_{ref\ (B)}$)	−2.166	<.0001	0.39	6	Log ($k_{ref\ (B)}$)	−1.659	<.0001	0.38
	Log ($k_{ref\ (G)}$)	−0.715	0.0179			Log ($k_{ref\ (G)}$)	−0.295	0.4889	
	Log ($k_{ref\ (T)}$)	−1.336	<.0001			Log ($k_{ref\ (T)}$)	−0.972	<.0001	
	Log K_f	−0.530	0.0029			Log F_s	0.538	0.0030	
	Log SOC	0.242	0.0773			Log SOC	−0.029	0.8036	

Assessment and conclusion by applicant:

The article investigates data of degradation and sorption tests performed for glyphosate on several Swedish agricultural soils. The analytical methods are not provided in detail, thus not allowing to check whether analytical methods could fulfil the requirements as set out for EU data generating methods including the appropriateness of LOD or LOQ. For the sorption experiment, no results are provided. No mass balances and measurement per sample date are provided for both experiments.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As indicated by the applicant, the lack of details in the study prevents using the endpoints measured in the study for the risk assessment. Efficacy of the extractions for example are not provided.

The article provides supportive information on the degradation and adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Alexa, E. et al

Data point:	CA 7.1.2.1.1/019
Report author	Alexa, E. et al.
Report year	2010
Report title	Studies on the biodegradation capacity of 14C–labelled glyphosate in vine plantation soils
Document No	ISSN 1459-0225
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate is among the most widely used broad spectrum herbicides in the world because they are highly efficacious, cost effective, practically non–toxic and degrade readily in the environment. The herbicide is inactivated and biodegraded by soil microbes, degradation rate depends on soil microbial activity and factors that affect this activity. Glyphosate degradation rates vary considerably across a wide variety of soil

types and microflora population types. The aim of this paper was to study the biodegradation capacity of glyphosate in soil samples collected from vine plantation from Timis county, Romania, belonging to Banat's University of Agricultural Science, Timisoara, in presence of organic and inorganic supplement, at different concentration levels. After addition of glyphosate–phosphonomethyl–14C–labeled, the accumulated 14CO2 (as % of total 14C) was monitored during 44 days. Investigated soil shows a high degradation capacity of over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic supplement causes a decrease of glyphosate biodegradation capacity to 10.77–12.87 % of total radioactivity, while in presence of straw the accumulated 14CO2 (as % of total 14C) during the 44 days ranged between 59.97 and 87.58 %. The amount of $^{14}CO2$ released reached the highest level in the first 4 days after herbicide application, both in control and experimental variants with organic and inorganic supplement (from 2.61 to 30.27 % of total radioactivity). By glyphosate addition the growth and multiplication of soil microorganisms, whose biomass is digested in the range of 9–12 days of treatment, according to the daily mineralization rate (DMR) values, is stimulated. Our results on the activity of microorganisms showed that glyphosate degradation in soil is mainly performed by micromyces.

Materials and methods

Chemicals and soil samples

Glyphosate-phosphonomethyl–14C– labeled (Sigma) lot number 012K9428/29, specific activity 2.2 mCi mmol–1 and commercially formulated glyphosate of isopropylamine ammonium salt (Roundup) were purchased from Monsanto, Romania. Liquid Scintillation Cocktail (Quicksafe A cocktail) was used in Triathler Liquid Scintillation Counting. All other reagents were of analytical reagent grade.

The soil characterized as cambic moderately gleyed chernozem were sampled in March 2010 from the vine plantation (Burgundy grape variety) of Banat's University of Agricultural Science in Timisoara (Western part of Romania). Sampling depth was between 0 and 10 cm. The glyphosate treatments and both organic and inorganic fertilizers are usually applied in grape–vine plantation. The soil samples were dried at room temperature for 48 h and crushed to pass a 2 mm sieve.

The basic physico–chemical soil characteristics and chemical composition of added inorganic and organic supplements were as follows:

soil: clay 42.1 %; sand 29.2 %; silt 28.7 %; pH in H2O 7.93; organic matter 3.95 %; N total 0.266 %; P 30 ppm; Fe 20,340 ppm; Cu 10 ppm; Mn 300 ppm; Zn 8 ppm

organic supplement: pH 6.5; Ntotal 14 %; organic matter 7.5 %; P 30 ppm; Zn 35.89 ppm; Cr 42.60 ppm; Ni 25.61 ppm; Cu: 31.51 ppm; Cd 2.01 ppm; Fe 487.7 ppm

inorganic supplement: Ntotal 15 %; P2O5 5 %; K2O 20 %; CaO 2 %; MgO 1 %; S 9 %; Cu 0.1 %; Fe 0.1 %; Mn 0.5 %; Zn 0.1 %

wheat straw: cellulose 35 %; lignin 18 %; ash 8 %; hemicellulose 35 %

14C–labelled glyphosate biodegradation radio-assay

Evaluation of 14C–labelled glyphosate biodegradation was done according to Getenga et al. using liquid scintillation counter Triathler (Finland) for radio–assay. In the incubation experiment, 25 g soil samples in duplicates were placed in biometer flasks. The soil was conditioned by being moistened to 85 % of the field water capacity. The biometer flask content is a plastic vial with soil treated with glyphosate, a vial containing 10 ml distillated water, which assures atmosphere saturation with water vapor and a plastic vial filled with 10 ml 0.2 M NaOH , to trap the 14CO2 released during mineralization by soil microorganisms. Non–labelled glyphosate solution in distilled water in concentration of 1.5 ppm was added to each soil sample and the initial radioactivity was done by glyphosate–phosphonomethyl–14C–labeled with specific activity 0.5 mCi. The soils were incubated at 20°C, in the dark for 44 days. In order to evaluate the biodegradation of 14C–labeled glyphosate during the incubation period, samples were taken every 4 days. The NaOH solution was mixed with 5 ml of Quicksafe A cocktail in a 20 ml scintillation vial before it was radio–assayed. After every sampling the vial was refilled with fresh 0.2 M NaOH. The amount of 14CO2 released during mineralization was quantified on the base of 14C disintegration number.

By adding the percentages at each sampling, the total amount of mineralized glyphosate depending on time is obtained. The mineralization curves of 14CO2 accumulated were compared during 44 days.

The experimental treatments were: Control – soil with glyphosate in concentration of 1.5 ppm; OSI – soil with glyphosate and addition of organic supplement 3.2 %; OSII - soil with glyphosate and addition of organic supplement 6.4 %; ISI – soil with glyphosate and addition of inorganic supplement 8 %; ISII – soil with glyphosate and addition of inorganic supplement 16 %; WSI – soil with glyphosate and addition of wheat straw 1 %; WSII – soil with glyphosate and addition of wheat straw 2 %.

Evaluation of microbial response parameters

Microbial communities in soils treated with glyphosate were evaluated using the method described by Seeley et al. 20. A soil sample (about 20 g) was treated with 1.5 ppm glyphosate unlabeled solution (Roundup) and incubated at 22 ± 3°C in an Erlenmeyer flask. Daily humidity was corrected so that it does not to fall below 75–80 % of the wet field capacity. After 3 and 10 days we determined the number of culturable microorganisms using the count plate method. For the quantitative determination of eubacterias we used Topping medium: yeast extract 0.25 %, peptone powder 0.25 %, agar 1.8 % and distillated water, pH 7.6. To quantify the number of actinomycetes we used Gause medium: KNO3 0.1 %, K2HPO4 0.05 %, MgSO4 0.05 %, NaCl 0.05 %, FeSO4 1 %, corn starch 2 %, agar 2 %, distillated water, pH 7, and for estimation of the micromycetes number we used Czapek Dox medium: NaNO3 0.3 %, K2HPO4 0.1 %, MgSO4 0.05 %, KCl 0.05 %, FeSO4 0.001 %, sucrose 0.3 %, agar 1.5 %, pH 5.5. To secure a microbial count the samples were diluted (in 0.1 % sodium pyrophosphate) and plated, and after incubation the colonies that develop were counted. The microbial count of the original samples was then determined by multiplying the average number of colonies that develop by the degree of dilution (dilution factor of the samples in the plate). Dilutions, expressed as negative exponents, were 10–5 for micromycetes and 10–7 for eubacterias and actinomycetes determinations. The results were expressed in colony forming units (CFU) per g soil (dry matter).

Results

^{14}C–glyphosate calibration was done on the basis of quench curve method. The curve establishes the relationship between a quench parameter (QP) and the counting efficiency. Quench parameter indicates a relative light production from the sample. In the Triathler the quench parameter (QP) is, in mathematical terms, the center of spectrum gravity in the counting window. The collective effect of quench is a reduction in the number of photons produced and, therefore, detected CPM (counts per minute). The Triathler uses parabolic regression to form the curve. First the quench curve was made by counting a set of standard samples with the same activity but variable quench. The Triathler prints the quench parameters and the corresponding efficiencies of the standards. When unknown samples are counted, the quench parameter is measured for each sample. Corresponding efficiency for the measured quench parameter is obtained from the curve and the DPM (desintegrations per minute or absolute radioactivity) corresponding value is calculated ($DPM = CPM \cdot Eff^{-1}$). The efficiency taken from the curve and an error percentage (err %), which is the difference of efficiencies (difference between measured eff. and the one taken from the quench curve, are indicated below.

Table 8.1.1.2-81: The data recorded for Triathler calibration

Sample	Time (s)	Counts	CPM	QP
1	300	1,094,190	218,838	44.144
2	300	550,900	110,180	42.995
3	300	489,535	97,907	34.861
4	300	108,650	21,730	28.142
5	300	27,095	5,419	23.558

Std DPM: 220,000.
Eff.= - 0.0006·qp2 + 0.0743·qp - 1.2895.

Table 8.1.1.2-82: The efficiencies obtained on the basis of data analysis

Eff	QP	Eff	err (%)
0.99472	44.14	0.99472	0.00
0.50082	43.00	0.50082	0.00
0.44503	34.86	0.44503	0.00
0.09877	28.14	0.09877	0.00
0.02463	23.56	0.02463	0.00

Results regarding 14C–glyphosate biodegradation

Experimental results regarding the amount of $^{14}CO_2$ (%) released reported to total initial radioactivity, in accordance with prelevation chart are represented in tables. The biodegradation degree of glyphosate in soil was estimated as ratio between the number of ^{14}C–glyphosate disintegration in the sample and the number of disintegration in the standard. From these data it can be observed that in both control and experimental variants with organic and inorganic supplement addition, the amount of $^{14}CO_2$ released recorded the maximum value in the first 4 days after herbicide application, ranging from 2.61 % to 30.27 % of total radioactivity. The biodegraded glyphosate amount decreases for all analyzed samples, being less than 1 % after 44 days. The experimental results are in accordance with previous data obtained, which show that the glyphosate biodegradation has only two phases, the initial rapid phase for about 20 days due to microorganisms action on free glyphosate in soil followed by a slow final phase when the microorganisms act on glyphosate adsorbed on the soil compounds. It can be observed that, for control, $^{14}CO_2$ resulting from the glyphosate decomposition reached maximum value after 4 days (30.27 %) and decreases with time advancing: 20.27 % after 8 days, 11.86 % after 12 days, 10.94 % after 16 days and only 3.94 after 20 days reaching 0.35 mg $^{14}CO_2$ after 44 days. In Figure 8.1.1.2-38 a–c the glyphosate mineralization curves expressed as accumulated $^{14}CO_2$ as % from total radioactivity are represented. Accumulated $^{14}CO_2$ in the case of control sample, without fertilizers, increased from 30.24 % after 4 days, to 50.54 % after 8 days from herbicide application, respectively, 80 % after first 24 days and slow growing to 85.96 % of total radioactivity after 44 days (Figure 8.1.1.2-38 a). The soil characteristics influence the degradation capacity of glyphosate in the presence of microorganisms. In the literature there are several papers describing that the adsorption of glyphosate by soils depends on cationic exchange capacity, clay content, pH and organic matter. Studies regarding the effect of pH on the adsorption of glyphosate by soils and clays agreed that an increase of pH decreased the adsorption of glyphosate. It was due to an increase in negative charge of glyphosate and mineral surface with an increase in pH value resulting in a decrease in the adsorption. The analyzed soil has a high content of clay (42.1 %), iron and pH in H_2O (7.93). The experimental results show a high glyphosate biodegradation capacity in control sample (85.96 % after 44 days) and availability of glyphosate to microorganisms, due to low level of glyphosate adsorption on soil particles, according to other studies.

Table 8.1.1.2-83: Impact of added supplement on 14CO2 release (% of total radioactivity)

Variant	Accumulated $^{14}CO_2$ (% of total ^{14}C) in the prelevation time (A-K)										
	A	B	C	D	E	F	G	H	I	J	K
Control	30.27	20.27	11.86	10.94	3.9	2.82	2.27	1.47	1.12	0.69	0.35
OSI	30.23	19.13	12.76	9.54	3.86	2.8	2.27	1.47	1.12	0.91	0.49
OSII	33.66	16.38	13.24	4.13	3.9	2.53	1.9	1.62	0.7	0.7	0.49
ISI	2.61	1.5	0.69	0.62	0.54	0.48	0.46	0.36	0.3	0.26	0.25
ISII	2.73	1.94	1.63	1.49	1.28	1.1	0.91	0.76	0.41	0.42	0.2
WSI	25.51	15.27	4.86	4.08	2.78	2.62	1.94	1.58	1.13	0.81	0.73
WSII	21.96	13.71	7.13	6.58	2.64	2.17	1.65	1.24	1.19	0.96	0.74

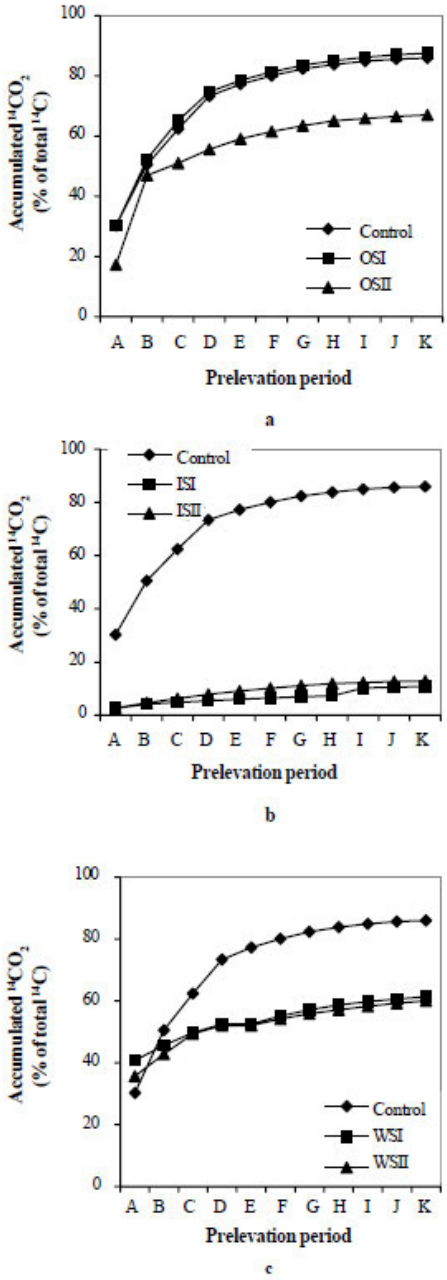
In Figure 8.1.1.2-38 a–c the glyphosate mineralization curves expressed as accumulated 14CO2 as % from total radioactivity, in the case of organic and inorganic supplement addition are represented. The experimental results obtained show significant differences between the amount of biodegradable glyphosate according to the type and amount of organic or inorganic fertilizer added. Addition of organic fertilizer at a rate of 3.2 % does not lead to significant changes in curve shape of glyphosate mineralization (Figure 8.1.1.2-38 b). Increasing the amount of organic fertilizer to 6.4 % leads to decrease in the amount of released 14CO2. Total accumulated 14CO2 after 44 days from the glyphosate application was 87.58 % for organic substrate addition OSI, respectively, 67 % in case of organic substrate addition of OSII. Our

results are in accordance with those of Getenga and Kengara, showing that compost addition does not stimulate intense mineralization of glyphosate by microbes.

Mineralization curves in Figure 8.1.1.2-38 b show the reduced availability of glyphosate to biodegradation in the presence of inorganic fertilizers. In case of mineral fertilizers addition in a proportion of 8 % of inorganic supplement, the amount of 14CO2 4 days after the herbicide administration was 2.61 % of the total radioactivity and decreased slowly reaching 0.25 % between 40 and 44 days. Biodegradation capacity of glyphosate in the presence of mineral fertilizers was much reduced compared to the control sample (Figure 8.1.1.2-38 b). The total amount of $^{14}CO_2$ released after 44 days was only 10.77 % in case of ISI and 12.87 % in case of ISII.

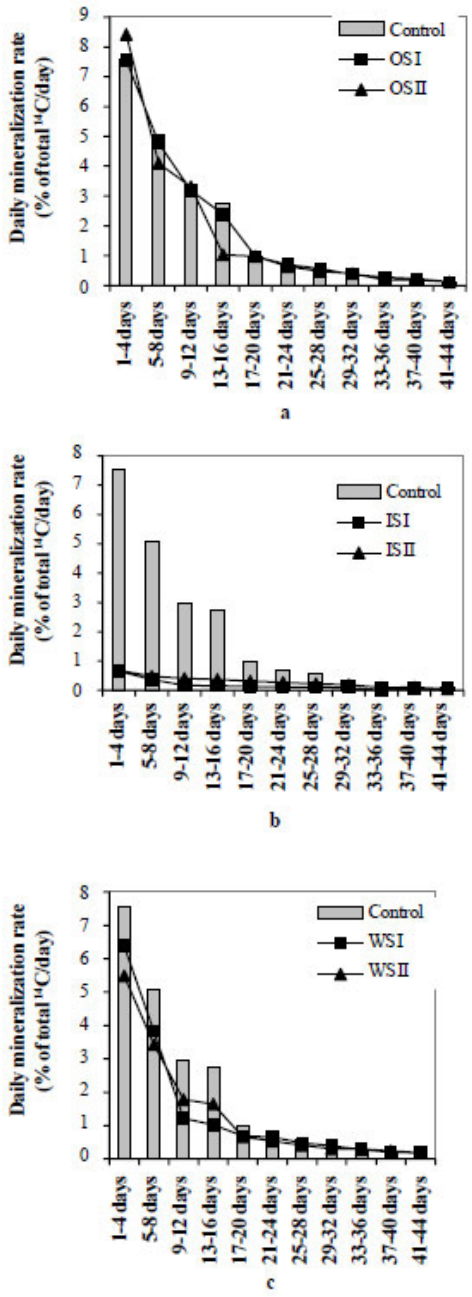
It can be observed that the biodegraded glyphosate percentage was between 2.61 to 2.73 % after the first 4 days and decreased to 0.2 % after 44 days of experimentation. Glyphosate contains functional groups of amine, carboxylate and phosphonate that can form strong coordination bonds with metal ions to give bidentate and tridentate complexes. Addition of inorganic fertilizers rich in metal ions leads to decrease in glyphosate biodegradation ability and reduces the amount of 14CO2 released. Cruz et al. studied the competitive adsorption between glyphosate and phosphate in different Brazilian soils. The results showed that on the clays glyphosate was not easily displaced by phosphate even in the ratio of 10.0 of phosphate/glyphosate. Our results are in accordance with those because in analyzed soil with high content of clays (42.1 %) the glyphosate is not displaced by phosphate ions. On the other hand, the addition of inorganic fertilizer rich in phosphate and nitrate led to micro–organisms orientation on nitrogen and phosphate source easily accessible, respectively, reduced availability of glyphosate for biodegradation. Thus, the amount of 14CO2 released is 10 times lower in variants fertilized with inorganic supplement (ISI, ISII). The increased content of mineral fertilizer, in the case of ISII, did not lead to significant changes regarding the release of 14CO2 from the glyphosate biodegradation. In WSI and WSII where wheat straw at a rate of 1 % and 2 % was added, there was a noticeable decrease in the amount of 14CO2 pursued as a result of glyphosate biodegradation compared with the control. Thus, after 4 days the percentage of released 14CO2 was 25.51 % in WSI and 21.96 % in WSII. After 8 days from the glyphosate application, the biodegradation capacity decreased to 4.86 and 7.13 %. 14CO2 total amount accumulated as a result of glyphosate biodegradation was 61.31 %, in the case of 1 % straw addition and 59.97 % to 2 % straw addition (Figure 8.1.1.2-38 c).

Figure 8.1.1.2-38: Mineralisation of 14C–glyphosate in soil with different supplements (a– control versus OS, b– control versus IS, c– control versus WS)



The daily mineralization rate (DMR) of glyphosate (Figure 8.1.1.2-39 a–c) in the case of different supplement addition was highest for all variants in the first 4 days of experiment, decreasing during incubation. If mineral fertilizers were added (ISI, ISII), the DMR value was much lower than in other cases. The explication is due to existing mineral compounds intake in inorganic fertilizers, compounds with which glyphosate forms complexes hard accessible for microbial metabolism but also, due to lack of energy substrate supporting the respiratory activity of microorganisms.

Figure 8.1.1.2-39: Daily mineralization rate of glyphosate (a– control versus OS, b– control versus IS, c– control versus WS)

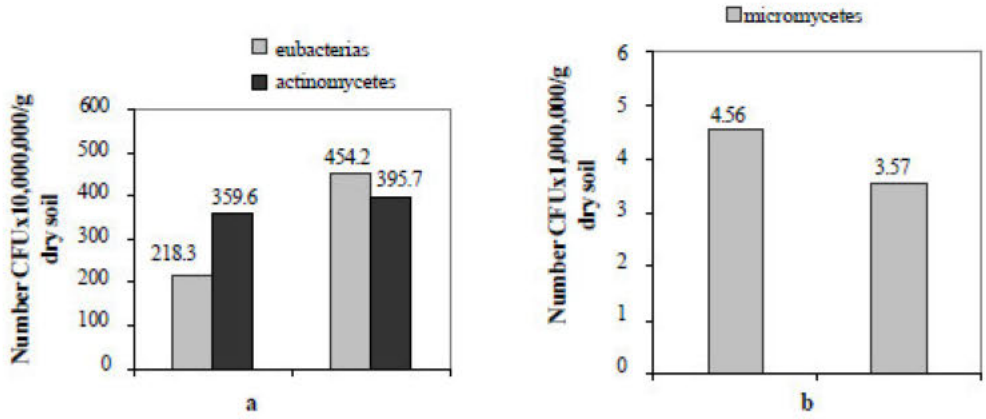


Besides, in the organic material addition, OSI, OSII, WSI and WSII, DMR value was highest in the first 4 days of experimentation. The highest values of 14CO2, corresponding to DMR, were determined in OS even exceeding the control (7.567 mg 14CO2), DMR value was higher than control value also for range of 9–12 days. This could be due to labelled carbon release from the microbial protoplasm which assimilates the labelled glyphosate, respectively of fungal biomass.

Results regarding microorganism activity in soil

Glyphosate remains unchanged in the soil for varying lengths of time, depending on soil texture and organic matter content. Soil microorganisms break down glyphosate and many can use glyphosate as a sole source of phosphorus. On the base of results regarding the number of culturable microorganisms existing in the soil with glyphosate (Figure 8.1.1.2-40 a, b) it can be observed that at 10 days after the treatment application, the eubacteria number increases from 218.3x105 to 454.2x105 CFU g–1 dry soil.

Figure 8.1.1.2-40: The variations of microorganisms number after 3 and 10 days since glyphosate addition in control a) eubacterias and actinomycetes, b) micromycetes



Conclusion

The soil characteristics influence the degradation capacity of glyphosate in the presence of microorganisms. The soil sampled from the vine plantation (Burgundy grape variety) of Banat's University of Agricultural Science in Timisoara shows a high degradation capacity, over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic substratum causes a decrease in glyphosate biodegradation capacity to 10.77 – 12.87 % of total radioactivity, while in presence of straw the accumulated 14CO2 (as % of total 14C) during the 44 days ranged between 59.97 – 87.58 %. The amount of 14CO2 released reached the highest level in the first 4 days after herbicide application both in the control and experimental variants with organic and inorganic substratum (from 2.61 to 30.27 % of total radioactivity). The growth and multiplication of soil microorganisms whose biomass is digested in the range of 9 – 12 days of treatment, according to the daily mineralization rate (DMR) values is stimulated by glyphosate addition. Our results on the activity of microorganisms have shown that glyphosate degradation in soil is mainly performed by micromyces.

Assessment and conclusion by applicant:

The article investigates the degradation of glyphosate in a European agricultural soil originating from vine in the laboratory. Only data on mineralisation are reported. Further data like mass balances, residues in soil and a half–life are not reported. The validity of the study cannot be evaluated due to missing information.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, only data on mineralisation are reported in the article. Further data useful for the analysis of the degradation of glyphosate in the conditions of the study are not reported.

The article provides supportive information on the degradation of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Al–Rajab, A., Schiavon, M

Data point:	CA 7.1.2.1.1/020
Report author	Al–Rajab, A., Schiavon, M.
Report year	2010
Report title	Degradation of 14C–glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils
Document No	DOI 10.1016/S1001-0742(09)60264-3 ISSN 1001-0742

Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate (N–phosphonomethyl glycine) is the most used herbicide worldwide. The degradation of 14C–labeled glyphosate was studied under controlled laboratory conditions in three french agricultural soils: a silt clay loam, a clay loam and a sandy loam soil. The kinetic and intensity of glyphosate degradation varied considerably over time within the same soil and among different types of soil. Our results demonstrated that the mineralization rate of glyphosate was high at the beginning of incubation and then decreased with time until the end of the experiment. The same kinetic was observed for the water extractable residues. The degradation of glyphosate was rapid in the soil with low adsorption capacity (clay loam soil) with a short half–life of 4 days. However, the persistence of glyphosate in high adsorption capacity soils increased, with half-live of 19 days for silt clay loam soil and 14.5 days for sandy loam soil. HPLC analyses showed that the main metabolite of glyphosate, aminomethylphosphonic acid (AMPA) was detected after three days of incubation in the extracts of all three soils. Our results suggested that the possibility of contamination of groundwater by glyphosate was high on a long–term period in soils with high adsorption capacity and low degrading activities and/or acid similar to sandy loam soil. This risk might be faster but less sustainable in soil with low adsorption capacity and high degrading activity like the clay loam soil. However, the release of non–extractable residues may increase the risk of contamination of groundwater regardless of the type of soil.

Materials and methods

Chemicals

[Phosphonomethyl–14C]–glyphosate was obtained from ARC–ISOBIO (Belgium) diluted in water. Its specific radioactivity was 385 GBq/mmol and its radiochemical purity 99 %. Non–radioactive glyphosate (purity 98.5 %) was obtained from CIL Cluzeau (France). AMPA, 10 ng/µL in water, was obtained from Dr. Ehrenstorfer GmbH (Germany). Sarcosine (N–methylglycine) C3H7NO2, purity 99 %, was obtained from Fluka (Germany). FMOC–chloride (purity 99 %), sodium tetraborate decahydrate (purity 99.5 %), potassium hydroxyde (purity 86 %), potassium dihydrogen phosphate (purity 99.5 %) were also obtained from Fluka (Germany). Acetonitrile was obtained from (SDS, France). All solvents were of high performance liquid chromatography (HPLC) grade.

Selected soils and treatments

Three cultivated soils from the Lorraine region in eastern France were selected on the basis of their texture and pH (Table 8.1.1.2-84). None of these soils had ever been exposed to glyphosate. Soil types were classified as rendzic leptosol, fluvic cambisol, and stagnic luvisol, hereafter referred to as: clay loam soil, sandy loam soil and silt clay loam soil, respectively. The surface layers (0–25 cm) of all three soils were sampled on the same day.

Soils were air dried and sieved to 2 mm maximum particle size. Soil samples (25 g) were placed in glass jars of 60 mm diameter by 40 mm high. Samples were prepared in triplicates for each soil and each sampling time. An aqueous solution of 0.51 mg glyphosate and 45.1 kBq (equivalent to 1800 g/ha) was added to each soil sample. The volume of aqueous solution was calculated for each soil to obtain samples with moisture content of 80 % of soil retention capacity.

Table 8.1.1.2-84: Principal characteristics of the soils (surface layers, 0-25 cm) used in this study

Soil	Clay (%)	pH (water)	OC[a] (%)	K_f[b]	Fe oxides[c] (g/kg)	Fe amorphous[d] (g/kg)	Total Cu[e] (mg/kg)	Total P_2O_5 (g/kg)
Sandy loam	10.5	5.1	0.82	34.5	9.73	2.89	7.89	1.24
Silt clay loam	30.6	6.3	1.45	33.6	40.05	8.52	29.80	3.24
Clay loam	34.9	7.9	1.91	16.6	33.16	2.51	14.11	2.74

[a] Organic carbon content; [b] K_f values obtained from Al-Rajab et al., 2008; [c] subtraction of extracted iron by sodium dithionite-citrate and by acid ammonium oxalate; [d] extracted iron by acid ammonium oxalate in darkness; [e] dissolved by HF.

Laboratory degradation studies

Each soil sample was placed in an individual airtight jar (1.5 L). A scintillation vial containing 10 mL water was placed in each jar to maintain a humid atmosphere and prevent desiccation of the soil. A second scintillation vial with 10 mL of 0.5 mol/L NaOH solution was also placed into each jar to trap any CO2, which evolved from the soil due to mineralization of organic matter and 14CO2. The jars were incubated in the dark at 20°C for 80 days. Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.

Evaluation of soil micro-organism activity

The total CO2 fixed by the NaOH was evaluated by titrating an aliquot (8 mL) with 0.2 mol/L HCl, in the presence of 3 mL of 20 % BaCl2 and thymolphtalein at 4 % in ethanol, on day 0, 1, 2, 3, 5, 8, 12, 17, 22, 30, 40, 65, and 80. On each sampling date, the replacement of the CO2 trapping solution by fresh solution allowed air renewal in the jars.

Estimation of mineralization of glyphosate

The amount of 14CO2 trapped by NaOH as a result of the mineralization of 14C–glyphosate was determined by liquid scintillation counting. NaOH (1 mL, in duplicates) of each sample received 10 mL Ultima Gold scintillation cocktail (LSC–cocktail) from Packard (USA) in a plastic scintillation vial. Radioactivity was measured during 10 min using a Packard Tri–Carb 1900 CA liquid scintillation counter (Packard, USA).

Residues in soil

Extractable residues of glyphosate were evaluated and analysis as follow. Soils samples in triplicates were removed from incubation for each soil on day 0, 1, 2, 3, 5, 8, 12, 17, 22, 30, 40, 65 and 80 after treatment. The soil of each sample (25 g) was transferred into a 250–mL PPCO (Nalgene, VWR, USA) centrifuge flask. The soil was extracted thrice with 100 mL distilled water (easily available residues) then 3 times with 100 mL of 0.1 mol/L KH2PO4. The samples were rotary shaken at (20 ± 2)°C for 2 hr, and then centrifuged at 5000 ×g for 20 min. The supernatants were combined, the volumes adjusted and radioactivity was determined using liquid scintillation as described above. The supernatants of each sample were filtered through Whatman 40 filter papers, and transferred into a round bottom glass bottle (1000 mL), and then frozen at –30°C for 48 hr before being freeze dried (Edwards–Modulyo–RUA). The freeze–dried extracts were dissolved in 7 mL distilled water and filtered through 0.2 µm using Minisart RC–25 filters (Sartorius, France), then the extracts were stored in freezer at –30°C till derivatization and analysis by HPLC.

Analysis

Derivatization of residues

This analysis was carried out only on the aqueous soil extracts. A 0.5 mL of 0.05 mol/L buffer borate was added to 3 mL of the aqueous solution to be analysed, then left to settle for 15 min. Then 3 mL ethyl ether were added and the solution was agitated vigorously for 2 min. The mixture was left to settle. After 15 min, 1.5 mL of the aqueous phase was removed and 0.25 mL acetonitrile added, followed by 0.25 mL of a solution of FMOC–Chloride in acetonitrile (1 g/L). The mixture was left to react for 60 min at ambient temperature. Two milliliter of ether ethyl was added and the solution was agitated vigorously for 2 min. The solution was left to settle for 1 hr and then the aqueous phase was recovered in a 2–mL vial for high performance liquid chromatography (HPLC) analysis.

Analysis of residues

The residues were analyzed by HPLC in a Varian chromatograph equipped with a fluorescence detector and a β–radioactivity detector (Flo–one β, Packard, USA) in the following operating conditions: Lichrosorb–NH2 column (5 µm, 4 mm × 250 mm) (CIL–Cluzeau, France) thermostated at 30°C, injection volume 50 µL, analysis time 22 min, flow rate 0.8 mL/min, elution KH2PO4 0.05 mol/L, pH 5.7, acetonitrile (70/30) (V/V). Detection was performed in the following conditions: (1) β–radioactivity detector: Scintillator Ultima–Flo, flow rate 1.2 mL/min, counting cell 500 µL, and (2) fluorescence detector: λ excitation 260 nm; λ emission 310 nm. Standards of the glyphosate (purity >98.5 %), AMPA (purity >98.5 %, CIL–Cluzeau, France) and sarcosine (N–methylglycine, purity >99 %, Fluka) were used for calibration (0, 10, 20, 50 and 100 µg/L). The retention time was 4.2 min for sarcosine, 6.6 min for AMPA, and 13.3 min for glyphosate.

Non–extractable radioactivity

After extraction by water and KH2PO4, all soil samples were air dried. Remaining non–extractable 14C–radioactivity was determined by combustion. An aliquot of 0.3 g was mixed with 0.15 mg cellulose powder and the sample was burnt at 900°C with a 307 Packard Oxidizer (Packard, USA). The released 14CO2 was trapped with 10 mL Carbosorb (Packard, USA) and the radioactivity was counted after the addition of 10 mL of Permafluor (Packard, USA).

Statistics

Statistical analyses were performed using Stat Box computer software (Grimmer Software version 6.4). Comparison of the means was done using the Newman–Keuls test at levels of 0.05, 0.01 and 0.001. Curves were plotted using SigmaPlot (Version 10, Systat Software Inc., USA). Data in figures represent the mean and standard deviation of triplicate samples.

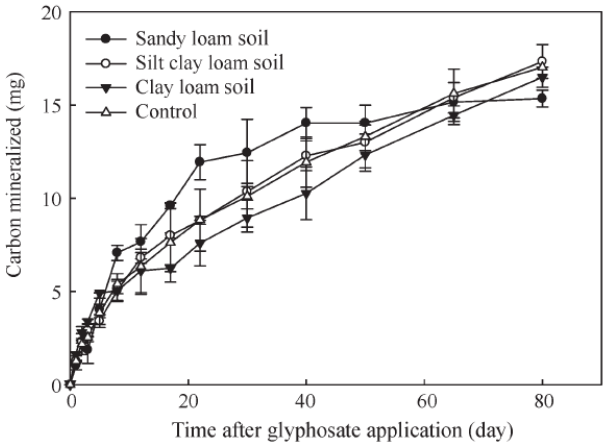
Results

Microbial activity

Total carbon mineralization of treated or untreated soils during the incubation was used as an indicator of the total microbial activity in the soils (Figure 8.1.1.2-41). Endogenous carbon was steadily mineralized in each soil during incubation and the intensity of mineralization differed slightly among soils between day 5 and day 50. During this period, mineralization was slightly faster in the sandy loam soil (14.4 mg carbon) than in the other two soils (13.73 mg for silt clay loam soil and 11.8 mg for clay loam soil). After 50 days, the slowdown in mineralization activity was more rapid for sandy loam soil than for the other two soils.

At the end of experiment (after 80 days of incubation), the total amount of carbon mineralized was similar for all three soils indicating that each soil presented significant microbial activity and that glyphosate had no toxic effect on soil micro–organisms.

Figure 8.1.1.2-41: Mineralization activity of microflora of three soils (clay loam, sandy loam and silt clay loam soils). The control is the average of mineralization activity for the three untreated soils



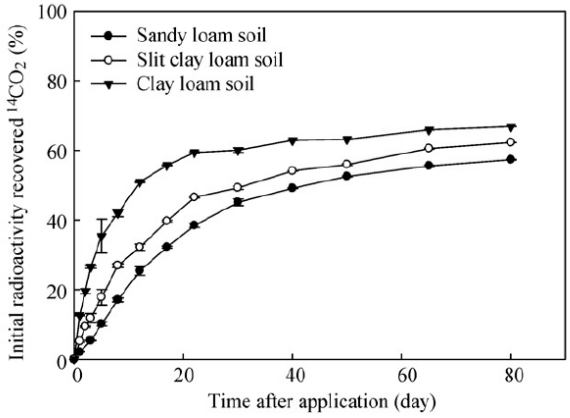
Mineralization of glyphosate

We observed an immediate and high rate of glyphosate degradation after its application on soil (Figure 8.1.1.2-42). The absence of lag phase indicates that the microflora of soil already had an enzymatic system capable of degrading glyphosate and as such did not need an adaptation period.

Mineralization of glyphosate after 17 days of incubation reached 32.2 % to 39.7 % of the initial amount applied to the two soils (sandy loam (pH 5.1) or silt clay loam (pH 6.3)). However, the mineralization rate was more rapid and intense for the clay loam soil (pH 7.9) with 48.4 % reached by 12 days of incubation. Thereafter, the mineralization of glyphosate declined gradually for all three soils. The endogenous activity of mineralization was comparable for the three investigated soils. The fast mineralization of glyphosate in clay loam soil appears due exclusively to a bioavailability more important than in other two soils.

We have previously shown that the adsorption of glyphosate in clay loam soil (Kf = 17) is lower than the other two soils (Kf = 34) (Al–Rajab et al., 2008). The half–lives of glyphosate derived from the mineralization rates were significantly different for the three soils, and were 42, 31, and 12 days for sandy loam, silt clay loam, and clay loam soils respectively. These results show that the degradation of glyphosate in biologically active agricultural soils could be influenced by the adsorption of glyphosate. Otherwise, the effect of organic matter content in the soil on mineralization of glyphosate was not clear under the conditions of this study.

Figure 8.1.1.2-42: Mineralization of 14C–glyphosate to 14CO2 in three soils incubated at 20 °C



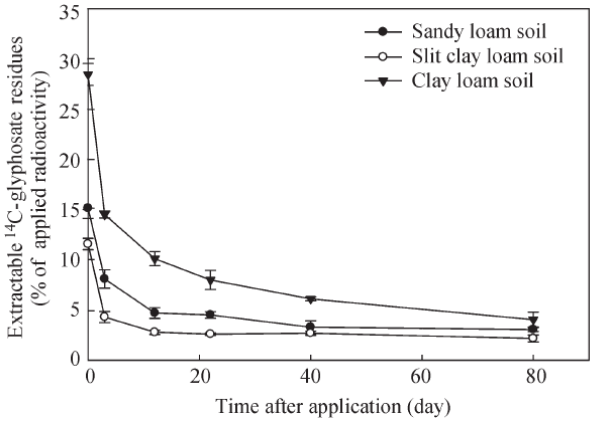
Glyphosate degradation products – Extractable residues

The soil was extracted separately three times with distilled water, then three times with 0.1 mol/L KH2PO4. The extraction rate of glyphosate residues with H2O is influenced by: (1) the degradation, which produces new products (metabolites) that differ in their water solubility and their reactivity with soil constituents; (2) by the process of adsorption–desorption, and (3) the formation of non–extractable residues over time; these sequestered residues are not available to be extracted by H2O.

The extraction rate of glyphosate with water is an indication of the accessibility of the residues for microbial degradation and/or their transfer to groundwater under natural conditions. The extraction of glyphosate residues with water is directly related to the Kf measured for these soils (Figure 8.1.1.2-43). The observed difference of glyphosate extractable residues with water between the sandy loam soil and silt clay loam soil (which have the same Kf value) is certainly related to their texture. For the sandy loam soil, the sandy texture and unstable structure results in a better accessibility to the extraction solution, which in turn leads to a greater extraction efficiency when compared to clay loam soil.

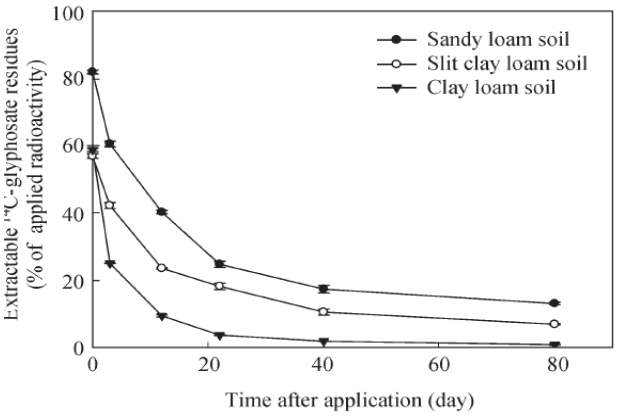
The extraction curves are opposite to those of the mineralization, with the same ranking of soils. These results indicate that the degrading activity of the microflora of soil is linked to the rate of glyphosate available for passage in the aqueous phase.

Figure 8.1.1.2-43: Evolution of extractable 14C–glyphosate residues with H2O from the three soils during incubation at 20 °C.



On the other hand, the extraction of glyphosate from soil with 0.1 mol/L KH2PO4 was more efficient than extraction with H2O. It did not seem affected by the level of bonds energy between the soil and residues of herbicide (Figure 8.1.1.2-44). In fact, in the sandy loam soil of Kf = 34, the percentage of glyphosate 14C–phosphonomethyl extracted at T0, immediately after treatment, was (81.9 ± 0.55) % of the initial amount applied (Figure 8.1.1.2-44). Thereafter, this value decreased slowly to reach (13.0 ± 0.41) % of the initial amount applied at the end of incubation. In contrast, in the silt clay loam soil, with similar value of Kf = 34, the percentage of extracted residues at day 0 was only (56.9 ± 0.7) %, which is similar to that obtained for the clay loam soil which has a different Kf value of 17. This difference may be due to the high clay content in these two soils (silt clay loam and clay loam) and their structures, which reduces the performance of extraction of KH2PO4. We can assume that the treatment in a dry soil may cause an entry of glyphosate into the microporisity of aggregates during the capillary invasion by the aqueous solution of treatment (Guimont et al., 2005). The size of this compartment would be defined at the time of treatment and may depend on the physicochemical and physical properties (size of microporal compartment), and the moisture rate of soil at application time. This availability to extraction decreased overtime, more quickly in the sandy loam soil than in the other two brown soils, and at the end of experiment it reached 13.0 %, 6.9 %, and 0.8 % of the initial amount for sandy loam, silt clay loam, and clay loam soils, respectively. The evolution of extraction rate with KH2PO4 over time in the three soils is related to the mineralization of residues and the rate that non–extractable residues become available for mineralization and extraction.

Figure 8.1.1.2-44: Evolution of extractable 14C–glyphosate residues with KH2PO4 from the three soils during incubation at 20 °C



Glyphosate degradation products – Degradation products

The analysis of water extracts by HPLC showed the appearance of two degradation products of glyphosate AMPA and/or sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co–eluted and unlabelled

organic compounds. This analysis showed only the very rapid onset of AMPA in the extracts and its predominance compared to glyphosate as of the day 12 of application for the clay loam soil.

The appearance of AMPA during incubation varied significantly depending on the speed of mineralization of glyphosate in each soil (Table 8.1.1.2-85). In sandy loam soil, there was only 12.7 % of AMPA present on day 3 after treatment, whereas 87.3 % of the initial radioactive glyphosate was present on the same day. Thereafter, the percentage of AMPA increased gradually overtime, reaching 58.9 % of residues after 22 days of incubation, and 91.1 % at the end of the experiment.

Table 8.1.1.2-85: Mass balance of glyphosate and AMPA in extracted residues during incubation over 80 days (%)

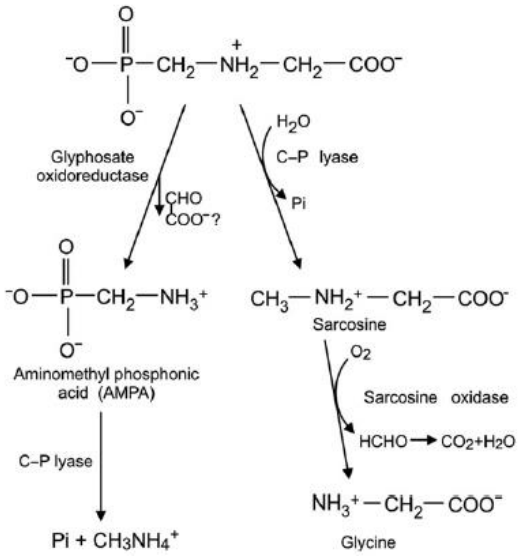
Incubation time (day)	Sandy loam soil		Silt clay loam soil		Clay loam soil	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
0	100	nd	100	nd	100	nd
3	87.3	12.7	79.7	20.3	51.5	48.5
12	71.0	29.0	58.5	41.5	40.2	59.8
22	41.1	58.9	25.6	74.4	12.0	88.0
40	22.3	77.7	22.5	77.5	5.6	94.4
80	8.9	91.1	14.9	85.1	0.9	99.1

AMPA: aminomethylphosphonic acid. nd: not detected.

The extractable residues of glyphosate with water are easily available to the degradation or transfer by water in soil. The half–life of glyphosate extractable with water was estimated and was found to vary depending on the biological activity of soil. It was 19 days for the sandy loam soil, 14.5 days for the silt clay loam soil and 4 days for the clay loam soil.

Together, our results suggest that the rupture of the –CH2–NH– bond giving rise to AMPA is easier than breaking the –CH2–PO3H2 bond that results in either sarcosine and phosphorus, or methylamine and phosphorus (Figure 8.1.1.2-45). The break of the –CH2–NH– bond may depend on the overall activity of the microflora and the retention of glyphosate by the soil; while the rupture of the –CH2–PO3H2 bond could be related to a more specific bacterial population. This difference in the rupture speed of these two links leads to some accumulation of AMPA in the soil (Figure 8.1.1.2-45).

Figure 8.1.1.2-45: Microbial degradation of glyphosate in soil through sarcosine or AMPA (Liu et al., 1991)

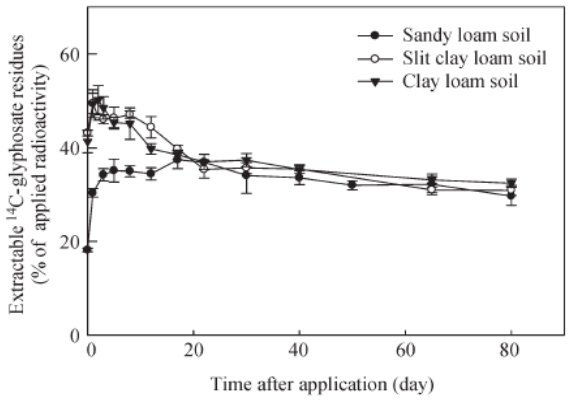


Non–extractable glyphosate residues

The non–extractable residues represent the fraction, which cannot be extracted from the soil by the series of KH2PO4 extractions (exhaustive extraction) (Figure 8.1.1.2-46). Upon application of glyphosate on a

sandy loam soil, we observed the formation of non–extractable residues at 18.1 % of the initial applied amount of herbicide. Subsequently, it progressed during 3 days to 35 %, staying stable until day 22, and then decreased very gradually over time until 30 % of initial applied amount of glyphosate was present at the end of experiment. In contrast, the formation of non–extractable residues for the clay loam and the silt clay loam soils was more intense and rapid than in the sandy loam soil. It reached 41.3 % and 43 % of the initial applied amount for the clay loam and silt clay loam soils respectively at day 0, and 49.4 % for both soils at day 1. For both soils, the rate stayed stable after day 2 until which decreased to 32.4 % and 30.9 %, respectively by the end of experiment. The rates of non–extractable residues seems specific for each soil, but are defined by day 3 after treatment.

Figure 8.1.1.2-46: Evolution of non–extractable residues in three soils during incubation time at 20 °C



The rate of non–extractable residues is probably dependent on the physico–chemical properties and physical aspects of the soils including the size of the microporal compartment. This rapid formation of non–extractable residues immediately after treatment with a maximum reached within 2 to 8 days after application is very specific for glyphosate and could probably be due to: (1) the high solubility of glyphosate in water (10.5 g/L) (Agri–tox, 2009), (2) the physico–chemical properties that allow glyphosate to immediately establish high energy bonds with the constituents of soil, (3) the physico–chemical properties of soils (texture, meso and microporisity), and/or (4) the treatment conditions.

The treatment of herbicide on a dry soil promotes the capillary invasion and the rapid transport of the solution of treatment in the microporisity intra aggregate (Guimont et al., 2005) subsequently making the glyphosate inaccessible to KH2PO4. Furthermore, the clayey texture promotes the importance of the microporosity. This explains the similar behaviour of clay loam and silt clay loam soils in the formation of non–extractable residues of glyphosate. In fact, these two soils have very different Kf values (17 and 34 respectively) but they have the same texture. These two soils, particularly the silt clay loam soil, differs strongly from the sandy loam soil which forms relatively a low rate of non–extractable residues and whose texture is sandy although having the same Kf (34) as the silt clay loam soil. We also noted that the initiation of the degradation of glyphosate did not affect the evolution of extractable residues rate. This implies that AMPA was not playing different role comparing to glyphosate. The very slow decrease of non–extractable residues showed that these residues can return by diffusion, and under the effect of a concentration gradient, to areas accessible to microorganisms to subsequently undergo mineralization. We note that from day 22 until the end of incubation the rates of non–extractable residues of glyphosate were similar for the three soils. The mineralization of glyphosate in three soils affects only the extractable fractions with water and KH2PO4 influenced by the forces adsorption defined by Kf.

The 14C mass balance for each sample revealed a deficit (loss) that fluctuated from (4 ± 2) % at day 0 (application of glyphosate) to (6.0 ± 3.4) % after 80 days of incubation independent of soil type and different sampling dates over time. These losses were probably partially caused by the handling of samples during analyses (extraction and concentration). Because of these low losses, results were corrected and returned to 100 % by distributing the deficit on the various compartments assessed in proportion to their respective importance.

Conclusion

We simultaneously monitored in controlled conditions the principal processes involved in 14C–glyphosate dissipation and their interactions in three agricultural soils over a period of 80 days. The results of this experiment showed that for agricultural soils with a significant and comparable biological activity, the fate of glyphosate and its potential in polluting water is closely related to the adsorption and the formation of non–extractable residues, which are dependent on soil texture and its moisture condition at the time of treatment. Our results showed that for a clay soil at basic pH, the glyphosate could be available to reach the groundwater in few days after treatment if the conditions are favourable for precipitation. Conversely, in the case of an acid sandy soil, the potential pollution of groundwater by glyphosate is greatly reduced by the strong adsorption of its residues in the soil. In case of rain following treatment, the risk of groundwater pollution by glyphosate will be low but may continue to be present for long time since the mineralization is slow. In this system, the silt clay loam soil is apparently less favourable for water pollution since it showed a strong adsorption of glyphosate and the formation of large amount of non–extractable residues. In the three investigated soils, a low level of water pollution (background) could be occurred over a long time by the sequestered residues of glyphosate, which are either gradually released into the soil solution, or circulated by the water through the soil.

Assessment and conclusion by applicant:

The article investigates the soil degradation of glyphosate in three agricultural soils from the EU. The test was performed with radio-labelled and non-radiolabelled test substance. For the part dealing with radiolabelled test substance, only mineralisation was followed after application to soil. Deviations in conduct were the use of air–tight test vessels not allowing for air exchange; no information whether the applied test solution was mixed with the soil; 14CO2 was passively (and potentially not quantitatively) collected; soil moisture was rather high (80 % of soil retention capacity). For the tests with non-radiolabelled test substance the details do not allow to assess the quality of the analytical method as EU data generating method including no LoD/LoQ provided. Only few results are reported quantitatively, mainly graphical plots; calculation method of DT50 not reported.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, the article is insufficiently detailed to allow to check the validity against current guidelines.

The article provides supportive information on the degradation of glyphosate and AMPA, but no reliable endpoints can be derived for use in risk assessment.

Bento et al, 2016

Data point:	CA 7.1.2.1.1/21, CA 7.1.2.1.4
Report author	Bento C. P. M. *et al.*
Report year	2016
Report title	Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness.
Document No	Science of the Total Environment, (2016) Vol. 572, pp. 301-311 https://doi.org/10.1016/j.scitotenv.2016.07.215
Guidelines followed in study	none
Deviations from current test guideline	not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

Full summary

The dissipation kinetics of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) were studied in loess soil, under biotic and abiotic conditions, as affected by temperature, soil moisture (SM) and light/darkness. Nonsterile and sterile soil samples were spiked with 16 mg/kg of glyphosate, subjected to three SM contents (20 % WHC, 60 % WHC, saturation), and incubated for 30 days at 5 °C and 30 °C, under dark and light regimes.
Glyphosate and AMPA dissipation kinetics were fit to single-first-order (SFO) or first-order-multicompartment (FOMC) models, per treatment combination. AMPA kinetic model included both the formation and decline phases. Glyphosate dissipation kinetics followed SFO at 5 °C, but FOMC at 30 °C. AMPA followed SFO dissipation kinetics for all treatments. Glyphosate and AMPA dissipation occurred mostly by microbial activity. Abiotic processes played a negligible role for both compounds. Under biotic conditions, glyphosate dissipation and AMPA formation/dissipation were primarily affected by temperature, but also by SM. Light regimes did not play a significant role. Glyphosate DT_{50} varied between 1.5 and 53.5 days, while its DT_{90} varied between 8.0 and 280 days, depending on the treatment. AMPA persisted longer in soil than glyphosate, with its DT_{50} at 30 °C ranging between 26.4 and 44.5 days, and its DT_{90} between 87.8 and 148 days.
The shortest DT_{50}/DT_{90} values for both compounds occurred at 30 °C and under optimal/saturated moisture conditions, while the largest occurred at 5 °C and reaching drought stress conditions. Based on these results, it has been concluded that glyphosate and AMPA dissipate rapidly under warm and rainy climate conditions. However, repeated glyphosate applications in fallows or winter crops in countries where cold and dry winters normally occur could lead to on-site soil pollution, with consequent potential risks to the environment and human health.

Materials and methods

Soil

The soil used in this study was a silty loam loess soil from Nagelbeek, in the region of Limburg, in the Netherlands. The soil was collected from the surface (0–10 cm) in a harvested wheat field, after removing the litter layer. Undisturbed soil samples, in triplicate, were collected at the surface with metal rings, to determine bulk density and the water retention curve. The soil was then sieved with a 2-mm sieve at field soil moisture, and immediately stored at 4 °C until use (no longer than three months as recommended by the OECD guideline 307). Since disturbed and sieved soil was used in this experiment, bulk density and the water retention curve were also determined in disturbed, slightly compacted, sieved soil. Prior to the experiment, a subsample of the soil was collected and oven-dried (105 °C) for 24 h to determine the initial soil moisture content. Background information on the history of glyphosate applications in this soil was not available. To check for any background residues of glyphosate and AMPA, the soil was analysed prior to the experiment. Soil properties and glyphosate and AMPA background residues are provided in the following table.

Table 8.1.1.2-86: Soil properties from the Dutch loess soil used in this study.

Parameters	Loess soil, Nagelbeek, Limburg region
Soil depth (cm)	0–10
Particle size distribution	
<0.002 mm (clay) (%)	26.5
0.002–0.05 mm (silt) (%)	59.2
>0.05 mm (sand) (%)	14.3
pH water	7.1
pH $CaCl_2$	6.7
Organic matter (OM) (g kg^{-1})	33.1
Organic carbon (OC) (g kg^{-1})	19.2
Inorganic carbon (g kg^{-1})	0.80
N total (g kg^{-1})	0.96
P total (g kg^{-1})	0.77
P available (mg P kg^{-1})	1.90
K available (mg K kg^{-1})	116
Mg available (mg Mg kg^{-1})	143
Na available (mg Na kg^{-1})	8.0
CEC (mmol + kg^{-1})	100
C/N ratio	9.0
Carbonates (%)	<0.2

Parameters	Undisturbed soil	Disturbed soil
Bulk density (g cm^{-3})	1.53 ± 0.05	0.84 ± 0.01
Soil saturation (pF0) (% w/w)	26.6 ± 1.9	47.7 ± 0.9
Soil water holding capacity (WHC) (pF1) (% w/w)	25.7 ± 1.7	39.1 ± 0.5
Soil field capacity (pF2) (% w/w)	24.8 ± 0.9	34.2 ± 0.8
Soil wilting point (pF4.2) (% w/w)	9.4 ± 0.6	5.2 ± 0.1
Glyphosate background (mg kg^{-1})	0.05 ± 0.03 (= LOQ)	
AMPA background (mg kg^{-1})	0.18 ± 0.07	

Facilities

This study was performed in a climate chamber (12 m^2) of the greenhouse facilities of Wageningen University, the Netherlands. Climate factors such as temperature, relative air humidity and light were controlled through automatic control panels. It was equipped with 2 long tables (each table was 0.85 × 3.80 m^2) divided by a central corridor. Above and along each table were 12 TL-D Xtra Polar 36 W/840 fluorescent lamps (Philips, the Netherlands), configured in 3 series, each series with 4 lamps in parallel. The light intensity produced by the array of lamps was dependent on temperature and distance from the source area. Luminosity above each table could be controlled separately.

Glyphosate dissipation and AMPA formation/dissipation

Preparation of glyphosate

Glyphosate (98 %) was dissolved in Millipore water to achieve a final stock solution with a concentration of 1040 mg/L. A concentration of glyphosate in soil of 16 mg/kg was used for all experiments. This concentration corresponded to a maximum application rate of 2.2 kg a.i./ha (recommended for chemical fallow against perennial weeds), assuming a soil depth of 1 cm.

Experimental design

This experiment employed a factorial design with 2 microbiological soil conditions × 2 temperatures × 3 soil moisture contents × 2 light regimes. The microbiological soil conditions used were nonsterile and sterile soil to test the effect of glyphosate dissipation and AMPA formation/dissipation under biotic and abiotic conditions, respectively. For temperature, 5 °C and 30 °C was chosen, to represent extreme climate conditions. The sterile soil treatment level was only tested at 30 °C. For soil moisture, three levels were chosen: 20 % of water holding capacity (WHC) (shortage of water), 60 % of WHC (optimal conditions) and saturation (excess of water, i.e. a soil moisture > 100 % WHC – corresponds to "Soil Saturation (pF0)" in Table 8.1.1.2-86). The two light regimes were absence and presence of light to test the role of photolysis under different temperatures and soil moisture contents on glyphosate dissipation and AMPA formation/dissipation. In order to represent natural conditions, light was applied for 12 h/day for those samples subject to light regimes. Real light intensity, determined as Photosynthetically Active Radiation (PAR: λ = 400–700 nm), was measured with a LI-190 quantum sensor (LI-COR, USA) at the table height (0.97 m), at both temperatures, giving an average PAR of 42 ± 7 μmol/m^2 s at 5 °C, and of 75 ± 9 μmol/m^2 s

at 30 °C. For dark conditions, a black and white poly panda film was installed around one of the tables in order to prevent any light from entering. The experiment totalized 18 treatments. Each treatment was done in triplicate, totalizing 21 samples per treatment (3 samples per treatment per sampling day; 7 sampling days in total). The treatment abbreviations used in this study were: 1) for microbiological soil conditions: NS – nonsterile soil, S – sterile soil; 2) for temperature: 5 – 5 °C, 30 – 30 °C; 3) for soil moisture: Sat – saturated soil, 60 – 60 % WHC, 20 – 20 % WHC; 4) for light regimes: L – presence of light, D – absence of light.

In this experiment, the relative air humidity inside the climate chamber was set to 70 ± 5 % at both temperatures. In the climate chamber, the samples were separated by dark and light conditions, but set randomly in each table. Before applying glyphosate, a pre-incubation period of 3 days was performed for the nonsterile soils at the corresponding testing temperatures (5 ± 1 °C and 30 ± 1 °C). After applying glyphosate, the samples were incubated for a maximum period of 30 days.

Non-sterile soil experiment was carried out by weighing 65 g (dry weight, d.w.) of nonsterile soil into plastic pots of 280 mL. The soil was slightly compacted using a small manual soil compactor. In order to guarantee as much as possible the same compaction in all samples, the soil compactor was set to fall 10 times by gravity at the pot height. Soils were then adjusted for the desired soil moisture contents by weighing the pots and adding Millipore water as needed, and pre-incubated. After the pre-incubation period, 1 mL of glyphosate solution was added to the soil. Soil moisture content was controlled at this stage and in a daily basis, and Millipore water was added when necessary. The pots were left open during the entire (pre-)incubation period at both temperatures. Control samples (without glyphosate) were used and prepared the same way.

For the sterile soil dissipation experiment, 15 kg of the stored soil were transferred to a heat-resistant plastic bag and sealed. The soil was autoclaved for 1 h at 121 °C and 18 psi, and then left at room temperature for 24 h. This procedure was repeated for 3 consecutive days. Simultaneously, several glass bottles containing Millipore water were sealed with lids equipped with septa, and autoclaved at the same conditions as soil. The sterilized water was used in the sterile soil dissipation experiment to prepare the glyphosate solution and to adjust the soil moisture content of the soil samples.

The sterile soil was then prepared as described for non-sterile soil above. Some additional steps were, however, performed. After weighing the soil, 1 mL of 1 g/kg NaN_3 solution was added to prevent microbial activity during the incubation period due to possible contamination of the soil with microorganisms or to poor sterilization. The soil was then thoroughly mixed to homogenise the NaN_3. After applying the glyphosate and adjust the soil moisture, the pots were sealed with 2 layers of cling film and rubber elastics to prevent soil contamination with microorganisms. Cling film was used to allow the contact of soil with light for those samples under light regimes. Soil moisture for these samples was controlled weekly.

The soils in each plastic pot (nonsterile and sterile) were collected at 0, 1, 2, 3, 5, 14 and 30 days after glyphosate application. The soil in each plastic pot was then thoroughly mixed, transferred to plastic bags and frozen at −18 °C until analysis.

Glyphosate and AMPA analysis

Chemicals

Glyphosate (98 %) and AMPA (98.5 %), were purchased from Sigma-Aldrich Co. (USA). Isotopically-labelled glyphosate (1,2-^{13}C, ^{15}N) and AMPA (^{13}C, ^{15}N) were used as internal standards and purchased from Dr. Ehrenstorfer (Augsburg, Germany). Mixed glyphosate and AMPA isotope-labelled standards (mix IS GLY/AMPA) of 5 µg/mL were prepared in Millipore water. Standard stock solutions of glyphosate and AMPA at concentrations of 2000 µg/mL were prepared in Millipore water. Mixed glyphosate and AMPA (mix GLY/AMPA) standard solutions of 100 µg/mL were prepared from glyphosate and AMPA stock solutions. All stock solutions and dilutions were stored at 4 °C until use.

Soil extraction

All samples were thawed and homogenised manually before extraction. Soil subsamples of 2 g were transferred to 50 mL centrifuge plastic tubes, and extracted with 10 mL of KOH 0.6 M, by shaking

mechanically in an end-over-end shaker for 1 h. The samples were then centrifuged at 3500 rpm for 15 min. Afterwards, 1 mL of the soil extract was transferred to a 10 mL plastic tube, and 80 µL of HCl 6 M were added to adjust the pH to approximately 9 before derivatisation.

Derivatisation

For the derivatisation step, 20 µL of 5 µg/mL mix IS GLY/AMPA were first added to the pH-adjusted soil extract. Then, 0.5 mL of borate buffer 5 % and 0.5 mL of FMOC-Cl 6.5 mM were added. The tubes were shaken for a few seconds in a vortex mixer and incubated for 30 min at room temperature, after which the reaction was stopped by adding 50 µL of formic acid. The tubes were shaken again for a few seconds in a vortex mixer, and 0.5 mL of the mixture were transferred to plastic LC vials integrated with 0.45 µm PTFE filters. Solvent standards were prepared from mix GLY/AMPA standard solutions in Millipore water. For each batch of samples, the solvent standards were derivatised the same way as the samples.

HPLC-MS/MS

Glyphosate and AMPA concentrations were determined by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), using an XBridge™ Shield RP C18 column, 3.5 µm particle size, 100 mm × 2.1 mm i.d. (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrople MS, Waters, the Netherlands).

Validation and quality control

Glyphosate and AMPA responses were normalised to their corresponding isotope-labelled internal standards, thereby correcting for any ion suppression or enhancement effects during the HPLC-MS/MS measurements. Solvent standards were always run at the beginning, middle and end of each batch of samples. Two standard calibration curves (CCs) were determined for each compound, based on the average normalised responses of the solvent standards, for each batch of samples. Linearity was satisfactory achieved for all CCs, with correlation coefficients > 0.99 and residuals within ± 20 %. An initial method validation was performed using two Argentinean loess soils from the pampas of Córdoba province. This involved analysis of duplicate blanks and six replicates of each soil spiked at 0.05, 0.2 and 0.5 mg/kg.
The average recovery and RSD obtained for glyphosate were 87 % and 10 %, respectively. The average recovery and RSD obtained for AMPA were 85 % and 12 %, respectively.
With each batch of samples analysed, spiked samples were included for quality control purposes. For this, besides the Argentinean loess soils, also a Dutch loess soil from the region of Limburg, and a Chinese loess soil from the Loess Plateau were used. Recoveries obtained for the different soils and at the different levels were consistent with those obtained during the initial validation.
The average recovery and RSD obtained for glyphosate for all quality control samples (n = 73) were 86 % and 13 %, respectively. For AMPA, the average recovery was 92 % and the RSD was 16 %.
Limits of detection (LODs) were determined considering a signal in the chromatogram at the lowest analyte concentration assayed that was 3 times the average of the background noise of blank soil samples (S:N = 3). The LODs for glyphosate and AMPA (n = 15) were 0.02 and 0.03 mg/kg, respectively. The limits of quantification (LOQ) were defined as the lowest concentration assayed and validated, which gave a satisfactory average recovery (70 – 120 %) and precision (≤ 20 % RSD). The LOQ was 0.05 mg/kg for both compounds (n = 28).

The analytical results for the soil were determined using the two corresponding CCs of each compound. In case the response of the sample was below the average response of the 0.2 µg/mL solvent standard, the CC with the lower concentration range was used for quantification. If not, the CC with the higher concentration range was used. With each batch of samples, two blank soil samples were fortified at 1 mg/kg and added as quality control samples to the batch. The quantification of the sample batch was considered satisfactory when the QC recoveries were between 70 % and 120 %. The analytes were considered identified when the retention time was the same as the isotopically labelled internal standards and the ion ratio of the 2 transitions measured for each compound deviated less than ± 30 % of the reference ion ratio as determined from the calibration standards.

Data analysis

All glyphosate and AMPA concentrations were converted to percentage of initially applied glyphosate prior to any data analysis, and are presented as such in this study. To determine and select the kinetic models that

best describe glyphosate dissipation and AMPA formation/dissipation for the different treatments applied, the FOCUS work group guidance document on degradation kinetics was followed. The kinetic models parameters, for both glyphosate and AMPA, were estimated using ModelMaker 4 (A.P. Benson). To select the model that best describes glyphosate dissipation and AMPA formation/dissipation, the indicators recommended by FOCUS to assess the goodness of fit and to compare the models were used (visual assessment of curve fits and residual plots, chi-square (χ^2) model errors, t-test).

In a first step, two kinetic models were used to fit glyphosate dissipation kinetics alone: single first-order (SFO) and first-order multicompartment (FOMC). After selecting the model that best describes glyphosate dissipation, AMPA data were added and the SFO model was used to fit AMPA formation/dissipation kinetics. The stepwise approach to fitting was followed, i.e. first glyphosate parameters initially determined were kept fixed and the model parameters for AMPA (C_0, *ffA* and *k*) were optimized and fitted; then, the fitted glyphosate and AMPA parameters were used as initial values, and were optimized and fitted altogether.

To determine the DT_{50} and DT_{90} values and to evaluate the effects of the different treatments on glyphosate dissipation and AMPA formation/dissipation, the statistical software SAS 9.3 was used.

Treatments effects: DT_{50}/DT_{90} values and glyphosate dissipation under biotic conditions

DT_{50} and DT_{90} values for glyphosate and AMPA were calculated using the FOMC equation for glyphosate and SFO for AMPA, FOMC was chosen in these calculations for glyphosate because its dissipation curves for the treatments at 5 °C did not show any differences between SFO and FOMC kinetic models, and the treatments at 30 °C followed FOMC. The formation of AMPA was taken into account in the equation to determine AMPA DT_{50} and DT_{90}. C_0 of glyphosate and AMPA, α and *ffA* parameters were fixed. Comparing the dissipation kinetics of glyphosate for the different treatments under biotic conditions was carried out by comparing the DT_{50} values. An ANOVA (analysis of variance) table was created to evaluate the main effects of factors temperature, light and soil moisture, and their interactions on glyphosate dissipation under biotic conditions, followed by pairwise comparisons using F-tests ($p < 0.05$, corrected by Bonferroni). The sums of squares for the ANOVA table were obtained as differences in residual sums of squares of two nested non-linear models, with and without the factor of interest. Multiple pairwise comparisons to test for differences between all DT_{50} values and between all DT_{90} values of glyphosate and AMPA were also performed using F-tests ($p < 0.05$, corrected by Bonferroni).

Treatments effects: biotic vs. abiotic and AMPA formation/dissipation under biotic conditions.

Linear regression analysis followed by F-tests ($p < 0.05$, corrected by Bonferroni when applicable) was applied to evaluate: 1) the effects of biotic (nonsterile) and abiotic (sterile) conditions, 2) the treatment effects on glyphosate dissipation and AMPA formation/dissipation within abiotic conditions, and 3) the treatment effects on AMPA formation/dissipation under biotic conditions. Because glyphosate dissipation and AMPA formation/dissipation follow nonlinear models, data were log-transformed before applying linear regression analysis. For glyphosate, a linear regression model with treatment dependent intercepts and linear and quadratic effects of time was applied. For AMPA, a linear model with treatment dependent intercepts and linear and quadratic effects of log(time) was applied. The linear and quadratic coefficients were used to evaluate the main effects of temperature, light and soil moisture, and their interactions, showing results in an ANOVA table.

Results

The dissipation behaviour of both compounds differed significantly between abiotic (sterile) and biotic (nonsterile) conditions, independently of the treatment combination. The experimental data show a visible negligible dissipation of glyphosate and formation of AMPA under abiotic conditions (6.9 ± 5.2 % of the initially applied glyphosate dissipated after 30 days; 0.7 ± 0.4 % of AMPA was formed after 30 days). Moreover, no significant effects of soil moisture and light/darkness were observed within abiotic conditions for glyphosate (p = 0.99). Nonetheless, as opposed to glyphosate, the statistical tests revealed that 4 out of 6 treatments under abiotic conditions formed some AMPA through time, although at an extremely slow pace (0.9 ± 0.3 % after 30 days, for the 4 treatments). The 4 treatments comprised the 3 treatments subject to light regimes and 30-SD-60. Under biotic conditions, glyphosate disappeared almost completely at 30 °C since only 3 % of the initially applied glyphosate remained in the soil. Exception was for treatment 30-NS-L-20 where only 77 ± 5 % was dissipated. At 5 °C, the lowest dissipation values were recorded (52 ± 12 %) for glyphosate.

Glyphosate and AMPA dissipation kinetic models

Under abiotic conditions, it was not possible to determine the kinetic parameters and DT_{50}/DT_{90} values for glyphosate and AMPA due to their negligible dissipation and formation. Under biotic conditions, the best fitting model for glyphosate was dependent on temperature. At 5 °C, the SFO model was the best fit. At 30 °C, the FOMC model was the best fit for glyphosate. Although the χ^2 error values were <15 % for the SFO fits, their residual plots indicated systematic deviations for the later sampling dates up to measured DT_{90} (graphs not shown).

For AMPA, the SFO model was used to fit the data since its formation/dissipation was reasonably well described by this model. However, its dissipation rates and DT_{50}/DT_{90} values could not be reliably determined for all treatments, because a plateau and decline phases were not reached during the incubation period.

Table 8.1.1.2-87: Glyphosate and AMPA dissipation kinetics parameters in nonsterile soil for the different treatments applied, by fitting single first-order (SFO) or Gustafson and Holden (FOMC) kinetic models. NS – nonsterile soil, 5 – 5 °C, 30 – 30 °C, Sat – saturated soil, 60 – 60 % WHC, 20 – 20 % WHC, L – presence of light, D – absence of light

Treatments	Glyphosate following SFO or FOMC					AMPA following SFO			
	$C_0 \pm$ SE (% of applied)	$k \pm$ SE (day^{-1}) (SFO)	$\alpha \pm$ SE (FOMC)	$\beta \pm$ SE (FOMC)	χ^2 error (%)	$C_0 \pm$ SE (% of applied)[a]	*ffA* ± SE	$k \pm$ SE (day^{-1})	χ^2 error (%)
5-NS-L-Sat	94.1 ± 1.0	0.03 ± 0.002*	–	–	1.6	5.8 ± 1.3	1.00 ± 0.17*	[b]	–
5-NS-L-60	98.8 ± 1.2	0.03 ± 0.002*	–	–	2.3	5.9 ± 1.4	0.71 ± 0.19*	[b]	–
5-NS-L-20	100 ± 1.1	0.01 ± 0.001*	–	–	2.0	4.9 ± 1.4	0.88 ± 0.42*	[b]	–
5-NS-D-Sat	95.1 ± 1.0	0.03 ± 0.002*	–	–	2.7	5.9 ± 1.2	0.64 ± 0.15*	[b]	–
5-NS-D-60	97.5 ± 1.1	0.03 ± 0.002*	–	–	1.5	6.0 ± 1.3	0.54 ± 0.17*	[b]	–
5-NS-D-20	102 ± 1.1	0.02 ± 0.001*	–	–	2.5	5.3 ± 1.4	0.82 ± 0.30*	[b]	–
30-NS-L-Sat	102 ± 2.7	–	1.9 ± 0.6*	3.3 ± 1.4*	2.8	11.5 ± 2.5	0.37 ± 0.05*	0.02 ± 0.004*	4.7
30-NS-L-60	109 ± 2.5	–	1.1 ± 0.2*	1.5 ± 0.4*	4.7	9.5 ± 2.4	0.43 ± 0.04*	0.03 ± 0.004*	4.3
30-NS-L-20	110 ± 3.4	–	1.4 ± 0.6*	12.8 ± 7.4*	6.0	7.3 ± 3.0	0.43 ± 0.13*	[b]	–
30-NS-D-Sat	101 ± 2.3	–	2.1 ± 0.7*	4.6 ± 1.9*	3.4	10.5 ± 2.1	0.40 ± 0.04*	0.02 ± 0.004*	3.7
30-NS-D-60	102 ± 3.1	–	2.4 ± 0.8*	6.3 ± 2.7*	4.3	10.1 ± 2.3	0.48 ± 0.05*	0.03 ± 0.005*	2.6
30-NS-D-20	110 ± 3.0	–	1.3 ± 0.3*	4.6 ± 1.7*	6.9	8.8 ± 3.0	0.47 ± 0.08*	0.02 ± 0.007*	6.1

[a] AMPA results were converted to equivalent mass of glyphosate, so C_0 for AMPA represents the initial concentration of AMPA in % of applied equivalent glyphosate.
[b] Plateau and decline phases for AMPA were not reached during the experimental period, thus it was not possible to reliably determine AMPA parameters, especially the dissipation rate (k).
* Estimated parameter is significantly different from zero (t-test; $p < 0.05$).

Treatments effects under biotic conditions

Under biotic conditions, glyphosate dissipation and AMPA formation/dissipation were primarily affected by temperature and secondly by soil moisture. Light regimes showed no significant effects on glyphosate dissipation and AMPA formation/dissipation. Exception was for AMPA for treatments 30-NS-L-20 vs. 30-NS-D-20, where AMPA formation/dissipation was significantly faster under dark conditions.

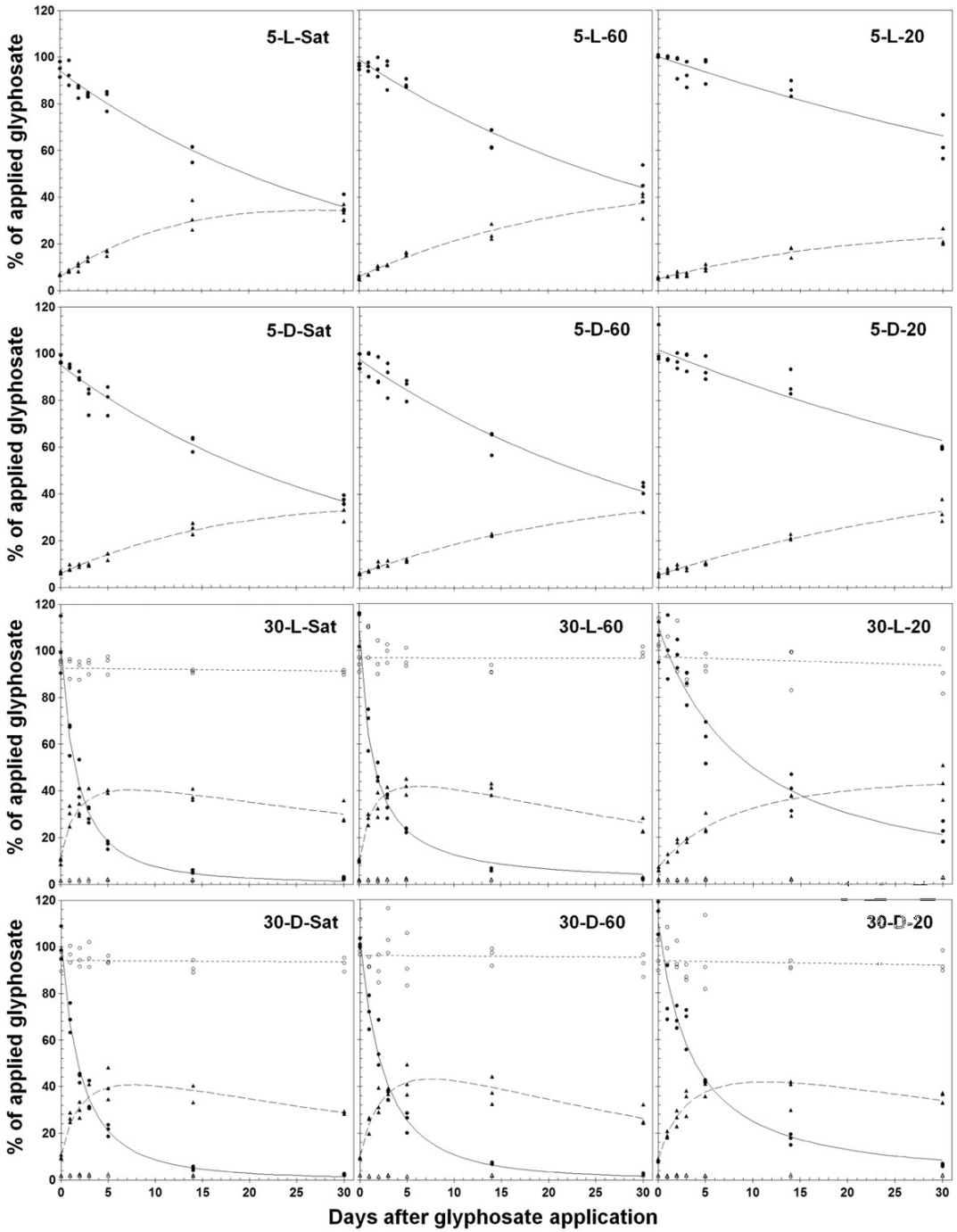


Figure 8.1.1.2-47: Dissipation kinetics of glyphosate (●) and AMPA (▲) in nonsterile (solid symbols) and sterile (open symbols) soil at 5 °C and 30 °C, under different soil moistures (Sat, 60, 20) and with light or in darkness (L, D). Symbols represent the experimental data and lines the theoretical kinetic models

The greater influence of temperature on glyphosate dissipation and AMPA formation/dissipation has been demonstrated by several ways: the much higher F-values for temperature; the significant differences between both temperatures for all treatments; the significant differences between both temperatures for all DT_{50} and DT_{90} values for glyphosate; and the immediate degradation of glyphosate to AMPA (C_0 of AMPA), which increased with increasing temperature. On the other hand, a much higher fraction of glyphosate (*ffA*) was degraded to AMPA at very low temperatures, although with higher variation as well.

The effect of soil moisture was more evident on glyphosate dissipation than on AMPA formation/dissipation. For glyphosate, the results show significant differences between all soil moisture contents tested, for both temperatures. For AMPA, only at 30 °C a significant slower formation and dissipation of AMPA occurred for soils reaching drought stress conditions (20 % WHC). The absence of

significant differences between the DT_{50}/DT_{90} values also demonstrates the lower influence of soil moisture on AMPA formation/dissipation.

DT_{50}/DT_{90} values
The DT_{50} and DT_{90} values of glyphosate under biotic conditions varied widely in this loess soil and they depended on the treatment combination. On average, glyphosate dissipated 8.4 times faster at 30 °C than at 5 °C. The variations in the range of DT_{50} and DT_{90} values of glyphosate for each temperature were mostly related to the influence of soil moisture. The wetter the soil the faster it was for glyphosate to dissipate from soil. Comparing high moisture soils with those reaching drought stress conditions (20 % WHC), on average, glyphosate DT_{50} was 2.9 times and 3.6 times shorter for soils at 60 % WHC and saturated, respectively, than for those at 20 % WHC. According to the results, glyphosate dissipation is, on average, 11 times faster at high temperatures for soils under saturated and optimal (60 % WHC) moisture conditions, but only 7 times faster when soil moisture conditions approach drought stress (≤ 20 % WHC). Based on the combined effect of temperature with soil moisture, glyphosate persists longer in this loess soil in the following order (from low to higher persistence): warm + moist < warm + dry < cold + moist < cold + dry.

DT_{50} and DT_{90} values were always larger for AMPA when compared to those of glyphosate at the same treatment conditions. However, this higher persistence of AMPA decreased with decreasing soil moisture (at 30 °C): AMPA persisted 21 times longer than glyphosate in saturated soils, 15 times longer in soils at 60 % WHC and 11 times longer in soils at 20 % WHC.

Discussion

Glyphosate dissipation
Glyphosate dissipates mostly by microbial activity. Abiotic processes were proven to have a negligible role on glyphosate dissipation. Under biotic conditions, temperature and soil moisture were the factors that mostly affected glyphosate dissipation. DT_{50} and DT_{90} values were much shorter at high temperatures combined with high moisture soils than those at low temperatures combined with soils reaching drought stress conditions. The presence of light, on the other hand, played a minor role. Nevertheless, this minor influence seems to depend on soil moisture. A positive influence (faster glyphosate dissipation) was observed for soils with high moisture contents (saturated and at 60 % WHC), but it was negative (slower glyphosate dissipation) for soils reaching drought stress conditions. Since photodegradation did not play a role on glyphosate dissipation under abiotic conditions, the observed effect suggests that light affected the microbial activity, but this seems unrelated with photodegradation.

The study indicates that glyphosate applied in cold conditions leads to a risk of slow dissipation and, therefore, longer presence in soils than often estimated in models for risk assessment.

Table 8.1.1.2-88: Glyphosate and AMPA DT_{50} and DT_{90} values in nonsterile soil, and incubation time (t_{Amax}) at which AMPA concentration peaks (C_{Amax}), for the different treatments. NS – nonsterile soil, 5 – 5 °C, 30 – 30 °C, Sat – saturated soil, 60 – 60 % WHC, 20 – 20 % WHC, L – presence of light, D – absence of light

Treatment	Glyphosate		AMPA			
	DT50[#] ± SE (days)	DT90[#] ± SE (days)	DT50[$] ± SE (days)	DT90[$] ± SE (days)	t_{Amax} (days)	C_{Amax} (% of applied)[a]
5-NS-L-Sat	17.8 ± 1.4 b	93.2 ± 10.1 b	[b]	[b]	>30	n.a.
5-NS-L-60	24.9 ± 2.1 b	130 ± 7.1 b	[b]	[b]	>30	n.a.
5-NS-L-20	53.5 ± 6.2 a	280 ± 40.4 a	[b]	[b]	>30	n.a.
5-NS-D-Sat	18.5 ± 1.4 b	96.5 ± 10.5 b	[b]	[b]	>30	n.a.
5-NS-D-60	22.2 ± 1.8 b	116 ± 13.1 b	[b]	[b]	>30	n.a.
5-NS-D-20	48.8 ± 5.3 a	255 ± 35.6 a	[b]	[b]	>30	n.a.
30-NS-L-Sat	1.5 ± 0.1 f	8.0 ± 0.7 f	34.6 ± 5.5 a	115 ± 18.1 a	8	40.4
30-NS-L-60	1.8 ± 0.1 e, f	9.4 ± 0.9 e, f	26.4 ± 3.4 a	87.8 ± 11.4 a	6	41.8
30-NS-L-20	10.6 ± 0.8 c	55.2 ± 5.6 c	[b]	[b]	>30	n.a.
30-NS-D-Sat	1.8 ± 0.1 e, f	9.2 ± 0.8 e, f	33.7 ± 5.2 a	112 ± 17.4 a	8	40.7
30-NS-D-60	2.2 ± 0.1 e	11.3 ± 1.0 e	31.6 ± 4.7 a	105 ± 15.5 a	8	43.1
30-NS-D-20	4.1 ± 0.3 d	21.5 ± 2.1 d	44.5 ± 8.1 a	148 ± 26.8 a	11	41.9

n.a. – not available.

Different lowercase letters within the same column mean significant differences between DT50s and between DT90s (F-test; $p < 0.05$ - corrected by Bonferroni).

[#] Glyphosate DT50 and DT90 values were determined using the FOMC model for all treatments, and by fixing C_0 and α, in order to allow for statistical comparisons.

[$] AMPA DT50 and DT90 values were determined by taking into account the FOMC model for glyphosate (for the formation phase of AMPA), and by fixing C_0 of AMPA, α and *ffA*.

[a] AMPA results were converted to equivalent mass of glyphosate, so C_{Amax} represents the peak concentration of AMPA in % of applied equivalent glyphosate.

[b] Plateau and decline phases for AMPA were not reached during the experimental period, thus it was not possible to reliably determine AMPA DT50 and DT90.

Table 8.1.1.2-89: Effect of temperature, light and soil moisture on glyphosate dissipation (DT50) and AMPA formation/dissipation under biotic conditions

A. ANOVA

	A1. Glyphosate (based on DT50)			A2. AMPA (based on linear regression)		
Source	df	Type III SS	F-value	df	Type III SS	F-value
Temperature (T)	1	23,540.60	638.71*	2	2.23	345.02*
Light (L)	1	78.00	2.12	2	0.004	0.64
Soil moist (SM)	2	7708.90	104.58*	4	0.20	15.66*
L × T	1	0.30	0.01	2	0.03	5.31*
SM × T	2	3133.50	42.51*	4	0.24	18.32*
L × SM	2	98.80	1.34	4	0.04	3.07*
L × SM × T	2	40.90	0.55	4	0.09	7.12*
Error	238	8771.90		216	0.70	

B. Pairwise comparisons between treatments with regard to SM × T (averaged over light levels)#

Temperature	B1. Glyphosate (based on DT50 ± SE; days)			B2. AMPA (based on linear regression)		
	Soil moisture			Soil moisture		
	Saturated	60% WHC	20% WHC	Saturated	60% WHC	20% WHC
5 °C	18.2 ± 1.0 a, A	23.6 ± 1.4 b, A	51.2 ± 4.2 c, A	a, A	a, A	a, A
30 °C	1.7 ± 0.1 a, B	2.0 ± 0.1 b, B	7.3 ± 0.4 c, B	a, B	a, B	b, B

Different lowercase letters within the same row mean significant differences between soil moisture levels; different capital letters within the same column mean significant differences between temperature levels (F-test, $p < 0.05$).

Note: Although the ANOVA table for AMPA (A2) shows significant differences with regard to L × T and L × SM × T, only the treatments 30-NS-L-20 vs. 30-NS-D-20 were significantly different from each other.

* Significant differences between treatment levels ($p < 0.05$).

\# p-Value was corrected by Bonferroni for multiple comparisons.

AMPA formation and dissipation
AMPA followed first-order (SFO) dissipation kinetics regardless temperature, soil moisture or light regimes. Similar to glyphosate, AMPA forms/dissipates mostly by microbial activity whereas abiotic processes play a minor role. Under biotic conditions, temperature and soil moisture were also the factors that mostly affected AMPA formation/dissipation, but the presence of light played a negligible role.

AMPA clearly persists longer in soil when temperatures are very low. Besides the inexistence of a decline phase for all treatments at 5 °C, the strong influence of temperature has also been demonstrated by the increasing initial concentration of AMPA (at day 0) with increasing temperature. This, though, also shows that glyphosate immediately degrades to AMPA within the first 2 h after applying glyphosate (average time between sampling and freezing the samples). The percentage of glyphosate being degraded to AMPA also seems to be strongly influenced by temperature. Much more AMPA has been formed at 5 °C than at 30 °C.
This suggests that the direct mineralisation of glyphosate or its degradation to other metabolites was reduced at low temperatures, while AMPA formation was beneficiated. Nevertheless, these *ffA* results at 5 °C need to be interpreted with care, since the plateau and decline phases were not reached at this temperature, which can result in an erroneous estimation and consequent misinterpretation of this parameter.

Even though no identification of soil microorganisms has been performed in the study, the *ffA* results suggest that the soil microorganisms degrading glyphosate to AMPA in the silty loam loess soil were more resistant and active at very low temperatures than those promoting the formation of other metabolites or its direct mineralisation.

Although soil moisture has been proven in the present study to influence AMPA formation/dissipation, its effect is less evident than for glyphosate. The stronger effect of soil moisture on glyphosate dissipation, when compared to AMPA, suggests that other dissipation processes of glyphosate, such as its degradation to other metabolites (e.g. sarcosine) or its direct mineralisation, are being more strongly affected by soil moisture than its formation to AMPA. The results also suggest that a lagphase might be occurring for AMPA during the first days of its formation or at least its dissipation occurs very slowly. This is based on the fact that the average *ffA* and C_{Amax} are very similar at 30 °C.

The results on DT_{50} and DT_{90} values at 30 °C also confirm the higher persistence of AMPA in this loess soil, when compared to those of glyphosate.

Conclusions

From the combined effect of temperature with soil moisture it has been concluded that glyphosate dissipates fast under warm and moist conditions, but it persists 30 times longer under cold and dry conditions. AMPA persists longer in the soil than glyphosate, even under warm and moist conditions. The type of dissipation kinetic model followed by glyphosate in loess soil depends on temperature, thus its DT_{90} values should be estimated using the appropriate kinetic model to avoid its underestimation. From a practical point of view, repeated glyphosate applications in fallows or winter crops in countries where cold and dry winters normally occur might increase the risk of on-site soil pollution due to accumulation of these chemicals.

Assessment and conclusion by applicant:

Supplementary information on the rate of degradation of glyphosate and rate of formation/dissipation of AMPA in loess soil as a function of temperature, soil moisture and light/darkness.

Assessment and conclusion by RMS:

This experimental study seems to be well performed. However the available information does not allow to check the validity against current guidelines (*e.g.* raw residues, mass balances, specificities of the compound used). Additionally, the organic content of the soil is very high and does not represent common EU agricultural soils. The conditions of the tests were all specific (20 or 60% WHC, 5 or 30°C).

The article provides supportive information on the degradation of glyphosate and AMPA in soil, but no reliable endpoints can be derived for use in risk assessment.

B.8.1.1.2.2. Rate of degradation in soil – Anaerobic conditions

The fate of glyphosate under laboratory anaerobic conditions was investigated in one soil in the course of one study which is considered valid to address the data point ([redacted], 2003, CA 7.1.1.2/003). The results of this study were evaluated according to the current FOCUS kinetic guidance ([redacted], 2020, CA 7.1.2.1.3/001).

Table 8.1.1.2-90: List of studies on anaerobic soil degradation with glyphosate (rate)

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)	Remark
CA 7.1.2.1.3/001	[redacted], 2020	-	Acceptable	Updated kinetic evaluation of [redacted], 2003, CA 7.1.1.2/003

Within the search for peer reviewed scientific literature (2010-2020), no article was identified that would provide information relevant to this data point.

[redacted], 2020

Data point:	CA 7.1.2.1.3/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from an anaerobic laboratory soil degradation study
Report No	112148-004
Guidelines followed in study	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
GLP/Officially recognised testing facilities	No, not applicable for this study type
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

The purpose of this assessment was to conduct a kinetic modelling evaluation for glyphosate and its major soil metabolite AMPA using results from an anaerobic laboratory soil degradation study ([redacted], 2003, CA 7.1.1.2/003). The aim of the evaluation was to derive trigger endpoints for glyphosate and AMPA.

Kinetic evaluation was performed with the data from the anaerobic phase, only.

1. Data pre-processing

The standard procedures recommended by FOCUS (2006, 2014) were followed for all residues to adjust the experimental data for kinetic modelling where necessary.

The residues used for the kinetic evaluation were based on the sum of results in the overlying water and soil extracts. Prior to day 28, glyphosate and AMPA were not quantified in the overlying water since there was overall less than 5 % of the total applied radioactivity found in the water samples. Only glyphosate was present in all subsequent water samples (with the exception of day 84 where AMPA residues of <1 % AR were found). Therefore, for the kinetic evaluation, it was assumed that the radioactivity in the water samples collected prior to day 28 only could be attributed to glyphosate, and the radioactivity in the water samples prior to day 28 was added to the amount of glyphosate in the respective soil extracts.

The initial amounts of glyphosate and AMPA were left at their originally measured values at day 0 since glyphosate was already applied in the aerobic phase.

Processed residue data for kinetic evaluation are presented with the kinetic assessment below.

2. Kinetic models and analysis

Kinetic models

Three kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model) and the double-first-order-in-parallel (DFOP) (FOCUS; 2006, 2014).

For the parent compound, the best-fit model was accepted for deriving trigger endpoints.

For the metabolite, a pathway fit was conducted using the appropriate kinetic model for trigger endpoints for the parent determination and SFO for the metabolite.

The kinetic endpoints for parent and metabolite are normally derived from the pathway fit but since no reliable endpoints could be derived from the pathway fit, trigger endpoints for the parent were derived from the parent-only fit.

Optimisation

The kinetic analysis was conducted using the software CAKE v3.3.

The data were fitted with the complete dataset and unconstrained initial concentration (M_0) for glyphosate and AMPA. Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M_0), degradation model parameters k, α, β or g, depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line

- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered

A statistical measure of the quality of a fit is given by the χ^2-test. The χ^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, for parent compounds, it is recommended that if the χ^2 error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. The guidance is less clear for metabolites due to the complexity of the curve fitting for multiple components, and so this criterion is a little more relaxed.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised parameters were significantly different from zero at a chosen significance level of 5 %. In case of metabolite data, a significance level of 10 % or higher may still be acceptable due to the inherent variability that often occurs in these types of data. This is particularly relevant for the degradation rate constants (k) of the SFO and DFOP kinetic models. For the FOMC kinetic model, only the significance of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a confidence interval on the optimised parameter estimates. The confidence interval should be relatively tight and not contain 0 to be considered statistically robust.

II. RESULTS AND DISCUSSION

The results of the kinetic evaluation of glyphosate and AMPA in anaerobic soil are presented in tables below.

Table 8.1.1.2-91: Processed residue data (% AR) of glyphosate and its metabolite AMPA in [redacted] (2003)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	57.99[1,2]	19.46[1]
0	57.48[1,2]	21.19[1]
3	54.55[2]	19.41
3	56.32[2]	19.76
7	54.05[2]	18.28
7	53.01[2]	18.62
14	48.20[2]	20.92
14	48.50[2]	20.49
28	44.96[2]	26.19
28	46.73[2]	25.24
56	37.00	30.56
56	46.00	22.54
84	38.31	29.60
84	37.21	30.89
120	37.93	29.93
120	40.18	26.96

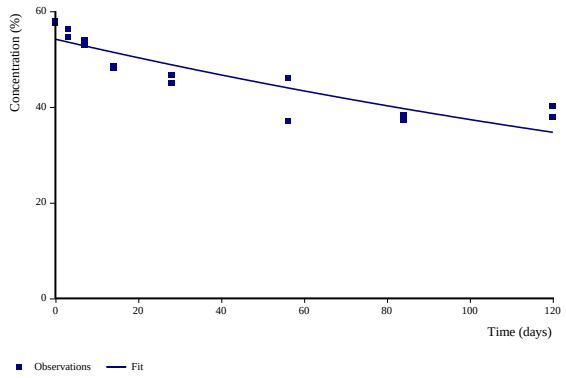
[1] Since the test item was applied in the aerobic phase, the measured values were used for M_0 and no corrections were made
[2] Total radioactivity in the overlying water for these samples accounted for <5 % applied activity. For the evaluation, it was assumed to be glyphosate and was included in total.

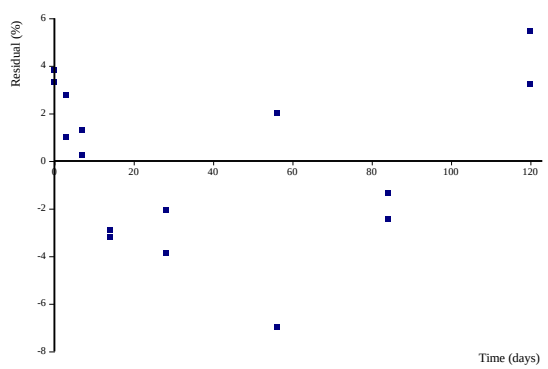
Table 8.1.1.2-92: Kinetic models and goodness-of-fit statistics of parent-only fits

Kinetic model	Visual assessment	M_0[1]	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	54.2	k: 0.0037	4.8	k: <0.001	k: 0.0026	k: 0.005	187	621
FOMC	Good	58.2	α: 0.1536 β: 7.248	1.8	-[2]	β: -2.956	β: 17.45	654	>1000
DFOP	Good	57.5	k_1: 0.0373 k_2: 7.98×10^{-10} g: 0.3344	1.6	k_1: 0.059 k_2: 0.5	k_1: -0.0109 k_2: -0.0043	k_1: 0.085 k_2: 0.004	>1000	>1000

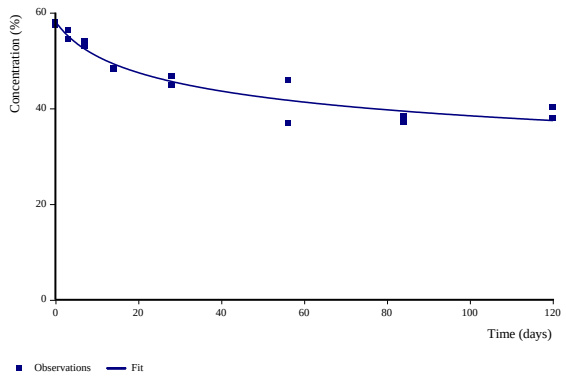
The anaerobic degradation of glyphosate in soil is best described by bi-phasic models.
Both bi-phasic models provide visually good fits. The estimate provided by the DFOP model for the slow phase degradation parameter (k_2) indicates that all visible degradation takes place during the fast phase. Hence k_2 is not significantly different from zero. Nonetheless, the DFOP model provides a slightly better statistical assessment than the FOMC model.
Conclusion: DFOP to be used in pathway fit for trigger endpoints

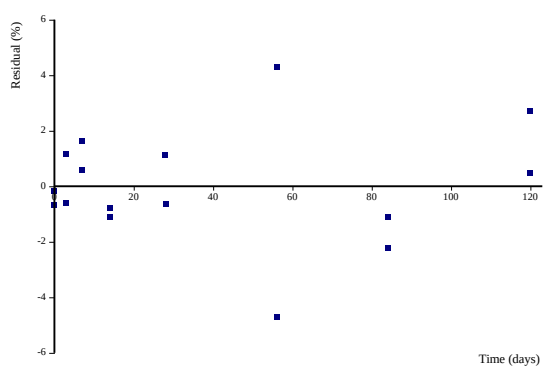
SFO



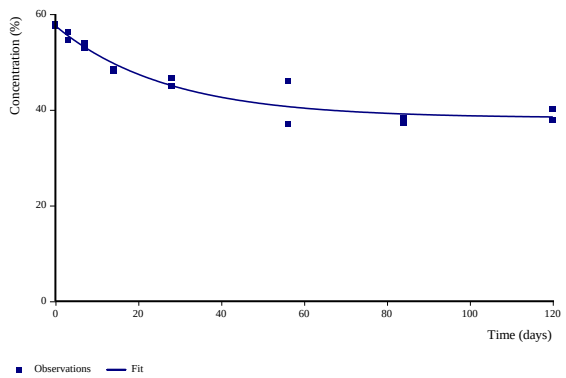


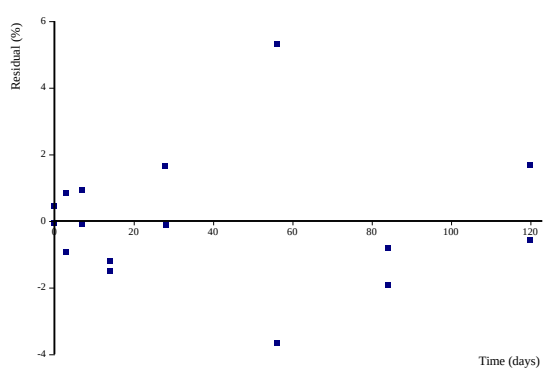
FOMC





DFOP





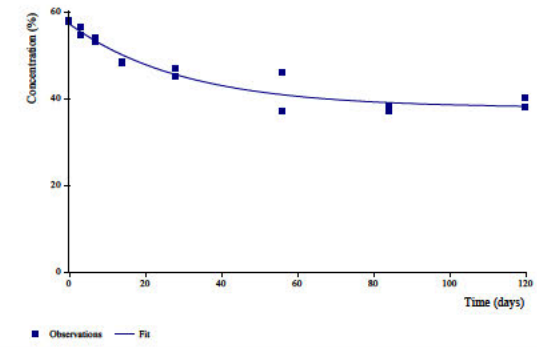
[1] Since glyphosate was applied in the aerobic phase, M_0 at day 0 were left at the measured values
[2] t-test not relevant for kinetic parameter β

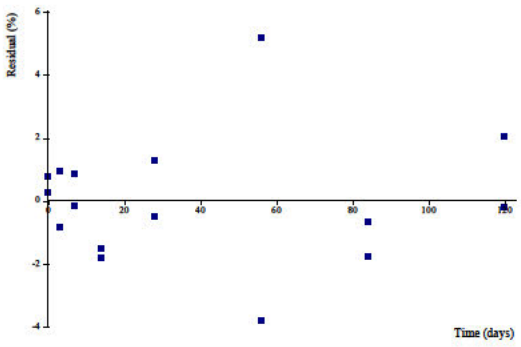
Table 8.1.1.2-93: Kinetic models and goodness-of-fit statistics of pathway fit

Kinetic model	Visual assess-ment	M_0[1]	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	57.2	k_1: 0.0331 k_2: 2.79x10^{-19} g: 0.3396	1.7	k_1: 0.0554 k_2: 0.5	k_1:-0.0081 k_2:-0.0044	k_1: 0.074 k_2: 0.004	>10000	>10000	-
AMPA: SFO	Accep-table	18.3	k: 1.31x10^{-16}	5.1	k: 0.5	k: -0.0029	k: 0.003	>10000	>10000	0.559 (±0.216)

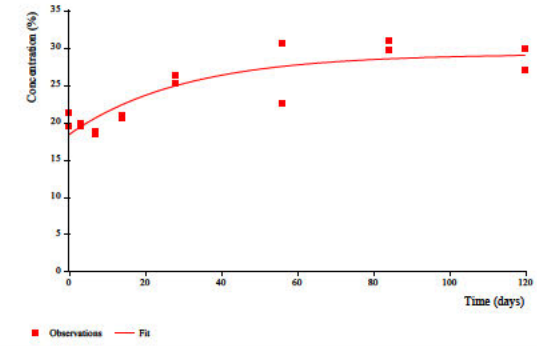
The measured data of glyphosate and AMPA are well described by the pathway fit. However, the degradation rate of AMPA is not significantly different from zero as no decline is observed. No reliable endpoints can thus be derived.
Conclusion: For glyphosate, estimated DT_{50} and DT_{90} are >1000 d each
For AMPA, no reliable endpoints could be derived

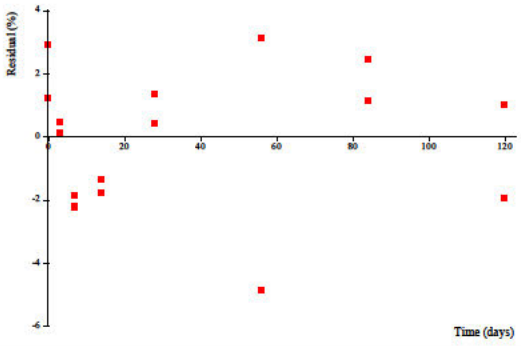
Glyphosate: DFOP





AMPA: SFO





[1] Since glyphosate was applied in the aerobic phase, M_0 at day 0 were left at the measured values

For glyphosate, estimated DT_{50} and DT_{90} are >1000 days (DFOP model). For AMPA no reliable endpoints could be derived.

Assessment and conclusion by applicant:

The kinetic evaluation was conducted according to current guidance and was therefore considered to be valid.

Assessment and conclusion by RMS:

RMS agrees with the assessment provided by the applicant. Glyphosate is not significantly degraded under anaerobic conditions.

The study is considered acceptable.

In the scientific literature review for glyphosate (2010-2019), one article was identified to provide further information relevant to the data point. This article was already summarized within the literature search for aerobic degradation.

Table 8.1.1.2-94: Anaerobic rate of degradation - relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status
CA 7.1.2.1.3/002	Kanissery *et al.*, 2015	Soil anaerobic degradation rate	Glyphosate	Reliable with restrictions

B.8.1.1.2.3. Rate of degradation in soil – photodegradation studies

Reliable data on photodegradation in soil of glyphosate were obtained in [redacted] (1993, CA 7.1.1.3/003). The results of this study were evaluated according to the current FOCUS kinetic guidance ([redacted], 2020, CA 7.1.1.3/001).

Table 8.1.1.2-95: kinetic degradation of glyphosate – photolysis in soil

Annex point	Study	Study type	Substance(s)	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021
CA 7.1.1.3/001	[redacted], 2020	Kinetic evaluation	Glyphosate	New study	Acceptable

Within the search for peer reviewed scientific literature (2010-2020), no article was identified that would provide information relevant to this data point.

[redacted], 2020

Data point:	CA 7.1.1.3/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from a soil photolysis study
Report No	112148-007
Guidelines followed in study	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: None
GLP/Officially recognised testing facilities	No, not applicable for this study type
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A kinetic evaluation of a soil photolysis laboratory study by [redacted] (1993, CA 7.1.1.3/003) was performed in order to derive the trigger (persistence) endpoints for glyphosate and its major soil metabolite AMPA. The evaluation was conducted according to FOCUS kinetics guidance (2006, 2014) using the fitting software CAKE.

1. Data pre-processing

In this assessment, the irradiated experiment was evaluated. The metabolite AMPA was included in the evaluation.

The standard procedures recommended by FOCUS (2006, 2014) were followed for all residues to adjust the experimental data for the kinetic modelling.

The initial amounts of glyphosate were set to the value of the material balance at day 0, thus assigning all radioactivity observed at day 0 to the parent compound and assuming that no degradation processes have yet taken place. Accordingly, the initial amounts of the metabolites were set to 0 in the pathway fits.

Processed residue data for kinetic evaluation are presented in the following table.

Table 8.1.1.2-96: Processed residue data of glyphosate and its metabolite AMPA

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	102.4[1]	0[2]
3	75.7	7.4
7	65.3	8.2
14	64.8	5.2
21	60.3	7.4
30	60.5	6.5

[1] Set to material balance
[2] Amounts of metabolite set to 0 at day 0

2. Kinetic models and analysis

Kinetic models

Four kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model), double-first-order-in-parallel (DFOP) and Hockey-stick (HS) (FOCUS; 2006, 2014).

Optimisation

The kinetic analysis was conducted using the software CAKE v3.3 (CAKE, 2016).

The data were directly fitted with the complete dataset and unconstrained initial concentration (M_0) for the substance. Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in Cake. Optimisations were carried out for the initial soil residue (M_0), degradation model parameters k, α, β, g or t_b, depending on the respective kinetic model selected.

The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. By default, the initial amount of metabolite was fixed to 0. The parameters were optimised by minimising the sum of squared differences between measured and calculated data.

The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly

scattered around the zero line

- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered

- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered

A statistical measure of the quality of a fit is given by the χ^2-test which considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, it is recommended that if the χ^2 error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. Depending on the complexity of the curve fitting for multiple components and the scattering of the experimental data, also fits with higher χ^2 error values may be acceptable if overall the measured data are well described by the fitted curve.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised parameters were significantly different from zero at a chosen significance level of 5 %. In case of metabolite data, a significance level of 10 % or higher may still be acceptable due to the inherent variability that often occurs in these types of data. This is particularly relevant for the degradation rate constants (k) of the SFO, DFOP and HS kinetic models. For the FOMC kinetic model, only the significance of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a confidence interval on the optimised parameter estimates. The confidence interval should be relatively tight and not contain 0 to be considered statistically robust.

II. RESULTS AND DISCUSSION

Table 8.1.1.2-97: Kinetic models and goodness-of-fit statistics of parent-only fits

Kinetic model	Visual assess-ment	M_0	Kinetic parameter	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	86.8	k: 0.0168	10.3	k: 0.035	k: -0.002	k: 0.036	41.4	137
FOMC	Good	102.4	α: 0.0991 β: 0.1185	2.0	-[1]	β: -0.240	β: 0.477	129	>1000
DFOP	Good	102.4	k_1: 0.4257 k_2: 0.0030 g: 0.3585	1.4	k_1: 0.021 k_2: 0.149	k_1: 0.033 k_2: -0.006	k_1: 0.818 k_2: 0.012	83.5	623
HS	Good	102.4	k_1: 0.1007 k_2: 0.0039 t_b: 4.342	1.2	k_1: 0.003 k_2: 0.055	k_1: 0.065 k_2: -0.002	k_1: 0.136 k_2: 0.010	69.8	482

Applicant's conclusion

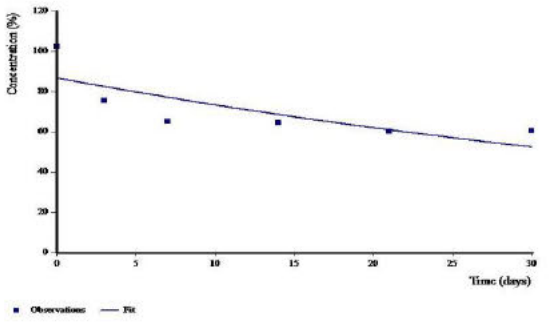
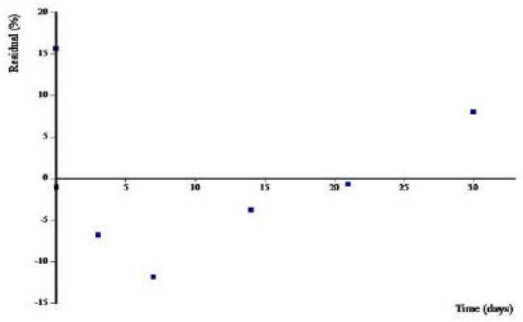
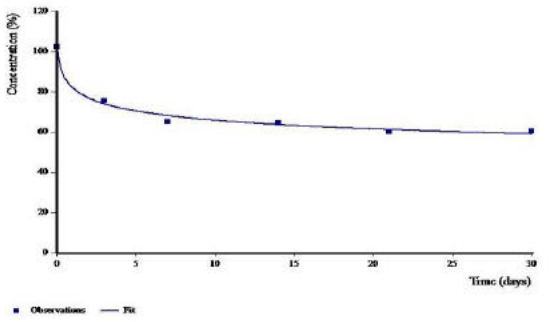
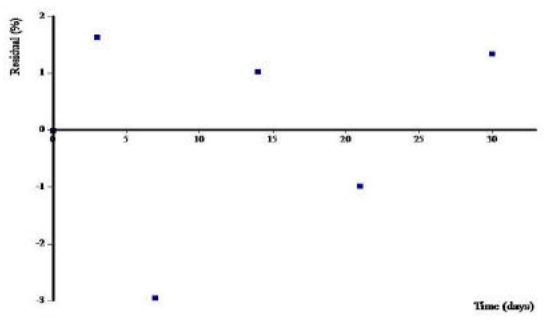
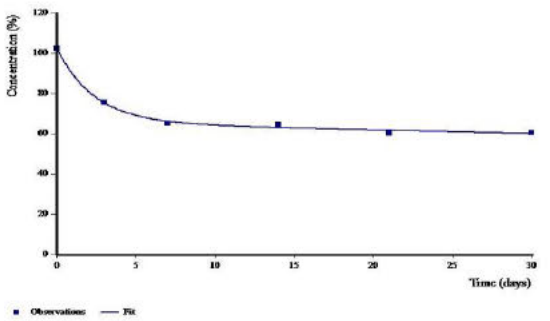
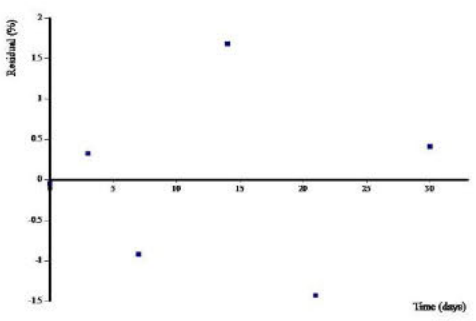
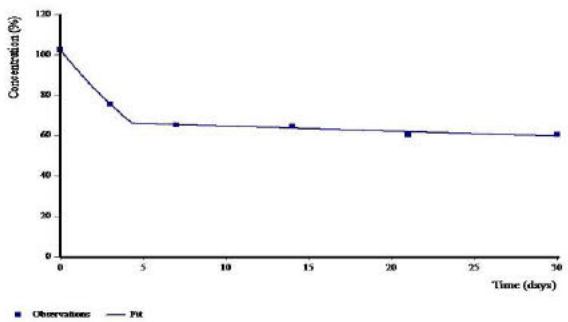
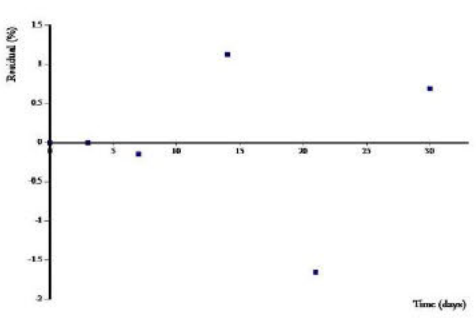
For the derivation of trigger endpoints, the kinetic evaluation was started by comparing SFO and biphasic models for the parent substance glyphosate. The SFO model showed visually poor results with systematic deviations and comparatively high scatter. As 10 % of the initially measured concentration was not reached within the study period and the DT_{90} was higher than the experimental period, FOMC is not a preferred fit for endpoint derivation. The DFOP and HS model show visually good fits with lowest χ^2 errors, more favourable for the HS fit, which reveals also the most significant t-test of the biphasic models.

Conclusion: HS to be used in pathway fit for trigger endpoints

RMS conclusion

The use of biphasic kinetic significantly improve the description of glyphosate degradation. All biphasic models provide good and similar visual fits. Confidence interval for β contains 0. T-test for k2 DFOP fails. T-test for

Table 8.1.1.2-97: Kinetic models and goodness-of-fit statistics of parent-only fits

k2 HS also fails but is slightly above the 5% significance level and is acceptable at 10% significance level. Considering that photodegradation rate is only derived for indicative purpose (not used in risk assessment), RMS agrees with the applicant that HS can be selected to be used in pathway fit for trigger endpoints.
SFO  
FOMC  
DFOP  
HS  

[1] t-test not relevant for kinetic parameter β

Table 8.1.1.2-98: Kinetic models and goodness-of-fit statistics of pathway fit

Kinetic model	Visual assess-ment	M_0	Kinetic parameter	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate HS	Good	102.4	k_1: 0.1017 k_2: 0.0040 tb: 4.282	1.2	k_1:<0.001 k_2: 0.012	k_1: 0.0828 k_2:-0.0008	k_1: 0.12 k_2:0.007	68.3	468	-
AMPA: SFO	Good	0.0	k: 0.0221	13.1	k: 0.0818	k: -0.0127	k: 0.057	31.3	104	0.244 (±0.046)

Applicant's conclusion

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. However, the degradation rate of AMPA is not significantly different from zero; therefore, no reliable endpoints can be derived.

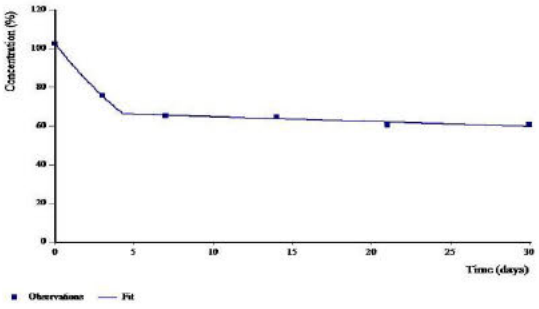
Conclusion: Parent-only HS fit to be used for deriving trigger endpoints for glyphosate

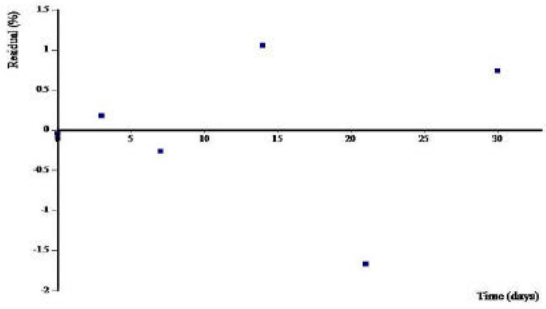
No trigger endpoints can be derived for AMPA

RMS conclusion

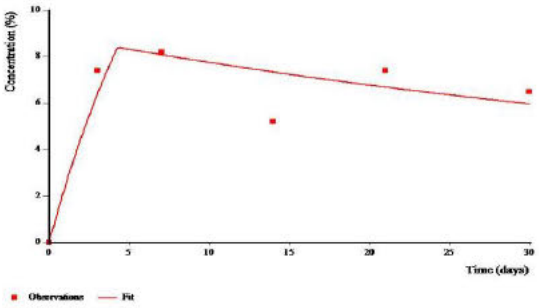
Agrees with the applicant. In addition the data are a bit scattered.

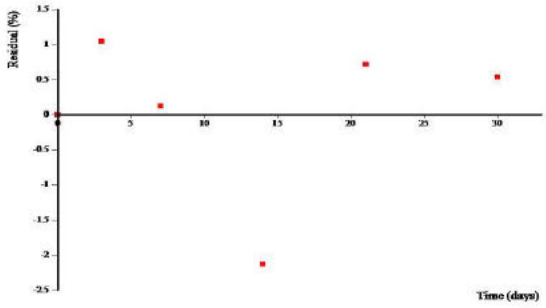
Glyphosate: HS





AMPA: SFO





Summary of trigger endpoints

For glyphosate, estimated trigger DT_{50} and DT_{90} are 69.8 and 482 days (HS model), respectively. For AMPA, no reliable endpoints could be derived.

Assessment and conclusion by applicant:

The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the study and the endpoints provided are considered valid.

Assessment and conclusion by RMS:

The kinetic analysis was performed according to FOCUS kinetic guidance and is considered acceptable. It is noted that initial concentration was not corrected for radiochemical purity, however no impact is expected considering the high radiochemical purity of >99.3%.

Updated kinetics should have also been provided for the control under dark conditions as some degradation was observed. This would enable to estimate the net soil photolysis rate.

However RMS reminds that it is commonly agreed that soil photolysis should be considered as a route of degradation study and not a rate of degradation study. Therefore the calculated DT_{50} values have no meaning for the risk assessment methodology. In addition, determining soil photolysis degradation rates is not an EU data requirement according to Regulation 283/2013. As such, no further information is deemed necessary.

B.8.1.1.3. Rate of degradation in soil – field studies

B.8.1.1.3.1. Soil dissipation studies

Field degradation of glyphosate was investigated in 19 existing field terrestrial dissipation studies, conducted in Europe, US and Canada. No new study was provided in this renewal dossier.

For field dissipation studies conducted in the US and Canada, an Ecoregion Crosswalk exercise was performed to evaluate their representativeness for European conditions ([redacted], 2020). When considered as non-relevant for European conditions, data from these field sites are not further considered for the assessment.

Table 8.1.1.3-1: List of existing soil dissipation studies - Glyphosate

Annex point	Study	Study type	Field location	Max. occurrence of AMPA (% applied glyphosate)**	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.1.2.2.1/002	[redacted], 2020	Ecoregion Crosswalk	-		-	Acceptable
CA 7.1.2.2.1/005	[redacted], 1993	Terrestrial field dissipation	Canada	28.64	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Acceptable for Ontario
CA 7.1.2.2.1/006	[redacted], 1993a	Terrestrial field dissipation	USA	32.42 30.02 37.30	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Acceptable for New York, Ohio and California
CA 7.1.2.2.1/009	[redacted], 1992b	Terrestrial field dissipation	Switzerland	35.17	Accepted	Acceptable
CA 7.1.2.2.1/010	[redacted], 1992c	Terrestrial field dissipation	Germany	23.31	Accepted	Acceptable
CA 7.1.2.2.1/011	[redacted], 1992d	Terrestrial field dissipation	Germany	46.89	Accepted	Acceptable
CA 7.1.2.2.1/004	[redacted], 1994	Terrestrial field dissipation	USA	-	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Not acceptable

Annex point	Study	Study type	Field location	Max. occurrence of AMPA (% applied glyphosate)**	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.1.2.2.1/008	[redacted], 1992a	Terrestrial field dissipation	Switzerland	-	Accepted	Not acceptable
CA 7.1.2.2.1/013	[redacted], 1992	Terrestrial field dissipation	Germany	-	Accepted	Not acceptable
CA 7.1.2.2.1/014	[redacted], 1992	Terrestrial field dissipation	Canada	-	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Not acceptable
CA 7.1.2.2.1/015	[redacted], 1990	Terrestrial field dissipation	USA	-	Not mentioned in RAR (2015), not accepted in DAR (2001)	Not acceptable
CA 7.1.2.2.1/016	[redacted], 1989a	Terrestrial field dissipation	USA	-	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Not acceptable
CA 7.1.2.2.1/017	[redacted], 1989b	Terrestrial field dissipation	USA	-	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)	Not acceptable
CA 7.1.2.2.1/018	[redacted], 1989c	Terrestrial field dissipation	USA	-	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Not acceptable
CA 7.1.2.2.1/020	[redacted], 1984	Terrestrial field dissipation	USA	-	Not mentioned in RAR (2015) nor in DAR (2001)	Not acceptable
CA 7.1.2.2.1/021 and CA 7.1.2.2.1/022 (Addendum)	[redacted], 1983 [redacted], 1988	Terrestrial field dissipation	USA	-	Not accepted in RAR (2015)	Not acceptable
CA 7.1.2.2.1/023	[redacted], 1983	Terrestrial field dissipation	Canada	-	Not mentioned in RAR (2015), reported as not required in DAR (2001)	Not acceptable
CA 7.1.2.2.1/024	[redacted], 1982	Terrestrial field dissipation	USA	-	Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)*	Not acceptable
CA 7.1.2.2.1/025	[redacted], 1979	Terrestrial field dissipation	Sweden, France	-	Not mentioned in RAR (2015) nor in DAR (2001)	Not acceptable

* These studies were not considered to derive endpoints in RAR (2015) since they were conducted outside EU and no information on the comparability of the weather conditions was available.
** Value reported only for sites considered acceptable to describe field dissipation of glyphosate in RAR 2021

[redacted], *2020*

Data point:	CA 7.1.2.2.1/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate: Ecoregion Crosswalk for Nineteen Terrestrial Field Dissipation Study Locations in North America
Report No	112148-005
Guidelines followed in study	No guideline followed
Deviations from current test guideline	Not applicable; evaluation was performed with OECD ENASGIPS tool recommended in OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016.
GLP/Officially recognised testing facilities	Not applicable
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes, pending data gap is addressed (see RMS comments)

I. METHODS

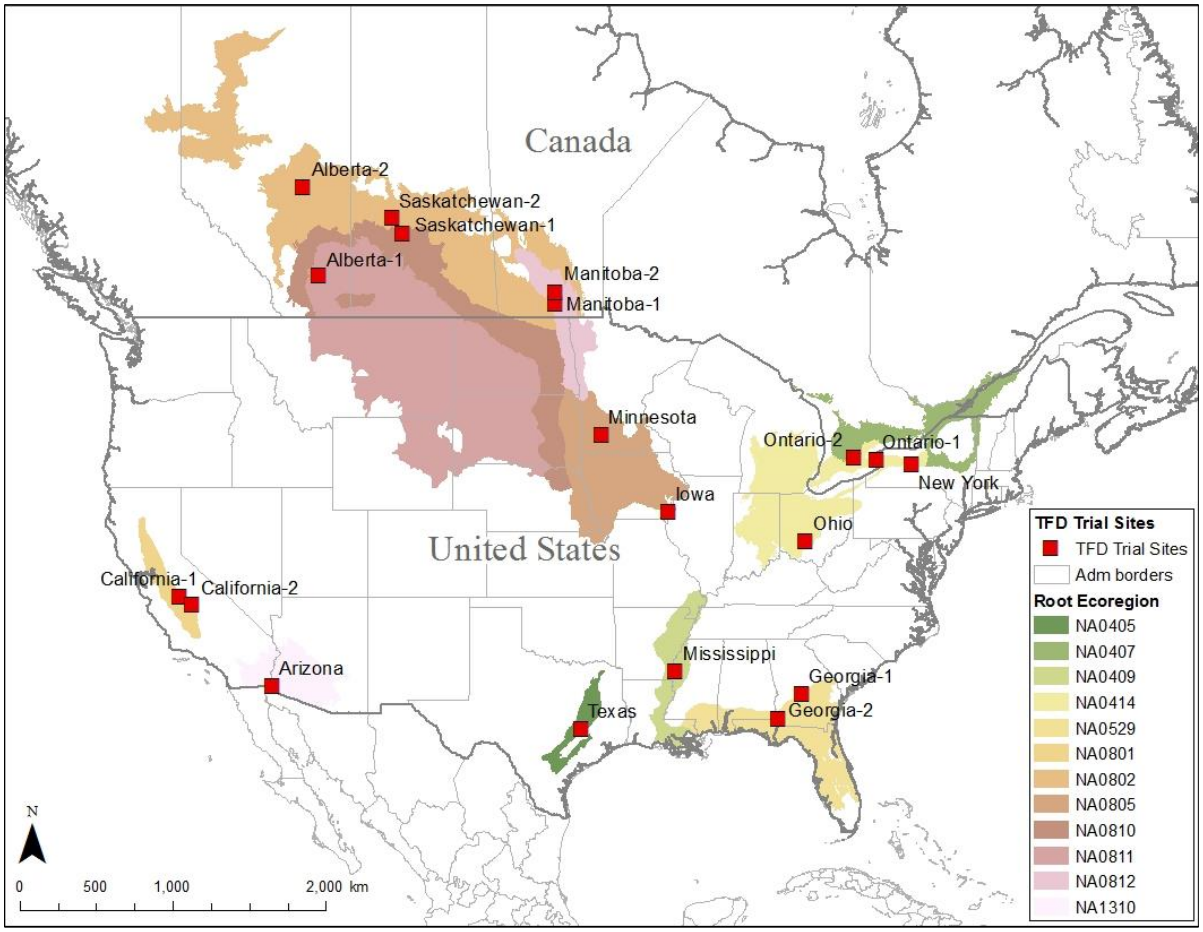
A. TRIAL SITE LOCATIONS

The similarity of 19 North American TFD trial sites was assessed. Eight of the assessed sites are located in Canada and eleven in the United States as presented in the table below. According to the OECD ENASGIPS tool (PMRA, 2015), the 19 TFD trial sites are assigned to twelve root ecoregions as presented in the figure below. These ecoregions reflect distinct combinations of regional environmental conditions and ecology, *e.g.* soil and climate characteristics.

Table 8.1.1.3-2: TFD trial sites in North America

Study	TFD Trial Site
[redacted], 1993	Ontario-2, CAN
	Alberta-2, CAN
	Manitoba-2, CAN
[redacted], 1993a	Arizona, USA
	California-1, USA
	Iowa, USA
	Georgia-1, USA
	Minnesota, USA
	Ohio, USA
	New York, USA
	Texas, USA
[redacted], 1992	Ontario-1, CAN
	Saskatchewan-2, CAN
	Saskatchewan-1, CAN
	Alberta-1, CAN
	Manitoba-1, CAN
[redacted], 1989a	California-2, USA
[redacted], 1989b	Mississippi, USA
[redacted], 1989c	Georgia-2, USA

Figure 8.1.1.3-1: Location of assessed glyphosate TFD trial sites in North America with associated root ecoregions (PMRA, 2015)



B. ECOREGION CROSSWALK

The ecoregion crosswalk was conducted using the ENASGIPS v3.0 (Europe-North America Soil Geographic Information for Pesticide Studies) application (PMRA, 2015). The model is recommended for conducting ecoregion crosswalks by the OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies published in 2016 (OECD, 2016).

For each Canadian and American TFD trial site, the respective root ecoregion was assigned based on the geographical coordinates using the ENASGIPS tool. The 'Holistic Ecoregions Similarity' tool implemented in ENASGIPS allows the user to identify similar ecoregions in Canada, North America, and Europe. A similarity score is calculated between each North American and all European ecoregions based on soil and climate parameters such as mean annual temperature, mean annual precipitation, soil pH, soil organic carbon, and soil texture. Similarity of each of the five parameters is scored separately, and then the five scores are combined with equal weighting into an overall Similarity Score. For the present assessment, the default similarity threshold value of 80 % was used.

The Holistic Ecoregion Similarity weights all five parameters equally, i.e. low to very low scores of an individual parameter might be compensated by the high to very high score of other individual parameters. Transferring trial site conditions to the opposite continent might therefore be questionable and require a closer look to the similarity score of individual parameters.

In addition to the holistic similarity approach, individual scores of soil and climate were evaluated in a refined assessment as applying the holistic similarity approach does not account for the high impact of temperature on degradation. Thus, holistic similarity results may also include ecoregions in Europe with very low and low temperature similarity. As a consequence, holistic matches were excluded from the final similarity results if temperature of the root ecoregion was not well represented by the comparison ecoregions in Europe.

II. RESULTS AND DISCUSSION

The 19 TFD trial sites are represented by twelve root ecoregions that cover large parts of central North America as well as parts at the south-eastern and south-western boundary of the United States. For three out of the eight root ecoregions, the area of the matching ecoregions covers >15 % of the total area of European ecoregions, for one root ecoregion the percentage area is about 4 %, and for four root ecoregions it is <2 % as presented in the table below.

Table 8.1.1.3-3: Root Ecoregions of 19 North American TFD trial sites and area covered by similar ecoregions in Europe (based on holistic approach, 80 % similarity)

Root Ecoregion	TFD Trial Site	Similar ecoregions Europe	
		Area[1] (km²)	Share[2] (%)
NA0414 - Southern Great Lakes forests (CA,USA)	New York Ohio Ontario-1	937,136	22.1
NA0407 - Eastern Great Lakes lowland forests (CA,USA)	Ontario-2	699,833	16.5
NA0801 - California Central Valley grasslands (USA)	California-1 California-2	647,759	15.2
NA0805 - Central tall grasslands (USA)	Iowa Minnesota	163,001	3.8
NA0811 - Northern short grasslands (CA,USA)	Alberta-1	42,624	1.0
NA0802 - Canadian Aspen forests and parklands (CA,USA)	Alberta-2 Saskatchewan-2	23,741	0.6
NA0810 - Northern mixed grasslands (CA,USA)	Saskatchewan-1	20,107	0.5
NA1310 - Sonoran desert (USA)	Arizona	2,720	0.1
NA0529 - Southeastern conifer forests (USA)	Georgia-1 Georgia-2	no similarity	-
NA0812 - Northern tall grasslands (CA,USA)	Manitoba-1 Manitoba-2	no similarity	-
NA0409 - Mississippi lowland forests (USA)	Mississippi	no similarity	-
NA0405 - East Central Texas forests (USA)	Texas	no similarity	-

[1] Area quantified with Lambert azimuthal equal-area (LAEA) coordinate map projection in ArcGIS v10.2.
[2] Share relative to area of the European Union

With the holistic approach, matching ecoregions (80 % similarity) were identified for eight of a total of twelve root ecoregions.

Individual scores of soil and climatic parameters were also assessed in their weight against each other. While soil conditions (pH, OC content and texture) reached high scores for the remaining eight ecoregions, individually and overall, temperature as the main driving parameter for the degradation of pesticides among the climatic parameters (temperature and precipitation) reached very low to low individual scores in some ecoregions. For four (NA0407, NA0414, NA0801, and NA0805) of the eight root ecoregions, individual matches of temperature reach 100 % for one or more European ecoregions. Thus, temperature characteristics of these root ecoregions are well represented by ecoregions in Europe.

NA0407-Eastern Great Lakes lowland forests

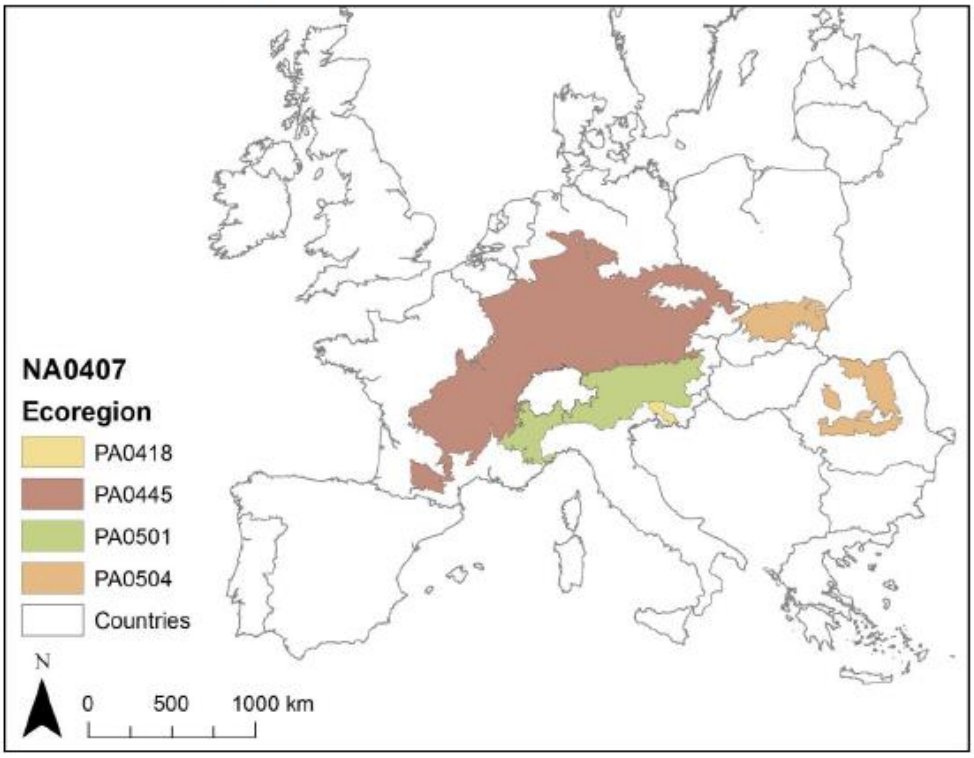
The trial site Ontario-2 ([redacted], 1993) is located within this root ecoregion. The ENASGIPS holistic similarity query identified four European ecoregions similar to the root ecoregion Eastern Great Lakes lowland forests based on the similarity scores summarized in the following table. The identified ecoregions cover large parts of Central Europe as well as regions in Eastern and Southeastern Europe.

Table 8.1.1.3-4: Similarity scores calculated by ENASGIPS for the root ecoregion Eastern Great Lakes lowland forests (NA0407)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class

PA0418 - Dinaric Mountains mixed forests (EU)	87	50	100	100	86	100
PA0445 - Western European broadleaf forests (EU)	81	44	63	100	100	100
PA0501 - Alps conifer and mixed forests (EU)	90	49	100	100	100	100
PA0504 - Carpathian montane forests (EU)	90	100	50	100	100	100
Average score	87	61	78	100	97	100

Figure 2 **European Ecoregions similar to root ecoregion *Eastern Great Lakes lowland forests* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



NA0414-Southern Great Lakes forests

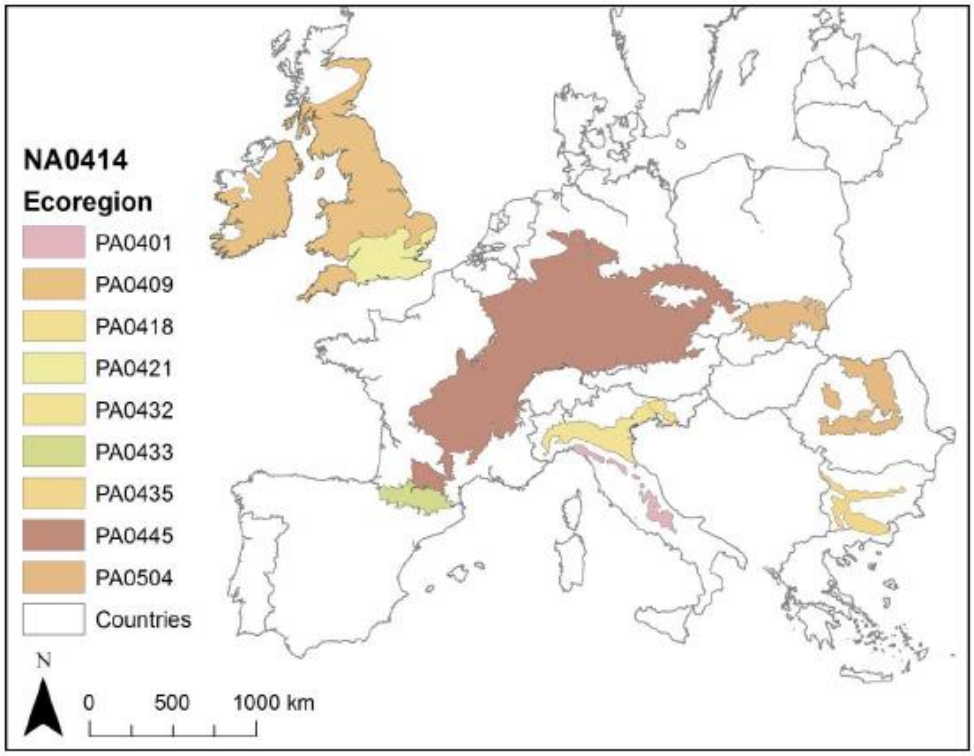
The trial sites New York, Ohio ([redacted], 1993a) and Ontario-1 ([redacted], 1992) are located within this root ecoregion. The ENASGIPS holistic similarity query identified nine European ecoregions similar to the root ecoregion Southern Great Lakes forests based on the similarity scores summarized in the following table. The identified ecoregions cover large parts of Central Europe as well as regions in Northwestern, South, and Southeastern Europe.

Table 8.1.1.3-5: Similarity scores calculated by ENASGIPS for the root ecoregion Southern Great Lakes forests (NA0414)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0401 - Appenine deciduous montane forests (EU)	81	60	61	100	86	100
PA0409 - Celtic broadleaf forests (EU)	83	100	97	17	100	100
PA0418 - Dinaric Mountains mixed forests (EU)	84	100	100	53	67	100

PA0421 - English Lowlands beech forests (EU)	83	100	48	77	100	88
PA0432 - Po Basin mixed forests (EU)	80	41	90	100	70	100
PA0433 - Pyrenees conifer and mixed forests (EU)	84	95	54	88	82	100
PA0435 - Rodope montane mixed forests (EU)	91	100	56	100	100	100
PA0445 - Western European broadleaf forests (EU)	92	100	91	74	97	100
PA0504 - Carpathian montane forests (EU)	80	52	74	76	100	100
Average score	84	83	75	76	89	99

Figure 3 **European Ecoregions similar to root ecoregion *Southern Great Lakes forests* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



NA0801-California Central Valley grasslands

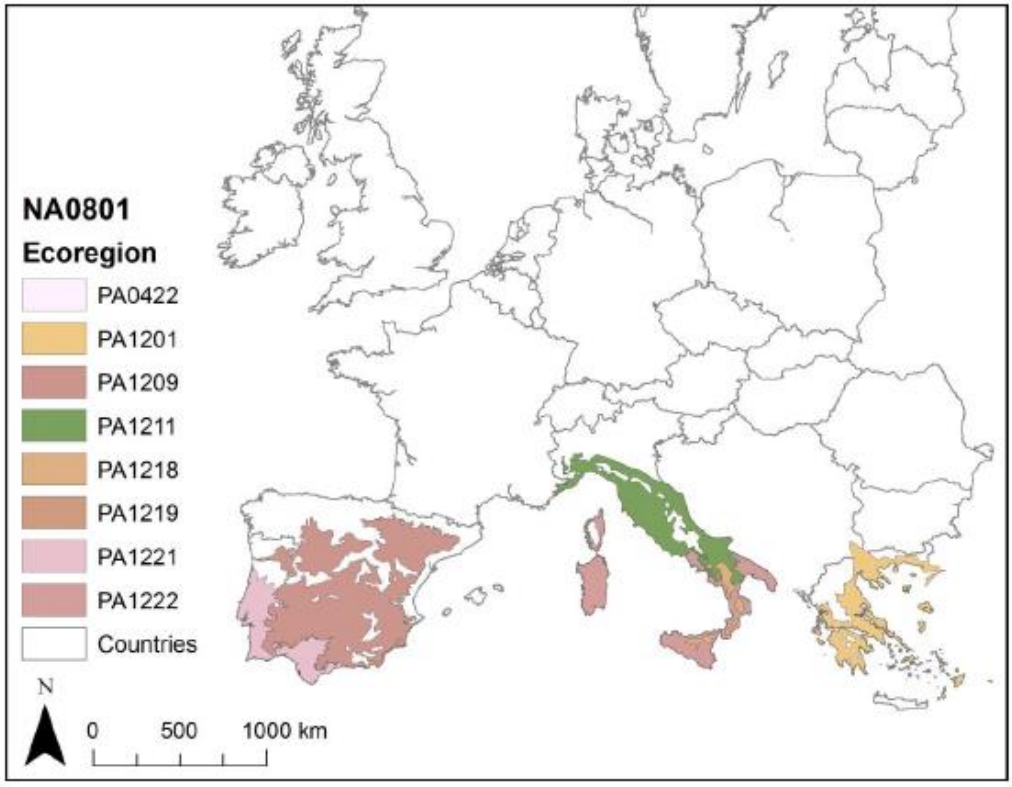
The trial sites California-1 ([redacted], 1993a) and California-2 ([redacted], 1989a) are located within this root ecoregion. The ENASGIPS holistic similarity query identified eight European ecoregions similar to the root ecoregion California Central Valley grasslands based on the similarity scores summarized in the following table. The identified ecoregions cover large parts of Southern and Southeastern Europe.

Table 8.1.1.3-6: Similarity scores calculated by ENASGIPS for the root ecoregion California Central Valley grasslands (NA0801)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0422 - Euxine-Colchic broadleaf forests (EU)	87	35	100	100	100	100
PA1201 - Aegean and Western Turkey	85	60	100	64	100	100

sclerophyllous and mixed forests (EU)						
PA1209 - Iberian sclerophyllous and semi-deciduous forests (EU)	84	44	100	85	89	100
PA1211 - Italian sclerophyllous and semi-deciduous forests (EU)	83	39	100	75	100	100
PA1218 - South Appenine mixed montane forests (EU)	90	50	100	100	100	100
PA1219 - Southeastern Iberian shrubs and woodlands (EU)	83	100	71	63	79	100
PA1221 - Southwest Iberian Mediterranean sclerophyllous and mixed forests (EU)	93	100	100	65	100	100
PA1222 - Tyrrhenian-Adriatic Sclerophyllous and mixed forests (EU)	97	100	100	84	100	100
Average score	88	66	96	80	96	100

Figure 4 **European Ecoregions similar to root ecoregion *California Central Valley grasslands* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



NA0805-Central tall grasslands

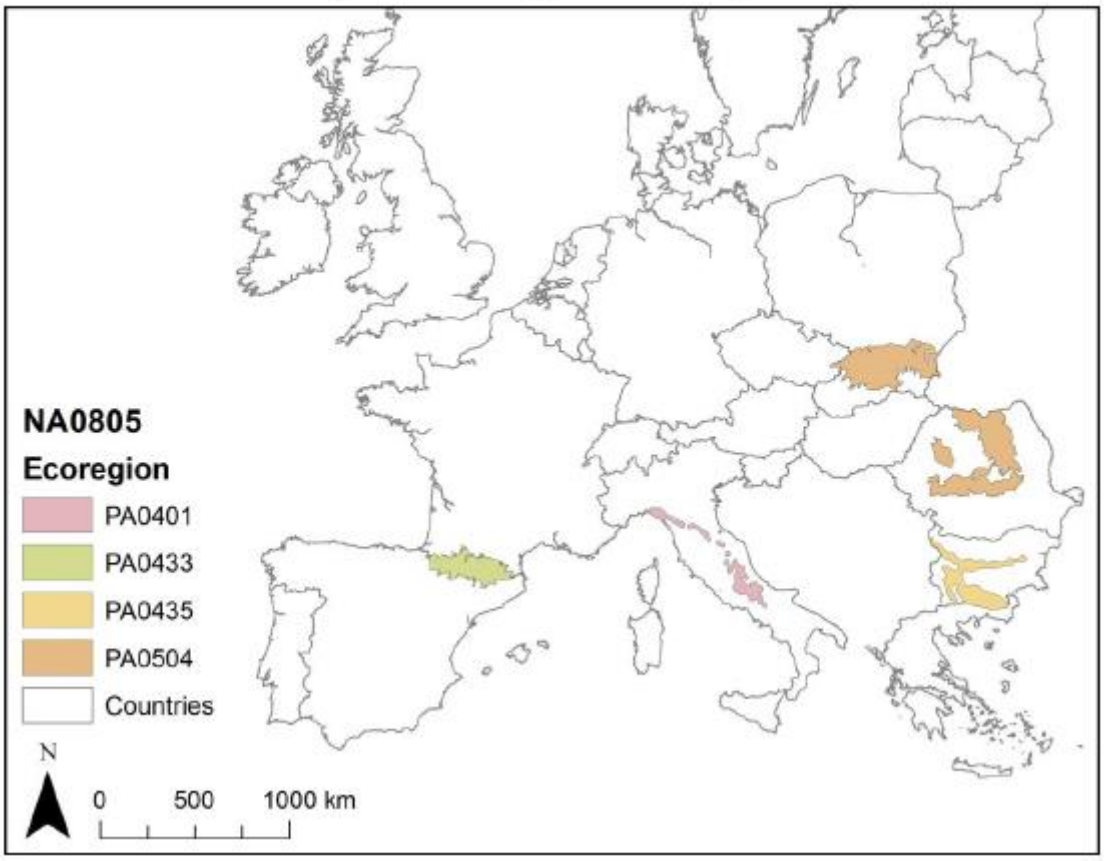
The trial sites Iowa and Minnesota ([redacted], 1993a) are located within this root ecoregion. The ENASGIPS holistic similarity query identified four European ecoregions similar to the root ecoregion Central tall grasslands based on the similarity scores summarized in the following table. The identified ecoregions cover parts of Southern, Southeastern and Eastern Europe.

Table 8.1.1.3-7: Similarity scores calculated by ENASGIPS for the root ecoregion Central tall grasslands (NA0805)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0401 - Appenine deciduous montane forests (EU)	91	62	100	100	93	100
PA0433 - Pyrenees conifer and mixed forests (EU)	90	100	89	100	65	97
PA0435 - Rodope montane mixed forests (EU)	98	100	100	92	100	100
PA0504 - Carpathian montane forests (EU)	86	82	100	86	62	100
Average score	91	86	97	95	80	99

Temperature similarity score of root ecoregion NA1310 with its solely similar ecoregion in Europe is 66 %, indicating a moderate similarity between the two ecoregions.

Figure 6 **European Ecoregions similar to root ecoregion *Central tall grasslands* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



NA1310-Sonoran desert

The trial site Arizona ([redacted] 1993) is located within this root ecoregion. The ENASGIPS holistic similarity query identified one European ecoregion similar to the root ecoregion Sonoran desert based on the similarity scores summarized in the following table. The identified ecoregion covers small parts of the coastal area in southern Spain.

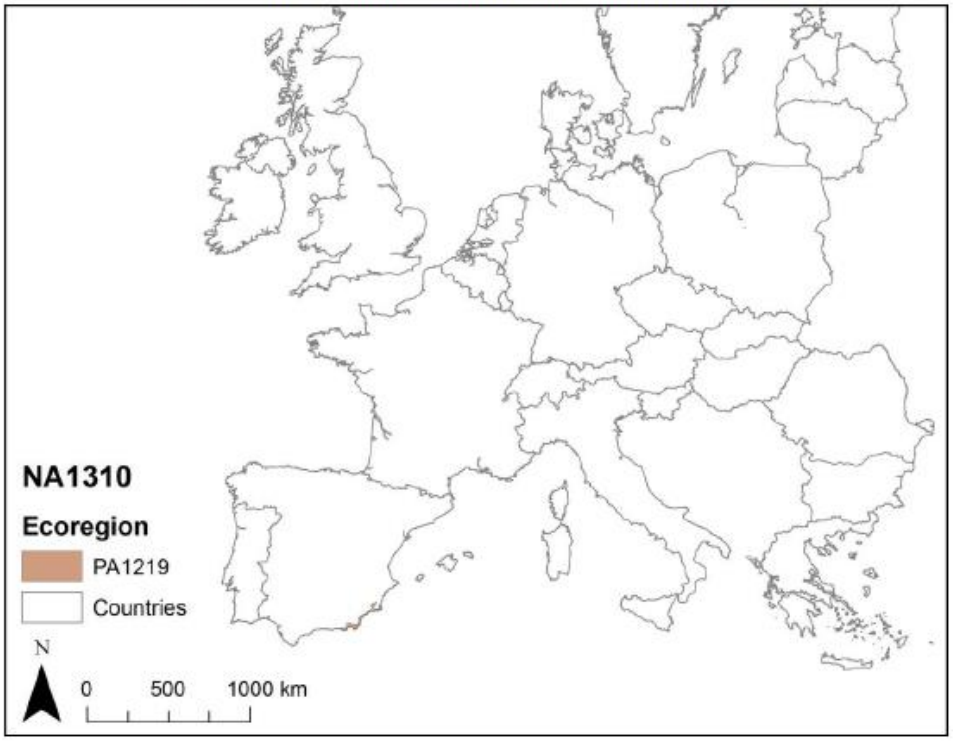
Table 8.1.1.3-8: Similarity scores calculated by ENASGIPS for the root ecoregion Sonoran desert (NA1310)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class

PA1219 - Southeastern Iberian shrubs and woodlands (EU)	86	66	100	100	100	66

For the remaining three root ecoregions, NA0802, NA0810, and NA0811, the scores for temperature similarity range from 7 to 33 % for individual matches, indicating pronounced differences in temperature conditions between root ecoregions and their corresponding ecoregions in Europe.

Figure 9 **European Ecoregions similar to root ecoregion *Sonoran desert* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



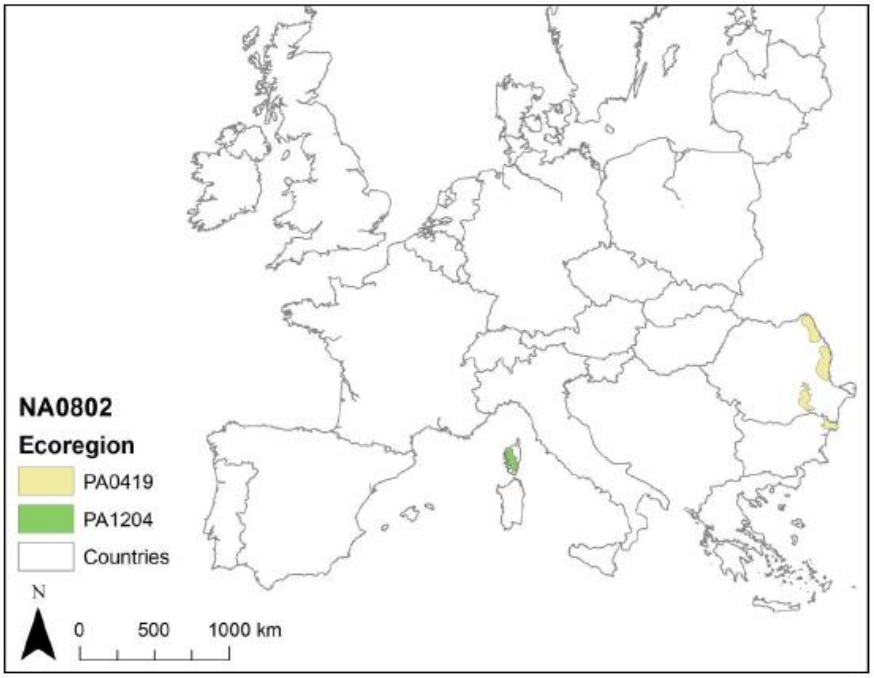
NA0802-Canadian Aspen forests and parklands

The trial sites Saskatchewan-2 ([redacted], 1992) and Alberta-2 ([redacted], 1993) are located within this root ecoregion. The ENASGIPS holistic similarity query identified two European ecoregions similar to the root ecoregion Canadian Aspen forests and parklands based on the similarity scores summarized in the following table. The identified ecoregions cover small parts of South and Southeastern Europe.

Table 8.1.1.3-9: Similarity scores calculated by ENASGIPS for the root ecoregion Canadian Aspen forests and parklands (NA0802)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0419 - East European forest steppe (EU)	80	10	92	100	96	100
PA1204 - Corsican montane broadleaf and mixed forests (EU)	81	7	100	100	100	100
Average score	81	9	96	100	98	100

Figure 5 **European Ecoregions similar to root ecoregion *Canadian Aspen forests and parklands* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



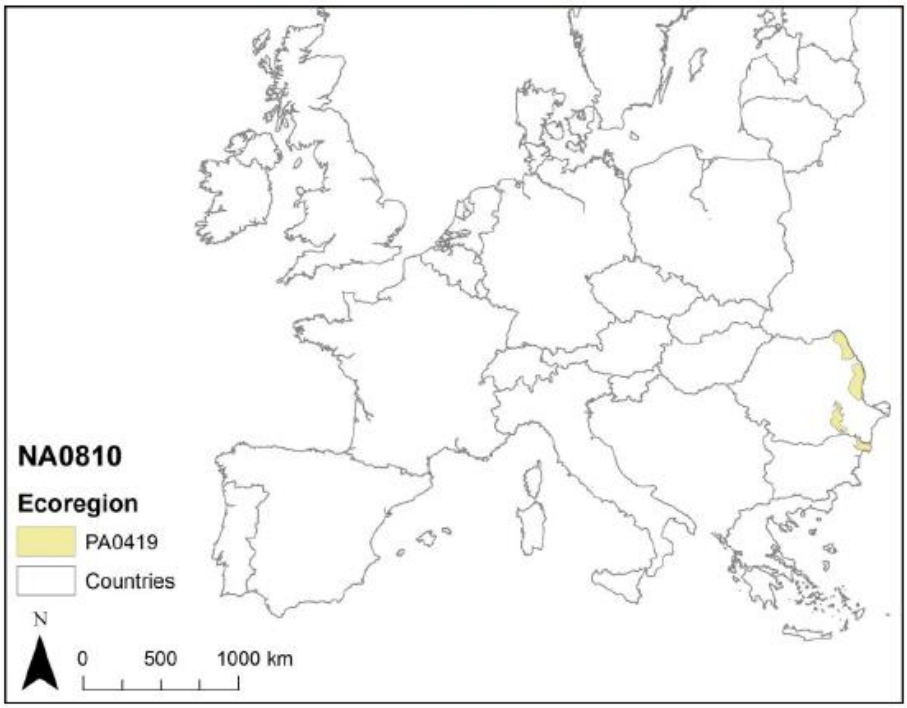
NA0810-Northern mixed grasslands

The trial site Saskatchewan-1 ([redacted], 1992) is located within this root ecoregion. The ENASGIPS holistic similarity query identified one European ecoregion similar to the root ecoregion Northern mixed grasslands based on the similarity scores summarized in the following table. The identified ecoregion covers small parts of Southeastern Europe.

Table 8.1.1.3-10: Similarity scores calculated by ENASGIPS for the root ecoregion Northern mixed grasslands (NA0810)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0419 - East European forest steppe (EU) 87	87	33	100	100	100	100

Figure 7 **European Ecoregions similar to root ecoregion *Northern mixed grasslands* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



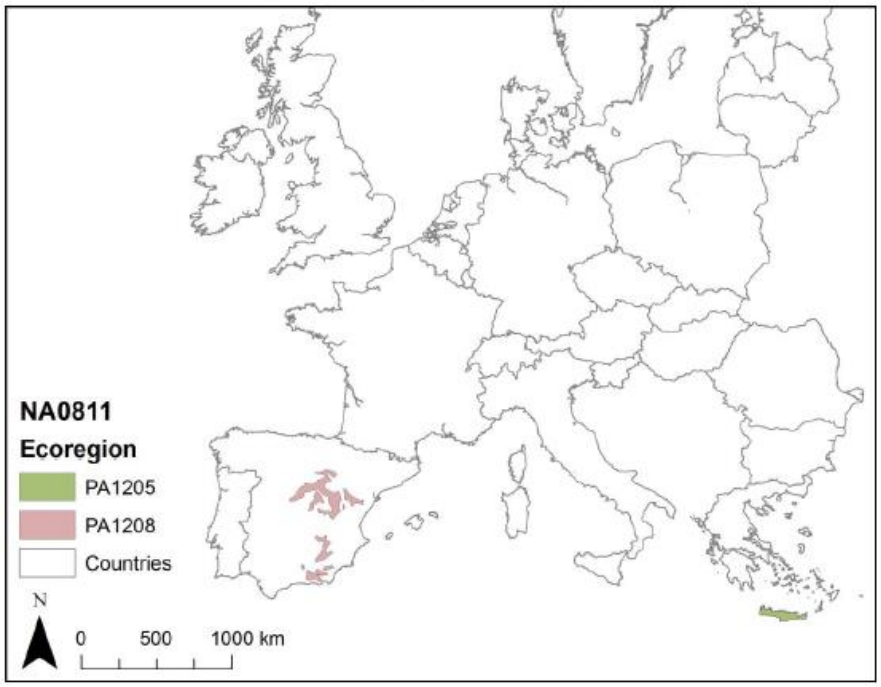
NA0811-Northern short grasslands

The trial site Alberta-1 ([redacted], 1992) is located within this root ecoregion. The ENASGIPS holistic similarity query identified two European ecoregions similar to the root ecoregion Northern short grasslands based on the similarity scores summarized in the following table. The identified ecoregions cover parts of Southwestern and Southeastern Europe.

Table 8.1.1.3-11: Similarity scores calculated by ENASGIPS for the root ecoregion Northern short grasslands (NA0811)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA1205 - Crete Mediterranean forests (EU)	81	12	92	100	100	100
PA1208 - Iberian conifer forests (EU)	86	30	100	100	100	100
Average score	84	21	96	100	100	100

Figure 8 **European Ecoregions similar to root ecoregion *Northern short grasslands* according to ENASGIPS (holistic similarity model with threshold > 80 %)**



III. CONCLUSIONS

In this report, an ecoregion crosswalk analysis was performed on 19 North American TFD trial sites which are represented by twelve root ecoregions. With the holistic similarity approach, matching ecoregions (80 % similarity) were identified for eight of a total of twelve root ecoregions (NA0407, NA0414, NA0801, NA0802, NA0805, NA0810, NA0811, and NA1310). This meant that six trial sites were not considered representative for European conditions based on the holistic similarity approach.

In addition to the holistic similarity approach, individual scores of temperature were evaluated in a refined assessment as temperature is known to be a main driving parameter for the degradation of pesticides. For three of the eight root ecoregions, the scores for temperature ranged from 7 to 33 % for individual matches. This indicates that the temperature conditions of the North American root ecoregions are not well represented by the European ecoregions and thus, the three root ecoregions NA0802, NA0810 and NA0811 representing four trial sites are considered not representative for European conditions.

Based on the refined ecoregion crosswalk analysis, similar soil and climate conditions were identified for five root ecoregions: NA0407, NA0414, NA0801, NA0805 and NA1310 comprising nine trial sites of the US and Canadian TFD studies available for glyphosate. These trials are considered representative for European conditions.

Table 8.1.1.3-12: Overview of TFD trial sites acceptable in European conditions

Root Ecoregion	TFD Trial Site	Study	Conclusion on similarity	Share relative to area ofEU (%)
NA0407 - Eastern Great Lakes lowland forests (CA,USA)	Ontario-2	[redacted] 1993	Sufficient similarity; considered represenative for European conditions for further evaluation	16.5
NA0414 - Southern Great Lakes forests (CA,USA)	New York	[redacted], 1993a		22.1
	Ohio	[redacted], 1993a		
	Ontario-1	[redacted], 1992		
NA0801 - California Central Valley grasslands (USA)	California-1	[redacted], 1993a		15.2
	California-2	[redacted], 1989a		
NA0805 - Central tall grasslands (USA)	Iowa	[redacted], 1993a		3.8
	Minnesota	[redacted], 1993a		

NA1310 - Sonoran desert (USA)	Arizona	[redacted], 1993a		0.1
NA0802 - Canadian Aspen forests and parklands (CA,USA)	Alberta-2	[redacted]n & [redacted], 1993	Insufficient similarity due to individual score of temperature	0.6
	Saskatchewan-2	[redacted], 1992		
NA0810 - Northern mixed grasslands (CA,USA)	Saskatchewan-1	[redacted], 1992		0.5
NA0811 - Northern short grasslands (CA,USA)	Alberta-1	[redacted], 1992		1.0
NA0405 - East Central Texas forests (USA)	Texas	[redacted], 1993a	No similarity due to score in holistic approach	0
NA0409 - Mississippi lowland forests (USA)	Mississippi	[redacted], 1989b		
NA0529 - Southeastern conifer forests (USA)	Georgia-1	[redacted], 1993a		
	Georgia-2	[redacted], 1989c		
NA0812 - Northern tall grasslands (CA,USA)	Manitoba-1	[redacted], 1992		
	Manitoba-2	[redacted] 1993		

Assessment and conclusion by applicant:

The evaluation was performed with OECD ENASGIPS tool recommended in OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016 and is therefore considered valid.

It is shown that 9 out of 18 field trials conducted in the US and Canada are representative for European conditions. Thus, residue data from these trials were used to derive endpoints for EU approval. The respective kinetic evaluation is summarised below ([redacted], 2020b, CA 7.1.2.2.1/003).

Assessment and conclusion by RMS:

Applicant provided an ecoregion crosswalk assessment based on the ENASGIPS holistic similarity approach. This approach was completed by further checking individually the similarity for temperature.

RMS notes that ENASGIPS tool allows for a GIS comparison (5 x 5 km resolution) based on annual average climate conditions and soil conditions. Based on its location, a root ecoregion was assigned to each field trial. Then, the characteristics (climate and soil properties) of the selected root ecoregion was compared to EU root ecoregions. A more accurate approach would have been to compare directly the actual climatic conditions and soil characterisation of the field sites with the EU root ecoregions.

A data gap is identified for providing a comparison of actual field sites properties instead of default root ecoregions of the trial soils.

Regarding Central tall grasslands (NA0805), Sonoran desert (NA1310), Canadian Aspen forests and parklands (NA0802), Northern short grasslands (NA0811) and Northern mixed grasslands (NA0810), the geographical extend of these European ecoregions was very limited throughout Europe (see map above) and correspond to a small percentage area of European soils (0.1 to 3.8%). For Central tall grasslands, which correspond to 3.8% European area, the geographical areas seem to correspond mainly to mountains, natural grasslands more than cultivated areas. It would have been relevant to consider in the comparison only the agricultural regions in Europe, by including for example the CORINE Land Cover layer.

Based on the available data, RMS considers that field sites corresponding to the ecoregions Eastern Great Lakes lowland forests (NA0407), Southern Great Lakes forests (NA0414) and California Central Valley grasslands (NA0801) are considered as representative of the European conditions. This corresponds to the following field sites: New York, Ohio and California-1 from [redacted]

1993a, Ontario-1 from [redacted] 1992, Ontario-2 from [redacted] 1993, and California-2 from [redacted] 1989a.

[redacted], *1993*

Data point:	CA 7.1.2.2.1/005
Report author	[redacted]
Report year	1993
Report title	The terrestrial field dissipation of Glyphosate in Canadian soil
Report No	MSL-12605
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: • Alberta and Manitoba sites not representative of EU conditions • weather data reported as monthly averages, only; distance of weather station from field site not reported • Sampling up to 45 cm only
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	Yes, for Ontario site only

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification: Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation: Roundup
Lot No. Alberta: LUL-9101-2706-F
Lot No. Manitoba, Ontario: PIT-8912-1385-A
Nominal concentration: 41 % as glyphosate salt
31 % as glyphosate

B. STUDY DESIGN

1. Test sites

Three test sites were selected, one in each of three representative provinces: Alberta, Manitoba, and Ontario. These three test sites encompass diverse climatological conditions, soil types, and geography which are representative of the wide range of conditions under which glyphosate would be used under normal agronomic practices.

Four test plots were established at each test site: one untreated (control) test plot and three replicate, treated test plots. The untreated (control) test plot was separated from the nearest treated test plot by a minimum of a 38 meter buffer zone. The replicate, treated test plots were separated by a minimum of a 10 meter buffer zone. The replicate treated test plots ranged in size from 45 to 60 m^2.

Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given below.

Characteristics of test soils

Parameter	Lamont, Alberta test site			Oakville, Manitoba test site			Ayr, Ontario test site		
Soil depth (cm)	0-15	15-30	30-45	0-15	15-30	30-45	0-15	15-30	30-45
Textural Class (USDA)	loam	loam	sandy clay loam	loam	sandy clay loam	sandy loam	loamy sand	sand	sand
Sand[1] (%)	50.0	50.0	52.0	44.0	50.0	68.0	80.0	88.0	92.0
Silt[2] (%)	28.0	28.0	20.0	32.0	26.0	14.0	16.0	8.0	6.0

Clay[3] (%)	22.0	22.0	28.0	24.0	24.0	18.0	4.0	4.0	2.0
pH[4]	6.5	6.5	6.6	7.3	7.8	7.9	6.8	7.3	7.7
OC (%)[5]	1.9	1.6	1.7	3.5	1.7	1.0	1.2	0.7	0.4
OM (%)	3.3	2.8	2.9	6.0	3.0	1.7	2.0	1.2	0.7
CEC (meq/100 g)	15.9	18.8	19.5	29.6	30.0	27.8	10.4	10.2	13.3
WHC at 1/3 bar (%)	30.0	29.5	31.2	35.9	32.1	22.5	16.3	13.4	10.9
Bulk Density (disturbed) (g/cm3)	1.07	1.05	1.05	1.00	1.04	1.13	1.33	1.37	1.42

[1] Sand (50 µm – 2 mm)
[2] Silt (2 µm – 50 µmm)
[3] Clay (< 2 µm)
[4] Medium not given
[5] Calculated from organic matter according to OC = OM x 0.58

The Ontario test site had a known two-year history of crop and pesticide use and had not been treated with Roundup herbicide or related chemistry during the two years preceding this study. Two-year crop and pesticide use histories were not reported for the Alberta and Manitoba test sites. However, the absence of detectable levels of glyphosate and AMPA in soil samples collected prior to test substance application at the Alberta and Manitoba test sites demonstrated that there were no glyphosate residues which could potentially compromise the integrity of this study. Test plots were maintained in a weed free condition by the use of paraquat herbicide at all three locations.

Weather data (air temperature, precipitations) were collected for each location from nearby, permanent, institutional weather recording stations. The climatological data indicate that climatic conditions at all test sites during the study were within the normal ranges and revealed no major deviations from expected weather patterns, with the exception of the Alberta test which received only about 60 % of the average 30 year historical precipitation. Soil temperature was measured on site at each sampling time.

2. Application

Single applications of Roundup herbicide were made to each bare ground, replicate test plot at each test site according to label directions using normal agronomic practices.

- At the Alberta test site, all three replicate test plots were treated at an application rate of **4.27 kg a.s./ha** using a total spray solution volume of 110 L/ha.
- At the Manitoba test site, all three replicate test plots were treated at an application rate of **4.27 kg a.s./ha** using a total spray solution volume of 122 L/ha.
- At the Ontario test site, the actual application rates and spray solution volumes for the .three replicate test plots were, respectively, **4.21, 4.07 and 4.27 kg a.s./ha** and 147.8, 142.9 and 150.0 L/ha.

Test substance application spray equipment was calibrated prior to test substance application at all three locations.

3. Sampling

Soil samples were randomly collected from both the treated and control test plots at each test site and sampling event. Early time point soil samples to define the dissipation of glyphosate were collected at 1,7,14, and 21 days after test substance application at all test sites, with the exception of the Ontario test site for which the 21 days after application samples were not collected.

Longer term time point samples were collected at approximately 1,2,3,12, and 16 months after test substance application at the Alberta test site, 1,2, 3, 4, 5, 12 and 17 months after application at the Manitoba test site, and 1, 2, 3, 4, 6, 12 and 18 months after application at the Ontario test site.

For the 0 days after application sampling at all three test sites and the sampling prior to application at the Alberta test site, samples were collected to a depth of 15 cm. For all other sampling events 10 soil

cores to a depth of 45 cm were randomly collected from each of three replicate test plots and the untreated control test plot. Soil cores were collected using "zero contamination" commercial soil coring equipment with removable acetate liners.

4. Specimen handling and preparation

All samples were frozen within 2 hours following collection and were maintained in frozen storage until they were shipped frozen to the Sponsor's testing facility via overnight air delivery. Following receipt at the Sponsor's testing facility, the 10 samples from each test plot at each test site were sectioned into 15 cm depth increments (e.g. 0-15 cm, 15-30 cm and 30-45 cm), thawed, and composited to afford 4 representative samples per depth increment per sampling event; i.e. one composited sample for each of the three replicate, treated test plots and one composited sample for the untreated (control) test plot. Following compositing, samples were refrozen within 4 hours and maintained in frozen storage until analysis. Untreated (control) soil cores were sectioned first. Sectioning was conducted from the bottom of the soil cores to the top to prevent contamination of samples.

5. Analytical methods

Glyphosate and AMPA were extracted from soil using a 0.5 N KOH solution. The extract solution was eluted through a Chelex 100 resin in the Fe(III) form, which retains glyphosate and AMPA due to chelation to Fe(III).

The retained glyphosate and AMPA iron salts are removed from the Chelex resin by elution with 6 N HCl. The isolated glyphosate and AMPA iron salts are then applied to a strong anion exchange resin and eluted with 6 N HCl to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness, to remove the HCl, the samples are re-dissolved in water and analysed by high pressure liquid chromatography (HPLC).

The chromatograph uses column switching and a o-phthalaldehyde post-column reactor with a fluorescence detector to separate and quantitate glyphosate and AMPA. In the post-column reactor, glyphosate is oxidised to a primary amine which then reacts with o-phthalaldehyde to form a fluorescence derivative. AMPA reacts directly with o-phthalaldehyde to form a second fluorescence derivative.

This method has been validated down to 0.05 mg/kg for both glyphosate and AMPA in 30 g soil samples and generally affords recoveries of glyphosate from fortified check samples which are greater than 70 %. AMPA recoveries are normally higher than glyphosate recoveries. The recoveries from check samples fortified over the range of 0.05 mg/kg to 2.00 mg/kg with both glyphosate and AMPA, averaged across all test sites, were 83.75 % and 81.88 %, respectively, for glyphosate and AMPA. The average recoveries of glyphosate ranged from a high of 96.61 % from soil from the Alberta test site to a low of 72.58 % from soil from the Manitoba test site. Average recoveries of AMPA ranged from a high of 87.71 % from soil from the Alberta test site to a low of 78.95 % from soil from the Ontario test site.

The limit of detection (LOD) was set at 0.01 mg/kg for glyphosate and 0.03 mg/kg for AMPA.

The stability of glyphosate and AMPA in soil was confirmed by a storage stability study (see [redacted], 1993a).

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate residues (mg/kg) analysis in Alberta soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT										
	Replicate	-1[5]	0	1	7	14	21	30	62	91	365	457
0-15	A	<LOD	1.081	0.680	0.616	0.393	0.384	0.220	0.212	0.252	0.178	0.128
	B	<LOD	1.036	0.624	0.801	0.331	0.375	0.679	0.170	0.416	0.147	0.119
	C	<LOD	1.125	0.482	0.499	0.265	0.273	0.519	0.176	0.013	0.113	0.005[4]
	Mean	<LOD	1.081	0.595	0.639	0.330	0.344	0.473	0.186	0.227	0.143	0.084
15-30	A	-	-	0.021	0.041	0.040	0.051	0.066	0.033	0.027	0.007[1]	0.005[1]

	B	-	-	0.069	0.026	0.020	0.031	0.045	0.026	0.034	0.010	<LOD
	C	-	-	0.033	0.020	0.021	0.034	0.040	0.030	0.211	0.009[1]	0.005[1]
	Mean	-	-	0.041	0.029[1]	0.027	0.039	0.050	0.030	0.031[2]	0.009[4]	0.003[4]
30-45	A	-	-	0.041	0.039	0.023	0.028	0.042	0.058	0.011	<LOD	0.009[1]
	B	-	-	0.033	0.058	0.019	0.025	0.042	0.005[1]	0.014	0.007[1]	0.005[1]
	C	-	-	0.035	0.084	0.022	0.027	0.034	0.010	0.006[1]	0.008[1]	0.043[3]
	Mean	-	-	0.036	0.060[1]	0.021	0.027	0.039	0.024	0.010	0.005[4]	0.007[4]

DAT: days after treatment
LOD = 0.01 mg/kg
1 The 15-30 and 30-45 cm depth interval samples are believed to have been inadvertently reversed during sample compositing.
2 Glyphosate residue levels of 0.027, 0.034, and 0.211 mg/kg were found in the three replicate samples for this depth interval and sampling event. The sample with the 0.211 mg/kg level was considered to be an outlier, and was not included in the calculation of the average residue level.
3 Glyphosate residue levels of 0.009, 0.005 and 0.043 mg/kg were found in the three replicate samples for this depth interval and sampling event. The sample with the 0.043 mg/kg level was considered to be an outlier and was not included in the calculation of the average residue level.
4 < LOD
5 Four values were measured, all being < LOD

Table 8.1.1.3-13: Results of AMPA residues (mg/kg) analysis in Alberta soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT										
	Replicate	-1 [2]	0	1	7	14	21	30	62	91	365	457
0-15	A	<LOD	0.035	0.024[1]	0.037	0.032	0.045	0.034	0.063	0.061	0.270	0.145
	B	<LOD	0.031	0.028[1]	0.056	0.043	0.057	0.085	0.049	0.121	0.104	0.240
	C	<LOD	0.037	0.020[1]	0.045	0.037	0.042	0.083	0.051	<LOD	0.136	<LOD
	Mean	<LOD	0.034	0.024[1]	0.046	0.037	0.048	0.067	0.054	0.061	0.170	0.128
15-30	A	-	-	<LOD	<LOD	<LOD	<LOD	0.016[1]	0.014[1]	<LOD	0.028[1]	<LOD
	B	-	-	0.017[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.019[1]	<LOD
	C	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.080[1]	<LOD	<LOD
	Mean	-	-	0.006[1]	<LOD	<LOD	<LOD	0.005[1]	0.005[1]	0.027[1]	0.016[1]	<LOD
30-45	A	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	0.056	<LOD	<LOD	<LOD
	B	-	-	<LOD	0.002[1]	<LOD	<LOD	0.013[1]	<LOD	<LOD	<LOD	<LOD
	C	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.064
	Mean	-	-	<LOD	0.001[1]	<LOD	<LOD	0.004[1]	0.019[1]	<LOD	<LOD	0.021[1]

[1] < LOD
[2] four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.03 mg/kg

Table 8.1.1.3-14: Results of glyphosate residues (mg/kg) analysis in Manitoba soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT													
	Rep.	-5	0	1	7	14	21	28	58	92	120	150	366	512
0-15	A	<LOD[2]	0.740	0.741	0.427	0.369	0.298	0.426	0.142	0.028	0.014	0.002[1]	0.009[1]	0.035
	B	<LOD	0.880	0.543	0.704	0.459	0.156	0.221	0.086	0.008[1]	0.013	0.001[1]	0.014	0.008[1]
	C	<LOD	0.783	0.497	0.495	0.481	0.338	0.271	0.062	0.009[1]	0.004[1]	0.022	<LOD	0.014
	Mean	<LOD	0.801	0.594	0.542	0.436	0.264	0.306	0.097	0.015	0.010	0.008[1]	0.008[1]	0.019
15-30	A	<LOD [3]	-	0.007[1]	0.010	0.012	0.020	0.015	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	0.050	0.006[1]	0.018	0.020	0.018	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	0.026	0.005[1]	0.016	0.025	0.018	<LOD	<LOD	<LOD	<LOD	<LOD	0.002[1]
	Mean	<LOD	-	0.028	0.007[1]	0.015	0.022	0.017	<LOD	<LOD	<LOD	<LOD	<LOD	0.001[1]
30-45	A	<LOD	-	0.020	0.007[1]	0.008[1]	<LOD	0.013	<LOD	<LOD	<LOD	<LOD	<LOD	0.003[1]

	B	<LOD	-	0.026	0.005[1]	0.012	0.019	<LOD	<LOD	<LOD	0.011	<LOD	<LOD	0.004[1]
	C	<LOD	-	0.008[1]	0.004[1]	0.012	0.014	0.016	<LOD	<LOD	<LOD	<LOD	<LOD	0.002[1]
	Mean	<LOD	-	0.018	0.005[1]	0.011	0.011	0.010	<LOD	<LOD	0.004[1]	<LOD	<LOD	0.003[3]

[1] < LOD
[2] five values were measured, all being < LOD
[3] four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.01 mg/kg
Rep. = Replicate

Table 8.1.1.3-15: Results of AMPA (mg/kg) residues analysis in Manitoba soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT												
	Replicate	-5	0	1	7	14	21	28	58	92	120	150	366	512
0-15	A	<LOD [2]	0.049	0.061	0.070	0.049	0.043	0.067	0.197	0.079	0.084	0.073	0.065	0.049
	B	<LOD	0.057	0.052	0.105	0.054	0.028[1]	0.046	0.143	0.061	0.087	0.067	0.072	0.031
	C	<LOD	0.050	0.043	0.075	0.064	0.075	0.054	0.155	0.060	0.074	0.074	0.033	0.027[1]
	Mean	<LOD	0.052	0.052	0.083	0.056	0.049	0.056	0.165	0.067	0.082	0.071	0.057	0.036
15-30	A	<LOD [3]	-	<LOD	<LOD	<LOD	0.012[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	0.012[1]	0.013[1]	<LOD	<LOD	<LOD	<LOD	<LOD	0.011[1]
	C	<LOD	-	0.011[1]	<LOD	<LOD	<LOD	0.012[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	0.004[1]	<LOD	<LOD	0.008[1]	0.008[1]	<LOD	<LOD	<LOD	<LOD	<LOD	0.004[1]
30-45	A	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.015[1]
	C	<LOD	-	<LOD	0.014[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	<LOD	0.005[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.005[1]

[1] < LOD
[2] five values were measured, all being < LOD
[3] four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.03 mg/kg

Table 8.1.1.3-16: Results of glyphosate residues (mg/kg) analysis in Ontario soil following treatment with Roundup at 4.18 kg/ha

Depth (cm)	DAT												
	Replicate	-1	0	1	7	14	28	57	86	129	177	364	537
0-15	A	<LOD [2]	0.678	0.493	0.496	0.425	0.173	0.051	0.039	0.025	0.029	0.027	0.001[1]
	B	<LOD	0.562	0.427	0.428	0.348	0.119	0.052	0.050	0.016	0.020	0.010	0.003[1]
	C	<LOD	0.773	0.686	0.562	0.442	0.160	0.064	0.052	0.034	0.035	0.054	0.010[1]
	Mean	<LOD	0.671	0.535	0.495	0.405	0.151	0.056	0.047	0.025	0.028	0.030	0.005[1]
15-30	A	<LOD [2]	-	<LOD	0.004[1]	<LOD	0.003[1]	<LOD	<LOD	<LOD	<LOD	<LOD	-0.002[1]
	B	<LOD	-	<LOD	0.004[1]	<LOD	0.001[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	0.009[1]	<LOD	0.004[1]	<LOD	<LOD	<LOD	<LOD	<LOD	-0.002[1]
	Mean	<LOD	-	<LOD	0.006[1]	<LOD	0.003[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-45	A	<LOD	-	<LOD	<LOD	<LOD	-0.001[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	0.003[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	<LOD	0.001[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[1] < LOD
[2] four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.01 mg/kg

Table 8.1.1.3-17: Results of AMPA residues (mg/kg) analysis in Ontario soil following treatment with Roundup at 4.18 kg/ha

Depth (cm)	DAT											
	Replicate	-1	0	1	7	14	28	57	86	129	177	364	537
0-15	A	<LOD [2]	0.118	0.105	0.116	0.137	0.119	0.082	0.133	0.084	0.163	0.099	0.008[1]
	B	0.028[1]	0.115	0.097	0.107	0.102	0.069	0.079	0.153	0.066	0.090	0.040	0.003[1]
	C	0.011[1]	0.114	0.112	0.118	0.137	0.092	0.093	0.147	0.120	0.140	0.178	0.024[1]
	Mean	0.013[1]	0.116	0.105	0.114	0.125	0.093	0.085	0.144	0.090	0.131	0.106	0.012[1]
15-30	A	<LOD [3]	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.015[1]	<LOD
	Mean	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.005[1]	<LOD
30-45	A	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

1 < LOD
2 four values were measured, being: < LOD, 0.028, 0.011 and 0.011
3 four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.03 mg/kg

B. Characterisation of residues

1. Alberta test site

The maximum average residue level of glyphosate in the 0 to 15 cm soil layer was 1.081 mg/kg at 0 days after treatment (DAT). Average glyphosate residues declined to 0.330 mg/kg by 14 DAT, increased to 0.473 mg/kg at 30 DAT, and then dissipated to 0.084 mg/kg at 457 DAT. Average glyphosate residues declined with depth and were below the LOD at 365 DAT for the 15-30 and 30-45 cm soil layers.

The average residue level of AMPA in the 0 to 15 cm soil layer was 0.034 mg/kg on the day of treatment and gradually increased to a maximum of 0.170 mg/kg by 365 DAT. AMPA residue levels were 0.128 mg/kg at the final sampling at 457 DAT. In the deeper soil layers the residue levels were below the LOD.

2. Manitoba test site

The average residue level of glyphosate in the 0 to 15 cm layer was 0.801 mg/kg at 0 DAT. Average glyphosate residues gradually dissipated to 0.019 mg/kg at 512 DAT. Average glyphosate residues greater than 0.01 mg/kg (the lower limit of detection) were only found in the 15-30 and 30-45 cm soil horizons for the 1, 14, 21 and 28 days after application sampling events. Average glyphosate residues declined with depth and were below the LOD at 58 DAT for the 15-30 and 30-45 cm soil layers.

The average AMPA residue level in the top 0-15 cm of soil measured 0.052 mg/kg at 0 DAT. Average AMPA residues reached a maximum concentration of 0.165 mg/kg at 58 DAT, and then declined to 0.036 mg/kg at 512 DAT. AMPA residues were less than 0.01 mg/kg in all soil samples taken below 15 cm.

3. Ontario test site

The maximum average residue level of glyphosate in the 0 to 15 cm soil layer was 0.671 mg/kg at 0 DAT, and declined steadily below LOD at 537 DAT. The average residue level of AMPA in the 0 to 15 cm soil layer measured 0.116 mg/kg at 0 DAT. Average AMPA residues reached a maximum of 0.144 mg/kg at 86 DAT, and then declined to 0.012 mg/kg at 537 DAT. Average glyphosate and AMPA residues were less than 0.01 mg/kg for all samples taken below 15 cm.

C. KINETICS

An Ecoregion Crosswalk exercise was performed ([redacted], 2020). The trial in Ontario was found to be representative for European conditions and included in kinetic evaluation ([redacted], 2020b).

III. CONCLUSIONS

Maximum average glyphosate residue levels in the 0-15 cm soil horizon were 1.081, 0.801 and 0.671 mg/kg at 0 DAT for the Alberta, Manitoba and Ontario test sites, respectively, and then dissipated to 0.084, 0.019 and below LOD, respectively, at the last sampling date. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate is degraded in soil. Maximum average AMPA residue levels in the 0-15 cm soil horizon were 0.170, 0.165, and 0.144 mg/kg and occurred at 365, 58, and 86 days after test substance application for the Alberta, Manitoba and Ontario test sites, respectively, and then dissipated to 0.128, 0.036, and below LOD, respectively, at the last sampling date.

The results of this study also demonstrate that glyphosate and AMPA possess very limited potential for vertical mobility in soil, consistent with previous laboratory and field studies. The results obtained from the Alberta test site may be consistent with low levels of vertical mobility of glyphosate in the soil profile. However, for this location, it has been determined that the maximum glyphosate residues in the 15-30 and 30-45 cm soil horizons resulting from vertical mobility of glyphosate are less than 0.060 and 0.039 mg/kg, respectively. For the Manitoba test site, average glyphosate residues greater than 0.01 mg/kg (the lower limit of detection) were found in the 15-30 and 30-45 cm soil horizons for the 1, 14, 21, and 28 days after application sampling events. However, these residues can be attributed to contamination during sampling rather than vertical mobility of glyphosate in the soil. At the Ontario location, no glyphosate residues greater than or equal to 0.01 mg/kg were found in the 15-30 or 30-45 cm soil horizons at any sampling time. For all three test sites, AMPA residues in the 15-30 and 30-45 cm soil horizons were always less than 0.03 mg/kg (limit of detection).

Assessment and conclusion by applicant:

The study provides detailed information on the dissipation behavior of glyphosate under Canadian field conditions at different testing conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the rather low sampling depth (45 cm), the missing plot management history and the reporting of monthly averaged weather data. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS:

The study design is globally consistent with OECD ENV/JM/MONO(2016)6.

The three test sites are Canadian soils. It was concluded from the ecoregion crosswalk [redacted], 2020) that only the Ontario site is considered as representative of European conditions. Therefore, the RMS assessment is focused on this site.

The soil samples were performed on 45cm depth only instead of 1 m as recommended by OECD guidance. However, the mobility of glyphosate and AMPA is known to be low and for Ontario site, only traces of either glyphosate or AMPA were already found in the 30-45cm layer depth. In this case then, the deviation is not considered as a major one.

Only monthly weather data are reported in the study instead of daily data. The distance of the weather station from field site is not reported in the study report. A data gap is identified for providing the distance of the weather station from the sites.

The study is considered acceptable and data from Ontario site can be used to derive endpoints.

[redacted], *1993a*

Data point:	CA 7.1.2.2.1/006
Report author	[redacted], M.E.
Report year	1993a
Report title	The terrestrial field dissipation of glyphosate: Final report
Report No	MSL-12651
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - Arizona, Georgia, Iowa, Minnesota, Texas sites not representative of EU conditions - Only 6 soil cores taken per subplot
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	Yes, for New York, California and Ohio sites

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:
Identification: Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation: Roundup
Lot No. Alberta: LUL-9101-2706-F
Nominal concentration: 41.0 % as glyphosate salt
30.4 % as glyphosate equivalent

B. STUDY DESIGN

1. Test sites

Eight test sites were selected, one in each of three representative provinces: Arizona, California, Georgia, Iowa, Minnesota, New York and Texas. These eight test sites encompass diverse climatological conditions, soil types, and geography which are representative of the wide range of conditions under which glyphosate would be used under normal agronomic practices. Two test plots were established at each test site: one untreated (control) test plot and one treated test plots. The treated test plot was divided in 3 subplots. The untreated (control) test plot was separated from the nearest treated test plot by a minimum of a 61 meter buffer zone. The replicate treated test plots ranged in size from 45 to 60 m². Soil cores were taken from the trial sites prior to application to determine the soil properties.

Table 8.1.1.3-18: Characteristics of test soil for Arizona test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	clay loam	clay loam	clay loam	loam
Sand (50 µm – 2 mm) (%)	37.3	27.3	25.3	41.3
Silt (2 µm – 50 µmm) (%)	29.2	39.2	38.0	32.0
Clay (< 2 µm) (%)	33.5	33.5	36.7	26.7
pH[1]	8.0	8.2	8.2	8.4
Organic carbon (%)[2]	0.5	0.6	0.4	0.2
Organic matter (%)	0.9	1.0	0.7	0.4
Cation exchange capacity (meq/100 g)	32.3	31.5	29.7	26.8
Water Holding Capacity at 1/3 bar (%)	27.2	26.2	28.6	27.9
Bulk Density (disturbed) (g/cm^3)	1.14	1.14	1.11	1.15
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	sandy loam	sandy loam	loamy sand	-

Sand (50 µm – 2 mm) (%)	53.3	69.3	83.3	-
Silt (2 µm – 50 µmm) (%)	38.0	24.0	12.0	-
Clay (< 2 µm) (%)	8.7	6.7	4.7	-
pH[1]	8.3	8.3	8.4	-
Organic carbon (%)[2]	0.6	0.06	0.2	
Organic matter (%)	1.0	0.1	1.3	-
Cation exchange capacity (meq/100 g)	20.9	18.9	18.9	-
Water Holding Capacity at 1/3 bar (%)	25.4	22.7	27.1	-
Bulk Density (disturbed) (g/cm^3)	1.28	1.28	1.19	-

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-19: Characteristics of test soil for California test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	loamy sand	loamy sand	loamy sand	sand
Sand (50 µm – 2 mm) (%)	79.3	83.3	83.3	88.5
Silt (2 µm – 50 µmm) (%)	15.2	11.2	11.2	8.0
Clay (< 2 µm) (%)	5.5	5.5	5.5	3.5
pH[1]	6.3	6.3	6.5	6.5
Organic carbon (%)[2]	0.1	0.1	0.0	0.2
Organic matter (%)	0.2	0.2	0.0	0.3
Cation exchange capacity (meq/100 g)	5.1	4.4	4.4	3.9
Water Holding Capacity at 1/3 bar (%)	10.3	9.1	7.9	12.4
Bulk Density (disturbed) (g/cm^3)	1.46	1.47	1.48	1.42
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	sand	loamy sand	loamy sand	-
Sand (50 µm – 2 mm) (%)	91.3	84.5	78.5	-
Silt (2 µm – 50 µmm) (%)	5.2	12.0	16.0	-
Clay (< 2 µm) (%)	3.5	3.5	5.5	-
pH[1]	6.9	7.0	7.1	-
Organic carbon (%)[2]	0.06	0.1	2.3	
Organic matter (%)	0.1	0.2	4.0	-
Cation exchange capacity (meq/100 g)	4.0	3.4	20.4	-
Water Holding Capacity at 1/3 bar (%)	10.5	12.7	16.5	-
Bulk Density (disturbed) (g/cm3)	1.43	1.37	1.35	-

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-20: Characteristics of test soil for Georgia test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	sand	loamy sand	sandy loam	sandy clay loam
Sand (50 µm – 2 mm) (%)	89.3	83.3	76.5	69.3
Silt (2 µm – 50 µmm) (%)	8.0	8.0	8.0	7.2
Clay (< 2 µm) (%)	2.7	8.7	15.5	23.5
pH[1]	6.8	5.8	4.8	4.9
Organic carbon (%)[2]	0.6	0.2	0.06	0.2
Organic matter (%)	1.1	0.4	0.1	0.3
Cation exchange capacity (meq/100 g)	3.2	2.8	4.4	6.0
Water Holding Capacity at 1/3 bar (%)	5.9	7.2	-	21.2
Bulk Density (disturbed) (g/cm^3)	1.56	1.47	1.40	1.27
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	sandy clay loam	silt loam	sandy clay loam	sandy clay loam
Sand (50 µm – 2 mm) (%)	69.3	67.3	69.3	67.3
Silt (2 µm – 50 µmm) (%)	7.2	5.2	5.2	7.2

Clay (< 2 µm) (%)	23.5	27.5	25.5	25.5
pH[1]	4.9	4.8	4.3	4.3
Organic carbon (%)[2]	0.1	0.06	0.0	0.06
Organic matter (%)	0.2	0.1	0.0	0.1
Cation exchange capacity (meq/100 g)	6.4	6.6	5.5	5.3
Water Holding Capacity at 1/3 bar (%)	22.5	23.9	23.7	22.6
Bulk Density (disturbed) (g/cm3)	1.27	1.26	1.28	1.26

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-21: Characteristics of test soil for Iowa test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	silty clay loam	silty clay loam	silty clay	silty clay loam
Sand (50 µm – 2 mm) (%)	13.3	13.3	9.3	9.3
Silt (2 µm – 50 µmm) (%)	58.0	53.2	50.0	51.2
Clay (< 2 µm) (%)	28.7	33.5	40.7	39.5
pH[1]	6.0	6.0	5.7	6.2
Organic carbon (%)[2]	1.4	1.0	0.7	043
Organic matter (%)	2.4	1.7	1.2	0.6
Cation exchange capacity (meq/100 g)	20.5	22.6	23.7	20.9
Water Holding Capacity at 1/3 bar (%)	35.2	37.8	41.3	47.6
Bulk Density (disturbed) (g/cm^3)	1.03	1.09	1.06	0.89
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	silty clay	silty clay loam	silty clay loam	silty clay loam
Sand (50 µm – 2 mm) (%)	11.3	9.3	13.3	9.3
Silt (2 µm – 50 µmm) (%)	45.2	53.2	49.2	53.2
Clay (< 2 µm) (%)	43.5	37.5	37.5	37.5
pH[1]	6.2	6.5	6.8	7.1
Organic carbon (%)[2]	0.2	0.4	0.1	0.1
Organic matter (%)	0.4	0.7	0.2	0.2
Cation exchange capacity (meq/100 g)	26.9	23.0	23.3	22.4
Water Holding Capacity at 1/3 bar (%)	42.2	44.1	44.4	45.6
Bulk Density (disturbed) (g/cm3)	1.12	1.01	0.99	0.89

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-22: Characteristics of test soil for Minnesota test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	loam	loam	loam	loam
Sand (50 µm – 2 mm) (%)	50.5	50.5	48.5	48.5
Silt (2 µm – 50 µmm) (%)	32.0	32.0	30.0	28.0
Clay (< 2 µm) (%)	17.5	17.5	21.5	23.5
pH[1]	6.5	6.8	7.1	7.6
Organic carbon (%)[2]	3.1	2.3	1.1	0.8
Organic matter (%)	5.3	3.9	1.9	1.3
Cation exchange capacity (meq/100 g)	7.0	22.3	20.1	20.7
Water Holding Capacity at 1/3 bar (%)	37.8	37.0	35.2	33.0
Bulk Density (disturbed) (g/cm^3)	1.08	1.11	1.15	1.15
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	loam	silt loam	sandy loam	sandy loam
Sand (50 µm – 2 mm) (%)	48.5	26.0	57.3	67.3
Silt (2 µm – 50 µmm) (%)	28.0	53.3	23.2	14.0
Clay (< 2 µm) (%)	23.5	20.7	19.5	18.7
pH[1]	7.9	8.1	8.2	8.3

Organic carbon (%)[2]	0.5	0.1	0.1	0.1
Organic matter (%)	0.8	0.2	0.2	0.2
Cation exchange capacity (meq/100 g)	28.1	26.1	26.8	22.2
Water Holding Capacity at 1/3 bar (%)	30.5	29.5	26.2	26.1
Bulk Density (disturbed) (g/cm^3)	1.11	1.20	1.16	1.07

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-23: Characteristics of test soil for New York test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	sandy clay loam	clay loam	clay loam	clay
Sand (50 µm – 2 mm) (%)	53.3	25.3	21.3	25.3
Silt (2 µm – 50 µmm) (%)	24.0	42.0	46.0	32.0
Clay (< 2 µm) (%)	22.7	32.7	32.7	42.7
pH[1]	5.8	6.4	7.3	7.3
Organic carbon (%)[2]	1.2	0.5	0.06	0.2
Organic matter (%)	2.1	0.8	0.1	0.3
Cation exchange capacity (meq/100 g)	10.6	13.6	25.9	29.3
Water Holding Capacity at 1/3 bar (%)	19.2	44.0	28.9	32.3
Bulk Density (disturbed) (g/cm3)	1.14	1.09	1.17	1.12
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	clay loam	loam	clay loam	loam
Sand (50 µm – 2mm) (%)	29.3	33.3	33.3	41.3
Silt (2 µm – 50 µmm) (%)	38.0	40.0	39.2	36.0
Clay (< 2 µm) (%)	32.7	26.7	27.5	22.7
pH[1]	7.5	7.6	7.8	8.1
Organic carbon (%)[2]	0.1	0.06	0.1	0.0
Organic matter (%)	0.2	0.1	0.2	0.0
Cation exchange capacity (meq/100 g)	28.8	25.5	24.6	24.3
Water Holding Capacity at 1/3 bar (%)	34.6	21.5	23.9	21.0
Bulk Density (disturbed) (g/cm3)	1.15	1.15	1.24	1.21

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-24: Characteristics of test soil for Ohio test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	loam	clay loam	clay	clay
Sand (50 µm – 2 mm) (%)	27.3	33.3	23.3	21.3
Silt (2 µm – 50 µmm) (%)	49.2	39.2	33.2	33.2
Clay (< 2 µm) (%)	23.5	27.5	43.5	45.5
pH[1]	7.8	7.6	7.5	7.7
Organic carbon (%)[2]	0.8	0.7	0.6	0.5
Organic matter (%)	1.3	1.2	1.1	0.9
Cation exchange capacity (meq/100 g)	17.6	12.2	18.6	21.0
Water Holding Capacity at 1/3 bar (%)	28.8	29.2	34.5	35.7
Bulk Density (disturbed) (g/cm^3)	1.10	1.06	1.11	1.11
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	clay loam	clay loam	loam	loam
Sand (50 µm – 2 mm) (%)	31.3	30.0	34.0	36.0
Silt (2 µm – 50 µmm) (%)	31.2	37.3	39.3	37.3
Clay (< 2 µm) (%)	37.5	32.7	26.7	26.7
pH[1]	8.0	8.2	8.4	8.4
Organic carbon (%)[2]	0.5	0.3	0.4	0.2
Organic matter (%)	0.8	0.5	0.7	0.3

Cation exchange capacity (meq/100 g)	20.9	26.4	30.1	23.3
Water Holding Capacity at 1/3 bar (%)	32.8	28.6	24.0	22.8
Bulk Density (disturbed) (g/cm^3)	1.13	1.21	1.26	1.26

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-25: Characteristics of test soil for Texas test site

Parameter	**Result**			
Soil depth (cm)	**0-15.2**	**15.2-30.5**	**30.5-45.7**	**45.7-61.0**
Textural Class (USDA)	silt loam	loam	loam	silt loam
Sand (50 µm – 2 mm) (%)	22.0	34.0	38.0	30.0
Silt (2 µm – 50 µmm) (%)	57.3	45.3	39.3	53.3
Clay (< 2 µm) (%)	20.7	20.7	22.7	16.7
pH[1]	8.2	8.3	8.3	8.3
Organic carbon (%)[2]	0.5	0.4	0.4	0.4
Organic matter (%)	0.9	0.7	0.7	0.6
Cation exchange capacity (meq/100 g)	26.7	26.2	27.6	25.0
Water Holding Capacity at 1/3 bar (%)	31.5	31.1	28.4	28.9
Bulk Density (disturbed) (g/cm^3)	1.25	1.26	1.31	1.27
Parameter				
Soil depth (cm)	**61.0-76.2**	**76.2-91.4**	**91.4-106.7**	**106.7-121.9**
Textural Class (USDA)	sandy loam	loam	loam	sandy loam
Sand (50 µm – 2 mm) (%)	56.0	48.0	51.3	59.3
Silt (2 µm – 50 µmm) (%)	33.3	37.3	39.2	33.2
Clay (< 2 µm) (%)	10.7	14.7	9.5	7.5
pH[1]	8.3	8.2	7.8	8.1
Organic carbon (%)[2]	0.2	0.3	0.2	0.3
Organic matter (%)	0.3	0.5	0.4	0.5
Cation exchange capacity (meq/100 g)	23.0	24.3	24.0	22.3
Water Holding Capacity at 1/3 bar (%)	25.8	26.9	25.2	23.4
Bulk Density (disturbed) (g/cm3)	1.26	1.26	1.28	1.23

[1] Medium not given
[2] Calculated from organic matter according to OC = OM x 0.58

All test sites had a known two-year history of crop and pesticide use, and none of the test sites had been treated with Roundup herbicide or related chemistry during the two years preceding this study.

Test plots were maintained in a weed free condition by hand weeding and/or the use of maintenance herbicides which were approved in advance. Irrigation was applied when necessary and where it was consistent with the local crop-growing practices or required to compensate for deficiencies of rainfall.

Weather data (depending on the location, mostly precipitations, air temperature, ground temperature and wind speed, solar radiation and evapotranspiration) was collected at each location, from test site research station instruments and/or from nearby, permanent, institutional weather recording stations (NOAA and others). The climatological data indicate that environmental conditions at all test sites during the study were within the normal conditions and revealed no major deviations from expected weather patterns. The prescribed sampling schedule was not significantly altered by climatological factors at any of the eight test sites, with the exception of the Iowa, Minnesota, and Texas test sites.

The final 18 months after treatment samples were not taken at the Iowa and Minnesota test sites due to frozen ground conditions. As a result of extensive flooding of the Texas test site on December 23-25, 1991, the Texas test site was terminated on March 6, 1992. Therefore, no sampling from the Texas test site occurred following the 6 months after application sampling event.

2. Application

Single applications of Roundup herbicide were made to each bare ground, replicate test plot at each test site according to label directions using normal agronomic practices. The average application rates used for this study was **9.07 kg a.s./ha** and ranged from a high rate of 9.90 kg a.s./ha at the California test

site to a low rate of 8.79 kg a.s./ha at the New York test site. The total test substance spray solution volume ranged from 137.9 L/ha to 246.1 L/ha. Test substance application spray equipment was calibrated prior to application.

3. Sampling

Soil samples were randomly collected from both the treated and control test plots at each test site and sampling event. Early time point soil samples to define the dissipation of glyphosate were collected at 1, 7, 14, and 21 days after test substance application at all test sites with the exception of the Minnesota and Texas test sites. In the case of the Minnesota test site, the 14 days after application sampling event occurred 15 days after application. For the Texas test site, the 7, 14 and 21 days after application sampling events occurred on 12, 15, and 28 days after application. Longer term time point samples were collected at approximately 1, 2, 3, 4, 6, 12, 15, and 18 months after test substance application, with the exception of the Iowa and Minnesota test sites.

For each sampling event, 18 soil core samples were collected from the treated test plot (6 from each of three subplots) to a depth of 121.9 cm. For the control test plot, 4 soil cores to a depth of 121.9 cm were collected at each sampling event. The untreated plot was always sampled first followed by the treated plot. With the exception of the 0-15 cm pre-excavation samples, soil samples at all sites were collected using "zero contamination" commercial soil coring equipment with removable acetate liners.

4. Specimen handling and preparation

The cores were cut into 15 cm sections in a clean area away from the field. Check (untreated) cores were sectioned first. Sectioning was performed from the bottom of the cores to the top of the cores to prevent contamination. Replicate soil cores for each sampling event for a given 15 cm depth increment were packaged together for storage and subsequent shipment to Monsanto. All samples were frozen within 4 hours of collection and were kept frozen during storage at the test site prior to shipment to Monsanto with one exception. The storage temperature for the 11 days after application (DAA) samples for the Texas location rose above freezing for approximately 24 hours due to equipment failure before the samples were transferred to another freezer and refrozen. All samples were shipped frozen to Monsanto, and all shipments were accompanied by an inventory list of the samples in the shipment that served as sample transfer document or chain-of-custody record.

5. Analytical methods

Glyphosate and AMPA were extracted from soil using a 0.5 N KOH solution. The extract solution was eluted through a Chelex 100 resin in the Fe(III) form, which retains glyphosate and AMPA due to chelation to Fe(III). The retained glyphosate and AMPA iron salts are removed from the Chelex resin by elution with 6 N HCl. The isolated glyphosate and AMPA iron salts are then applied to a strong anion exchange resin and eluted with 6 N HCl to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness to remove the HCl, the samples are re-dissolved in water and analysed by high pressure liquid chromatography (HPLC). The chromatograph uses column switching and an o-phthalaldehyde post-column reactor with a fluorescence detector to separate and quantitate glyphosate and AMPA. In the post-column reactor, glyphosate is oxidised to a primary amine which then reacts with o-phthalaldehyde to form a fluorescence derivative. AMPA reacts directly with o-phthalaldehyde to form a second fluorescence derivative. This method has been validated down to 0.05 mg/kg for both glyphosate and AMPA in 30 g soil samples.

Due to the varying degrees of glyphosate adsorption to different soil types, glyphosate recoveries from fortified check samples vary with soil type, and obtaining consistent recoveries of glyphosate is occasionally difficult. Nonetheless, the analytical method used generally affords recoveries of glyphosate from fortified check samples which are greater than 70 %. AMPA recoveries are normally higher than glyphosate recoveries. The recoveries from check samples fortified over the range of 0.05 mg/kg to 5.00 mg/kg with both glyphosate and AMPA, averaged across all test sites, were 77.8 % and 85.4 %, respectively, for glyphosate and AMPA. The average recoveries of glyphosate ranged from a high of 88.8 % from soil from the Georgia test site to a low of 65.0 % from soil from the Iowa test site. Average recoveries of AMPA ranged from a high of 87. 5% from soil from the Minnesota test site to a low of 81.0 % from soil from the Iowa test site.

The limit of detection (LOD) was set at 0.02 mg/kg for glyphosate and 0.04 mg/kg for AMPA.

The stability of glyphosate and AMPA in soil was confirmed by a storage stability study (see [redacted], 1993b).

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-26: Results of glyphosate residues (mg/kg) analysis in Arizona soil following treatment with Roundup at 9.05 kg/ha

Depth	DAT														
(cm)	Rep.	-1	0	1	7	14	21	28	64	92	122	184	364	462	553
0-15.2	A	-	1.34	2.45	2.23	0.63	1.05	1.28	0.41	0.16	0.16	0.04	0.03	<LOQ	0.01
	B	-	1.27	2.57	2.67	0.53	4.50	0.57	0.37	0.13	0.12	0.02	0.04	<LOQ	-
	C	-	3.11	1.16	1.79	0.60	0.19	0.45	0.24	0.13	0.10	0.10	0.04	-	-
15.2-30.5	A	-	0.01	0.01	0.01	-	0.01	-	0.01	-	-	-	-	-	-
	B	-	0.01	0.01	0.18	-	-	-	0.01	-	-	-	-	-	-
	C	-	0.02	-	0.06	-	0.03	-	-	-	-	0.08	0.01	0.03	-
30.5-45.7	A	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
	B	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	0.02	-	-	-	-	-	-	-	-	-	0.01
45.7-61.0	A	-	-	-	-	-	-	-	-	-			-	-	-
	B	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-
	C	0.01	-	-	-	-	0.15	-	-	0.01	-	-	-	-	-
61.0-76.2	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	-													
	B	-													
	C	<LOQ													
91.4-106.7	A	<LOQ													
	B	0.01													
	C	-													
106.7-121.9	A	-													
	B	<LOQ													
	C	-													

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-27: Results of metabolite AMPA (mg/kg) residues analysis in Arizona soil following treatment with Roundup at 9.05 kg/ha

Depth	DAT														
(cm)	Rep.	-1	0	1	7	14	21	28	64	92	122	184	364	462	553
0-15.2	A	-	0.07	0.16	0.21	0.21	0.46	0.47	0.48	0.26	0.37	0.27	0.14	0.08	0.07
	B	-	0.08	0.11	0.23	0.16	1.16	0.37	0.52	0.27	0.34	0.15	0.14	0.05	0.05
	C		0.17	0.15	0.24	0.17	0.07	0.19	0.38	0.30	0.36	0.35	0.22	<LOQ	0.04
15.2-30.5	A	-	-	-	-	-	-	-	0.04	-	-	-	-	-	0.03
	B	-	-	-	0.02	-	-	-	0.03	-	-	-	-	-	-
	C	0.01	-	-	0.01	-	-	-	-	-	-	0.09	-	-	-
30.5-45.7	A	-	-	-	-	0.02	-	-	-	-	-	-	-	-	0.01
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
45.7-61.0	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	0.18	-	-				-	-	-
61.0-76.2	A	-													
	B	-													
	C	-													

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	28	64	92	122	184	364	462	553
76.2-91.4	A	-													
	B	-													
	C	-													
91.4-106.7	A	<LOQ													
	B	-													
	C														
106.7-121.9	A	-													
	B	-													
	C	-													

"-": not found
Blank: not measured
Rep. = Replicate
DAT: days after treatment

Table 8.1.1.3-28: Results of glyphosate residues (mg/kg) analysis in California soil following treatment with Roundup at 9.90 kg/ha

Depth (cm)	DAT														
	Rep.	-8	0	1	7	14	21	29	61	91	123	183	365	456	550
0-15.2	A	-	1.36	2.11	1.47	0.56	0.44	0.58	0.48	0.19	0.05	0.07	0.04	0.03	-
	B	-	0.72	1.97	1.40	0.94	0.65	0.62	0.52	0.10	0.04	0.07	0.04	0.04	0.04
	C		1.27	1.75	0.85	1.78	0.45	0.67	0.46	0.17	0.06	0.09	0.04	0.04	0.03
15.2-30.5	A	-	0.18	0.04	0.04	-	0.03	0.03	<LOQ	0.02	0.02	-	-	0.02	-
	B	-	0.38	0.04	0.04	-	0.05	0.03	-	0.01	0.01	-	0.01	-	-
	C		0.33	0.06	-	-	0.04	-	0.06	0.02	<LOQ	-	0.01	-	-
30.5-45.7	A	-	0.24	0.01	0.01	0.01	0.03	0.02	-	-	0.02	-	<LOQ	-	-
	B	0.01	0.23	0.01	0.01	0.01	0.01	0.03	-	0.01	0.02	-	<LOQ	-	-
	C	0.01	0.17	0.02	0.01	0.01	0.01	<LOQ	-	0.01	0.04	-	-	-	-
45.7-61.0	A	<LOQ	0.12	0.01	<LOQ	0.01	0.01	0.01		0.01	-	-			
	B	0.01	0.06	<LOQ	<LOQ	-	<LOQ	0.01		-	-	-			
	C		0.11	<LOQ	<LOQ	0.01	0.02	<LOQ		0.01	-	-			
61.0-76.2	A	-	0.05												
	B	0.01	0.03												
	C	0.01	0.03												
76.2-91.4	A	<LOQ	-												
	B	0.01	-												
	C	-	-												
91.4-106.7	A	-	-												
	B	-	-												
	C		-												
106.7-121.9	A	-													
	B	-													
	C	0.01													

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-29: Results of metabolite AMPA (mg/kg) residues analysis in California soil following treatment with Roundup at 9.90 kg/ha

Depth (cm)	DAT														
	Rep.	-8	0	1	7	14	21	29	61	91	123	183	365	456	550
0-15.2	A	-	0.16	0.15	0.34	0.20	0.26	0.33	0.34	0.25	0.25	0.20	0.26	0.31	0.25
	B	-	0.11	0.14	0.35	0.24	0.35	0.38	0.34	0.25	0.16	0.23	0.27	0.34	0.33
	C		0.13	0.13	0.23	0.63	0.33	0.33	0.33	0.26	0.19	0.19	0.32	0.40	0.34
15.2-30.5	A	-	-	-	-	-	0.03	0.04	0.04	0.04	0.04	-	0.03	0.06	0.07
	B	-	-	-	-	0.02	0.02	0.01	0.011	0.03	0.05	-	0.02	0.04	0.02
	C		-	-	-	0.02	0.02	0.01	0.05	-	0.03	-	0.04	0.02	0.03

30.5-45.7	A	-	0.02	-	-	-	-	-	-	-	-	-	0.02	-	0.02
	B	-	-	-	-	-	-	-	-	-	-	-	0.02	-	0.01
	C	-	0.02	-	-	-	-	-	-	-	-	-	-	-	0.02
45.7-61.0	A	-	0.03	-	-	-	0.02			-	-	-			
	B	-	-	-	-	0.04	-			-	-	-			
	C		0.03	-	-	-	<LOQ			-	-	-			
61.0-76.2	A	-	-												
	B	-	-												
	C	-	-												
76.2-91.4	A	-	-												
	B	-	-												
	C		-												
91.4-106.7	A	0.01	-												
	B	0.03	-												
	C		-												
106.7-121.9	A	-													
	B	-													
	C	-													

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-30: Results of glyphosate residues (mg/kg) analysis in Georgia soil following treatment with Roundup at 8.95 kg/ha

Depth	DAT														
(cm)	Rep.	-2	0	1	7	14	21	31	61	94	123	188	368	459	550
0-15.2	A	-	3.36	1.79	1.61	1.21	0.76	0.73	0.38	0.24	0.11	0.15	0.12	0.05	0.03
	B	-	2.81	3.11	1.64	1.54	1.01	0.48	0.60	0.14	0.08	0.24	0.10	0.06	0.03
	C		3.02	2.91	1.66	1.32	1.05	0.48	0.33	0.18	0.10	0.14	0.07	0.05	0.04
15.2-30.5	A	0.01	-	-	0.01	0.02	0.02	0.02	-	-	0.03	-	0.01	0.02	-
	B	0.01	-	0.02	0.01	0.01	0.02	0.02	-	-	-	-	<LOQ	-	-
	C		-	0.01	0.01	0.01	0.02	0.02	-	-	-	-	<LOQ	-	0.01
30.5-45.7	A	-	-	-	0.02	0.01	0.01	0.01	-	<LOQ	-	<LOQ	<LOQ	-	-
	B	-	-	0.02	0.03	0.01	0.01	-	<LOQ	<LOQ	-	0.01	<LOQ	-	-
	C	0.01	-	0.02	0.03	0.01	0.01	0.01	-	-	0.01	0.01	<LOQ	-	-
45.7-61.0	A	-	-	-	-	0.02	-	-		0.01	0.01	-			
	B	-	-	-	-	-	-	-		-	0.02	0.02			
	C		-	-	0.01	-	-	-		0.02	0.02	-			
61.0-76.2	A	-													
	B	0.01													
	C														
76.2-91.4	A	-													
	B	-													
	C	0.01													
91.4-106.7	A	<LOQ													
	B	-													
	C	-													
106.7-121.9	A	-													
	B	0.01													
	C														

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-31: Results of metabolite AMPA (mg/kg) residues analysis in Georgia soil following treatment with Roundup at 8.95 kg/ha

Depth (cm)	DAT														
	Rep.	-2	0	1	7	14	21	31	61	94	123	188	368	459	550
0-15.2	A	0.02	0.07	0.06	0.30	0.27	0.21	0.47	0.47	0.62	0.39	0.56	0.49	0.23	0.18
	B	0.02	0.05	0.09	0.27	0.32	0.30	0.27	0.86	0.38	0.26	0.50	0.44	0.28	0.28
	C		0.07	0.08	0.26	0.30	0.34	0.29	0.48	0.39	0.32	0.52	0.38	0.26	0.27
15.2-30.5	A	-	-	-	-	-	-	-	0.02	-	0.01	-	0.02	-	0.06
	B	-	-	-	-	-	-	-	-	-	-	-	0.02	-	0.02
	C		-	-	-	-	-	-	-	-	-	-	0.04	-	-
30.5-45.7	A	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
	B	-	-	0.08	-	-	-	-	-	-	-	-	0.02	-	0.03
	C	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-
45.7-61.0	A	-	-	-	-	0.02	-	-		-	-	-			
	B	-	-	-	-	-	-	-		-	-	-			
	C		-	-	0.01	-	-	-		0.01	-	-			
61.0-76.2	A	-													
	B	-													
	C														
76.2-91.4	A	-													
	B	-													
	C	-													
91.4-106.7	A	-													
	B	-													
	C	-													
106.7-121.9	A	-													
	B	-													
	C														

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-32: Results of glyphosate residues (mg/kg) analysis in Iowa soil following treatment with Roundup at 8.90 kg/ha

Depth (cm)	DAT													
	Rep.	-1	0	1	7	14	21	29	62	92	123	190	366	458
0-15.2	A	0.01	2.29	1.59	1.69	1.47	1.76	1.25	1.21	0.33	2.71	0.40	0.52	0.55
	B	0.01	1.36	2.70	2.75	1.42	1.09	0.78	3.42	1.07	0.54	0.83	1.09	0.30
	C	0.01	2.02	2.74	1.54	2.23	1.61	2.63	1.45	0.58	0.48	1.19	0.87	0.51
15.2-30.5	A	-	0.05	0.08	0.06	0.14	0.08	0.12	0.02	0.03	0.07	0.04	0.01	0.03
	B	-	0.04	0.01	0.08	0.10	0.06	0.10	0.03	0.11	0.03	0.03	<LOQ	<LOQ
	C		0.02	0.04	0.05	0.11	0.08	0.12	0.03	0.23	-	0.04	0.01	0.03
30.5-45.7	A	-	0.05	<LOQ	0.02	0.04	0.03	0.07	0.01	<LOQ	<LOQ	0.06	<LOQ	0.01
	B	0.01	0.01	0.03	0.02	0.02	0.02	0.03	<LOQ	<LOQ	0.04	0.04	<LOQ	-
	C		-	-	0.02	0.02	0.02	0.03	<LOQ	<LOQ		0.04	-	-
45.7-61.0	A	-	-	-	-	0.01	-	-		0.02	0.01	-		
	B	-	-	0.04	-	0.01	-	0.01		0.02	-	0.01		
	C		-	-	-	0.01	-	-		0.02	0.01	0.01		
61.0-76.2	A	-												
	B	-												
	C	0.01												
76.2-91.4	A	<LOQ												
	B	-												
	C	-												
91.4-106.7	A	-												
	B	-												
	C	-												
106.7-121.9	A	-												
	B	-												

	C	-												

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-33: Results of metabolite AMPA (mg/kg) residues analysis in Iowa soil following treatment with Roundup at 8.90 kg/ha

Depth	DAT													
(cm)	Rep.	-1	0	1	7	14	21	29	62	92	123	190	366	458
0-15.2	A	-	0.05	0.05	0.12	0.10	0.16	0.11	0.27	0.08	0.62	0.16	0.38	0.61
	B	-	0.04	0.07	0.16	0.10	0.09	0.06	0.53	0.22	0.16	0.40	0.67	0.41
	C		0.05	0.08	0.09	0.14	0.19	0.34	0.27	0.09	0.17	0.33	0.56	0.73
15.2-30.5	A	-	-	-	-	0.03	0.02	0.02	0.01	0.04	0.03	-	0.02	0.05
	B	-	-	-	-	0.03	-	0.02	0.02	0.07	0.05	0.04	0.01	-
	C		-	-	-	-	0.02	0.03	-	0.12		0.05	0.02	<LOQ
30.5-45.7	A	-	-	-	-	-	-	0.02	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	0.02
	C		-	-	-	-	-	-	-	0.02	-		-	<LOQ
45.7-61.0	A	-	-	-	-	-	-	-		-	-	<LOQ	-	-
	B	-	-	-	-	-	-	-		-	-	0.01	-	-
	C		-	-	-	-	-	-		-	-	0.01	-	-
61.0-76.2	A	-												
	B	-												
	C													
76.2-91.4	A	-												
	B	-												
	C													
91.4-106.7	A	-												
	B	-												
	C	0.04												
106.7-121.9	A	-												
	B	-												
	C	-												

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-34: Results of glyphosate residues (mg/kg) analysis in Minnesota soil following treatment with Roundup at 9.02 kg/ha

Depth	DAT													
(cm)	Rep.	-3	0	1	7	15	21	35	71	95	129	179	372	475
0-15.2	A	-	1.19	1.59	1.82	2.41	1.45	0.40	0.35	0.46	0.26	0.22	0.05	0.05
	B	-	0.92	0.90	1.04	1.50	0.79	0.49	0.15	0.19	0.15	0.08	0.03	0.09
	C	0.03	1.01	1.64	2.09	1.54	2.09	0.91	0.17	0.17	0.19	0.10	0.05	0.03
15.2-30.5	A	-	0.02	0.06	0.05	0.03	0.03	0.01	0.02	0.01	0.01	0.01	-	-
	B	-	0.02	0.06	0.10	0.10	0.02	-	<LOQ	0.01	0.02	-	-	-
	C		-	0.03	0.07	0.04	0.02	<LOQ	<LOQ	0.01	0.02	0.02	-	-
30.5-45.7	A	-	<LOQ	0.01	-	0.02	-	-	-	-	0.01	-	-	-
	B	-	-	-	-	0.02	-	-	-	-	0.01	0.01	-	-
	C	-	-	-	0.01	-	-	-	-	-	-	-	<LOQ	-
45.7-61.0	A	-	-	0.01	-	-	-	-						
	B	0.01	-	-	-	-	-	-						
	C		-	-	-	-	-	-						
61.0-76.2	A	-												
	B	-												
	C	0.01												

76.2-91.4	A	-												
	B	-												
	C													
91.4-106.7	A	-												
	B	-												
	C													

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-35: Results of metabolite AMPA (mg/kg) residues analysis in Minnesota soil following treatment with Roundup at 9.02 kg/ha

Depth	DAT													
(cm)	Rep.	-3	0	1	7	15	21	35	71	95	129	179	372	475
0-15.2	A	-	0.15	0.26	0.23	0.24	0.31	0.27	0.66	0.62	0.42	0.45	0.19	0.21
	B	-	0.17	0.13	0.25	0.21	0.17	0.30	0.27	0.37	0.39	0.25	0.11	0.35
	C		0.15	0.20	0.29	0.22	0.34	0.51	0.26	0.29	0.28	0.28	0.16	0.14
15.2-30.5	A	-	-	-	-	-	-	-	-	0.03	0.02	0.02	-	-
	B	-	0.03	-	-	0.02	-	-	-	0.02	-	0.02	-	-
	C		-	-	-	-	-	-	-	0.02	-	0.02	-	0.01
30.5-45.7	A	-	-	-	-	-	-	-	-	0.03	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	0.02
	C	-	-	-	-	-	-	-	-	-	-	-	-	0.02
45.7-61.0	A	-	0.04	-	-	-	-	-						
	B	-	-	-	-	-	-	-						
	C		-	-	-	-	-	-						
61.0-76.2	A	-												
	B	-												
	C	-												
76.2-91.4	A	-												
	B	-												
	C													
91.4-106.7	A	-												
	B	-												
	C													

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-36: Results of glyphosate residues (mg/kg) analysis in New York soil following treatment with Roundup at 8.79 kg/ha

Depth	DAT														
(cm)	Rep.	-1	0	1	7	14	21	30	61	90	120	180	362	453	546
0-15.2	A	-	4.65	2.48	1.45	4.84	3.71	2.04	1.70	2.18	1.26	1.47	0.40	0.79	0.38
	B	-	1.64	2.81	1.62	3.84	5.57	4.47	1.34	2.28	0.71	0.88	0.88	0.72	0.84
	C	0.02	1.95	2.34	2.41	5.05	4.27	1.44	1.82	1.48	1.48	1.62	0.26	-	0.64
15.2-30.5	A	<LOQ	-	-	0.03	0.01	0.03	0.20	0.03	-	0.02	0.02	0.02	0.03	-
	B	-	-	0.01	0.01	0.03	-	0.02	0.02	0.11	0.04	0.05	0.01	0.06	0.01
	C	0.02	0.05	0.01	-	0.03	-	0.06	0.03	0.09	0.02	0.01	0.03	0.06	0.03
30.5-45.7	A	<LOQ	-	-	-	<LOQ	<LOQ	<LOQ	<LOQ	0.02	0.03	0.03	-	-	<LOQ
	B	-	-	-	0.01	-	0.01	-	0.01	0.02	-	0.02	<LOQ	0.02	-
	C	-	-	-	-	-	-	-	0.01	0.03	0.02	0.02	-	0.01	0.02
45.7-61.0	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-	-	-	0.01	0.01	0.02			
	B	<LOQ	<LOQ	<LOQ	<LOQ	-	0.01	0.01	-	0.01	0.02	0.02			
	C		<LOQ	<LOQ	-	<LOQ	-	0.01	-	-	-	0.02			
	A	-													

61.0-76.2	B	-													
	C	0.01													
76.2-91.4	A	-													
	B	-													
	C	-													
91.4-106.7	A	-													
	B	-													
	C	-													
106.7-121.9	A	-													
	B	-													
	C														

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-37: Results of metabolite AMPA (mg/kg) residues analysis in New York soil following treatment with Roundup at 8.79 kg/ha

Depth	DAT														
(cm)	Rep.	-1	0	1	7	14	21	30	61	90	120	180	362	453	546
0-15.2	A	0.02	0.17	0.10	0.12	0.39	0.39	0.41	0.23	0.54	0.30	0.46	0.19	0.28	0.21
	B	0.03	0.09	0.10	0.14	0.36	0.44	0.62	0.27	0.58	0.29	0.25	0.27	0.56	0.49
	C	0.04	0.08	0.08	0.20	0.55	0.44	0.26	0.32	0.32	0.41	0.57	0.14	-	0.37
15.2-30.5	A	0.03	0.06	0.02	0.05	0.04	0.06	0.08	0.04	-	0.03	0.03	0.03	0.08	-
	B	-	-	0.01	-	0.06	-	0.04	0.02	0.06	0.06	0.05	0.02	0.09	0.06
	C	0.05	-	-	-	0.08	0.02	0.05	0.03	0.06	0.04	0.03	0.03	0.10	0.10
30.5-45.7	A	<LOQ	-	-	-	0.01	0.01	0.02	-	0.04	0.04	0.05	-	-	-
	B	-	-	-	0.04	0.01	0.02	-	0.02	0.03	-	-	-	-	-
	C	-	-	-	-	0.01	-	-	-	0.04	0.04	0.04	-	-	-
45.7-61.0	A	-	-	<LOQ	<LOQ	-	-	-	-	-	-	-			
	B	0.01	-	-	<LOQ	-	-	-	-	-	-	-			
	C		<LOQ	0.01	-	<LOQ	-	-	-	-	-	-			
61.0-76.2	A	-													
	B	-													
	C														
76.2-91.4	A	-													
	B	-													
	C														
91.4-106.7	A	-													
	B	-													
	C	-													
106.7-121.9	A	-													
	B	-													
	C														

"-": not found
Blank: not measured

Rep. Replicate
DAT= days after treatment

Table 8.1.1.3-38: Results of glyphosate residues (mg/kg) analysis in Ohio soil following treatment with Roundup at 9.12 kg/ha

Depth	DAT														
(cm)	Rep.	-1	0	1	7	14	21	30	61	90	121	177	365	455	545
0-15.2	A	-	2.30	2.32	0.70	0.51	0.73	0.44	0.12	0.07	0.05	0.02	0.03	0.02	-
	B	0.01	1.45	1.34	0.49	0.37	0.75	0.78	0.15	0.09	0.03	0.02	0.03	0.02	0.02
	C	0.02	2.29	1.83	0.78	0.65	0.62	0.56	0.09	0.08	0.09	0.06	0.01	0.02	0.01
	A	<LOQ	<LOQ	<LOQ	<LOQ	0.011	0.03	0.02	-	-	-	-	0.01	-	-

15.2-30.5	B	-	<LOQ	0.01	<LOQ	0.03	0.02	0.03	-	0.02	-	-	<LOQ	-	-
	C	<LOQ	<LOQ	<LOQ	<LOQ	0.01	0.02	-	0.02	0.02	-	-	-	-	-
30.5-45.7	A	-	-	-	<LOQ	-	-	-	-		-	-	-	-	-
	B	-	-	<LOQ	<LOQ	-	0.01	-	-	<LOQ	-	-	<LOQ	-	-
	C		-	-	<LOQ	-	0.02	-	0.01	<LOQ	-	-	-	-	-
45.7-61.0	A	-													
	B														
	C														
61.0-76.2	A	-													
	B	0.01													
	C														
76.2-91.4	A	<LOQ													
	B	-													
	C	-													
91.4-106.7	A	-													
	B	-													
	C														
106.7-121.9	A	-													
	B														
	C														

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-39: Results of metabolite AMPA (mg/kg) residues analysis in Ohio soil following treatment with Roundup at 9.12 kg/ha

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	30	61	90	121	177	365	455	545
0-15.2	A	-	0.29	0.43	0.56	0.33	0.69	0.45	0.34	0.22	0.18	0.18	0.12	0.10	0.05
	B	-	0.28	0.28	0.47	0.34	0.58	0.74	0.41	0.32	0.17	0.14	0.15	0.08	0.07
	C	0.02	0.45	0.33	0.65	0.61	0.53	0.46	0.37	0.25	0.38	0.30	0.10	0.09	0.08
15.2-30.5	A	-	-	-	<LOQ	0.04	0.04	-	-	-	0.03	-	-	0.03	-
	B	-	-	<LOQ	<LOQ	0.04	0.05	0.05	0.03	-	-	-	0.02	-	-
	C	-	-	-	-	0.03	-	-	0.04	0.03	-	-	-	-	-
30.5-45.7	A	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-
	C		-	-	-	-	-	-	-	-	-	-	-	-	-
45.7-61.0	A	-													
	B														
	C														
61.0-76.2	A	-													
	B	-													
	C														
76.2-91.4	A														
	B	-													
	C	-													
91.4-106.7	A	-													
	B	-													
	C														
106.7-121.9	A	-													
	B														
	C														

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-40: Results of glyphosate residues (mg/kg) analysis in Texas soil following treatment with Roundup at 8.80 kg/ha

Depth (cm)	DAT											
	Rep.	-1	0	1	11	14	27	30	61	91	122	183
0-15.2	A	-	1.46	1.68	0.95	-	0.04	0.16	0.02	0.02	0.02	<LOQ
	B	-	1.58	1.49	1.21	-	0.15	0.19	0.01	0.03	<LOQ	-
	C	0.04	2.75	1.64	1.17	0.04	0.21	0.11	0.01	0.02	<LOQ	-
15.2-30.5	A	-	0.06	0.05	0.01	0.02	-	-	-	-	-	-
	B	-	0.05	0.02	-	-	<LOQ	-	-	0.01	-	0.01
	C	0.02	0.07	0.01	0.01	-	-	-	<LOQ	-	-	-
30.5-45.7	A	-	-	-	-	0.01	-	-	-	-	-	0.04
	B	-	-	-	-	-	-	-	<LOQ	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
45.7-61.0	A	-	-	-	-	-	-	-				
	B	-	-	-	-	-	-	0.01				
	C		-	-	-	-	-	-				
61.0-76.2	A	-										
	B	0.01										
	C											
76.2-91.4	A	0.01										
	B											
	C											
91.4-106.7	A	-										
	B											
	C											
106.7-121.9	A	-										
	B											
	C											

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

Table 8.1.1.3-41: Results of metabolite AMPA (mg/kg) residues analysis in Texas soil following treatment with Roundup at 8.80 kg/ha

Depth (cm)	DAT											
	Rep.	-1	0	1	11	14	27	30	61	91	122	183
0-15.2	A	0.02	0.10	0.13	0.20	-	-	0.27	0.12	0.08	0.14	0.02
	B	-	0.10	0.11	0.31	-	0.24	0.34	0.15	0.16	0.06	0.02
	C	-	0.12	0.13	0.29	-	0.22	0.20	0.07	0.07	0.08	0.02
15.2-30.5	A	-	0.03	0.02	-	-	-	-	-	-	0.02	-
	B	-	0.02	-	-	-	-	-	<LOQ	0.02	-	-
	C	-	0.02	-	-	-	-	-	-	-	-	-
30.5-45.7	A	-	0.02	-	-	-	-	-	-	0.03	-	0.04
	B	-	-	-	-	0.03	-	-	-	0.03	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
45.7-61.0	A	-	-	-	-	-	-	-				
	B	-	-	-	-	-	-	-				
	C		-	-	-	-	-	0.04				
61.0-76.2	A	-										
	B	-										
	C											
76.2-91.4	A	-										
	B											
	C											
91.4-106.7	A	0.03										
	B											
	C											
	A	-										

106.7-121.9	B											
	C											

"-": not found
Blank: not measured
Rep. = Replicate
DAT= days after treatment

B. Characterisation of residues

1. Arizona test site

Residues of glyphosate averaged less than 0.05 mg/kg in all soil samples taken below 15.2 cm, with two exceptions. The 15.2-30.5 cm sample at 7 DAT contained an average glyphosate residue level of 0.08 mg/kg. In addition, one of the three replicate samples from the 45.7-61.0 cm sample at 21 DAT contained 0.15 mg/kg glyphosate. However, the other two replicate samples from the 45.7-61.0 cm depth at 21 DAT failed to contain detectable amounts of glyphosate. Since only one of the three replicate samples had measurable glyphosate residues and all the other samples that bracket this sample by depth and time contained no glyphosate residues, contamination of this sample is suspected. These residues were also attributed to contamination during sampling rather than vertical mobility due to the absence of supporting residues in the 15.2-30.5 cm and 30.5-45.7 cm soil horizons at 21 days after treatment and the absence of residues below 30.5 cm in subsequent soil samples.

AMPA residues were below LOD in all soil samples taken below 15.2 cm except for 0.18 mg/kg, which was detected in the same 45.7-61.0 cm soil sample at 21 DAT described above which contained 0.15 mg/kg glyphosate. Since the other two replicate samples from this depth interval and sampling event did not contain detectable amounts of AMPA, contamination of this sample was suspected.

2. California test site

The average residue level of glyphosate in the 0-15.2 cm layer was 1.12 mg/kg at 0 DAT. Average glyphosate residues increased to a maximum of 1.94 mg/kg at 1 DAT, and then gradually dissipated to less than 0.02 mg/kg at 550 DAT. The average AMPA residue level in the top 15.2 cm of soil measured 0.13 mg/kg at 0 DAT. Average AMPA residues reached a maximum concentration of 0.36 mg/kg at 14 DAT, and then declined to 0.31 mg/kg at 550 DAT.

California was one of two locations at which pre-excavation of the top 15.2 cm of the soil was not performed prior to soil core sampling. Consequently, the day zero samples showed evidence of contamination at lower depths. Residues of glyphosate were found in all the 0 DAT samples analysed to a depth of 61.0 cm. For day 0, the average glyphosate residues were 0.30 mg/kg in the 15.2-30.5 cm layer, 0.21 mg/kg in the 30.5-45.7 cm layer, 0.10 mg/kg in the 45.7-61.0 cm layer, and 0.04 mg/kg in the 61.0-76.2 cm layer. These residues were attributed to contamination during sampling rather than vertical mobility due to the depth of the residues at such an early time point following test substance application and the absence of supporting concentrations of glyphosate below 30.5 cm in subsequent soil samples. In all other samples below 15.2 cm, glyphosate residues were equal or less than 0.05 mg/kg. AMPA residues were less than 0.05 mg/kg in all soil samples taken below 15.2 cm.

3. Georgia test site

The maximum average residue level of glyphosate in the 0 to 15.2 cm soil layer was 3.06 mg/kg at 0 DAT, and declined steadily to 0.03 mg/kg at 550 DAT. The average residue level of AMPA in the top 15.2 cm measured 0.06 mg/kg at 0 DAT. Average AMPA residues reached a maximum of 0.60 mg/kg at 61 DAT, and then declined to 0.24 mg/kg at 550 DAT. Average glyphosate and AMPA residues were less than 0.05 mg/kg for all samples taken below 15.2 cm, demonstrating that glyphosate and AMPA did not move vertically in the soil profile at this test site.

4. Iowa test site

The average residue level of glyphosate in the 0 to 15.2 cm layer was 1.89 mg/kg at 0 DAT, and reached a maximum concentration of 2.34 mg/kg at 1 DAT. Average glyphosate residues then declined slowly to 0.45 mg/kg at 458 DAT. The average AMPA residue level in the top to 15.2 cm was 0.05 mg/kg at

0 DAT. Average AMPA residues rose to 0.36 mg/kg at 62 DAT, declined slightly to 0.31 mg/kg at 366 DAT, and increased again to 0.58 mg/kg at 458 DAT sampling.

Average glyphosate residues ranging from 0.05 to 0.12 mg/kg were found in the 15.2-30.5 cm layer of all sampling events between 7 and 123 DAT, except for the 62 DAT sampling. Glyphosate residues averaged less than 0.05 mg/kg for all samples taken below 30.5 cm with one exception; an average glyphosate residue level of 0.05 mg/kg was found in the 30.5-45.7 cm soil horizon at 190 days after treatment. However, this residue was attributed to contamination during sampling rather than vertical mobility due to the absence of supporting residues in the 15.2-30.5 cm soil horizon at 190 days after treatment and the absence of residues below 30.5 cm in subsequent soil samples. AMPA residues were less than 0.05 mg/kg in all soil samples taken below 30.5 cm except for an average level of 0.08 mg/kg found in the 15.2-30.5 cm layer at 92 DAT.

Iowa was the second of two locations, at which pre-excavation of the top 30.5 cm of the soil was not performed prior to soil core sampling. Since Iowa did have more instances and generally higher residues of glyphosate and AMPA in the 15.2-30.5 cm soil layer than other locations, it was postulated that contamination during sampling contributed, at least in part, to the residues found in the 15.2-30.5 cm layer.

The 18 months after application sampling was not taken at the Iowa because the ground was frozen too hard to permit sampling.

5. Minnesota test site

The average residue level of glyphosate in the 0 to 15.2 cm layer measured 1.04 mg/kg at 0 DAT. The maximum average glyphosate residue was 1.82 mg/kg at 15 DAT, after which it rapidly decreased to 0.27 mg/kg by 95 DAT and then continued to decline at a slower rate to 0.06 mg/kg at 475 DAT. The average AMPA residue level in the 0 to 15.2 cm soil layer was 0.16 mg/kg at 0 DAT. Average AMPA residues peaked at 95 DAT, reaching 0.43 mg/kg, and then declined to 0.23 mg/kg at 475 DAT. Average glyphosate residues of 0.05, 0.07 and 0.06 mg/kg were found in the 15.2 to 30.5 cm layer at the 1, 7 and 15 DAT sampling events, respectively. Glyphosate residues averaged less than 0.05 mg/kg for all other samples taken below 15.2 cm. AMPA residues were less than 0.05 mg/kg in all soil samples taken below 15.2 cm.

The 18 months after application sampling was not taken at the Minnesota test site because the ground was frozen too hard to permit sampling.

6. New York test site

The average residue level for glyphosate in the 0 to 15.2 cm horizon was 2.75 mg/kg at 0 DAT. The maximum average glyphosate residue was 4.58 mg/kg at 14 DAT, after which average residues decreased steadily and were measured at 0.50 mg/kg on day 453. Average glyphosate residues were 0.62 mg/kg at 546 DAT, the final sampling. Average residue levels of AMPA were measured at 0.11 mg/kg on 0 DAT and increased to 0.48 mg/kg at 90 DAT. After 90 DAT, AMPA residues declined to 0.20 mg/kg at 362 DAT and then rose again to 0.36 mg/kg at 546 DAT.

Average glyphosate residue levels of 0.09 and 0.07 mg/kg were found in the 15.2-30.5 cm layers at 30 and 90 DAT, respectively. With the exception of these samples, no average glyphosate residues above 0.05 mg/kg were found below the 0 to 15.2 cm layer. AMPA was detected in the 15.2-30.5 cm layer on 14 and 30 days after treatment when the average residue levels reached 0.06 mg/kg at both times. AMPA was also detected in the 15.2-30.5 cm layer on 453 and 546 days after application when the average residue levels reached 0.09 and 0.05 mg/kg, respectively. No other AMPA residues averaging 0.05 mg/kg or greater were measured below 15.2 cm.

7. Ohio test site

The maximum average residue level of glyphosate in the 0 to 15.2 cm layer was 2.01 mg/kg at 0 DAT. Glyphosate residue levels decreased steadily and rapidly to less than LOD at 545 DAT. Average AMPA residues were measured at 0.34 mg/kg at the 0 DAT sampling. The highest average AMPA residue level of 0.60 mg/kg was found at 21 DAT. After this time, the AMPA levels decreased steadily to 0.07 mg/kg at 545 DAT. Residues of glyphosate and AMPA averaged less than 0.05 mg/kg in all soil samples taken

below 15.2 cm at all sampling times. These results demonstrate that glyphosate and AMPA did not move vertically in the soil profile at this test site.

8. Texas test site

The maximum average residue level of glyphosate in the 0 to 15.2 cm soil layer was 1.93 mg/kg at 0 DAT. Average glyphosate residue levels decreased rapidly to less than 0.05 mg/kg at 14 DAT, the increased to 0.15 mg/kg at 30 DAT, and then decreased to less than 0.05 mg/kg at all other sampling times. The average AMPA residue level at the 0 DAT sampling was measured at 0.11 mg/kg. The highest average AMPA residue level of 0.27 mg/kg was found at both 11 and 30 DAT. After this time, AMPA residue levels decreased to less than 0.05 mg/kg at 183 DAT. Residues of glyphosate averaged less than 0.05 mg/kg in all soil samples taken below 15.2 cm except for the 15.2 to 30.5 cm depth sample at 0 DAT which contained an average glyphosate residue level of 0.06 mg/kg. No AMPA residues were found to exceed 0.05 mg/kg below 15.2 cm for any sampling times.

The test plots and surrounding areas at the Texas location were flooded with approximately three feet of water for three days (December 23, 24, and 25 1991) due to a record rainfall during December 1991. As a result, all sampling from this location was stopped after the flood, and no 12, 15 or 18 months after treatment samples were collected. Due to the very rapid degradation of glyphosate at this location, the existing sampling events through six months after treatment were sufficient to define the dissipation of glyphosate.

C. KINETICS

An Ecoregion Crosswalk exercise was performed (see [redacted], 2020, CA 7.1.2.2.1/002). The trials in New York, Ohio, California, Iowa, Minnesota and Arizona were found to be representative for European conditions and included in kinetic evaluation ([redacted], 2020b, CA 7.1.2.2.1/003).

III. CONCLUSIONS

Maximum average glyphosate residue levels in the 0-15.2 cm soil horizon were 2.23, 0.62, 3.06, 2.34, 1.82, 4.58, 2.01 and 1.93 mg/kg occurred at 7, 0, 0, 1, 15, 14, 0 and 0 days after test substance application for the Arizona, California, Georgia, Iowa, Minnesota, New York, Ohio and Texas test sites, respectively, and then dissipated close to or below LOD, respectively, at the last sampling date. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate is degraded in soil. Maximum average AMPA residue levels in the 0-15 cm soil horizon were 0.56, 0.36, 0.60, 0.36, 0.43, 0.48, 0.60 and 0.27 mg/kg and occurred at 21, 14, 61, 62, 95, 90, 21 and 11 days after test substance application for the Arizona, California, Georgia, Iowa, Minnesota, New York, Ohio and Texas test sites, respectively, and then dissipated close to or below LOD, respectively, at the last sampling date with exception of the New York test site with a AMPA concentration of 0.36 mg/kg at 546 DAT.

The results of this study demonstrate that glyphosate and AMPA had little propensity to leach through the soil. Glyphosate degradation was typically rapid. The AMPA metabolite residue levels initially increased as glyphosate degraded, and then declined as it also degraded, demonstrating that it was a non-persistent metabolite. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate degraded in soil. As glyphosate degraded, the levels of AMPA rose and reached a maximum concentration between days 11 and 95 at seven of eight test sites. AMPA has also been demonstrated to dissipate with time. Average AMPA residue levels decreased from a maximum of 0.27 mg/kg, found at both the 11 and 30 days after treatment sampling events, to 0.02 mg/kg at 6 months after application at the Texas test site. At the Georgia and Ohio test sites, the average AMPA residue levels in the 0-15.2 cm soil horizon decreased from a maximum of 0.60 mg/kg at 6 and 21 days after treatment, respectively, to 0.24 mg/kg and 0.07 mg/kg, respectively, at 18 months after treatment.

The results of this study demonstrate that glyphosate and AMPA possess very limited potential for vertical mobility in soil. Average glyphosate and AMPA residues greater than 0.05 mg/kg (the lower limit of method validation) were never detected below 30.5 cm in the soil profile with three exceptions. For the sampling event on the day of test substance application at the California test site, average glyphosate residues of 0.21 mg/kg and 0.10 mg/kg, respectively, where found in the 30.5-45.7 cm and 45.7-61.0 cm soil horizons. However, these residues were attributed to contamination during sampling rather than vertical mobility of glyphosate due to the depth of the residues at such an early time point following test substance application and the absence of supporting concentrations of glyphosate below

30.5 cm in subsequent soil samples. In addition, average glyphosate and AMPA residues of 0.05 and 0.06 mg/kg, respectively, were found in the 45.7-61.0 cm soil horizon at 21 days after treatment at the Arizona test site and an average glyphosate residue level of 0.05 mg/kg was found in the 30.5-45.7 cm soil horizon at 190 days after treatment at the Iowa test site. These residues were also attributed to contamination during sampling rather than vertical mobility due to the absence of supporting residues in the 15.2-30.5 cm and 30.5-45.7 cm soil horizons at 21 days after treatment at the Arizona test site, the absence of residues in the 15.2-30.5 cm soil horizon at 190 days after treatment at the Iowa test sites, and the absence of residues below 30.5 cm in subsequent soil samples from both test sites. Lack of pre-excavation at the Iowa test site may be responsible for the residues found at lower depths at that location.

Assessment and conclusion by applicant:

The study provides detailed information on the dissipation behavior of glyphosate under field conditions at different testing conditions according to the relevant guideline. It is considered valid to address the data point.

Assessment and conclusion by RMS:

The study design is globally consistent with OECD ENV/JM/MONO(2016)6.

The eight test sites are US soils. It was concluded from the ecoregion crosswalk ([redacted], 2020) that only the New York, Ohio and California sites are considered as representative of European conditions. Therefore, the RMS assessment is focused on these sites.

The field history is available in the study report for two years before the test. No crop was grown on the California site, the site was treated with alachlor in 1989 (no treatment in 1990). Grass was present on the New York site, no pesticide had been applied. Grass, orchard grass and corn had been cultivated in 1989 and 1990 on the Ohio test site. Applications of Lorsban and Pounce (permethrin) were performed in 1989, of Tandem (lambda-cyhalothrin and thiamethoxam) and Bladex (cyanazine) in 1990. No glyphosate had therefore been applied on these sites the two previous year before test.

During the study, the sites were also treated with different pesticides, none of them including glyphosate.

Only 6 soil cores were taken per subplot, instead of 10 recommended.

The distance of the weather station from the site (when not onsite) is not indicated in the report. A data gap is identified for providing the distance of the weather stations from New York, Ohio and California sites ([redacted], 1993a).

The study is considered acceptable and data from New York, Ohio and California sites can be used to derive endpoints.

[redacted], 1992b

Data point:	CA 7.1.2.2.1/009
Report author	[redacted]
Report year	1992b
Report title	Field soil dissipation rate determination of Glyphosate 360 (Egerkingen, Switzerland)
Report No	280416
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. Teil IV, 4-1, Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.

Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available - Verification of application rate was not conducted - Sampling up to 30 cm only - No information on transport and processing - Weather data recorded daily but only example of one month daily data or every monthly means are available in the study report
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification: Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt

Tested formulation: Glyphosate 360

Lot No.: 229-Jak-24-1/F

Nominal concentration: 360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Egerkingen, Switzerland. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 100 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m^2 area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m^2.

Soil cores were taken from the trial sites prior to application to determine the soil properties.

Table 8.1.1.3-42: Soil characteristics of the test plots

Parameter		Result
Particle Size Analysis (USDA) (%) [1]	sand	34.2
	silt	28.8
	clay	37.1
Soil Type		clay loam
Organic Carbon (%)		1.55
Organic Matter (%) [2]		2.67
pH-Value (KCl)		7.33
pH (H_2O)[3]		7.79
Cation Exchange Capacity(meq/100 g)		31.3
Max. water holding capacity (g H_2O/100 g soil dw)		69.6
Biomass before application (mg microb. C/100 g dry soil)		187.0
Biomass 62d after application (mg microb. C/100 g dry soil)		200.7
Biomass 202d after application (mg microb. C/100 g dry soil)		211.0

[1] Due to rounding differences the sum may not correspond to 100 percent.

[2] Calculated from organic carbon according to OM = OC / 0.58.

[3] calculated by RMS considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)[5]

[5] EFSA Journal 2017;15(10):4982, source of the formula: Boesten et al. 2012

Daily weather data during the entire study from September 1990 to March 1991 was recorded using the weather station "Wynau", about 7 km straight line from the trial site. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported.

Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. After harvest on August 1990, the field was ploughed and afterwards milled by means of a tiller.

2. Application

Applications at the plots were conducted on 5th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution. The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.6 min application on the 22 m² plot. 4735 mL of the application solution were used corresponding to an actual application rate of 3874.1 g a.s./ha. Stability of the application solution was assessed before and after application with mean values of 92.6 % and 93.5 %.

3. Sampling

Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30, 62 and 202 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30 62 and 202 days DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation

At the test facility the core specimens were thawed and cut into segments of 0-10 cm. 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted], 1995 (CA 7.1.2.2.1/012).

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount of glyphosate or AMPA in bi-distilled water. Six fortification levels from 0.02 mg/kg up to 2.0 mg/kg were prepared. The mean recovery for glyphosate was 79.3 % with a relative standard deviation of 25.2 %. The mean recovery of AMPA was 78.9 % with a relative standard deviation of 15.2 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-43: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)]	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
-1	0 - 10	0.040	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	1.317	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
7	0 - 10	0.637	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
15	0 - 10	0.637	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.472	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
62	0 - 10	0.440	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
202	0 - 10	0.091	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 8.1.1.3-44: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	as glyphosate eq.	Concentration (mg/kg) as AMPA	as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.096	0.146	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.115	0.175	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.235	0.358	< LOQ	< 0.030

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	as glyphosate eq.	Concentration (mg/kg) as AMPA	as glyphosate eq.
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.302	0.460	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
62	0 - 10	0.328	0.500	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
202	0 - 10	0.217	0.330	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 1.317 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.091 mg/kg at DAA 202. In the deeper soil segments, 10-20 cm and 20-30 cm, no concentrations above the limit of quantification were found. Hence it follows that glyphosate was not leached from the top layer. The maximum concentration of the metabolite AMPA (0.328 mg/kg) was observed in the soil layer 0-10 cm, 62 days after application. This value corresponds to 0.500 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3530.5 g a.s./ha. This treatment resulted in a residue level of 1.317 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.091 mg/kg on DAA 202. In the deeper soil segments 10-20 cm and 20-30 cm, no concentrations above the limit of quantification were found. The maximum concentration of the metabolite AMPA (0.328 mg/kg) was observed in the soil layer 0-10 cm, 62 days after application. This value corresponds to 0.500 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS

The following deviations from OECD ENV/JM/MONO(2016)6 are identified.

Meteorological data were recorded daily but only monthly means/minimum/maximum are reported in the study report.

No verification of application rate was done. For information, based on a default bulk density of 1.5 g/cm^3 (no measured value), the initial measured concentration including glyphosate and AMPA can be roughly estimated to 2194 g/ha, which represents around 57% of the intended application rate (3874 g/ha).

At each sampling time, a sample consisted of 20 cores. The 20 cores from same date and soil layer were mixed for analysis. No replicate is available at any time. According to OECD guidance, 10 cores for different subplots should have been sampled; the 10 cores from all subplots should not be mixed.

The soil samples were performed on 30 cm depth only instead of 1 m. However, the mobility of glyphosate and AMPA is known to be low and no quantifiable amounts of either glyphosate or AMPA were found in the 20-30 cm layer depths.

Despite the above deviations, the study is considered acceptable.

[redacted] *1992c*

Data point:	CA 7.1.2.2.1/010
Report author	[redacted]
Report year	1992c
Report title	Field soil dissipation rate determination of Glyphosate 360 (Bad Krozingen, Germany)
Report No	280427
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. Teil IV, 4-1,Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available - Verification of application rate was not conducted - Sampling up to 30 cm only - No information on transport and processing - Weather data recorded daily but only example of one month daily data or every monthly means are available in the study report - Study was terminated at decline of a.s. to ca. 16 % of initial
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification: Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt

Tested formulation: Glyphosate 360

Lot No.: 229-Jak-24-1/F

Nominal concentration: 360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Bad Krozingen, Germany. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 150 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m^2 area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m^2.

Soil cores were taken from the trial sites prior to application to determine the soil properties.

Table 8.1.1.3-45: Soil characteristics of the test plots

Parameter		Result
Particle Size Analysis (USDA) (%) [1]	sand	55.0
	silt	27.1
	clay	17.9
Soil Type		sandy loam
Organic Carbon (%)		0.36
Organic Matter (%) [2]		0.62
pH-Value (KCl)		6.0
pH (H_2O)[3]		6.6
Cation Exchange Capacity(meq/100 g)		8.9
Max. water holding capacity(g H_2O/100 g soil d)		32.3
Biomass before application (mg microb. C/100 g dry soil)		19.5
Biomass 61d after application (mg microb. C/100 g dry soil)		47.1

[1] Due to rounding differences the sum may not correspond to 100 percent.
[2] Calculated from organic carbon according to OM = OC / 0.58
[3] calculated by RMS considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)[6]

Daily weather data during the entire study from September to November 1990 was recorded using the weather stations "Schallstadt-Mengen" (for temperature and precipitation) and "Bremgarten" (for sunlight hours), about 7 km and 4 km straight line from the trial site, respectively. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported.

Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. After harvest on August 1990, the field was ploughed and afterwards harrowed.

2. Application

Applications at the plots were conducted on 4th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution.

The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.4 min application time on the 22 m^2 plot. 4480 mL of the application solution were used corresponding to an actual application rate of 3665.5 g a.s./ha.

The stability of the application solution was tested in the field dissipation studies RCC 273565 and RCC 280416. The solutions were considered to be stable under the application conditions.

3. Sampling

Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30 and 61 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30 and 61 days DAA. Soil cores were taken by means

6 EFSA Journal 2017;15(10):4982, source of the formula: Boesten et al. 2012

of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation

At the test facility the core specimens were thawed and cut into segments of 0-10 cm, 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted], 1995 (CA 7.1.2.2.1/012).

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount of glyphosate or AMPA in bidistilled water. Six fortification levels from 0.05 mg/kg up to 2.5 mg/kg were prepared. The mean recovery of glyphosate was 81.3 % with a relative standard deviation of 7.8 %. The mean recovery of AMPA was 86.2 % with a relative standard deviation of 5.4 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-46: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
-1	0 - 10	< LOQ	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	2.456	< LOQ

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
7	0 - 10	0.893	< LOQ
	10 - 20	0.046	< LOQ
	20 - 30	< LOQ	< LOQ
15	0 - 10	0.812	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.436	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
61	0 - 10	0.390	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 8.1.1.3-47: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	as glyphosate eq.	Concentration (mg/kg) as AMPA	as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.253	0.385	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.233	0.355	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.266	0.405	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.300	0.457	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
61	0 - 10	0.425	0.647	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 2.456 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.390 mg/kg at DAA 61. Only small quantities of glyphosate (0.046 mg/kg) were found in the 10-20 cm soil segment, 7 days after application, no residues were encountered above LOQ in the 20-30 cm layer. Hence it follows that glyphosate was not leached from the top layer. The maximum concentration of the metabolite AMPA (0.425 mg/kg) was observed in the soil layer 0-10 cm, 61 days after application. This value corresponds to 0.647 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3665.5 g a.s./ha. This treatment resulted in a residue level of 2.456 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.390 mg/kg on DAA 61 at approximately 84 % glyphosate decline compared to initial. Glyphosate was not prone to leaching; as a maximum of 0.046 mg a.s./kg was found in the 10-20 cm layer. No residues > LOQ were encountered in the 20-30 cm layer. The maximum concentration of the metabolite AMPA (0.425 mg/kg) was observed in the soil layer 0-10 cm, 61 days after application. This value corresponds to 0.647 mg equivalents glyphosate/kg soil. No AMPA concentrations above the determination limit were found in deeper soil layers.

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS

The following deviations from OECD ENV/JM/MONO(2016)6 are identified.

Meteorological data were recorded daily but only monthly means/minimum/maximum are reported in the study report.

No verification of application rate was done. For information, based on a default bulk density of 1.5 g/cm^3 (no measured value), the initial measured concentration including glyphosate and AMPA can be roughly estimated to 4262 g/ha, when the intended application rate was 3665 g/ha.

At each sampling time, a sample consisted of 20 cores. The 20 cores from same date and soil layer were mixed for analysis. No replicate is available at any time. According to OECD guidance, 10 cores for different subplots should have been sampled; the 10 cores from all subplots should not be mixed.

The soil samples were performed on 30 cm depth only instead of 1 m. However, the mobility of glyphosate and AMPA is known to be low and no quantifiable amounts of either glyphosate or AMPA were found in the 20-30 cm layer depths.

Despite the above deviations, the study is considered acceptable.

[redacted], *1992d*

Data point:	CA 7.1.2.2.1/011
Report author	[redacted]
Report year	1992d
Report title	Field soil dissipation rate determination of Glyphosate 360 (Menslage, Germany)
Report No	RCC 280438
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. Teil IV, 4-1,Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available - Verification of application rate was not conducted - Sampling up to 30 cm only - No information on transport and processing

	- Weather data recorded daily but only example of one month daily data or every monthly means are available in the study report
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification: Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt

Tested formulation: Glyphosate 360

Lot No.: 229-Jak-24-1/F

Nominal concentration: 360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Menslage, Germany. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 150 m from the treated plot. In each of these plots, a 22 m² area was constructed. The 22 m² area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m².

Soil cores were taken from the trial sites prior to application to determine the soil properties.

Table 8.1.1.3-48: Soil characteristics of the test plots

Parameter		Result
Cation Exchange Capacity(meq/100 g)		4.9
Particle Size Analysis (USDA) (%) [1]	sand	90.6
	silt	2.1
	clay	7.2
Soil Type		sandy soil
Organic Carbon (%)		0.25
Organic Matter (%) [2]		0.43
pH-Value (KCl)		4.73
pH (H_2O)[3]		5.6
Max. water holding capacity (g H_2O/100 g soil dw)		33.3
Biomass before application (mg microb. C/100 g dry soil)		11.2
Biomass 60d after application (mg microb. C/100 g dry soil)		24.6
Biomass 271d after application (mg microb. C/100 g dry soil)		18.8

[1] Due to rounding differences the sum may not correspond to 100 percent.
[2] Calculated from organic carbon according to OM = OC / 0.58
[3] calculated by RMS considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)[7]

Daily weather data during the entire study from September 1990 to July 1991 was recorded using the weather stations "Löningen" (for temperature and sunlight) and "Menslage-Borg" (for precipitation), about 10 km and 0.2 km straight line from the trial site, respectively. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported.

7 EFSA Journal 2017;15(10):4982, source of the formula: Boesten et al. 2012

Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. Prior to application, the grown corn was cut and the soil hoed.

2. Application

Applications at the plots were conducted on 7th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution.

The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.3 min application time on the 22 m² plot. 4480 mL of the application solution were used corresponding to an actual application rate of 3665.5 g a.s./ha. The stability of the application solution was tested in the field dissipation studies RCC 273565 and RCC 280416. The solutions were considered to be stable under the application conditions.

3. Sampling

Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30, 60, 192, 271 and 315 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30, 60, 192, 271 and 315 days DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation

At the test facility the core specimens were thawed and cut into segments of 0-10 cm. 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted], 1995 (CA 7.1.2.2.1/012).

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent

compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount of glyphosate or AMPA in bidistilled water. Eight fortification levels from 0.05 mg/kg up to 2.5 mg/kg were prepared. The mean recovery of glyphosate was 74.7 % with a relative standard deviation of 16.0 %. The mean recovery of AMPA was 78.7 % with a relative standard deviation of 19.3 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-49: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
-1	0 - 10	< LOQ	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	2.659	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	0.030[1]	< LOQ
7	0 - 10	1.319	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
15	0 - 10	0.580	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.678[2]	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
60	0 - 10	0.506	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
192	0 - 10	0.277	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
271	0 - 10	0.281	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
315	0 - 10	0.122	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

[1] Peak is probably not caused by glyphosate; therefore, this concentration is not used for interpretation

[2] Sample was re-analysed since the peak is probably not caused by glyphosate; consequently, the result of the second analysis is presented (result of first analysis: 0.299 mg/kg).

Table 8.1.1.3-50: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	as glyphosate eq.	Concentration (mg/kg) as AMPA	as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-

Table 8.1.1.3-50: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	as glyphosate eq.	Concentration (mg/kg) as AMPA	as glyphosate eq.
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.094	0.143	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.224	0.341	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.312	0.475	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.374[1]	0.569	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
60	0 - 10	0.515	0.784	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
192	0 - 10	0.416	0.633	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
271	0 - 10	0.853	1.299	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
315	0 - 10	0.417	0.635	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

[1] Sample was re-analysed due to unsatisfactory result; consequently, the result of the second analysis is presented (result of the first analysis: 0.241 mg/kg)
Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 2.659 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.122 mg/kg at DAA 315. No residues above the limit of quantification (0.02 mg/kg) were found in the soil layers 10-20 and 20-30 cm. Hence it follows that no glyphosate was leached from the top layer. The maximum concentration of the metabolite AMPA (0.853 mg/kg) was observed in the soil layer 0-10 cm, 271 days after application. This value corresponds to 1.299 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3665.5 g a.s./ha. This treatment resulted in a residue level of 2.659 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.122 mg/kg on DAA 315. No residues above the limit of quantification (0.02 mg/kg) were found in the soil layers 10-20 and 20-30 cm. Hence it follows that glyphosate was not leached from the top layer. The maximum concentration of the metabolite AMPA (0.853 mg/kg) was observed in the soil layer 0-10 cm, 271 days after application. This value corresponds to 1.299 mg glyphosate equivalent/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS

The following deviations from OECD ENV/JM/MONO(2016)6 are identified.

Meteorological data were recorded daily but only monthly means/minimum/maximum are reported in the study report.

No verification of application rate was done. For information, based on a default bulk density of 1.5 g/cm^3 (no measured value), the initial measured concentration including glyphosate and AMPA can be roughly estimated to 4203 g/ha, when the intended application rate was 3665 g/ha.

At each sampling time, a sample consisted of 20 cores. The 20 cores from same date and soil layer were mixed for analysis. No replicate is available at any time. According to OECD guidance, 10 cores for different subplots should have been sampled; the 10 cores from all subplots should not be mixed.

The soil samples were performed on 30 cm depth only instead of 1 m. However, the mobility of glyphosate and AMPA is known to be low and no quantifiable amounts of either glyphosate or AMPA were found in the 20-30 cm layer depths.

Despite the above deviations, the study is considered acceptable.

[redacted], *1994*

Data point:	CA 7.1.2.2.1/004
Report author	[redacted]
Report year	1994
Report title	Touchdown: Field dissipation study for terrestrial uses, Champaign, Illinois, 1988-1989 residue data to support registrations of products containing Glyphosate-trimesium as active ingredient
Report No	RR 93-027B
Guidelines followed in study	US EPA 164-1
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - Soil treated with a product containing glyphosate before and during the study - Treatment on cropped plots
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type:	terrestrial field dissipation
	Test item:	Touchdown, a.s.: glyphosate-trimesium
	Test sites:	one locations in USA, planted with wheat

	Soil characterization (upper soil layer): Silty clay loam OM: 3.6 % pH: 6.0 (water) Moisture content: 29.45% WMC at 1/3 bar; 12.80% WMC at 15 bar Application rate: 9 kg a.s./ha, single application Application method: tractor-mounted CO_2 presurized broadcast spray applicator; to wheat and pigweed cover Application timing: 27 May 1988, 24 days after planting, ground cover: 50 – 60 % Sampling times: 12 events, -1, 0, 1, 3, 7, 14, 28, 61, 90, 171, 382, 517 DAT Sampling method: core samplers Sampling depths: 0 – 8.9 cm, 8.9 – 39.4 cm, 39.4 - 100.3 cm Tillage: no cultural practices after application of the herbicide Sample storage: frozen directly after sampling and kept frozen until sample preparation Workup and analysis: Mixing by hand extraction with ammonium hydroxide and potassium phosphate Derivatization with trifluoroacetic anhydride and heptafluorobutanol analysis by GC/MSD, LOQ = 0.05 mg/kg Recovery in fortified samples: Glyphosate: mean: 85 %, coeff. of var. (CV): 13 % AMPA: mean: 85 %, CV: 15 %
Short description of results:	Residues: Glyphosate: (0 – 8.9 cm depth) 2.5 mg/kg (0 DAT) Max.: 2.9 mg/kg (1 DAT) <0.05 mg/kg (517 DAT) AMPA: (0 – 8.9 cm depth) Max.: 0.44 mg/kg (90 DAT) 0.17 mg/kg (517 DAT) No residues of glyphosate and AMPA >0.05 mg/kg were found in any other soil layer Half-life times: calculated based on a least-squares fit of the linear transformation of a exponential function (for the first soil layer): Glyphosate: 79 days (r = 0.969) AMPA: 419 days (r = 0.843) Glyphosate and AMPA show no evidence to leach below the 8.9 cm soil layer.
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid due to the following deficiencies: • The test was conducted on cropped plots • High application rate (9 kg/ha)

Assessment and conclusion by RMS

As mentioned by the applicant, the applications were performed on cropped plots in this study. The soil history of treatments is reported in the study, ROUNDUP containing glyphosate had been applied

the year before testing (along with other pesticides such as LASSO and ATRAZINE). During the study, the soil was also treated with different pesticides for maintenance, among which ROUNDUP was applied on august 1988.

The study is therefore not considered acceptable.

[redacted], *1992a*

Data point:	CA 7.1.2.2.1/008
Report author	[redacted]
Report year	1992a
Report title	Field soil dissipation rate determination of Glyphosate 360 (Diegten, Switzerland)
Report No	273565
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prufung von Pflanzenschutzmitteln. Teil IV, 4-1,Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available - Verification of application rate was not conducted - Sampling up to 30 cm only - No information on transport and processing - Weather data recorded daily but only example of one month daily data or every monthly means are available in the study report - Contamination of the untreated plot
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification: Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation: Glyphosate 360
Lot No.: 229-Jak-24-1/F
Nominal concentration: 360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Diegten, Switzerland. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 100 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m^2 area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m^2.

Soil cores were taken from the trial sites prior to application to determine the soil properties.

Table 8.1.1.3-51: Soil characteristics of the test plot

Parameter		Result
Cation Exchange Capacity(meq/100 g)		31.0
Particle Size Analysis (USDA) (%) [1]	sand	47.6
	silt	13.3
	clay	39.2
Soil Type		sandy clay

Table 8.1.1.3-51: Soil characteristics of the test plot

Parameter	Result
Organic Carbon (%)	1.61
Organic Matter (%) [2]	2.78
pH-Value (KCl)	7.1
pH (H_2O)[3]	7.6
Max. water holding capacity (g H_2O/100 g soil dw)	70.1
Biomass before application (mg microb. C/100 g dry soil)	180.0
Biomass 62d after application (mg microb. C/100 g dry soil)	170.0
Biomass 282d after application (mg microb. C/100 g dry soil)	240.5

[1] Due to rounding differences the sum may not correspond to 100 percent.

[2] Calculated from organic carbon according to OM = OC / 0.58

[3] calculated by RMS considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)[8]

Daily weather data during the entire study from September 1990 to June 1991 was recorded using the weather station "Rünenberg" (altitude: 610 m), about 7 km straight line from the trial site. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported.

Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. After harvest on July 1990, the field was ploughed and afterwards meadow was sown. Immediately before application, the plots were milled by means of a tiller (almost no grass was obtained at this time).

2. Application

Applications at the plots were conducted on 5th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution. The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.5 min application time on the 22 m² plot. 4315 mL of the application solution were used corresponding to an actual application rate of 3530.5 g a.s./ha. Stability of the application solution was assessed before and after application with mean values of 86.9 % and 88.0 %.

3. Sampling

Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30, 62, 194 and 282 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30 62, 194 and 282 DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation

At the test facility the core specimens were thawed and cut into segments of 0-10 cm. 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil

[8] EFSA Journal 2017;15(10):4982, source of the formula: Boesten et al. 2012

layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted], 1995.

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount in bidistilled water. Seven fortification levels from 0.02 mg/kg up to 3.0 mg/kg were prepared for each compound. The mean recovery for glyphosate was 82.2 % with a relative standard deviation of 21.9 %. The mean recovery of AMPA was 84.1 % with a relative standard deviation of 16.1 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-52: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
-1	0 - 10	< LOQ	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	2.065	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
7	0 - 10	1.033	0.065
	10 - 20	0.054	0.028
	20 - 30	LOQ	< LOQ
15	0 - 10	0.586	0.038
	10 - 20	< LOQ	0.029
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.245	< LOQ
	10 - 20	0.028	0.057
	20 - 30	0.025	0.026
62	0 - 10	0.308	< LOQ

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
194	0 - 10	0.175	0.039
	10 - 20	0.039	0.031
	20 - 30	0.028	0.037
282	0 - 10	0.066	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 8.1.1.3-53: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	as glyphosate eq.	Concentration (mg/kg) as AMPA	as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.266	0.344	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.362	0.551	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.211	0.321	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.181	0.276	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	0.024	< 0.037
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
62	0 - 10	0.343	0.522	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
194	0 - 10	0.337	0.513	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	0.036	0.055	< LOQ	< 0.030
282	0 - 10	0.238	0.362	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. Characterisation of residues

The highest level of residue was observed with 2.065 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.066 mg/kg at DAA 282. Only small quantities of glyphosate were found in the soil segments 10-20 cm. In the 20-30 cm soil layers, concentrations close to the limit of quantification were found. Hence it follows that only small quantities of glyphosate leached from the top layer. The maximum concentration of the metabolite AMPA (0.362 mg/kg) was observed in the soil layer 0-10 cm, 7 days after application. This value corresponds to 0.551 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers, except for a concentration of 0,036 mg/kg in the 20-30 cm soil segment after 194 days.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3530.5 g a.s./ha. This treatment resulted in a residue level of 2.065 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.066 mg/kg on DAA 282. Only small quantities of glyphosate were found in the soil segments 10-20 cm. In the 20-30 cm soil layers residues were generally below LOQ or close to LOQ for single samplings. Hence it follows that only small quantities of glyphosate leached from the top layer. Thus, leaching is not expected to present a relevant decline process and to impact on degradation kinetics. The maximum concentration of the metabolite AMPA (0.362 mg/kg) was observed in the soil layer 0-10 cm, 7 days after application. This value corresponds to 0.551 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers, except for a concentration of 0.036 mg/kg in the 20-30 cm soil segment after 194 days.

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS

The following deviations from OECD ENV/JM/MONO(2016)6 are identified.

Meteorological data were recorded daily but only monthly means/minimum/maximum are reported in the study report. There is no information on transport and processing of the samples.

No verification of application rate was done. For information, based on a default bulk density of 1.5 g/cm^3 (no measured value), the initial measured concentration including glyphosate and AMPA can be roughly estimated to 3614 g/ha, which is a little above the intended rate of 3530.5 g/ha.

At each sampling time, a sample consisted of 20 cores. The 20 cores from same date and soil layer were mixed for analysis. No replicate is available at any time. According to OECD guidance, 10 cores for different subplots should have been sampled; the 10 cores from all subplots should not be mixed.

Samples for this study were taken to a depth of 30 cm only, instead of 1 m. Glyphosate and AMPA are low mobile in soil, therefore no significant amounts are expected to be found in deeper layers. However, RMS notes that at some sampling dates, some residues of glyphosate are measured in the deepest sampled layer (20-30 cm). Analysis of deeper layers would have been needed.

In addition, some glyphosate residues are measured in the untreated plot, at days 7, 15, 30 and 194. These correspond to the sampling dates at which glyphosate was detected in the 10-30 cm layer depths. This would tend to indicate contamination during the sampling. As a consequence, RMS considers that the measured concentrations in this study are uncertain and should not be considered further.The study is not considered acceptable.

[redacted], *1992*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.2.1/013
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Soil Dissipation Study (Germany 1990-1992)

Report No	RJ1294B
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - Only 5 cores per subplot - No replicate residue data available - Sampling up to 30 cm only, and results presented up to 20 cm - Reported weather data do not cover the whole study period for Buchen and Kleinzecher sites - Only monthly weather data reported for Unzhurst and Rohrbah sites from December 1990 - No information on transport and processing - analytical method not validated - residues were corrected for external procedural recoveries based of fortified results, with not uniform handling of the data - only average recoveries of fortified controls reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate as **glyphosate-trimesium** (ICIA 0224)
Product:	Sulfosate (YF7712A), 48 % SL formulation
Lot No.:	D4875/160
Nominal concentration:	48 % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

The study (field work and analysis) was carried out in Germany between April 1990 and June 1992. Test sites were chosen to present typical soils and climates of proposed use areas of the product. Pesticide history was reported over three years for each trial.

At each of the trial sites the area was divided into two plots, a treated plot and an untreated or control plot, separated by a buffer zone. The size of the treated and control plots for all trials was 2.5 m x 22 m (except trial RS-9027/G2 where the size was 2.5 m x 20 m). Each of the treated plot areas was subdivided into four sub-plots from which a total of 20 core samples (generally 5 from each subplot) were taken.

At each of the trial sites (except for Buchen (RS-9027/B1) and Wang-Inzkofen (RS-9027/G2)) at least one soil pit was dug and samples were taken from three horizons to a depth of at least 90 cm in all cases. Between 0.5 and 1 kg of soil was then bulked from each horizon and sent to Jealott's Hill Research Station, Bracknell, UK, for physico-chemical characterization.

Table 8.1.1.3-54: Soil characteristics of the Buchen (RS-9027/B1) test site

Parameter		Horizon		
Soil depth (cm)		0-30	30-60	60-100
Cation Exchange Capacity (meq/100g)		6.5	5.5	3.5
Particle Size Analysis (USDA) (%)	sand	80	80	81
	silt	14	12	15
	clay	6	8	4
Soil Type		Loamy Sand		
Organic matter (%)		2.8	2.1	0.8

Organic carbon (%) [2]	1.624	1.218	0.464
Soil pH [3]	6.4	6.5	6.7
Soil Bulk Density (g/L) [1]	1.4	-	-
Field capacity (% soil moisture at 1/3 bar)	12.72	9.33	6.95

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM x 0.58
[3] Medium not stated

Table 8.1.1.3-55: Soil characteristics of the Kleinzecher (RS-9027/B2) test site

Parameter		Horizon		
Soil depth (cm)		0-30	30-60	60-100
Cation Exchange Capacity (meq/100g)		7.7	8.7	10.4
Particle Size Analysis (USDA) (%)	sand	66	68	62
	silt	21	15	19
	clay	13	17	19
Soil Type		Sandy loam		
Organic matter (%)		1.9	1.2	0.2
Organic carbon (%) [2]		1.102	0.696	0.116
Soil pH		7.0	7.0	7.3
Soil Bulk Density (g/L) [1]		1.6	-	-
Field capacity (% soil moisture at 1/3 bar)		13.71	14.60	15.19

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-56: Soil characteristics of the Unzhurst (RS-9027/E1) test site

Parameter		Horizon		
Soil depth (cm)		0-30	30-60	60-90
Cation Exchange Capacity (meq/100g)		6.6	6.1	6.2
Particle Size Analysis (USDA) (%)	sand	48	53	44
	silt	39	31	37
	clay	13	16	19
Soil Type		Loam	Sandy clay loam	Loam
Organic matter (%)		1.8	0.6	0.3
Organic carbon (%) [2]		1.044	0.348	0.174
Soil pH		6.7	5.4	5.3
Soil Bulk Density (g/L) [1]		1.4	-	-
Field capacity (% soil moisture at 1/3 bar)		15.57	16.50	16.88

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-57: Soil characteristics of the Rohrbach (RS-9027/E2) test site

Parameter		Horizon		
Soil depth (cm)		0-25	25-35	35-105
Cation Exchange Capacity (meq/100g)		12.7	12.1	5.4
Particle Size Analysis (USDA) (%)	sand	12	13	15
	silt	77	60	70
	clay	11	27	15
Soil Type		Silt Loam		
Organic matter (%)		1.8	0.5	0.1
Organic carbon (%) [2]		1.044	0.290	0.058
Soil pH		8.5	8.5	8.7
Soil Bulk Density (g/L) [1]		1.3	-	-
Field capacity (% soil moisture at 1/3 bar)		23.10	21.28	18.95

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-58: Soil characteristics of the Herrngiersdorf (RS-9027/G1) test site

Parameter		Horizon [2]	
Soil depth (cm)		upper	lower
Cation Exchange Capacity (meq/100g)		14.4	9.3
Particle Size Analysis (USDA) (%)	sand	23	21
	silt	47	58
	clay	30	21
Soil Type		Clay loam	Silt loam
Organic matter (%)		2.8	0.8
Organic carbon (%) [3]		1.624	0.464
Soil pH		8.0	8.4
Soil Bulk Density (g/L) [1]		1.5	-
Field capacity (% soil moisture at 1/3 bar)		24.31	21.18

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] The soil horizons were not measured. The two horizons were sampled from a pit dug to a depth of 1 m
[3] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-59: Soil characteristics of the Wang-Inzkofen (RS-9027/G2) test site

Parameter		Horizon
Soil depth (cm)		0-30
Cation Exchange Capacity (meq/100g)		14.0
Particle Size Analysis (USDA) (%)	sand	25
	silt	51
	clay	24
Soil Type		Silt loam
Organic matter (%)		2.1
Organic carbon (%) [2]		1.218
Soil pH		7.2
Soil Bulk Density (g/L) [1]		1.6
Field capacity (% soil moisture at 1/3 bar)		24.53

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM x 0.58

Long-term air temperatures (daily or monthly mean), precipitation data as well as sunshine hours (as sum daily or monthly) were reported. No information on irrigation was reported.

Details on weather data are presented in the table below.

Table 8.1.1.3-60: Weather station and reporting time

Test site	Study duration (from date of application)	Reporting time	Weather station
Buchen (RS-9027/B1)	11/04/1990-30/07/1991	Daily, April 1990 – September 1991	Mölln-Grambek (T, N), Lübeck-Blankensee (S)
Kleinzecher (RS-9027/ B2)	10/08/1990-28/02/1992	Daily, July 1990 – December 1991	Mölln-Grambek (T, N), Lübeck-Blankensee (S)
Unzhurst (RS-9027/E1)	03/05/1990-18/11/1991	Daily: April 1990 –November 1990 Monthly: December 1990 – November 1991	Rheinau-Freistett (T, N, S)
Rohrbach (RS-9027/E2)	25/07/1990-27/02/1992	Daily: July 1990 – November 1990 Monthly: December 1990 – February 1992	Daily: Bad Bergzabern (T, S), Landau/Pfalz (N) Monthly: Bad Bergzabern (T, N, S)
Herrngiersdorf (RS-9027/G1)	08/05/1990-31/10/1991	Daily, April 1990 – December 1991	Regensburg (T, N, S)
Wang-Inzkofen (RS-9027/G2)	02/07/1990-02/01/1992	Daily, July 1990 – January 1992	Freising-Weihenstephan

T: Temperature; N: Precipitation; S: Sunshine

2. Application

Glyphosate-trimesium (as YF7712A) was applied on bare soil as 48 % SL formulation (sulfosate) at each trial, at a nominal application rate of 4.80 kg a.s./ha (ICIA 0224). One batch of spray solution was mixed to cover the entire plot. The application was made in all cases using a band held CO2 pressurised sprayer equipped with a 2.5 m boom. A single application was made at each trial site. Conditions during application are detailed in the table below.

Table 8.1.1.3-61: Conditions during application

Treatment No.	Buchen (RS-9027/B1)	Kleinzecher (RS-9027/ B2)	Unzhurst (RS-9027/E1)	Rohrbach (RS-9027/E2)	Herrngiersdorf (RS-9027/G1)	Wang-Inzkofen (RS-9027/G2)
Application date	11.04.1990	10.08.1990	03.05.1990	25.07.1990	08.05.1990	02.07.1990
Application equipment	Hand held CO_2 pressurised sprayer equipped with a 2.5 m boom					
Spray pressure (bar at boom)	2	2	3.6	3.6	3.0	3.0
Mean application volume actual (L/plot)	2.4	2.6	2.2	2.3	2.1	2.0
Nominal application rate (kg/ha)	4.8	4.8	4.8	4.8	4.8	4.8
Actual application rate (kg a.s./ha)	5.2	5.7	4.8	5.0	4.6	4.8
Mean air temperature (°C)	11	17	19	11	20	18
Mean wind speed (m/s)	calm	2	1-3	1	1	-
Wind direction	calm	NW	NE	NE	West	No wind
Relative air humidity (%)	80	80	low	medium	55	55
Cloud cover (%)	30	30	0	0	30	100
Ground cover (%)	0	0	0	0	0	0
Wetness of soil surface	dry	moist	dry	dry	dry, crumbly	dry, crumbly

3. Sampling

Samples of untreated soil were taken from each site (30 cm cores with 2.3 cm internal diameter). Treated soil was sampled directly after application, using 10 cm cores with a 5 cm internal diameter. At subsequent intervals, up to approximately 19 months, soil was sampled using a 30 cm x 2.3 cm internal diameter corer. For each trial, at each interval, 20 cores were taken (usually five per sub-plot) in order to obtain a representative sample. All soil samples were taken using a zero contamination corer with plastic inserts.

4. Specimen handling and preparation

All soil samples were frozen in dry ice within two hours of sampling and transferred to a deep freezer within 16 hours of sampling. The samples were maintained frozen at <-20 °C and shipped frozen to Jealotts Hill Research Station for analysis.

For the day 0 samples, where a nominal depth of 10 cm was sampled, the twenty cores were bulked together for analysis. For the pre-application samples and all other time intervals, soil was sampled to a depth of 30 cm. These cores were sectioned into three horizons: 0-10 cm, 10-20 cm and 20-30 cm. Soil from each horizon, from each of the twenty cores was then bulked together for analysis. Control soil taken from the untreated plot was sectioned into profiles and bulked as indicated above. All soil was then air-dried for approximately 24 hours, sieved and then stones and debris were removed.

5. Analytical procedures

Samples were analysed between February 1992 and May 1992 for residues of glyphosate (N phosphonomethylglycine (PMG)), derived from glyphosate-trimesium and also the metabolite AMPA (aminomethylphosphonic acid) using ICI Americas Residue Analytical Method WRC 85-34. The method is summarised below:

Glyphosate (PMG) and AMPA were extracted from soil samples using 0.5 M ammonium hydroxide. After centrifugation, an aliquot of the supernatant was filtered and taken to dryness using a rotary evaporator. After re-dissolving the residue in 0.05 M borate buffer the glyphosate (PMG) and AMPA were then derivatised with 9-fluorenylmethyl chloroformate. The derivatives were determined by HPLC using an S5-SAX column and fluorescence detection.

The extraction solution was modified to 0.25 M ammonium hydroxide + 0.10 M monobasic potassium phosphate for trial Rohrbach (RS-9027/E2).

Residues were quantified by external standardisation and were corrected for recovery values generated by analysis of fortified control samples. Sample residue values have not been corrected for control values or for recovery values greater than 100%.

The conditions for high-performance liquid chromatographic (HPLC) determination of glyphosate (PMG) and AMPA residues were optimised for the soil matrix.

The limit of detection for PMG and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Recoveries from fortified untreated soil with glyphosate (PMG) and AMPA during the course of analysis reported in this study were as follows. Recoveries from soil fortified between 0.05 and 2.5 mg/kg (n=39) of glyphosate (PMG) ranged from 63 to 94 %; the mean was 81 %, and the coefficient of variation was 12 %. Recoveries from soil fortified between 0.05 and 2.5 mg/kg (n=39) of AMPA ranged from 53 to 111 %, the mean was 89 %, and the coefficient of variation was 15 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-62: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Buchen test site (RS-9027/B1)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD 2	< 2	≤ 0.06 2
	10 - 20	< LOD	< 2	< LOD
0	0 – 10	2.5 [2]	85	0.12 [2]
7	0 – 10	2.2	86	0.13
	10 - 20	< LOD	< 2	< LOD
14	0 – 10	1.9	77	0.20
	10 - 20	< LOD	< 2	< LOD
28	0 – 10	1.5	59	0.23
	10 - 20	< LOD	< 2	< LOD
61	0 – 10	0.75	30	0.30
	10 - 20	< LOD	< 2	< LOD
91	0 – 10	0.60	24	0.51
	10 - 20	< LOD	< 2	< LOD
121	0 – 10	0.23	10	0.18
	10 - 20	< LOD	< 2	< LOD
182	0 – 10	0.27	11	0.38
	10 - 20	< LOD	< 2	< LOD
240	0 – 10	0.18	7	0.31
	10 - 20	< LOD	< 2	< LOD
322	0 – 10	0.16	6	0.20
	10 - 20	< LOD	< 2	< LOD
475	0 – 10	0.15	6	0.33

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
	10 - 20	< LOD	< 2	< LOD

[1] DAA = Days after application

[2] Mean, where a sample has been analysed more than once

Residues have been corrected for external recovery values. Mean recovery in this soil: 75% (CV 10%) for PMG and 71% (CV 13%) for AMPA

Table 8.1.1.3-63: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Kleinzecher test site (RS-9027/B2)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD [2]	< 2	< LOD [2]
	10 - 20	< LOD	< 2	< LOD
0	0 – 10	2.0 [2]	67	0.12 [2]
7	0 – 10	1.9	74	0.25
	10 - 20	< LOD	< 2	< LOD
14	0 – 10	1.4	55	0.28
	10 - 20	< LOD	< 2	< LOD
28	0 – 10	1.0	40	0.29
	10 - 20	< LOD	< 2	< LOD
61	0 – 10	0.82	32	0.37
	10 - 20	< LOD	< 2	0.07
91	0 – 10	0.45	18	0.25
	10 - 20	< LOD	< 2	0.09
119	0 – 10	0.54	22	0.31
	10 - 20	< LOD	< 2	0.06
201	0 – 10	0.44	18	0.41
	10 - 20	< LOD	< 2	0.05
244	0 – 10	0.39	15	0.39
	10 - 20	< LOD	< 2	0.06
298	0 – 10	0.16	7	0.30
	10 - 20	< LOD	< 2	0.06
479	0 – 10	0.08	3	0.33
	10 - 20	< LOD	< 2	0.09
567	0 – 10	< LOD	< 2	0.24
	10 - 20	< LOD	< 2	0.07

[1] DAA = Days after application

[2] Mean , where a sample has been analysed more than once

Residues have been corrected for external recovery values. Mean recovery in this soil: 85% (CV 11%) for PMG and 93% (CV 9%) for AMPA

Table 8.1.1.3-64: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Unzhurst test site (RS-9027/E1)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD [2]	< 2	< LOD [2]
	10 - 20	< LOD	< 2	< LOD
0	0 – 10	3.2 [2]	100	0.07 2
7	0 – 10	1.8	76	0.14
	10 - 20	< LOD	< 2	< LOD
13	0 – 10	1.8	73	0.20
	10 - 20	< LOD	< 2	< LOD
27	0 – 10	1.4	55	0.17
	10 - 20	< LOD	< 2	< LOD
57	0 – 10	0.48	20	0.36
	10 - 20	< LOD	< 2	< LOD

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
90	0 – 10	0.34	15	0.40
	10 - 20	< LOD	< 2	< LOD
117	0 – 10	0.22	9	0.36
	10 - 20	< LOD	< 2	< LOD
187	0 – 10	0.15	6	0.35
	10 - 20	< LOD	< 2	< LOD
251	0 – 10	0.14	6	0.40
	10 - 20	< LOD	< 2	< LOD
314	0 – 10	0.12	5	0.35
	10 - 20	< LOD	< 2	< LOD
418	0 – 10	0.07	3	0.26
	10 - 20	< LOD	< 2	< LOD
564	0 – 10	n.a.	-	n.a.
	10 - 20	n.a.	-	n.a.

n.a. = defrosted on arrival, therefore not analysed
[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than onceResidues have been corrected for external recovery values. Mean recovery in this soil: 96% (CV 14%) for PMG and 87% (CV 11%) for AMPA

Table 8.1.1.3-65: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Rohrbach test site (RS-9027/E2)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 2	< LOD
	10 - 20	≤ LOD	< 2	< LOD
0	0 – 10	2.1[2]	65	≤ 0.05[2]
7	0 – 10	2.0	78	0.22
	10 - 20	< LOD	< 2	< LOD
14	0 – 10	1.5	58	0.31
	10 - 20	< LOD	< 2	< LOD
28	0 – 10	1.0	39	0.30
	10 - 20	< LOD	< 2	< LOD
56	0 – 10	0.29	12	0.45
	10 - 20	< LOD	< 2	< LOD
85	0 – 10	0.11	5	0.42
	10 - 20	< LOD	< 2	< LOD
231	0 – 10	< LOD	< 2	0.37
	10 - 20	< LOD	< 2	< LOD
282	0 – 10	< LOD	< 2	0.35
	10 - 20	< LOD	< 2	< LOD
418	0 – 10	< LOD	< 2	0.17
	10 - 20	< LOD	< 2	< LOD
582	0 – 10	< LOD	< 2	0.13
	10 - 20	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once
Residues have been corrected for external recovery values. Mean recovery in this soil: 74% (CV 10%) for PMG and 91% (CV 10%) for AMPA

Table 8.1.1.3-66: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Herrngiersdorf test site (RS-9027/G1)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 3	< LOD
	10 - 20	< LOD	< 3	< LOD

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
0	0 – 10	1.9[2]	62	0.05[2]
6	0 – 10	1.3	61	0.21
	10 - 20	< LOD	< 3	< LOD
13	0 – 10	0.94	46	0.16
	10 - 20	< LOD	< 3	< LOD
28	0 – 10	0.90	45	0.23
	10 - 20	< LOD	< 3	< LOD
58	0 – 10	0.27	14	0.23
	10 - 20	< LOD	< 3	< LOD
90	0 – 10	0.16	8	0.23
	10 - 20	< LOD	< 3	< LOD
125	0 – 10	0.09	4	0.22
	10 - 20	< LOD	< 3	< LOD
168	0 – 10	< LOD	< 3	0.14
	10 - 20	< LOD	< 3	< LOD
330	0 – 10	< LOD	< 3	0.15
	10 - 20	< LOD	< 3	< LOD
464	0 – 10	< LOD	< 3	0.06
	10 - 20	< LOD	< 3	< LOD
541	0 – 10	< LOD	< 3	< LOD
	10 - 20	< LOD	< 3	< LOD

[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once
Residues have been corrected for external recovery values. Mean recovery in this soil 82% (CV 16%) for PMG and 103% (CV 5%) for AMPA

Table 8.1.1.3-67: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Wang-Inzkofen test site (RS-9027/G2)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD 2	< 3	0.07 2
	10 - 20	< LOD	< 3	< LOD 2
0	0 – 10	2.3 [2]	78 [2]	0.21 [2]
7	0 – 10	1.2	62	0.30
	10 - 20	< LOD	< 3	< LOD
15	0 – 10	0.87	42	0.38
	10 - 20	< LOD	< 3	< LOD
29	0 – 10	0.81	40	0.46
	10 - 20	< LOD	< 3	< LOD
58	0 – 10	0.39	19	0.36
	10 - 20	< LOD	< 3	< LOD
94	0 – 10	0.23	11	0.39
	10 - 20	< LOD	< 3	< LOD
114	0 – 10	0.21	10	0.41
	10 - 20	< LOD	< 3	< LOD
275	0 – 10	0.11	6	0.32
	10 - 20	< LOD	< 3	< LOD
414	0 – 10	0.06	3	0.26
	10 - 20	< LOD	< 3	< LOD
549	0 – 10	< LOD	< 3	0.19
	10 - 20	< LOD	< 3	< LOD

[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once
Residues have been corrected for external recovery values. Mean recovery in this soil: 84% (CV 12%) for PMG and 99% (CV 9%) for AMPA

B. CHARACTERISATION OF RESIDUES

No residues of glyphosate (PMG) were measured in any trial, on any occasion, in the soil horizon below 10 cm. No residues of AMPA above the LOD were measured in any trial, on any occasion, in the soil horizon below 10 cm except for trial Kleinzecher (RS-9027/B2) where no residues were measured up until the 28 day sample, and then after this time, the residues were not greater than 0.09 mg/kg.

Initial residues of glyphosate (PMG) measured in the soil at day 0 were greater than 78 % for three out of the six trials. The other three trials gave recoveries of 62, 65 and 67 %. There is no reason evident from the field or weather data for the lower recoveries found and only one recovery (65%, Rohrbach, RS-9027/E2) was substantiated by the day 0 recovery of TMS+ (67%).

Glyphosate (PMG) degrades fairly rapidly and falls below 0.05 mg/kg by the end of four trials. In the other two trials, Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027G2), the residue values measured at the end of the trials were 0.15 mg/kg (< 7 % of applied) and 0.07 mg/kg (< 4 % of applied), respectively.

For all trials in this study, residues of AMPA in the soil increased as the glyphosate (PMG) residue decreased and then declined again to between 0.06 mg/kg and 0.33 mg/kg at the end of the trials.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

From the data it can be concluded that for the typical climatic conditions and soil types studied, glyphosate-trimesium dissipates fairly rapidly. Since no residue greater than 0.10 mg/kg of glyphosate (PMG) or AMPA was determined in any sample taken from a depth greater than 10 cm it can be concluded that neither glyphosate-trimesium or its metabolite, leach or therefore present any potential groundwater contamination risk.

For all trials in this study, residues of AMPA in the soil increased as the glyphosate (PMG) residue decreased and then declined again to between 0.06 mg/kg and 0.33 mg/kg at the end of the trials.

Assessment and conclusion by applicant:

The study provides valuable information on the dissipation behavior of glyphosate under field conditions from a variety of different test site conditions. As the representative formulation of the current submission does not contain the trimesium cation, the trimesium findings were neglected for further consideration. The study is considered valid to address the data point.

Assessment and conclusion by RMS

Glyphosate trimesium was applied on the field site. As mentioned by the applicant, trimesium findings are not considered for this renewal report.

According to section B5, the analytical method used in this study is not considered acceptable. As a consequence, results cannot be validated.

The following additional deviations from OECD ENV/JM/MONO(2016)6 are identified.

Initial measured concentrations of glyphosate were below 70% of nominal application rate for 3 test sites.

At each sampling time, 5 cores were taken in each of the 4 subplots. The 20 cores from same date and soil layer were mixed for analysis. No replicate is available at any time.

The study indicates that the soils were sampled to a 30 cm depth, even though only 0-10 and 10-20 cm layers are reported and no indication of the results in the 20-30 cm layer depth is provided for any

of the sites. The mobility of glyphosate and AMPA is known to be low and no quantifiable amounts of either glyphosate or AMPA were found in the 10-20 cm layer depth, except for Kleinzecher site. On this site, AMPA was quantified in the 10-20 cm layer from 61 days to the end of the study. In this case, results for lower soil depth should have been provided to ensure that no AMPA residue is found in 20-30 cm or deeper. Additionally, it is acknowledged that the residues measured in the 10-20 cm layer can be considered as small (slightly above the LOD). However, when considering the total layer 0-20 cm, the contribution of 10-20 cm layer is not negligible. Therefore, for this site it cannot be easily concluded that the impact of potential additional residues in the 20-30 cm layer will be negligible.

For Buchen and Kleinzecher sites, the reported weather data do not cover the whole study period. For Unzhurst and Rohrbach sites, daily weather data are reported only until November 1990. Monthly data are reported for the remaining study period.

RMS highlights that the residues in this study were quantified by external standardisation and corrected for external recoveries of fortified control samples. This approach has been criticized by the applicant in the laboratory degradation study [redacted], 1991 (see point B 1.1.1) and considered as a major deviation. It was not mentioned by the applicant in the case of this study report, although exactly the same information is provided in the study report.

Regarding this correction for external recovery, the procedure used is not clearly explained in the study report. From RMS understanding, the following issues are noted:

- the measured residues were corrected for external recovery values when measured recovery of glyphosate was below 100%, while no correction was done in other cases. Therefore the data were handled differently.
- Uncorrected raw data are not available. In addition, the individual recoveries were reported to be used but only the mean recoveries (presented above under each table of results) were indicated in the study. Therefore the procedure is not reproducible, and it is not possible to back calculate the raw data and to identify which data were corrected and which were not.
- the mean recoveries and coefficient of variation are in the recommended range (70-110%, with CV < 20%). However, since individual recoveries are not presented, it is not possible to check for possible outliers. Some procedural recoveries are indicated under appendix 3 but it is not known to what soils they refer, nor if they are the "external recoveries" used for correction.

As a consequence, this prevents validating the correction procedure and the data are considered quite uncertain.

Considering the use of a non-validated analytical method and the issues regarding the correction procedure, the study is not considered acceptable.

[redacted], *1992*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.2.1/014
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Soil Dissipation Study (Canada, 1988-1990)
Report No	RJ1225B
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - Only Ontario site representative on EU conditions - No replicate residue data available for all sampling dates

	- Sampling up to 30 cm only, and results presented up to 20 cm - No information on transport and processing - analytical method not validated - residues were corrected for external procedural recoveries based of fortified results, with not uniform handling of the data - only average recoveries of fortified controls reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	No

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate as glyphosate-trimesium (ICIA 0224)
Product:	Sulfosate (TF 1242 or YF7712A), 48 % SL formulation
Lot No.:	WHD0401
Nominal concentration:	48 % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

Prior to application, each of the Canadian trial site was cultivated, the surface levelled and the surface trash removed, by hand raking. At each of the trial sites the area was divided into two plots, a treated plot and an untreated (or control) plot, separated by a buffer zone. The treated plots were generally (in three out of five trials) 15 m x 15 m square and the smallest plot area was 9 m x 12 m. Control plots were generally smaller, ranging from 36 m2 (3 m x 12 m) up to 180 m2 (12 m x 15 m). Each of the treated plot areas was subdivided into at least three sub-plots from which a total of 30 core samples (generally 10 from each subplot) were taken. Pesticide use history over three years prior to the study were reported.

At each of the trial sites at least one soil pit was dug and samples were taken from at least two horizons to a depth of greater than 22.5 cm in all cases. Between 0.5 and 1 kg of soil was then bulked from each horizon and sent to Jealott's Hill Research Station, Bracknell, UK for physico-chemical characterisation.

Table 8.1.1.3-68: Soil characteristics of the St. Davids, Ontario (CA-SD-88-01) test site

Parameter		Horizon		
Soil depth (cm)		0-30	30-50	50 +
Cation Exchange Capacity (meq/100g)		15.8	25.3	12.0
Particle Size Analysis (USDA) (%)	sand	11	1	14
	silt	49	19	46
	clay	41	80	40
Soil Type		Silty clay	Clay	Silty clay loam
Organic matter (%)		4.3	3.8	0.8
Organic carbon (%) [1]		2.494	2.204	0.464
Soil pH [2]		7.9	7.9	7.7
Field capacity (% soil moisture at 1/3 bar)		30.63	43.44	26.77

[1] Calculated from organic matter according to OC = OM x 0.58

[2] Medium not stated

Table 8.1.1.3-69: Soil characteristics of the Carman, Manitoba (CA-SD-88-02) test site

Parameter	Horizon	
Soil depth (cm)	0-15	15-30
Cation Exchange Capacity (meq/100g)	10.8	10.4

Particle Size Analysis (USDA) (%)	sand	80	81
	silt	10	9
	clay	10	10
Soil Type		Loamy sand	
Organic matter (%)		2.9	2.6
Organic carbon (%) [1]		1.682	1.508
Soil pH		7.8	8.1
Field capacity (% soil moisture at 1/3 bar)		10.26	10.34

[1] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-70: Soil characteristics of the Grandora, Saskatchewan (CA-SD-88-03) test site

Parameter		Horizon		
Soil depth (cm)		0-30	30-50	50 +
Cation Exchange Capacity (meq/100g)		15.3	15.5	15.8
Particle Size Analysis (USDA) (%)	sand	42	36	45
	silt	30	34	29
	clay	28	30	27
Soil Type		Clay loam		
Organic matter (%)		3.3	2.0	1.0
Organic carbon (%) [1]		1.914	1.160	0.580
Soil pH		7.1	7.9	8.6
Field capacity (% soil moisture at 1/3 bar)		21.44	22.51	20.01

[1] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-71: Soil characteristics of the Speers, Saskatchewan (CA-SD-88-04) test site

Parameter		Horizon		
Soil depth (cm)		0-12	12-24	24 +
Cation Exchange Capacity (meq/100g)		22.0	16.7	17.5
Particle Size Analysis (USDA) (%)	sand	12	7	8
	silt	55	60	59
	clay	34	33	33
Soil Type		Silty clay loam		
Organic matter (%)		9.1	2.0	0.9
Organic carbon (%) [1]		5.278	1.160	0.522
Soil pH		7.1	7.8	8.2
Field capacity (% soil moisture at 1/3 bar)		34.71	24.68	24.49

[1] Calculated from organic matter according to OC = OM x 0.58

Table 8.1.1.3-72: Soil characteristics of the Brooks, Alberta (CA-SD-88-05) test site

Parameter		Horizon	
Soil depth (cm)		0-15	15-30
Cation Exchange Capacity (meq/100g)		13.2	13.6
Particle Size Analysis (USDA) (%)	sand	36	39
	silt	42	38
	clay	22	23
Soil Type		Loam	
Organic matter (%)		1.7	1.7
Organic carbon (%) [1]		0.986	0.986
Soil pH		7.6	7.3
Field capacity (% soil moisture at 1/3 bar)		18.84	18.56

[1] Calculated from organic matter according to OC = OM x 0.58

Meteorological records were obtained from local stations close to the trial sites, except for Alberta site for which no data were reported. Air temperature and precipitation were measured. Copies of these daily weather records for the study period are stored in the ICI Agrochemicals GLP Archives, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY under Study No: 88JH140.

Examination of these weather records showed that no extraordinary conditions were experienced during the dissipation period at each site.

Details on weather data are presented in the table below.

Table 8.1.1.3-73: Weather station and reporting time

Test site	Reporting time	Weather station
St. Davids, Ontario	September 1988 – July 1989	5 km from trial site
Carman, Manitoba	From July 1988 onwards	Environment Canada, climate reference station located at Morden, Manitoba, approximately 30 km from test site
Grandora and Speers, Saskatchewan	From July 1988 onwards	Saskatchewan Research Council, climate reference station located at Saskatoon Airport, approximately 12 km from Grandora test site
Brooks, Alberta	Not available in study report	Not available in study report

2. Application

Glyphosate-trimesium was applied on bare soil as sulfosate (TF 1242 or YF7712) as 48 % SL formulation to each trial, at a nominal application rate of 5.76 kg a.s./ha. Actual application rates are detailed in the table below. One batch of spray solution was mixed to cover the entire plot, then divided into three or 4 portions. The application was made in all cases using a hand-held CO2 pressurised sprayer equipped with a 3 m boom. Depending on the site size 3 to 5 passes were necessary for the application of the test compound. The sprayers were calibrated before use, unsprayed solution was collected and a sample was stored frozen for analysis. Conditions during application are detailed in the table below.

Table 8.1.1.3-74: Conditions during application

Treatment No.	St. Davids, Ontario	Carman, Manitoba	Grandora, Saskatchewan	Speers, Saskatchewan	Brooks, Alberta
Application date	30.09.1988	23.09.1988	23.09.1988	20.09.1988	26.09.1988
Application equipment	hand held CO2 pressurised sprayer equipped with a 3 m boom				
Nozzle type	Teejet 8003, flat fan	Teejet 8002, flat fan	Teejet 8001, flat fan	Teejet 8001, flat fan	Teejet 8001 LP, flat fan
Spray pressure	35 PSI	275 kPa	276 kPa	275 kPa	245 kPa
Number of passes	4	3	5	5	5
Actual application volume (mL) per test site	5008	1362	1720	1660	2475
Nominal application rate (kg a.s./ha)	5.76	5.76	5.76	5.76	5.76
Actual application rate (kg a.s./ha)	6.41	6.48	7.50	7.24	5.76
Mean air temperature (°C)	12-15	15	-1	7	5
Mean wind speed (m/s)	0-1	1.4	0.8	1.4	1.3
Wind direction	SW-NE	W/E	SW	E/SE	N-S
Relative air humidity (%)	70-80	55	82	85	85
Cloud cover (%)	0	0	30	100	95
Ground cover (%)	0	0	0	0	0
Wetness of soil surface	moist	dry	dry	dry	dry
Soil surface description	uniform, slightly crusted	fine	fine	slightly cloddy	granular

3. Sampling

Prior to application, samples of soil were taken from each site (30 cm cores with 2.5 cm internal diameter). Treated soil was sampled at day 0 and one day and three days after application, using 10 cm cores with a 5 cm internal diameter. At subsequent intervals, up to approximately 20 months, soil was sampled using a 30 cm x 2.5 cm internal diameter corer. For each trial at each interval, 30 cores were taken (usually 10 cores per sub-plot), in order to obtain a representative sample. All soil samples were taken using a zero contamination corer with plastic inserts.

4. Specimen handling and preparation

All soil samples were frozen within two to four hours of sampling. The samples were maintained frozen at < -15 °C and shipped frozen to Jealotts Hill Research Station for analysis.

The samples were received deep frozen at Jealott's Hill between October 1988 and October 1990 and were stored at <-15°C in the Residue and Environmental Chemistry Laboratory deep freeze until required for analysis.

For the 0, 1 and 3 day samples, where a nominal depth of 10 cm was sampled, the cores taken from the sub-plots in the treated plot were bulked separately for analysis. In detail, 10 cores from three sub-plots each, were bulked for the trials in Manitoba (Carman), Saskatchewan (Grandora and Speers) and Alberta (Brooks). In Ontario (St. Davids) where there were four treated sub-plots, 6 to 8 cores were bulked per sub-plot. For the pre-application samples and all other time intervals, soil was sampled to a depth of 30 cm. These cores were sectioned into three horizons 0-10 cm, 10-20 cm and 20-30 cm. Soil from each horizon was then bulked from the sub-plots as indicated above. Control soil taken from the untreated plot was sectioned into profiles as indicated above. All soil was then air-dried for up to 48 hours, sieved and then stones and debris removed.

5. Analytical procedures

Samples were analysed for residues of glyphosate (N phosphonomethylglycine (PMG)), and the metabolite AMPA (aminomethylphosphonic acid) using ICI Americas Residue Analytical Method WRC 85-34. The method is summarised below:

Glyphosate and AMPA were extracted from soil samples using 0.5 M ammonium hydroxide. After centrifugation, an aliquot of the supernatant was filtered and taken to dryness using a rotary evaporator. After re-dissolving the residue in 0.05 M borate buffer the glyphosate and AMPA were then derivatised with 9-fluorenylmethyl chloroformate. The derivatives were determined by HPLC using an S5-SAX column and fluorescence detection.

The conditions for high-performance liquid chromatographic (HPLC) determination of glyphosate and AMPA residues were optimised for the soil matrix.

Residues were quantified by external standardisation and were corrected for recovery values generated by analysis of fortified control samples. Sample residue values have not been corrected for control values or for recovery values greater than 100%.

The limit of determination for glyphosate and AMPA was 0.05 mg/kg.

Some preliminary analysis of spray solutions for each trial was carried out for trimesium (TMS+) (using a method based on the analytical procedures described earlier). Since the data were semiquantitative only, the full data were not reported and the data were used only in order to confirm the application rates. The investigations made, suggested that > 80 % of nominal applied was recovered for all trials with the exception of Speers. It was not possible to obtain a representative result from the Speers tank-mix sample.

Recoveries from fortified untreated soil with glyphosate (PMG) and AMPA were done for each trial separately and are as follows. Recoveries from soil fortified with glyphosate (PMG) ranged from 71 to 93 %; the coefficients of variation ranged from 14 to 22 %. Recoveries from soil fortified with AMPA ranged from 82 to 90 %, the coefficients of variation ranged from 14 to 19 %.

II. RESULTS AND DISCUSSION

A. DATA

All residues have been corrected for external recovery values except for control values or recovery values greater than 100%.

Table 8.1.1.3-75: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the St. Davids test site (CA-SD-88-01) - Ontario

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 2	< LOD
	10 - 20	< LOD	< 2	< LOD
0 [3]	0 – 10	2.2 [2] (1.5/1.7/2.6/3.2)	56 [2] (38/43/66/76)	0.14 [2] (0.13/0.13/0.19/0.11)
1 [3]	0 – 10	2.0 [2] (1.7/1.9/1.8/2.5)	50 [2] (47/48/47/60)	0.15 [2] (0.13/0.15/0.21/0.12)
3 [3]	0 – 10	0.83 [2] (0.78/0.84/0.86/n.a.)	22 [2] (21/21/23/-)	0.34 [2] (0.32/0.37/0.32/n.a.)
7	0 – 10	0.73	19	0.28
	10 - 20	< LOD	< 2	< LOD
14	0 – 10	0.61	16	0.41
	10 - 20	< LOD	< 2	< LOD
31	0 – 10	0.33	9	0.31
	10 - 20	< LOD	< 2	< LOD
60	0 – 10	0.19	5	0.31
	10 - 20	< LOD	< 2	< LOD
207	0 – 10	0.15	4	0.27
	10 - 20	< LOD	< 2	< LOD
297	0 – 10	< LOD	< 2	0.06
	10 - 20	< LOD	< 2	< LOD
391	0 – 10	< LOD	< 2	0.06
	10 - 20	< LOD	< 2	< LOD
577	0 – 10	< LOD	< 2	< LOD
	10 - 20	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)
Residues have been corrected for external recovery values. Mean recovery for this soil: 71% for glyphosate (CV 14%), 82% for AMPA (CV 15%).

Table 8.1.1.3-76: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Carman test site (CA-SD-88-02)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 2	< LOD
	10 - 20	< LOD	< 2	< LOD
0 [3]	0 – 10	1.6 [2] (1.8/1.6/1.5)	45[2] (49/43/43)	0.10 [2] (0.09/0.10/0.12)
1 [3]	0 – 10	1.6 [2] (1.7/1.7/1.4)	45 [2] (48/47/40)	0.10 [2] (0.10/0.13/0.07)
3 [3]	0 – 10	1.4 [2] 1.2/1.2/1.8)	38 [2] (33/32/50)	0.08 [2] (0.07/0.060.10)
7	0 – 10	1.6	39	0.07
	10 - 20	< LOD	< 2.0	< LOD
14	0 – 10	1.5	37	0.10
	10 - 20	< LOD	< 2	< LOD
215	0 – 10	0.37	10	0.26

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
	10 - 20	< LOD	< 2	< LOD
308	0 – 10	< LOD	< 2	0.08
	10 - 20	< LOD	< 2	< LOD
360	0 – 10	< LOD	< 2	0.05
	10 - 20	< LOD	< 2	< LOD
577	0 – 10	< LOD	< 2	0.07
	10 - 20	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)
Residues have been corrected for external recovery values. Mean recovery in this soil: 93% for PMG (CV 16%), 86% for AMPA (CV 12%).

Table 8.1.1.3-77: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Grandora test site (CA-SD-88-03)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 2	< LOD
	10 - 20	< LOD	< 2	< LOD
0 [3]	0 – 10	2.9 [2] (2.8/2.5/3.4)	69 [2] (61/58/89)	0.1 [2] (0.09/0.10/0.13)
1 [3]	0 – 10	2.5 [2] (2.5/2.6/2.4)	63 [2] (62/63/63)	0.14 [2] (0.13/0.15/0.14)
3 [3]	0 – 10	2.9 [2] (2.7/2.9/3.0)	76 [2] (70/77/79)	0.10 [2] (0.09/0.10/0.10)
7	0 – 10	1.5	35	0.17
	10 - 20	< LOD	< 2.0	< LOD
11	0 – 10	1.5	37	0.20
	10 - 20	< LOD	< 2	< LOD
27	0 – 10	1.5	35	0.25
	10 - 20	< LOD	< 2.	< LOD
212	0 – 10	0.91	25	0.36
	10 - 20	< LOD	< 2.	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)
Residues have been corrected for external recovery values. Mean recovery us: 80% for PMG (CV 20%), 87% for AMPA (CV 16%).

Table 8.1.1.3-78: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Speers test site (CA-SD-88-04)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 2	< LOD
	10 - 20	< LOD	< 2	< LOD
0 [3]	0 – 10	3.4 [2] (2.9/3.2/4.1)	72 [2] (62/6888)	< 0.08 [2] (<0.06/<0.06/0.12)
1 [3]	0 – 10	2.2 [2] (3.2/1.6/2.5)	50 [2] (63/33/56)	< 0.06 [2] (0.07/<LOD/<LOD)
3 [3]	0 – 10	3.5 [2] (3.6/3.6/3.4)	70 [2] (67/71/70)	0.15 [2] (0.14/0.15/0.15)
9	0 – 10	3.2	68	0.27
	10 - 20	< LOD	< 2	< LOD
14	0 – 10	2.3	47	0.24
	10 - 20	< LOD	< 2	< LOD

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
30	0 – 10	2.0	39	0.22
	10 - 20	< LOD	< 2	< LOD
232	0 – 10	1.2	26	0.46
	10 - 20	< LOD	< 2	< LOD
308	0 – 10	0.21	4	0.38
	10 - 20	< LOD	< 2	< LOD
359	0 – 10	< LOD	< 2	0.29
	10 - 20	< LOD	< 2	< LOD
615	0 – 10	< LOD	< 2	0.32
	10 - 20	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)
Residues have been corrected for external recovery values. Mean recovery: 84% for PMG (CV 22%), 85% for AMPA (CV 19%).

Table 8.1.1.3-79: Summary of residues (mg/kg) and (% of applied) for glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Brooks test site (CA-SD-88-05)

DAA[1]	Soil depth (cm)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 2	< LOD
	10 - 20	< LOD	< 2	< LOD
0	0 – 10	3.0 [2] (2.9/3.1/3.2)	106 [2] (90/98/130)	0.07 [2] (0.07/0.07/0.07)
1 3	0 – 10	2.2 [2] (2.5/2.3/1.9)	74 [2] (77/70/73)	0.06 [2] (0.06/0.06/<LOD)
3 3	0 – 10	1.9 [2] (2.3/2.0/1.5)	63 [2] (70/61/56)	0.06 [2] (0.07/0.07/<LOD)
7 3	0 – 10	1.9	60	0.10
	10 - 20	< LOD	< 2	< LOD
16	0 – 10	2.2	72	0.20
	10 - 20	< LOD	< 2	< LOD
29	0 – 10	1.4	45	0.17
	10 - 20	< LOD	< 2	< LOD
218	0 – 10	1.1	36	0.33
	10 - 20	< LOD	< 2	< LOD
323	0 – 10	0.65	20	0.42
	10 - 20	< LOD	< 2	< LOD
361	0 – 10	0.21	7	0.34
	10 - 20	< LOD	< 2	< LOD
575	0 – 10	0.20	6	0.53
	10 - 20	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)
Residues have been corrected for external recovery values. Mean recovery: 81% for PMG (CV 14%), 90% for AMPA (CV 14%).

B. CHARACTERISATION OF RESIDUES

Degradation of glyphosate (PMG) at all of the sites appeared to show some dependence on temperature. Applications were not made on any of the sites until on or following the 20th September. Evidence for some initial degradation was then seen on most sites, but with temperatures falling below 0 °C in October and November 1988, degradation appeared to slow or even cease until the temperature had

started to rise again in the spring of the following year. No residues of glyphosate or AMPA greater than 0.06 mg/kg were found in any trial, on any occasion, in the soil horizon below 10 cm.

Residues of glyphosate (PMG) in the top layer ranged from 1.6 to 3.4 mg/kg at day 0 and decreased to < LOD in three trials after 297 to 615 days. Since the Grandora trial was terminated after 250 days, in the last sampling at day 212, 0.91 mg/kg glyphosate remained in the top layer. In the Brooks trial 0.2 mg/kg glyphosate remained after 575 days.

Residues of AMPA (0.36 mg/kg and 0.32 mg/kg, i.e. < 15% of the original day 0 glyphosate residues) remained at the termination of the Grandora and Speers trials respectively. In the Brooks trial similarly to glyphosate, the AMPA residue in the soil had not dissipated by the termination of the trial. 0.53 mg/kg of AMPA remained (equivalent to < 20 % of the day 0 PMG concentration). At St. Davids and Carman, AMPA had declined to < LOD and 0.07 mg/kg respectively at study termination.

C. KINETICS

An Ecoregion Crosswalk exercise was performed ([redacted], 2020, CA 7.1.2.2.1/002), and depending on its outcome, new kinetic calculations based on more recent guidance becomes necessary, therefore the kinetic information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020b, CA 7.1.2.2.1/003).

III. CONCLUSIONS

From the data it can be concluded that for the typical climatic conditions and soil types studied, glyphosate-trimesium dissipates fairly rapidly. Since no residue greater than 0.06 mg/kg of glyphosate (PMG) or AMPA was determined in any sample taken from a depth greater than 10 cm it can be concluded that neither glyphosate-trimesium or its metabolite, leach or therefore present any potential groundwater contamination risk.

For all of the trials, residues of AMPA in the soil increased as the glyphosate (PMG) residue decreased and then declined again to between 0.07 mg/kg and 0.53 mg/kg at the end of the trials. No residue significantly greater than the limit of determination was found at the end of the trial.

Assessment and conclusion by applicant:

The study provides valuable information on the dissipation behavior of glyphosate from a variety of different test site conditions. As the representative formulation of the current submission does not contain trimesium cation, the trimesium findings were neglected for further consideration.

The study is considered valid to address the data point.

Assessment and conclusion by RMS

Glyphosate trimesium was applied on the field site. As mentioned by the applicant, trimesium findings are not considered for this renewal report.

The five test sites are Canadian soils. It was concluded from the ecoregion crosswalk ([redacted], 2020) that only the Ontario site is considered as representative of European conditions. The three other sites are therefore not commented below.

According to section B5, the analytical method used in this study is not considered acceptable. As a consequence, results cannot be validated.

The following additional deviations from OECD ENV/JM/MONO(2016)6 are identified.

At most of the sampling times, the cores taken from each subplot (for a same layer) were mixed before analysis. Then, no replicate analysis is available.

The study indicates that the soils were sampled to a 30cm depth, even though only 0-10 and 10-20 cm layers are reported. Since the mobility of glyphosate and AMPA is known to be low and no quantifiable amounts of either glyphosate or AMPA were found in the 10-20 cm layer depth, the absence of analysis of the 20-30 cm layer can be accepted.

Initial recovery in Ontario soil was low, 56% of the applied substance had been measured in the 0-10cm layer. The study report indicates that this may be due to the clay content of the soil which renders the extractability more difficult.

RMS highlights that the residues in this study were quantified by external standardisation and corrected for external recoveries of fortified control samples. This approach has been criticized by the applicant in the laboratory degradation study [redacted], 1991 (see point B 1.1.1) and considered as a major deviation. It was not mentioned by the applicant in the case of this study report, although exactly the same information is provided in the study report.

Regarding this correction for external recovery, the procedure used is not clearly explained in the study report. From RMS understanding, the following issues are noted:

- the measured residues were corrected for external recovery values when measured recovery of glyphosate was below 100%, while no correction was done in other cases. Therefore the data were handled differently.
- Uncorrected raw data are not available. In addition, the individual recoveries were reported to be used but only the mean recoveries (presented above under each table of results) were indicated in the study. Therefore the procedure is not reproducible, and it is not possible to back calculate the raw data and to identify which data were corrected and which were not.
- the mean recoveries and coefficient of variation are in the recommended range (70-110%, with CV < 20%). However, since individual recoveries are not presented, it is not possible to check for possible outliers.

Consequently, this prevents validating the correction procedure and the data are considered quite uncertain.

Considering the use of a non validated analytical method and the issues regarding the correction procedure, the study is not considered acceptable.

[redacted], 1990

Data point:	CA 7.1.2.2.1/015
Report author	[redacted]
Report year	1990
Report title	Dissipation of Glyphosate and Aminomethylphosphonic acid in forestry sites
Report No	MSL-9940
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: Aerial application on forestry
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not mentioned in RAR (2015), not accepted in DAR (2001)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type:	terrestrial field dissipation
	Test item:	Roundup
	Test sites:	Three sites in USA, forestry use,

Chassell: harvested, foreseen for conifers.
Corvallis: mixed conifers and hardwoods
Cuthbert: blank, foreseen for planting of conifers

Soil types (0 – 15 cm depth):
Chassell: sandy loam, pH 4.8, OM 2.5 %
Corvallis: sandy clay loam, pH 5.8, OM 7.2 %
Cuthbert: sandy loam, pH 5.4, OM 0.8 %

Application rate: 4.2 kg a.s./ha, single application
Application method: Aerial application by helicopter, over the forests
Application timing: late August – mid of September
Sampling periods and considered compartments:
One month for water (flowing water, non-flowing pond)
One year for sediment
One year for exposed soil and soil under litter
One year for litter
One year for foliar and herbaceous vegetation
Sampling times: -9, -1, 0, 1, 3 , 7, 14, 28/30, 58-63, 120-122, 180-187, 321-346, 365, (398-409) DAT
Sampling method:
plant material by gloved hands
soil samples by core sampler
water: grab sampling (plastic bottles)
sediment: soil core sampler
Sampling depth (soil): 0 - 15.2 cm depth and 15.2 – 30.4 cm depth

Sample storage: frozen directly after sampling and kept frozen until sample preparation
Workup and analysis:
<u>Soil and plant material:</u>
Grounding when frozen with dry ice, thawing overnight, mixing
Extraction with chloroform and HCl.
Elution through Chelex column chromatography
Anion exchange chromatography
Analysis by HPLC
LOD = 0.05 mg/kg for soil, foliage, vegetation (both, glyphosate and AMPA)
LOD = 0.1 mg/kg for leaf litter (both, glyphosate and AMPA)
<u>Sediment:</u>
Same procedure as for soil, extraction is different:
Extraction with KOH.
<u>Water:</u>
Thawing of frozen water samples
Concentration and drying of samples
Mixing with HPLC buffer and EDTA.
Filtering through a membrane filter
Analysis by HPLC, LOD = 0.001 µg/L (both, glyphosate and AMPA)

Recovery in fortified samples:

	Glyphosate	AMPA
Pond water	97.08 %	94.72 %
Stream water	105.10 %	100.23 %
Pond sediment	51.05 – 93.66 %	59.52 – 85.05 %
Stream sediment	79.64 – 89.96 %	79.12 – 86.34 %
Soil	72.89 – 91.41 %	70.99 – 89.60 %
Foliage	92.77 %	86.73 %
Vegetation	94.09 %	90.67 %
Leaf litter	84.36 %	86.59 %

Short description of results:
Residues
<u>Glyphosate</u>:

Chassell site:
Pond water: 1.678 µg/L (0 DAT), <0.001 µg/L (30 DAT0)
Stream water: 1.237 µg/L (0 DAT) , <0.001 µg/L (30 DAT)
Pond sediment: max. 1.92 mg/kg (60 DAT), 0.99 mg/kg (398 DAT)
Stream sediment: max. 0.69 mg/kg (DAT 7), <0.05 mg/kg (335 DAT)
Foliage: 1272.62 mg/kg (0 DAT), 0.24 mg/kg (335 DAT)
Vegetation: 628.63 mg/kg (0 DAT), <0.05 mg/kg (335 DAT)
Leaf litter: 322.4 mg/kg (0 DAT), 0.11 mg/kg (398 DAT)
Exposed soil (0 – 15 cm): max. 4.67 mg/kg (14 DAT), <0.05 mg/kg (398 DAT)
Exposed soil (15 – 30 cm): always <0.05 mg/kg
Soil under litter (0 – 15 cm): max. 1.4 mg/kg (7 DAT), 0.1 mg/kg (398 DAT)
Soil under litter (15 – 30 cm): max. 0.09 mg/kg (60 DAT), <0.05 mg/kg (398 DAT)

Corvallis site:
Pond water: 0.09 µg/L (0 DAT, 0.002 µg/L (28 DAT)
Stream water: 0.035 µg/L (0 DAT), 0.001 µg/L (28 DAT)
Pond sediment: max. 19.42 mg/kg (28 DAT), 1.21 mg/kg (409 DAT)
Stream sediment: max. 0.11 mg/kg (DAT 180), <0.05 mg/kg (346 DAT)
Foliage: 652.19 mg/kg (0 DAT), 13.42 mg/kg (63 DAT)
Vegetation: 47.37 mg/kg (7 DAT), 0.44 mg/kg (346 DAT)
Leaf litter: 590.07 mg/kg (63 DAT), 0.19 mg/kg (409 DAT)
Exposed soil (0 – 15 cm): max. 0.15 mg/kg (122/180 DAT), <0.05 mg/kg (409 DAT)
Soil under litter (0 – 15 cm): max. 0.07 mg/kg (63 DAT), <0.05 mg/kg (409 DAT)
Soil (both, 15 – 30 cm): always <0.05 mg/kg, except 346 DAT sample of exposed soil (0.07 mg/kg).

Cuthbert site:
Pond water: 0.983 µg/L (0 DAT), 0.001 µg/L (30 DAT)
Stream water: 0.031 µg/L (0 DAT) , <0.001 µg/L (30 DAT)
Pond sediment: max. 0.26 mg/kg (0 DAT), <0.05 mg/kg (400 DAT)
Stream sediment: max. 0.18 mg/kg (1 DAT), 0.07 mg/kg (181 DAT)
Foliage: 760.01 mg/kg (0 DAT), <0.05 mg/kg (321 DAT)
Vegetation: 360.5 mg/kg (0 DAT), <0.05 mg/kg (321 DAT)
Leaf litter: max. 262.11 mg/kg (30 DAT), 8.41 mg/kg (120 DAT)
Exposed soil (0 – 15 cm): max. 1.87 mg/kg (3/7 DAT), <0.05 mg/kg (400 DAT)
Soil under litter (0 – 15 cm): max. 0.14 mg/kg (30 DAT),<0.05 mg/kg (400 DAT)
Soil (both, 15 – 30 cm): always <0.05 mg/kg.

AMPA:
Chassell site:
Pond water: 0.035 µg/L (3 DAT), <0.001 µg/L (30 DAT)
Stream water: 0.003 µg/L (1 DAT) , <0.001 µg/L (30 DAT)
Pond sediment: max. 1.37 mg/kg (30 DAT), 1.09 mg/kg (398 DAT)
Stream sediment: max. 0.38 mg/kg (14 DAT), <0.05 mg/kg (335 DAT)
Foliage: max. 2.65 mg/kg (0 DAT), <0.05 mg/kg (335 DAT)
Vegetation: max. 2.21 mg/kg (0 DAT), <0.05 mg/kg (335 DAT)
Leaf litter: max. 17.5 mg/kg (3 DAT), 0.13 mg/kg (398 DAT)
Exposed soil (0 – 15 cm): max. 0.51 mg/kg (187 DAT), <0.05 mg/kg (398 DAT)
Soil under litter (0 – 15 cm): max. 0.68 mg/kg (30 DAT), 0.12 mg/kg (398 DAT)
Soil (both, 15 – 30 cm): always <0.05 mg/kg

Corvallis site:

Pond water: 0.002 µg/L (0/14 DAT), <0.001 µg/L (28 DAT)
Stream water: 0.002 µg/L (1 DAT) , <0.001 µg/L (28 DAT)
Pond sediment: max. 1.85 mg/kg (28 DAT), 0.56 mg/kg (409 DAT)
Stream sediment: max. 0.18 mg/kg (63 DAT), 0.11 mg/kg (346 DAT)
Foliage: max. 2.16 mg/kg (7 DAT), 0.64 mg/kg (63 DAT)
Vegetation: max. 0.2 mg/kg (63 DAT), <0.05 mg/kg (346 DAT)
Leaf litter: max. 4.4 mg/kg (63 DAT), 0.19 mg/kg (409 DAT)
Exposed soil (0 – 15 cm): max. 0.32 mg/kg (346 DAT), <0.05 mg/kg (409 DAT)
Soil under litter (0 – 15 cm): max. 0.14 mg/kg (346 DAT), 0.07 mg/kg (409 DAT)
Soil (both, 15 – 30 cm): always <0.05 mg/kg, except 346 DAT sample of exposed soil (0.11 mg/kg).

Cuthbert site:
Pond water: 0.014 µg/L (0 DAT), 0.002 µg/L (30 DAT)
Stream water: always <0.001 µg/L
Pond sediment: max. 0.13 mg/kg (321 DAT), <0.05 mg/kg (400 DAT)
Stream sediment: always <0.05 mg/kg
Foliage: max. 1.66 mg/kg (7 DAT), <0.05 mg/kg (321 DAT)
Vegetation: max. 1.06 mg/kg (7 DAT), <0.05 mg/kg (321 DAT)
Leaf litter: max. 9.09 mg/kg (3 DAT), 1.79 mg/kg (120 DAT)
Exposed soil (0 – 15 cm): max. 0.18 mg/kg (400 DAT)
Soil under litter (0 – 15 cm): max. 0.23 mg/kg (181 DAT), 0.13 mg/kg (400 DAT)
Soil (both, 15 – 30 cm): always <0.05 mg/kg

Half-life times: not calculated

No evidence for leaching of glyphosate into the 15 – 30 cm soil horizon.

Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:

The study is considered invalid due to the following deficiencies:

- Forestry sites
- Aerial application
- Application to vegetation – high variance and uncertainty in dissipation data (falling leaves etc.).
- Disturbance of sites by forest management.
- Daily weather data only provided for nearest available weather stations, not for the site itself.

Assessment and conclusion by RMS

As mentioned by the applicant, application was done by aerial application on forestry.

The study is not considered acceptable.

[redacted], *1989a*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.2.1/016
Report author	[redacted]
Report year	1989a
Report title	ICIA 0224 – Field Dissipation Study for Terrestrial Uses, California, 1987-1988, Residue Data to Support Registration of TOUCHDOWN
Report No	WRC 89-37

Guidelines followed in study	U.S. EPA 164-1 None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No information on site management and pesticide use history - Treated area was not divided into subplots - Only 4-5 cores per plot - No replicates available, except for 0-7.6 cm layer - No information on sample transport and processing - Analytical method not validated
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation:	Touchdown 4-LC
Lot No.:	WCH 1105
Nominal concentration:	41.8 wt. % or 479 g/L (4 lb per gallon) glyphosate-trimesium
Measured concentrations:	40.1 wt. % Glyphosate-trimesium 27.5 wt. % Glyphosate (CMP) 14.2 wt. % Trimesium (TMS)

B. STUDY DESIGN

1. Test sites

The test site was located near Orange Cove, California, which is near ICI´s America´s Western Research Station at Visalia, California, about 30 km (20 miles) from the local climate weather station in Fresno, California ([redacted], Elevation: 1072 m (327 feet)). The non-replicated treatment plot had a size of 26 x 6 m, 158 m^2 (85 by 20 feet), containing one treated and one control plot.

Table 8.1.1.3-80: Soil characteristics of the Californian test site

Parameter		Horizon			
Soil depth (cm)		0-30.5	30.5-61.0	61.0-91.4	91.4-121.9
Soil depth (inch)		0-12	12-24	24-36	36-48
Cation Exchange Capacity (meq/100g)		7.0	6.8	7.7	7.9
Particle Size Analysis (USDA) (%) [1]	sand	66	68	60	64
	silt	21	19	29	25
	clay	13	13	11	11
Soil Type		Sandy loam			
Organic matter (%)		0.6	0.2	0.2	0.1
Organic carbon (%) [2]		0.348	0.116	0.116	0.058
Soil pH [3]		7.1	7.7	7.7	7.6
Soil Bulk Density (g/L) [b)]		Not indicated in the study report			
Field capacity (% soil moisture at 1/3 bar)		10.85	10.64	11.17	11.57

[1] Due to rounding differences the sum may not correspond to 100 %
[2] Calculated from organic matter according to OC = OM x 0.58
[3] medium not stated

Long-term daily air temperatures and precipitation data as well as annual average air temperatures and total annual precipitation was provided from the weather station in Fresno, California. Reported daily

parameters include minimum, maximum and mean air temperatures, total daily precipitation, average wind speed and direction, sky cover (sunrise – sunset) and peak wind. Additionally, monthly average soil (at 20.3 cm depth) and air temperature data are available from Madera, approximately 16-32 km (10-20 miles) away from the test site. Irrigation was applied and recorded prior to application and at weekly intervals throughout the test period in amounts typical for the area. In areas of natural rainfall, historical weekly rainfall records were obtained from the nearest weather station. If necessary, irrigation was applied to bring the total (rainfall plus irrigation) to 110 % of the historical weekly average.

2. Application

The test site received one application with TOUCHDOWN 4-LC (batch WCH 1105) containing 4.48 kg (4 lb/gallon) of active ingredient glyphosate. Application was conducted on 7 July 1987 with a tractor mounted boom sprayer to bare soil, consisting of fine clods. The formulation was not incorporated. The plots were not cultivated or fertilized before application. During application the air temperature was 29.4 °C (85 °F), soil temperature was 26.6 °C (80 °F), relative humidity was 53 %, and the air movement was 8 km/h (5 mph).

3. Sampling

Soil samples were taken at the following depths: 0 to 7.6 cm, 7.6 to 15.2 cm, 15.2 to 22.9 cm, 22.9 to 30.5 cm, 30.5 to 61.0 cm, 61.0 to 91.4 cm and 91.4 to 121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 1, 3, 7, 14, 31, 59, 205 and 366 after application.

Five separate field samples were randomly collected from both check (untreated control) and treated areas at each soil sampling time. A 10.2 cm length (4 inch) of 7.6 cm (3 inch) diameter aluminium tube was inserted into the ground to a 7.6 cm (3 inch) depth. The 0 to 7.6 cm (0 to 3 inch) depth soil sample was collected by removal of the soil inside the aluminium tube. The 7.6 to 121.9 cm (3 to 48 inch) soil sample was collected with a 2.54 cm (1 inch) diameter hydraulic soil probe; the probe contained an acetate liner to prevent contamination of the soil.

4. Specimen handling and preparation

Soil samples were chilled at the time of collection, transported to the Research Station and frozen. Frozen samples were shipped via overnight express courier or commercial refrigerated truck to ICI America's Western Research Center (WRC) analytical laboratory and arrived frozen. Samples were subdivided into the various appropriate lengths and stored at -20 °C until analysis. Lower depths increments (7.6 to 15.2 cm and below) were mixed to one combined sample, 0 to 7.6 cm samples were kept separately.

Storage stability in soil was assessed in a separate study (RRC 86-61 "Frozen Storage Stability of Touchdown in Soil"), while the results are summarised within the present report. The data indicate that ICIA 0224 residues (glyphosate and AMPA) in sandy loam soil (from Orange Cove, California) are stable, under the frozen storage conditions for at least two years. During this study no field-treated sample was stored in excess of 366 days (12 months).

5. Analytical procedures

CMP (glyphosate) and AMPA were analysed by liquid chromatography using RCC method 85-34 (“Determination of SC-0224 Anion Residues in Crops, Water, and Soil by Liquid Chromatographie”). The method is described briefly in the following. Soil samples were extracted with 0.5 M ammonium hydroxide. The extracts were purified by using a cation-exchange column. The CMP and its metabolite AMPA were eluted from the column, derivatised with 9-fluorenylmethylchloroformate, and determined by using an HPLC equipped with an anion-exchange column and a fluorescence detector.

For every set of field samples extracted, one untreated control sample and one fortified control sample were concurrently extracted. If the set was composed of more than ten samples, one control and one fortified control were concurrently extracted for each subset of ten field samples. Untreated control samples contained no residues above the 0.05 mg/kg detection limit for soil.

The limit of detection for CMP and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Recoveries from fortified untreated soil with CMP and AMPA during the course of analysis reported in this study as follows. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of CMP ranged from 70 to 118 %; the mean was 89 %, and the coefficient of variation was 15 %. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of AMPA ranged from 64 to 120 %; the mean was 90 %, and the coefficient of variation was 17 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-81: Summary of residues (mg/kg) for glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.s,/ha

DAA[1]	Soil depth (cm)	CMP	AMPA
0	0 – 7.6	2.1/2.2 [2]	0.06/0.06 [2]
		2.5	0.10
		2.6.	0.05
		3.3	0.09
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
1	0 – 7.6	3.2/3.2 [2]	0.08/0.08 [2]
		2.2	0.07
		2.8	0.07
		1.7	0.06
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	n.a.	n.a.
3	0 – 7.6	1.8/1.7 [2]	0.15/0.15 [2]
		1.6	0.14
		1.5	0.15
		1.7	0.12
		2.0	0.15
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
7	0 – 7.6	1.1/1.3 [2]	0.13/0.15 [2]
		1.3	0.20
		0.9	0.30
		1.0	0.30
		0.8	0.30
	7.6 – 15.2	0.2	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
14	0 – 7.6	1.00/1.10 [2]	0.19/0.21 [2]
		0.34	0.26
		0.81	0.18
		0.64	0.17
		0.72	0.24
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD
31	0 – 7.6	0.29/0.27 [2]	0.36/0.34 [2]
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD

DAA[1]	Soil depth (cm)	CMP	AMPA
59	0 – 7.6	< LOD/< LOD [2]	0.13/0.15 [2]
		0.060	0.24
		0.130	0.24
		0.090	0.21
		< LOD	0.30
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD
205	0 – 7.6	< LOD/< LOD [2]	0.10/0.10 [2]
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
366	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD

[1] DAA = Days after application
[2] analysed in duplicate
n.a. = not analysed

B. CHARACTERISATION OF RESIDUES

Glyphosate (CMP) amounted to 2.7 mg/kg on the day of application and decreased to 0.08 mg/kg on day 59; thereafter no residues > LOQ were encountered. A 0.20 mg/kg CMP anion residue value was found on day 7 at 7.6-15.2 cm depth (3 to 6 inch). Significant amounts of residues were found only at the 0 to 7.6 cm soil depth (0 to 3 inch); these residues completely dissipated by day 203.

It can be concluded that AMPA is formed following the application of TOUCHDOWN 4-LC. Residue levels increased to about 0.35 mg/kg after 31 days and began to decline during the remaining period. AMPA is a very small, highly polar molecule, capable of binding tightly to soil. The 0.10 to 0.35 mg/kg residuals at 0 to 7.6 cm (0 to 3 inch) at days 31 to 205, may represent AMPA that is tightly bound to the soil and not capable of undergoing rapid dissipation. AMPA residues were not detected in the 366 day soi1 samples. AMPA residues were found only in the upper 0 to 7.6 cm (0 to 3 inch) layer, thus, it can be concluded that AMPA does not leach.

C. KINETICS

An Ecoregion Crosswalk exercise was performed (see [redacted], 2020, CA 7.1.2.2.1/002). The trial in California was found to be representative for European conditions and included in kinetic evaluation ([redacted], 2020b, CA 7.1.2.2.1/003).

III. CONCLUSIONS

ICIA 0224 (as measured by trimesium and glyphosate residues) dissipated rapidly in sandy loam soil in California after application of TOUCHDOWN 4-LC formulation.

ICIA 0224 did not leach or migrate prior to its environmental degradation. Except for one 0.20 mg/kg glyphosate residue at 7 days and the 7.6 to 15.2 cm soil depth (3 to 6 inch), all residues were found in the 0 to 7.6 cm (0 to 3 inch) soil depth samples.

AMPA was formed as an intermediate degradate in the course of carbon recyclisation/mineralisation of glyphosate. From an initial 2.7 mg/kg glyphosate residue, the maximum amount of AMPA residue found was 0.35 mg/kg. It appeared that most of the AMPA was rapidly further degraded, but a small amount (0.1 mg/kg) became bound to the soil and unavailable for rapid degradation. AMPA was not found below the 0 to 7.6 cm (0 to 3 inch) soil depth sampled. AMPA was not detected in the 366 day soil samples.

Assessment and conclusion by applicant:

The study was performed according to the respective guideline in force in 1987. There are minor deviations to current guideline requirements. Nevertheless, the study provides valuable information on the dissipation behavior of glyphosate under field conditions. As the representative formulation of the current submission does not contain trimesium cation, the trimesium findings were neglected for further consideration.

The study is considered valid to address the data point.

Assessment and conclusion by RMS

Glyphosate trimesium was applied on the field site. As mentioned by the applicant, trimesium findings are not considered for this renewal report.

The test site was assessed in ecoregion crosswalk study from [redacted], 2020. It is considered as representative of European conditions.

According to section B5, the analytical method used in this study is not considered acceptable. As a consequence, results cannot be validated.

The following additional deviations from OECD ENV/JM/MONO(2016)6 are identified.

- No information on pesticide use and site history is available.
- Four to five cores were taken from a single treated plot. For the soil layers deeper than 7.6 cm (3 inch), the cores were mixed to one combined sample, then no replicates are available. For the uppermost soil layer (0-7.6 cm), the individual cores were analysed separately; in addition, one of the samples was further divided in two subsamples and analysed in duplicate.

Considering that the analytical method used is not validated, the study is not acceptable.

[redacted], *1989b*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.2.1/017
Report author	[redacted]
Report year	1989b
Report title	ICIA 0224 – Field Dissipation Study for Terrestrial Uses, Mississippi, 1987-1988, Residue Data to Support Registration of TOUCHDOWN
Report No	WRC 89-40
Guidelines followed in study	U.S. EPA 164-1
Deviations from current test guideline	None From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - Site not representative of EU conditions - No information on site management and pesticide use history - Treated area was not divided into subplots - Only 5 cores per plot - No replicate residue data available except for 0-7.6 cm layer - No information on sample transport and processing - Analytical method not validated
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)

Acceptability/Reliability: No

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification: Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation: Touchdown 4-LC
Lot No.: WCL 1402
Nominal concentration: 41.8 wt. % or 479 g/L (4 lb per gallon) glyphosate-trimesium
Measured concentration: 41.4 wt. % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

The test site was located in Leland, Mississippi, which is near ICI´s America´s Southern Research Station at Leland, Mississippi. The local climate weather station (mid-south Agricultural Weather service Center) is located at Stoneville, Mississippi. The non-replicated treatment plot had a size of 12 x 15 m, 186 m^2 (40 by 50 feet), containing one treated and one control plot.

Table 8.1.1.3-82: Soil characteristics of the Mississippi test site

Parameter		Horizon			
Soil depth (cm)		0-30.5	30.5-61.0	61.0-91.4	91.4-121.9
Soil depth (inch)		0-12	12-24	24-36	36-48
Cation Exchange Capacity (meq/100g)		7.1	10.6	9.6	13.5
Particle Size Analysis (USDA) (%) [1]	sand	23	12	22	12
	silt	62	67	59	56
	clay	15	21	19	32
Soil Type		silt loam	silt loam	silt loam	silty clay loam
Organic matter (%)		0.7	0.6	0.3	0.7
Organic carbon (%) [2]		0.406	0.348	0.174	0.406
Soil pH [3]		6.9	7.0	7.1	7.2
Soil Bulk Density (g/L) [b)]		Not indicated in the study report			
Field capacity (% soil moisture at 1/3 bar)		21.5	22.4	25.0	31.2

[1] Due to rounding differences the sum may not correspond to 100 %
[2] Calculated from organic matter according to OC = OM x 0.58
[3] medium not stated

Long-term daily air temperatures and precipitation data as well as soil temperature and wind speed was provided from the weather service Center at Stoneville, Mississippi. Reported daily parameters include minimum and maximum air temperatures, minimum and maximum soil temperatures (at depths of 5.1, 10.2, 20.3 and 50.8 cm (2, 4, 8 and 20 inches), total daily precipitation, evaporation and wind speed. Daily rainfall was measured and irrigation was applied and recorded at 14-day intervals throughout the test period to bring the total (rainfall plus irrigation) to 110 % of the historical weekly average.

2. Application

The test site received one application with TOUCHDOWN 4-LC (batch WCL 1402) containing 4.48 kg (4 lb/gallon) of active ingredient. Application was conducted on 7 July 1987 with a tractor mounted boom sprayer to bare soil, consisting of dry small clods. The formulation was not incorporated. The plots were not cultivated or fertilized before application. During application the air temperature was 34.4 °C (94 °F), soil temperature was 30.0 °C (86 °F), relative humidity was 45 %, and the air movement was 3.2 km/h (2 mph) from southwest.

3. Sampling

Soil samples were taken at the following depths: 0 to 7.6 cm, 7.6 to 15.2 cm, 15.2 to 22.9 cm, 22.9 to 30.5 cm, 30.5 to 61.0 cm, 61.0 to 91.4 cm and 91.4 to 121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 1, 3, 7, 14, 28, 57, 199, 380 and 542 after application.

Five separate field samples were randomly collected from both check (untreated control) and treated areas at each soil sampling time. A 10.2 cm length (4 inch) of 7.6 cm (3 inch) diameter aluminium tube was inserted into the ground to a 7.6 cm (3 inch) depth. The 0 to 7.6 cm (0 to 3 inch) depth soil sample was collected by removal of the soil inside the aluminium tube. The 7.6 to 121.9 cm (3 to 48 inch) soil sample was collected with a 2.54 cm (1 inch) diameter hydraulic soil probe; the probe contained an acetate liner to prevent contamination of the soil.

4. Specimen handling and preparation

Soil samples were chilled at the time of collection, transported to the Research Station and frozen. Frozen samples were shipped via overnight express courier or commercial refrigerated truck to ICI America's Western Research Center (WRC) analytical laboratory and arrived frozen. Samples were subdivided into the various appropriate lengths and stored at -20 °C until analysis. Lower depths increments (7.6 to 15.2 cm and below) were mixed to one combined sample, 0 to 7.6 cm samples were kept separately.

Storage stability in soil was assessed in a separate study (RRC 86-61 "Frozen Storage Stability of Touchdown in Soil"), while the results are summarised within the present report. The data indicate that ICIA 0224 residues (glyphosate and AMPA) in silty clay loam soil (from Leland, Mississippi) are stable, under the frozen storage conditions for at least two years. During this study no field-treated sample was stored in excess of 170 days (5.7 months).

5. Analytical procedures

CMP (glyphosate) and AMPA were analysed by liquid chromatography using RCC method 85-34 (“Determination of SC-0224 Anion Residues in Crops, Water, and Soil by Liquid Chromatographie”). The method is described briefly in the following. Soil samples were extracted with 0.5 M ammonium hydroxide. The extracts were purified by using a cation-exchange column. The CMP and its metabolite AMPA were eluted from the column, derivatised with 9-fluorenylmethylchloroformate, and determined by using an HPLC equipped with an anion-exchange column and a fluorescence detector.

For every set of field samples extracted, one untreated control sample and one fortified control sample were concurrently extracted. If the set was composed of more than ten samples, one control and one fortified control were concurrently extracted for each subset of ten field samples. Untreated control samples contained no residues of above the 0.05 mg/kg detection limit for soil.

The limit of detection for glyphosate and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Additional recovery data for method validation are contained in the residue method reports (RCC reports No. 85-33 and 85-34), included in the present study report.

Recoveries from fortified untreated soil with glyphosate and AMPA during the course of analysis reported in this study are as follows. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of CMP ranged from 60 to 94 %; the mean was 79 %, and the coefficient of variation was 16 %. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of AMPA ranged from 74 to 108 %; the mean was 93 %, and the coefficient of variation was 9 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-83: Summary of residues (mg/kg) for glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.s,/ha

DAA[1]	Soil depth (cm)	CMP	AMPA
0	0 – 7.6	3.1/3.2 [2]	0.09/0.09 [2]
		2.2	0.08
		2.5	0.09
		2.5	0.09

DAA[1]	Soil depth (cm)	CMP	AMPA
		3.3	0.10
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
1	0 – 7.6	1.7/1.8 [2]	0.08/0.09 [2]
		3.0	0.12
		2.3	0.11
		2.6	0.13
		2.3	0.12
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
3	0 – 7.6	2.0/1.5 [2]	0.24/0.21 [2]
		2.6	0.26
		2.4	0.25
		1.6	0.18
		1.4	0.17
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
7	0 – 7.6	1.4/0.90 [2]	0.32/0.25 [2]
		1.10	0.26
		1.20	0.26
		1.00	0.24
		1.10	0.29
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
14	0 – 7.6	0.55/0.45 [2]	0.35/0.32 [2]
		0.77	0.41
		0.40	0.27
		0.78	0.32
		0.31	0.27
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD
28	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD
57	0 – 7.6	< LOD/< LOD [2]	0.12/0.12 [2]
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD
199	0 – 7.6	< LOD/< LOD [2]	0.068/0.078 [2]
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
380	0 – 7.6	< LOD/< LOD [2]	0.09/< LOD [2]
	15.2 – 22.9	< LOD	< LOD

DAA[1]	Soil depth (cm)	CMP	AMPA
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
542	0 – 7.6	< LOD	0.058
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD

[1] DAA = Days after application
[2] analysed in duplicate
n.a. = not analysed

B. CHARACTERISATION OF RESIDUES

Glyphosate (CMP) amounted to 2.7 mg/kg on the day of application and decreased to 0.55 mg/kg on day 14; thereafter no residues > LOQ were encountered. No residues were found in the lower soil depths of 7.6 to 121.9 cm (3 to 48 inch).

It can be concluded that AMPA is formed following the application of TOUCHDOWN 4-LC. Residue levels increased to about 0.32 mg/kg after 14 days and began to decline during the remaining period. AMPA is a very small, highly polar molecule, capable of binding tightly to soil. AMPA residues were not detected in the 28 day soi1 samples. The 0.12 to 0.06 mg/kg residuals at 0 to 7.6 cm (0 to 3 inch) at days 57 to 542 may represent AMPA that is tightly bound to the soil and not capable of undergoing rapid dissipation. AMPA residues were found only in the upper 0 to 7.6 cm (0 to 3 inch) layer, thus, it can be concluded that AMPA does not leach.

C. KINETICS

An Ecoregion Crosswalk exercise was performed ([redacted], 2020, CA 7.1.2.2.1/002) and the trial is not considered reprentative for European conditions. Therefore, a new kinetic evaluation of the data is not performed.

III. CONCLUSIONS

ICIA 0224 (as measured by trimesium and glyphosate residues) dissipated rapidly in silty loam soil in Mississippi after application of TOUCHDOWN 4-LC formulation.

ICIA 0224 did not leach or migrate prior to its environmental degradation. All glyphosate residues were found in the 0 to 7.6 cm (0 to 3 inch) soil depth samples.

AMPA was formed as an intermediate degradate in the course of carbon recyclisation/mineralisation of glyphosate. From an initial 2.7 mg/kg glyphosate residue, the maximum amount of AMPA residue found was 0.32 mg/kg. It appeared that most of the AMPA was rapidly further degraded, but a small amount (0.1 mg/kg) became bound to the soil and unavailable for rapid degradation. AMPA was not found below the 0 to 7.6 cm (0 to 3 inch) soil depth sampled.

Assessment and conclusion by applicant:

The study was performed according to the respective guideline in force in 1987. There are minor deviations to current guideline requirements. Nevertheless, the study provides valuable information on the dissipation behavior of glyphosate under field conditions. As the representative formulation of the current submission does not contain trimesium cation, the trimesium findings were neglected for further consideration.

Since the trial is not considered representative for European conditions, the study is considered as supportive information.

Assessment and conclusion by RMS

Glyphosate trimesium was applied on the field site. As mentioned by the applicant, trimesium findings are not considered for this renewal report.

The test site was assessed in ecoregion crosswalk study from [redacted], 2020. It is not considered as representative of European conditions. No further assessment was therefore performed by RMS (the same deviations as the ones identified in [redacted] 1989a are listed in the header for information).

It is also highlighted that according to section B5, the analytical method used in this study is not considered acceptable.

The study is not considered acceptable.

[redacted], *1989 c*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation are not reported below for easiness of reading.

Data point:	CA 7.1.2.2.1/018
Report author	[redacted].
Report year	1989c
Report title	ICIA 0224 – Field Dissipation Study for Terrestrial Uses, Georgia, 1987-1988, Residue Data to Support Registration of TOUCHDOWN
Report No	WRC 89-23, Protocol No. RP-87-27
Guidelines followed in study	U.S. EPA 164-1
Deviations from current test guideline	None From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - Site not representative of EU conditions - No information on site management and pesticide use history - Treated area was not divided into subplots - Only 3 cores per plot - No information on sample transport and processing - Analytical method not validated
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	No

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	
Identification:	Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation:	Touchdown 4-LC
Lot No.:	WCL 1402
Nominal concentration:	41.8 wt. % or 479 g/L (4 lb per gallon) glyphosate-trimesium
Measured concentration:	41.4 wt. % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

The test site was located near Donalsonville, Georgia. The non-replicated plot had a size of 22 x 5 m, 120 m^2 (72 by 18 feet), containing one treated and one control plot.

An overview of the soil characterization is given below.

Table 8.1.1.3-84: Soil characteristics of the Georgia test si

Parameter		Horizon			
Soil depth (cm)		0-30.5	30.5-61.0	61.0-91.4	91.4-121.9
Soil depth (inch)		0-12	12-24	24-36	36-48
Cation Exchange Capacity (meq/100g)		2.9	1.6	1.3	3.1
Particle Size Analysis (USDA) (%) [1]	sand	86	88	88	72
	silt	9	7	7	4
	clay	5	5	5	24
Soil Type		Loamy sand	Sand	Sand	Sandy clay loam
Organic matter (%)		1.2	0.4	0.1	0.1
Organic carbon (%) [2]		0.696	0.232	0.058	0.058
Soil pH [3]		6.5	5.1	5.3	5.5
Soil Bulk Density (g/L) [b)]		Not indicated in the study report			
Field capacity (% soil moisture at 1/3 bar)		3.8	4.1	3.4	9.2

[1] Due to rounding differences the sum may not correspond to 100 %
[2] Calculated from organic matter according to OC = OM x 0.58
[3] Medium not stated

Long-term daily air temperatures and precipitation data was provided from the Southern Agricultural Research Inc. in Donalsonville, Georgia. Reported daily parameters include minimum and maximum air temperatures, minimum and maximum soil temperatures at 7.6 cm below ground level (3 inches), total daily precipitation, evaporation and relative humidity. Irrigation was applied and recorded prior to application and at weekly intervals throughout the test period in amounts typical for the area. If necessary, irrigation was applied to bring the total (rainfall plus irrigation) to 110 % of the historical weekly average.

Prior to the test, the site was cultivated with bahiagrass and bermudagrass pasture. To maintain bare soil in the plots, a herbicide mixture with residual soil activity was applied (Atrazine + Dual) in order to prevent grass re-establishing in plots. The tankmix was applied after 3 passes with disk (to destroy "turf") and prior to final two diskings.

2. Application

The test site received one application with TOUCHDOWN 4-LC (batch WCL 1402) containing 4.48 kg (4 lb/gallon) of active ingredient. Application was conducted on 12 August 1987 with a carbon dioxide-charged backpack sprayer with a four-nozzle boom to bare dry soil, previously in bahiagrass and bermudagrass pasture. The formulation was not incorporated. The plots were not cultivated or fertilized before application. During application the air temperature was 30.6 °C (87 °F), soil temperature was 35.6 °C (96 °F), relative humidity was 59 %, and the atmospheric condition was calm.

3. Sampling

Soil samples were taken at the following depths: 0 to 7.6 cm, 7.6 to 15.2 cm, 15.2 to 22.9 cm, 22.9 to 30.5 cm, 30.5 to 61.0 cm, 61.0 to 91.4 cm and 91.4 to 121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 2, 5, 7, 14, 33, 58, 182, and 369 after application.

Three separate field samples were randomly collected from both check (untreated control) and treated areas at each soil sampling time. A 10.2 cm (4 inch) length of 15.2 cm (6 inch) diameter aluminium tube was inserted into the ground to a 7.6 cm (3 inch) depth. The 0 to 7.6 cm (0 to 3 inch) depth soil sample was collected by removal of the soil inside the aluminium tube. The 7.6 to 121.9 cm (3 to 48 inch) soil sample was collected with a 2.54 cm (1 inch) diameter hydraulic soil probe; the probe contained an acetate liner to prevent contamination of the soil. On day 5 sample collection was only possible from the top layer, soil probe samples below the 0 to 7.6 cm (0 to 3 inch) horizon could not be physically collected because of the saturated soil after cumulative rainfall (89 mm (3.5 inches) over 4 days).

4. Specimen handling and preparation

Soil samples were chilled at the time of collection, transported to a freezer within two hours of sampling and frozen. Frozen samples were shipped via overnight express courier or commercial refrigerated truck to ICI America's Western Research Center (WRC) analytical laboratory and arrived frozen. Samples were subdivided into the various appropriate lengths and stored at -20 °C until analysis.

Storage stability in soil was assessed in a separate study (RRC 86-61 "Frozen Storage Stability of Touchdown in Soil"), while the results are summarised within the present report. The data indicate that ICIA 0224 residues (glyphosate and AMPA) in a fine sand soil are stable, under the frozen storage conditions for at least two years. During this study no field-treated sample was stored in excess of 133 days (4.4 months).

5. Analytical procedures

CMP (glyphosate) and AMPA were analysed by liquid chromatography using RCC method 85-34 ("Determination of SC-0224 Anion Residues in Crops, Water, and Soil by Liquid Chromatographie"). The method is described briefly in the following. Soil samples were extracted with 0.5 M ammonium hydroxide. The extracts were purified by using a cation-exchange column. The CMP and its metabolite AMPA were eluted from the column, derivatised with 9-fluorenylmethylchloroformate, and determined by using an HPLC equipped with an anion-exchange column and a fluorescence detector.

For every set of field samples extracted, one untreated control sample and one fortified control sample were concurrently extracted. If the set was composed of more than ten samples, one control and one fortified control were concurrently extracted for each subset of ten field samples. Untreated control samples contained background equivalent to 0.01-0.04 mg/kg of TMS and 0.01-0.08 mg/kg AMPA. There was no background interference for CMP analysis.

The limit of detection for glyphosate and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Additional recovery data for method validation are contained in the residue method reports (RCC reports No. 85-33 and 85-34), included in the present study report.

Recoveries from fortified untreated soil with glyphosate and AMPA in the course of analysis reported in this study are as follows.

Recoveries from soil fortified between 0.05 and 2.0 mg/kg of CMP ranged from 68 to 115 %; the mean was 81 %, and the coefficient of variation was 14 %. Recoveries from soil fortified between 0.05 and 2.0 mg/kg of AMPA ranged from 70 to 118 %; the mean was 82 %, and the coefficient of variation was 15 %.

II. RESULTS AND DISCUSSION

A. DATA

The table below summarises the mean residues of soil samples from the 0 to 7.6 cm depth (0 to 3 inch) for glyphosate (CMP) and AMPA over one year. The coefficients of variations of the replicate analyses were calculated over 58 days to assess uniform application of the test compound. The coefficient of variation reflects the less then optimal application achieved by use of a backpack sprayer as contrasted to a tractor-mounted boom.

Table 8.1.1.3-85: Mean residues (mg/kg) for glyphosate (CMP) and AMPA and coefficient of variations (%) in the top layer (0 to 7.6 cm)

DAA	Mean residue (mg/kg)		Coefficient of variation (%)	
	CMP	AMPA	CMP	AMPA
0	1.2	0.09	13	0
2	0.55	0.26	82	62
5	0.27	0.37	56	30
7	0.19	0.42	17	7
14	0.08 [1]	0.21	n.c.	n.c.
33	< LOD	0.29	n r.	34

Table 8.1.1.3-85: Mean residues (mg/kg) for glyphosate (CMP) and AMPA and coefficient of variations (%) in the top layer (0 to 7.6 cm)

DAA	Mean residue (mg/kg)		Coefficient of variation (%)	
	CMP	AMPA	CMP	AMPA
58	< LOD	0.083	n r.	35
182	< LOD	0.02 [1]	n r.	n.c.
369	< LOD	< LOD	n r.	n r.
Mean	-		40	

n.a. = not analysed
n.c. = not calculated
n r. = not relevant
[1] Only one of three samples was > LOD

A summary of the residues for glyphosate (CMP) and AMPA for all soil layers is presented below.

Table 8.1.1.3-86: Summary of residues (mg/kg) for glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.i,/ha

DAA[1]	Soil depth (cm)	CMP	AMPA
0	0 – 7.6	1.4/1.1 [2]	0.10/0.08 [2]
		1.0	0.09
		1.2	0.09
	7.6 – 15.2	0.25	< LOD
		< LOD	< LOD
		< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
		< LOD	< LOD
		< LOD	< LOD
2	0 – 7.6	0.25/0.23 [2]	0.16/0.14 [2]
		1.06	0.44
		0.35	0.19
	7.6 – 15.2	< LOD	< LOD
		< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
		< LOD	< LOD
		< LOD	< LOD
5 [3]	0 – 7.6	0.12/0.12 [2]	0.24/0.26 [2]
		0.41	0.47
		0.28	0.40
7	0 – 7.6	0.18/0.16 [2]	0.40/0.50 [2]
		0.18	0.40
		0.23	0.40
	7.6 – 15.2	< LOD	< LOD
		< LOD	< LOD
	15.2 – 22.9	n.a	< LOD
		< LOD	< LOD
		< LOD	< LOD
14 [4]	0 – 7.6	< LOD/< LOD [2]	0.16/0.17 [2]
		n.a.	n.a.
		0.13	0.25
	7.6 – 15.2	< LOD	< LOD
		< LOD	< LOD
		< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
		< LOD	< LOD
		< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD

Table 8.1.1.3-86: Summary of residues (mg/kg) for glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.i,/ha

DAA[1]	Soil depth (cm)	CMP	AMPA
33	0 – 7.6	< LOD/< LOD [2]	0.25/0.25 [2]
		< LOD	0.22
		< LOD	0.41
	7.6 – 15.2	< LOD	< LOD
		< LOD	< LOD
		< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
		< LOD	< LOD
		< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD
58	0 – 7.6	< LOD/< LOD [2]	0.10/0.10 [2]
		< LOD	0.10
		< LOD	0.05
	7.6 – 15.2	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD
182	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]
		< LOD	0.07
		< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
369	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]
	15.2 – 22.9	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD

[1] DAA = Days after application
[2] Analysed in duplicate
[3] Soil probe samples below the 0 to 7.6 cm (0 to 3 inch) horizon could not be collected because of the saturated soil after cumulative rainfall (89 mm (3.5 inches) over 4 days)
[4] Horizons below 3 inch were sampled at day 15 after application
n.a. = not analysed

B. CHARACTERISATION OF RESIDUES

Glyphosate (CMP) amounted to 1.2 mg/kg on the day of application and decreased to 0.08 mg/kg on day 14; thereafter no residues > LOQ were encountered. A 0.12 mg/kg CMP anion residue value was found on day 0, attributed to inadvertrant contamination, at 7.6 to 15.2 cm depth (3 to 6 inch). Significant amounts of residues were found only at the 0 to 7.6 cm soil depth (0 to 3 inch); these residues completely dissipated by day 33.

It can be concluded that AMPA is formed following the application of TOUCHDOWN 4-LC. Residue levels increased to about 0.42 mg/kg after 7 days and began to decline during the remaining period. AMPA is a very small, highly polar molecule, capable of binding tightly to soil. The 0.21 to 0.06 mg/kg residuals at 0 to 7.6 cm (0 to 3 inch) at days 14 to 182 may represent AMPA that is tightly bound to the soil and not capable of undergoing rapid dissipation. AMPA residues were not detected in the 369 day soi1 samples. AMPA residues were found only in the upper 0 to 7.6 cm (0 to 3 inch) layer, thus, it can be concluded that AMPA does not leach.

C. KINETICS

An Ecoregion Crosswalk exercise was performed [redacted], 2020, CA 7.1.2.2.1/002) and the trial is not considered reprentative for European conditions. Therefore, a new kinetic evaluation of the data is not performed.

III. CONCLUSIONS

ICIA 0224 (as measured by trimesium and glyphosate residues) dissipated rapidly in Lucy loamy sand in Georgia after application of TOUCHDOWN 4-LC formulation.

ICIA 0224 did not leach or migrate prior to its environmental degradation. Except for one 0.12 mg/kg residue of CMP at the day of application in the 7.6 to 15.2 cm soil depth (3 to 6 inch), attributed to inadvertent contamination, all residues were found in the 0 to 7.6 cm (0 to 3 inch) soil depth samples.

AMPA was formed as an intermediate degradate in the course of carbon recyclisation/mineralisation of glyphosate. From an initial 1.2 mg/kg glyphosate residue, the maximum amount of AMPA residue found was 0.42 mg/kg. It appeared that most of the AMPA was rapidly further degraded, but a small amount (0.1 mg/kg) became bound to the soil and unavailable for rapid degradation. AMPA was not found below the 0 to 7.6 cm (0 to 3 inch) soil depth sampled. AMPA was not detected in the 369 day soil samples.

Assessment and conclusion by applicant:

The study was performed according to the respective guideline in force in 1987. There are minor deviations to current guideline requirements. Nevertheless, the study provides valuable information on the dissipation behavior of glyphosate under field conditions. As the representative formulation of the current submission does not contain the trimesium cation, the trimesium findings were neglected for further consideration.

Since the trial is not considered representative for European conditions, the study is considered as supportive information.

Assessment and conclusion by RMS

Glyphosate trimesium was applied on the field site. As mentioned by the applicant, trimesium findings are not considered for this renewal report.

The test site was assessed in ecoregion crosswalk study from [redacted], 2020. It was not considered as representative of European conditions. No further assessment was therefore performed by RMS (the same deviations as the ones identified in [redacted] 1989a are listed in the header for information).

It is also highlighted that according to section B5, the analytical method used in this study is not considered acceptable.

The study is not considered acceptable.

[redacted], *1984*

Data point:	CA 7.1.2.2.1/020
Report author	[redacted]
Report year	1984
Report title	Dissipation of Glyphosate in U.S. field soils following multiple applications of Roundup herbicide
Report No	MSL-3352
Guidelines followed in study	None
GLP/Officially recognised testing facilities	No
Previous evaluation	Not mentioned in RAR (2015), not accepted in DAR (2001)

Acceptability/Reliability: No

Short description of study design and observations:

Study type: Terrestrial field dissipation
Test item: Roundup

Test sites:
16 trials in orchards and vineyard sites at 9 locations in USA (Alabama, Florida, Virginia, New York, Washington, Michigan, 2x Oregon
Five locations with bare soil in USA (California, Florida, Illinois, New York, Wisconsin)

Soil types: fine sand, gravel loam, sandy loam, sandy clay loam, silty loam, clay loam
Information about pH of organic matter content not given

Application: multiple applications (method not given)
Orchards & vineyards: total application of 6.7 to 134.5 kg Roundup/ha over 1 to 6 years, 1st application spring or autumn
Bare soil: 4 x 4.2 kg glyphosate/ha within 1 year, 1st application in autumn

Sampling (method not given):
Orchards & vineyards: one or multiple samples per plot until 7 to 613 days after last application
Bare soil: multiple samples per plot until 159 to 412 days after last application; one trial with incomplete sampling was excluded from further assessment.
Sampling depth: 0 - 15.2 cm depth and 15.2 - 30.4 cm depth (the latter not for all sites)

Sample storage: frozen at day of sampling and kept frozen until sample preparation
Workup and analysis: analysis was done for glyphosate, AMPA and N-nitrosoglyphosate (NNG)
Air drying, mixing and removing of stones and foreign matter, soil moisture adjusted to 10 - 20 %
Twofold extraction with 0.5 M ammonium hydroxide
Primary cleanup with anion exchange chromatography
Glyphosate and AMPA are quantified by HPLC
NNG is quantified with a Griess postcolumn reactor and an absorbance detector
LOD = 0.05 mg/kg for glyphosate and AMPA
LOD = 0.02 mg/kg for NNG

Recovery in fortified samples:
Glyphosate: mean = 78 %
AMPA: mean = 76 %
NNG: mean = 75 %

All results were corrected for average analytical recoveries.

Short description of results:

<u>Orchards and vineyards</u>
(residues after 7 to 476 days after last application)
Glyphosate: non-detectable to 10 % of total applied amount for most trials; up to 48 % of total applied amount for one location, assumed to be caused by unrecorded treatments or sampling deficiencies
AMPA: 1.4 - 54 % of total applied glyphosate equivalents, but <20 % for 12 out of 16 plots
NNG: not detected for 7 of 9 locations, up to 0.09 mg/kg for two locations (confirmed by secondary analytical method).

<u>Bare soil</u>

	(residues at last sampling date, 159 to 412 days after last application) Glyphosate: <0.05 - 0.87 mg/kg AMPA: ≤0.16 - 0.52 mg/kg NNG: not detected Half-life times: not calculated
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid due to the following deficiencies: No soil characterization (only soil type) No climate and weather data provided No information on soil history provided Multiple applications No sampling documentation (only sampling protocol provided) No information on sampling method Number of sampling times insufficient No day 0 samples taken for some locations

Assessment and conclusion by RMS

Considering the above deviations, the study is not considered acceptable.

[redacted] *1983 and* [redacted] *1988*

Data point:	CA 7.1.2.2.1/021
Report author	[redacted]
Report year	1983
Report title	Dissipation of Glyphosate in U.S. field soils following direct application of Roundup herbicide
Report No	MSL-3210
Guidelines followed in study	Not stated
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

Data point:	CA 7.1.2.2.1/0022
Report author	[redacted]
Report year	1988
Report title	Addendum to MSL 3210 - Dissipation of Glyphosate in U.S. field soils following direct application of Roundup herbicide
Report No	MSL-8081
Guidelines followed in study	US EPA Guideline 164-1
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: Terrestrial field dissipation Test item: Roundup Test sites: 15 locations in USA (North Dakota, Illinois, 2x Colorado, Idaho, Indiana, Kentucky, Ohio, Oklahoma, Tennessee, Texas, California, North Carolina, Minnesota, Florida)

	Soil types: Four clay loam, one loamy sand, two silt loam, one silt clay loam, three sandy loam, one loam and three sandy clay loam; Soil pH: 5.25 - 8.15 (medium not stated) OM: 0.5 - 7 % Application rate: 2.2, 4.5 & 8.9 kg a.s./ha, single application Application method: CO_2 pressured sprayer; directly to the soil; at 8 locations, soil was tilled after application; no information about crops given Application timing: April/May for 9 locations, Jun to Aug for 4 locations, Sep/Oct for 2 locations Sampling times: 4 events between 0 and 100 DAT (4 locations); to 5 – 7 events between day 0 and 377 DAT (11 locations) Sampling method: core samplers or shovels (not stated at which locations) Sampling depth: 0 - 15.2 cm, only, for 1 - 3 months; later additionally 15.2 - 30.4 cm Sample storage: frozen directly after sampling and kept frozen until sample preparation Workup and analysis: analysis was done for glyphosate, AMPA and N-nitrosoglyphosate (NNG). Pre-processing of samples: mixing and adjustment of soil moisture to 10-20% Twofold extraction with 0.5 M ammonium hydroxide solution Primary cleanup with anion exchange chromatography Glyphosate and AMPA are quantified by HPLC NNG is quantified with a Griess postcolumn reactor and an absorbance detector LOD = 0.05 mg/kg for glyphosate and AMPA LOD = 0.02 mg/kg for NNG Recovery in fortified samples: Glyphosate: mean = 85.5 % AMPA: mean = 80.2 % NNG: mean = 75.0 %
Short description of results:	Residues: Glyphosate: day-0 recovery (mg/kg): <LOD – 3.7 (2.2 kg/ha applied) <LOD – 2.43 (4.5 kg/ha applied) <LOD – 12.6 (8.9 kg/ha applied) Time when 90% dissipation was reached 10 – 291 days (2.2 kg/ha applied) 18 – 301 days (4.5 kg/ha applied) 12 – 291 days (8.9 kg/ha applied) AMPA: highest residues in the range of 0.2 – 0.8 mg/kg, observed after one year after application in only 8 of the total 42 plots NNG: not detected in any soil sample Glyphosate and AMPA were infrequently observed in the lower soil layer, indicating that their presence was not from leaching but as an artefact of sampling. Half-life times: calculated considering a two-compartment-model and by regression analysis (bi-phasic):

	2 – 174 days, (independent of application rate) mean of 34 days (2.2 kg/ha applied) mean of 37 days (4.5 kg/ha applied) mean of 44 days (8.9 kg/ha applied) The dissipation of glyphosate was not dependent on the application rate. In addendum, half-life times were re-calculated considering a log-transformation approach: all locations: mean of 57 days, range: 13 - 159 days excluding 3 sites due to consideration of outliers: mean of 45 days, range: 13 – 124 days
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid due to the following deficiencies: • No soil characterization (only soil type, pH and OM) • Some test plots were tilled after application • 2 locations not sampled at day 0 • Number of sampling times insufficient for some locations • No sampling documentation (only sampling protocol provided) • Some locations sampled with shovels (not stated which) • Unclear soil treatment history

Assessment and conclusion by RMS

Considering the above deviations, the study is not considered acceptable.

[redacted], *1983*

Data point:	CA 7.1.2.2.1/023
Report author	[redacted]
Report year	1983
Report title	Roundup herbicide dissipation in cool climate forest soil and leaf litter
Report No	MSL-2950
Guidelines followed in study	None
GLP/Officially recognised testing facilities	No
Previous evaluation	Not mentioned in RAR (2015), reported as not required in DAR (2001)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: terrestrial field dissipation in forest soil and leaf litter Test item: Roundup Test site: one location in British Columbia, Canada, cool climate, forest soil (Douglas fir) Soil type: "red mineralized forest soil" Application rate: 1.7 and 3.4 kg a.s./ha, single application Application method: hand-held CO_2 pressurized sprayer Three replicate plots; appl. to leaf litter and to bare soil (without plant matter) and untreated control per plot. Application timing: 23 September 190 Sampling times: six events, at 0, 15, 28, 58, 247, 344 DAT Sampling method: manually from a 400 cm² area (0 DAT), pipe core sampler (all other samplings) Sampling depth: Soil: 0 – 6 cm depth, 7 – 12 cm depth

	Litter: 0 – 6 cm depth Sample storage: frozen with dry ice within 4 hours after collection and during shipment and storage Workup and analysis: - Soil: air drying, sieving (#8 mesh standard sieve), mixing in a mechanical mixer - Raw leaf litter: blending at high speed with dry ice - Samples frozen again after processing - Extraction of samples with ammonium hydroxide - Primary cleanup with anion exchange chromatography - Further cleanup and separation with cation exchange chromatography - Analysis by HPLC, limit of sensitivity: 0.05 mg/kg - Duplicate analysis of each sample Recovery in fortified samples: Soil: - Glyphosate: mean: 84 % - AMPA: mean: 72 % Litter: - Glyphosate: mean: 83 % - AMPA: mean: 71 %
Short description of results:	Residues: Glyphosate: 0 DAT recovery soil (mg/kg), average over 3 plots: 0-6 cm: - 21.2 (1.7 kg/ha appl. rate) - 31.5 (3.4 kg/ha appl. rate) 7-12 cm: - 1.06 (1.7 kg/ha appl. rate) - 1.17 (3.4 kg/ha appl. rate) litter (mg/kg), average over 3 plots: - 7.60 (1.7 kg/ha appl. rate) - 28.2 (3.4 kg/ha appl. rate) 344 DAT soil (mg/kg), average over 3 plots: 0-6 cm: - 1.48 (1.7 kg/ha appl. rate) - 8.63 (3.4 kg/ha appl. rate) 7-12 cm: - 0.16 (1.7 kg/ha appl. rate) - 0.83 (3.4 kg/ha appl. rate) litter (mg/kg), average over 3 plots: - 0.11 (1.7 kg/ha appl. rate) - 0.53 (3.4 kg/ha appl. rate) AMPA: soil: 0-6 cm: max. 0.89 mg/kg at 15 DAT 7-12 cm: max. 0.14 mg/kg at 344 DAT litter: max. 3.96 mg/kg, observed at 15 DAT Half-life times: no reliable half-life according SFO could be calculated but 50% of the initial concentration dissipates within 2 months or faster. Glyphosate and AMPA remain mostly in the leaf litter or 0 – 6 cm soil layer.

Reasons for why the study is not considered relevant/reliable or not considered as key study by the aplicant:	The study is considered invalid due to the following deficiencies: - Field trials conducted on forest soil - No soil characterization (only soil type and OM) - No soil management history - No climate and weather documentation - Evidence for not evenly sprayed products provided - Number of sampling times insufficient - Day 0 samples not immediately after application - Sampling depth only 12 cm - Overall: documentation is very poor/unreadable

Assessment and conclusion by RMS

Considering the above deviations, the study is not considered acceptable.

[redacted], *1982*

Data point:	CA 7.1.2.2.1/024
Report author	[redacted]
Report year	1982
Report title	Dissipation of Glyphosate in field soils following minimum till application of Roundup alone or in tank mix combinations with Lasso ME, Atrazine, Dyanap or Metribuzin.
Report No	MSL-2422
Guidelines followed in study	None
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, Accepted in DAR (2001) but not considered to derive endpoints in RAR (2015)
Acceptability/Reliability:	No

Short description of study design and observations:

Study type: Terrestrial field dissipation
Test item: Roundup (solo or in tank mix combinations with Lasso ME, Atrazine, Dyanap or Metribuzin)

Test sites: 6 locations in USA: Holdenville (Oklahoma), Shawnee (Oklahoma), Tumbleton (Alabama), Mankato (Minnesota), Adel (Iowa), Olathe (Kansas);
2 experiments each (solo and tank mix)

Soil types: Two loam, two silty clay loam, one silty clay, one sandy loam
Soil pH not given
OM 0.8 % - 6.5 %

Application rate: 5 kg a.s./ha, single application, pre-emergence
Solo and in tank mix with Lasso ME and atrazine to corn (n = 2)
Solo and in tank mix with Lasso ME and Dyanap to peanuts (n = 3)
Solo and in tank mix with Lasso ME and metribuzin to soybeans (n = 1)

Application method: CO_2 sprayer
Application timing: Beginning to mid of May
Sampling times: three to five events, between day 0 and 336 DAT; day 0 sampling at 0 or 1 DAT
Sampling method: not reported
Sampling depth: 0 - 15.2 cm and 15.2 – 30.4 cm
Tillage: minimum tillage

	Sample storage: no information provided Workup and analysis: analysis was done for glyphosate, AMPA and N-nitrosoglyphosate (NNG), which could theoretically be formed from glyphosate in a nitrosating medium. Pre-processing of samples: mixing and adjustment of soil moisture to 10-20% Threefold extraction with 0.5 M ammonium hydroxide solution Primary cleanup with anion exchange chromatography Further purification and separation with cation exchange chromatography. Glyphosate and AMPA are quantified by GLC-FPD NNG is quantified with liquid chromatograph equipped with a Partisil SAX analytical column, a postcolumn Griess reactor and a 546 nm absorbance detector LOD = 0.05 mg/kg for glyphosate and AMPA LOD = 0.02 mg/kg for NNG Recovery of externally fortified samples: Glyphosate: 65.1 - 72.9 % AMPA: 69.0 – 72.1 % NNG: 66.0 – 75.5 % All residues data were corrected for recoveries of fortified samples but not for soil moisture content.
Short description of results:	Residues: Glyphosate: day-0 recovery at 0 - 15.2 cm: 0.38 – 5.88 mg/kg for plots with application of Roundup solo (n = 6) 0.08 – 4.67 mg/kg for tank mix plots (n = 6) AMPA: At 4 locations increasing to last sampling date, at 2 locations peak concentration at 43 and 92 DAT. Maximum concentration: 1.23 mg/kg NNG: not detected in any soil sample Half-life times (estimated with computer program “HALFLI”): Mean of 38.6 days (27.3 – 55.5 days), for Roundup solo plots (n = 6) Mean of 35.3 days (31.8 & 38.8 days), for Roundup + Lasso ME + Dyanap (n = 2) Mean of 37.5 days (48.8 & 26.3 days), for Roundup + Lasso ME + atrazin (n = 2) 32.5 days, for Roundup + Lasso ME + metribuzin (n = 1) Glyphosate and AMPA were occasionally observed in the 15 to 30 cm layer.
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid due to the following deficiencies: • No soil characterization (only soil type and OM) • No climate and weather data provided • No information on soil history provided • The test plots were cropped • Tank mixtures applied • Insufficient number of sampling times • Residue data were corrected for recoveries of fortified samples but not for soil moisture • Day 0 samples not taken immediately after application • Sampling method and sample storage conditions not provided

Assessment and conclusion by RMS

Considering the above deviations, the study is not considered acceptable.

[redacted], *1979*

Data point:	CA 7.1.2.2.1/025
Report author	[redacted]
Report year	1979
Report title	Field soil dissipation studies of Roundup conducted in Sweden and France
Report No	MLL30033
Guidelines followed in study	None
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not mentioned in RAR (2015) nor in DAR (2001)
Acceptability/Reliability:	No

Short description of study design and observations:

Study type: terrestrial field dissipation
Test item: Roundup, containing 360 g glyphosate /L

Test sites: 4 locations in France, planted with vines (total 8 trials)
1 location in France, planted with maize (total 2 trials)
6 locations in Sweden, forestry cultivated (total 10 trials)

France:
Soil types: clay loam, sandy clay loam, clay sand, loamy sand; pH 6.7 – 8
Application rate: vines: 4.3 and 8.6 kg a.s./ha, maize: 2.15 & 4.3 kg a.s./ha, single application
Application method: Knapsack sprayer; application on existing vegetation
Application timing: end of May to begin of July
Sampling times: six events, between 0 and 70 DAT
Sampling method: core samplers, 10 cm depth, 3 replicates (pooled)

Sweden:
Soil types: clay loam, sandy gravel, brown soil, podsol; pH 4.6 – 6.6
Application rate: 2 and 4 kg a.s./ha, single application
Application method: Knapsack sprayer; application on existing vegetation
Application timing: end of July to mid of August
Sampling times: six events, between 1 and 828 DAT
Sampling method: core samplers, 5 cm depth, 3 replicates (pooled)

All locations:
Sample storage: -20 °C
Workup and analysis: extraction with water, analysis by GC-FPD, LOD = 0.05 mg/kg
Recovery in fortified samples:
Glyphosate: mean: 73 %, range: 52 – 96 %
AMPA: mean: 68 %, range: 33 – 99 %

Short description of results:

Residues:
Glyphosate: France: day-0 recovery: 35 – 93 % of applied amount, final sampling (61 – 70 DAT): 2 – 16 % of applied amount
Sweden: day-0 recovery: 10.3 – 81 % of applied amount, final sampling (818 – 827 DAT): ≤2 % of applied amount

	AMPA: maximum 13% of applied amount, observed around 20 to 50 DAT with subsequent decline (except two trials in France) Half-life times: calculated according to SFO by regression analysis; France: 11.0 – 30.1 days, Sweden: 13.6 – 36.1 days (independent of application rate).
Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid due to the following deficiencies: • Trials were conducted in existing cultures (vines, maize, forestry) • Application was made onto existing vegetation • Sampling depth was only 5 to 10 cm • No weather data is reported • Soils are characterized insufficiently (only type, pH, Corg) • Analytical procedure is described insufficiently

Assessment and conclusion by RMS

Considering the above deviations, the study is not considered acceptable.

B.8.1.1.3.2. Storage stability studies

In addition, three existing storage stability studies are provided to support the stability of glyphosate and AMPA in soil (point B.8.1.1.1.2).

Table 8.1.1.3-87: Storage stability studies

Annex point	Study	Study type	Substance(s)	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.1.2.2.1/007	[redacted], 1993b	Storage stability study	Glyphosate and AMPA	Supportive	Acceptable
CA 7.1.2.2.1/012	[redacted], 1995	Storage stability study	Glyphosate; AMPA	Supportive	Acceptable
CA 7.1.2.2.1/019	[redacted], 1986	Storage stability study	Glyphosate-Trimesium	Supportive	Not acceptable

[redacted], 1993b

Data point:	CA 7.1.2.2.1/007
Report author	[redacted]
Report year	1993b
Report title	Storage stability of Glyphosate and AMPA in Soil and Stream sediment
Report No	MSL-12682
Guidelines followed in study	None
Deviations from current test guideline	Not relevant
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, Supportive in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

During the course of the study, three different lots of glyphosate and four different lots of AMPA were used as presented below.

1. Test Material:

Identification:	Glyphosate
Lot No.:	PIT-90001-1524-A
Chemical purity:	≥ 98.8 %
Lot No.:	PIT-8906-666-A
Chemical purity:	99.7 %
Lot No.:	RUD-9203-3961-A
Chemical purity:	99.8 %

Identification:	AMPA
Lot No.:	SIG-8912-1253-A
Chemical purity:	≥ 97.4 %
Lot No.:	SIG-8912-1253-A-5
Chemical purity:	99 %
Lot No.:	PIT-8912-1385-A
Chemical purity:	99.1 %
Lot No.:	PIT-8912-1385-A-2
Chemical purity:	99.1 %

2. Soil:

For this study, soil from Georgia and Iowa and one stream sediment from Oregon were used. These matrices are representative of the soils and sediment to which glyphosate would be applied under normal agronomic practices. The samples were taken from test sites that had a known two-year history of crop and pesticide use, and none of the test sites had been treated with Roundup herbicide or related chemistry during the two years preceding this study.

Soil characterization data for each soil type are presented in the table below.

Table 8.1.1.3-88: Characteristics of test soils

Parameter	Results		
Soil	Georgia soil (Climax)	Iowa soil (Danville)	Oregon sediment (Corvallis)
Textural Class (USDA)	Sandy loam	Silt loam	Sandy clay loam
Sand (50 µm – 2 mm) (%)	76	20	56
Silt (2 µm – 50µmm) (%)	14	54	23
Clay (< 2 µm) (%)	10	26	21
pH [1]	4.7	6.0	5.8
Organic matter (%)	1.1	4.4	7.2
Organic carbon (%) [2]	0.64	2.55	4.18
CEC (meq/100 g)	2.3	17.8	18.2
WHC at 1/3 bar (%)	7.02	33.65	40.37
Bulk Density (disturbed) (g/cm^3)	1.29	1.17	0.99

[1] medium not stated

[2] Calculated from organic matter according to OC=OM x 0.58

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions and sampling

Untreated control soil from Climax, Georgia and Danville, Iowa and sediment from Corvallis, Oregon were pre-weighed as 30.0 g aliquots into 250 mL polypropylene centrifuge bottles. The uniquely labelled sample bottles were capped securely to prevent loss of moisture and placed into closed

cardboard boxes. The boxes were transferred to a restricted access freezer and stored at a temperature < -17 °C in the dark. The closed boxes excluded light from the samples and provided a small degree of insulation from temperature changes in the freezer due to door openings.

At approximately every six months, three unfortified samples from each location were removed from frozen storage, thawed, fortified, and then returned to frozen storage. In the case of the Georgia soil and Oregon sediment, fortifications were made 0, 65, 247, 429, 611, 793 and 975 days prior to analysis. In the case of the Iowa soil, fortifications were made 0, 67, 249, 431, 613, 795 and 977 days prior to analysis. Fortifications were made by pipetting the test solution directly onto the soil matrix at a level of 1.0 mg/kg each of glyphosate and AMPA. Immediately after fortification, the samples were re-capped securely and taped to further prevent the lids from loosening.

Samples were removed from frozen storage and analysed in sets consisting of unfortified samples, method recovery samples (day 0 samples fortified at the time of analysis) and fortified storage stability samples for each fortification interval. Of the three samples fortified at each time point, only two were analysed; the third was kept frozen. The method recovery samples served as day 0 analyses for comparison to the stored fortified samples.

After the initial chromatographic analysis, the sample extracts for all samples were returned to refrigerated storage (3 – 6 °C) in the dark. After a period ranging from 35 to 42 days, four random sample extracts from each of the three locations were reanalysed to determine if there were any gross changes in recovery due to sample extract storage. With average changes of -1.8 % for glyphosate and 3.5 % for AMPA between initial analyses and analyses after storage, stability in sample extracts was demonstrated.

2. Analytical procedures

Glyphosate and AMPA were extracted from soil using a 0.5 N KOH solution. The extract solution was eluted through a Chelex 100 resin in the Fe(III) form, which retains glyphosate and AMPA due to chelation to Fe(III). The retained glyphosate and AMPA iron salts are removed from the Chelex resin by elution with 6 N HCl. The isolated glyphosate and AMPA iron salts are then applied to a strong anion exchange resin and eluted with 6 N HCl to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness, to remove the HCl, the samples are re-dissolved in water and analysed by high pressure liquid chromatography (HPLC). The chromatograph uses column switching and an o-phthalaldehyde post-column reactor with a fluorescence detector to separate and quantitate glyphosate and AMPA. In the post-column reactor, glyphosate is oxidised to a primary amine which then reacts with o-phthalaldehyde to form a fluorescence derivative. AMPA reacts directly with o-phthalaldehyde to form a second fluorescence derivative.

This method has been validated down to 0.05 mg/kg for both glyphosate and AMPA in 30 g soil samples.

Due to the varying degrees of glyphosate adsorption to different soil types, glyphosate recovery from fortified check samples varies with soil type, and obtaining consistent recoveries of glyphosate is occasionally difficult. Nonetheless, the analytical method used generally affords recoveries of glyphosate from fortified check samples that are greater than 70 %. The percentage recoveries from samples fortified on the day of analysis (day 0) with both glyphosate and AMPA averaged across all three soil matrices were 79.44 % and 77.45%, respectively.

II. RESULTS AND DISCUSSION

A. DATA

Summary tables of residues for untreated control and fortified frozen field samples are presented below. Analyses of duplicate samples (uncorrected for recovery) are reported for all time points.

Table 8.1.1.3-89: Summary of residues (mg/kg) of glyphosate and AMPA in Georgia soil after frozen storage

Fortification rate (mg/kg)	Days in storage	glyphosate (mg/kg)				AMPA (mg/kg)			
		Rep. 1	Rep. 2	Mean	in %	Rep. 1	Rep. 2	Mean	in %
0	0	0	0	0		0	0.0067	0.003	
1	0	0.8001	0.8365	0.818		0.7778	0.8152	0.797	
1	65	0.8246	0.8314	0.828	101.19	0.8046	0.8115	0.808	101.45

1	247	0.7866	0.8351	0.811	97.93	0.7588	0.7905	0.775	95.87
1	429	0.7928	0.8411	0.817	100.75	0.7689	0.8098	0.789	101.90
1	611	0.7376	0.7431	0.740	90.62	0.7100	0.7278	0.719	91.07
1	793	0.6768	0.7284	0.703	94.90	0.6639	0.6983	0.681	94.74
1	975	0.7770	0.7928	0.785	111.71	0.7623	0.7730	0.768	112.71

Table 8.1.1.3-90: Summary of residues (mg/kg) of glyphosate and AMPA in Iowa soil after frozen storage

Fortification rate (mg/kg)	Days in storage	glyphosate (mg/kg)				AMPA (mg/kg)			
		Rep. 1	Rep. 2	Mean	in %	Rep. 1	Rep. 2	Mean	in %
0	0	0	0	0		0	0	0	
1	0	0.7205	0.7940	0.757		0.6868	0.7692	0.728	
1	67	0.7333	0.8109	0.772	101.96	0.7210	0.7912	0.756	103.86
1	249	0.7712	0.8346	0.803	103.99	0.7554	0.8401	0.798	105.51
1	431	0.7211	0.7408	0.731	91.04	0.7486	0.7734	0.761	95.39
1	613	0.6969	0.6974	0.697	95.38	0.7688	0.7761	0.772	101.50
1	795	0.6100	0.6759	0.643	92.23	0.6983	0.7698	0.734	95.03
1	977	0.6717	0.6804	0.676	105.15	0.7881	0.8066	0.797	108.62

Table 8.1.1.3-91: Summary of residues (mg/kg) of Glyphosate and AMPA in Oregon sediment after frozen storage

Fortification rate (mg/kg)	Days in storage	glyphosate (mg/kg)				AMPA (mg/kg)			
		Rep. 1	Rep. 2	Mean	in %	Rep. 1	Rep. 2	Mean	in %
0	0	0	0	0		0	0	0	
1	0	0.7943	0.8208	0.808		0.7944	0.8033	0.799	
1	65	0.8723	0.8975	0.885	109.58	0.8361	0.8504	0.843	105.56
1	247	0.7909	0.8321	0.812	91.71	0.7997	0.8110	0.805	95.51
1	429	0.5946	0.6863	0.640	78.92	0.6216	0.7014	0.662	82.14
1	611	0.7122	0.7623	0.737	115.11	0.7663	0.8567	0.812	122.68
1	793	0.5816	0.6712	0.626	84.96	0.6714	0.7891	0.730	89.99
1	975	0.6931	0.7249	0.709	113.19	0.7944	0.8779	0.836	114.50

B. CHARACTERISATION OF RESIDUES

The results from all three soil matrices show that average recoveries of glyphosate and AMPA residues fortified at 1.0 mg/kg generally range from 0.65 to 0.85 mg/kg.

The average recoveries of the Georgia soil fortified on the date of extraction (day 0 samples) were 0.82 and 0.80 mg/kg for glyphosate and AMPA, respectively. The average recoveries of the Iowa soil fortified on day 0 were 0.76 and 0.73 mg/kg for glyphosate and AMPA, respectively. The average recoveries of the Oregon sediment fortified on the date of extraction were 0.81 and 0.80 mg/kg for glyphosate and AMPA, respectively.

After 975 days amounts of 0.79 and 0.77 mg/kg glyphosate and AMPA, respectively, were found in the Georgia soil. In the Iowa soil 0.68 and 0.80 mg/kg glyphosate and AMPA, respectively, were found after 977 days and in the Oregon sediment amounts of 0.71 mg/kg glyphosate and 0.84 mg/kg AMPA were found after 975 days.

Statistical analysis of the data included fitting the data to a simple first-order decay model and testing the hypothesis that the slope is equal to zero.

The results showed a small but statistically significant decrease in the amount of glyphosate recovered with increasing time in storage for all three soil matrices. AMPA also showed a statistically significant decrease in the Georgia soil. No statistical trend was found in the Iowa soil and Oregon sediment indicating stability of the AMPA residues in those two matrices.

Based on the respective first-order decay models the percentage remaining after 500 days ranges from 88 to 94 % for glyphosate and 95 % or above for AMPA.

The observed decrease of glyphosate recovery with increasing storage time is postulated to be due to increased adsorption of glyphosate to the soil and binding to metallic cations. If microbial, chemical, or photolytic degradation were the cause of the decreased glyphosate recoveries, the AMPA residues would be expected to increase with increasing time, which is not the case. Moreover, since the samples were stored in the dark at < - 17 °C, photodecomposition and microbial degradation can be eliminated.

III. CONCLUSION

The results of this study demonstrate that glyphosate had a small but statistically significant decrease in recovery with increasing storage time for the soils and sediment used. AMPA was determined to be stable in one of the two soils and the sediment, but it also had a small but statistically significant decrease in recovery with increasing storage time in the Georgia sandy loam soil tested. Glyphosate loss in soil at less than -17 °C in the dark is typically very slow with the percentage remaining after 500 days ranging from 88 to 94 %. The AMPA metabolite loss is slower, yet, with 95 % or greater of the AMPA still extractable at 500 days.

Assessment and conclusion by applicant:

It was shown that glyphosate and AMPA residues in soil are stable for up to three years when stored at -17 °C. The study is considered as supportive information.

Assessment and conclusion by RMS:

The study is considered acceptable.

This study shows that glyphosate and AMPA were stable when stored at -17°C for more than 975 days, with recoveries of 78.92-115.11% initial rate for glyphosate in all three soils and 82.14-122.68% for AMPA.

[redacted] *1995*

Data point:	CA 7.1.2.2.1/012
Report author	[redacted]
Report year	1995
Report title	Storage stability of Glyphosate and AMPA in soil
Report No	303625
Guidelines followed in study	Biologische Bundesanstalt (BBA) Richtlinie Teil IV, Reihe 2: Rückstandsanalytik (1986), BBA-Merkblatt Nr. 58. Rückstandsuntersuchungen - Richtlinien zur Durchführung der Analysen (1983) Industrieverband Agrar (IVA) Guidelines „Rückstandsversuche“
Deviations from current test guideline	Not relevant
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, Supportive in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate Analytical standard
Lot No.: 185-ff-131
Chemical purity: 99.5%

Identification: AMPA

Lot No.: 108F3811
Chemical purity: Appr. 99 %

2. Soil:

The soil control samples were obtained from a local farmer (CH-4457 Diegten) in the Swiss Jura, and derived from RCC project 273565 (please refer to [redacted], 1992a, CA 7.1.2.2.1/008). The soil cores were of a sandy clay soil type (organic carbon content = 1.6 %), taken from a depth of 0-30 cm, and the moisture content was determined to be 20.3 %. The control samples were stored deep frozen until the storage stability test.

B. STUDY DESIGN

1. Experimental conditions

To prepare the storage stability test samples, the deep frozen untreated soil sample (about 1 kg) was thawed to room temperature. Afterwards, analytical size portions of 25 g wet soil were taken and transferred into 50 ml plastic screw-top bottles on August 08, 1991. These samples were immediately fortified with 240 µl of the glyphosate stock solution or 130 µl of the AMPA stock solution, corresponding to concentration levels of 1.0 mg glyphosate/kg wet soil and 0.5 mg AMPA/kg wet soil, respectively. To achieve a nearly homogeneous distribution, the fortification solution was slowly injected by circular movements of the microliter syringe.

Additionally, two control samples were stored for each time interval under equal conditions as the storage stability test samples.

Immediately after fortification, the plastic bottles were put in storage in a deepfreeze compartment (at about -20 °C) in the dark until the analyses were performed. Samples were taken for analysis one week after preparation of storage stability sample (day 7), and about six months (day 188), about nine months (day 292), and about one year (day 404) later. At each time interval, the storage stability test sample and the corresponding control sample were removed from the freezer and analysed for glyphosate and its metabolite AMPA.

For method validation, at least one procedural recovery at a level of 1.0 mg glyphosate/kg wet soil or 0.5 mg AMPA/kg wet soil was freshly prepared per sample series by fortifying untreated control samples with calculated amounts of glyphosate or AMPA solutions. These fortified samples were analysed according to the same analytical procedures as the storage stability samples. The procedural recoveries provided were an indication of the method efficiency on that day.

2. Analytical procedures

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bi-distilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the co-eluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Procedural Recoveries

This method of analysis was validated with recovery experiments. Stock solutions were prepared by dissolving an appropriate amount of glyphosate or AMPA in 0.001 mol/L EDTA solution. These stock solutions were diluted with 0.001 mol/L EDTA solution to yield concentrations of 10 µg/ml. Fortified samples were prepared by adding calculated volumes of the latter solutions to the analytical material of untreated control samples based on the lowest concentrations successfully used in RCC project 273565 (please refer to [redacted], 1992a, CA 7.1.2.2.1/008).

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Residues for glyphosate and AMPA after frozen storage are presented in the tables below.

Summary of residues (mg/kg) of glyphosate in sandy clay soil after frozen storage

DAT	Control	Procedural recoveries		Storage stability sample	
	Residue (mg/kg)	Residue (mg/kg)	Recovery (%)	Residue (mg/kg)	Recovery (%)
0	< 0.02	0.916 (1.017) [2]	90.1	0.916 (1.017) [2]	90.1
188	< 0.02	0.676 (1.000) [2]	67.6	0.571 (1.017) [2]	56.1
292	0.048 [1]	0.747 (1.000) [2]	74.7	1.137 (1.017) [2]	111.8
404	< 0.02	0.821 (1.000) [2]	82.2	0.775 (1.017) [2]	76.2
Mean			78.6		83.6

[1] Sample was assumed to be contaminated during analytical procedure. The storage stability was not correct for the control sample

[2] Fortification level in brackets

Summary of residues (mg/kg) of AMPA in sandy clay soil after frozen storage

DAT	Control	Procedural recoveries		Storage stability sample	
	Residue (mg/kg)	Residue (mg/kg)	Recovery (%)	Residue (mg/kg)	Recovery (%)
0	< 0.02	n.a. [1]	n.a. [1]	0.401 (0.519) [2]	77.3
188	< 0.02	0.278 (0.500) [2]	55.7	0.306 (0.519) [2]	58.9
292	< 0.02	0.396 (0.500) [2]	79.2	0.440 (0.519) [2]	84.9
404	< 0.02	0.400 (0.500) [2]	80.0	0.381 (0.519) [2]	73.4
Mean			73.6		71.6

[1] na = not evaluated due to technical reasons

[2] Fortification level in brackets

B. Characterisation of residues

The recovery percentages for glyphosate in soil storage stability samples were calculated to be 90.1 % one week after sample fortification, 56.1 % after about six months, 111.8 % after about nine months, and 76.2 % after about one year of storage time. The overall mean recovery was determined to be 83.6 % with a relative standard deviation of 28.1 % (n=4).

The recovery percentages for AMPA in soil storage stability samples were calculated to be 77.3 % one week after sample fortification, 58.9 % after about six months, 84.9 % after about nine months, and 73.4 % after about one year of storage time. The overall mean recovery was determined to be 73.6 % with a relative standard deviation of 14.8 % (n=4).

No residues of glyphosate or AMPA above the limit of determination of 0.02 mg/kg were found in the control samples, except for the glyphosate control sample analysed after about nine months (292 days). This control sample was assumed to be contaminated during the analytical procedure.

The efficiency of the analytical method on the day of analysis was determined with freshly prepared procedural recoveries performed at the fortification levels of the stored samples, namely 1.0 mg/kg for glyphosate and 0.5 mg/kg for AMPA. The mean procedural recovery for glyphosate was 78.6 % with a relative standard deviation of 12.3 % (n=4). The mean procedural recovery for AMPA was 71.6 % with a relative standard deviation of 19.3 % (n=4).

The recoveries of glyphosate and AMPA were not corrected for control values and the storage stability results were not corrected for procedural recoveries or control values.

III. CONCLUSIONS

In conclusion, the results indicate that glyphosate and AMPA are stable in the tested soil for at least 404 days at about -20 °C. The recoveries of stored fortified samples were nearly identical to that of the procedural fortification samples.

Assessment and conclusion by applicant:

It was shown that glyphosate and AMPA residues in soil are stable for up to one year storage time under deep frozen conditions. The relative standard deviation is fairly high due to the low recovery on day 188. Due to the improved recovery at day 292 and 404, the overall conclusion is seen as sufficiently reliable. The study is considered as supportive information.

Assessment and conclusion by RMS:

Overall recoveries are acceptable for this storage stability study. The recoveries after 6 months were low 56.1% and 58.9% AR for glyphosate and AMPA, respectively No explanation is given in the study report. Considering that recoveries are acceptable on the next sampling dates (292 and 404 days), RMS considers that glyphosate and AMPA can be considered as stable to storage at -20°C for up to 404 days.

[redacted], *1986*

Data point:	CA 7.1.2.2.1/019
Report author	[redacted]
Report year	1986
Report title	Frozen storage stability of SC-0224 in soil
Report No	RRC 86-61
Guidelines followed in study	None
Deviations from current test guideline	Not relevant
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, Supportive in RAR (2015)
Acceptability/Reliability:	No

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation: Touchdown 4-LC

2. Soil:

Test substances are field treated samples containing Touchdown residues. Test commodities are soils of three different types: St. Johns fine sand, sandy loam and silty clay loam.

Table 8.1.1.3-92: Characteristics of test soils

Parameter	Results		
Soil	St. Johns fine sand	Sandy loam	Silty clay loam
Test site location	Sanford, Florida	Orange cove, California	Libon, Iowa
pH [1]	5.4	6.9	n.i.

Organic matter (%)	0.5	2.2	6.0
Organic carbon (%) [2]	0.29	1.28	3.48

[1] Medium not stated
[2] Calculated from organic matter according to OC=OM x 0.58
n.i. = not indicated

B. STUDY DESIGN

1. Experimental conditions

The three test soils were field-treated with Touchdown 4-LC at a rate of 6.72 kg a.s./ha (6.0 lb/acre) applied in water via mechanical sprayers at post-emergence tests sites in Sandford, Florida (St. Johns Fine sand), Orange cove, California (Sandy loam) and Libon, Iowa (Silty clay loam). Several 2.54 cm (1 inch) core samples were taken, composited and then frozen until time of analysis. Untreated controls and untreated controls fortified at the time of extraction were analysed to obtain recovery data. Control and fortified samples were prepared for each oil type. Samples were prepared for each soil type and analysed annually in triplicate for each test compound: AMPA and glyphosate (CMP).

2. Sampling

Field treated samples were stored in freezers at -20 °C inside sealed plastic bags. Subsamples were taken as needed from the composited soil stored in the plastic bag (0 days, 1 and 2 years after application).

3. Analytical procedures

Four different analytical test methods are described as indicated in the following table. Test methods for determination of residues for glyphosate and AMPA in soil are the same for RRC 83-44 and RRC 85-34. Test method RRC 83-44 describes additional clean-up steps at different pH values. However, methods RRC 83-44 was not used for the storage stability test; therefore no details on this method are given. Further, while the used methods describe analysis also in other commodities, this summary only describes the relevant methods for analysis in soil.

Table 8.1.1.3-93: Summary of test methods used for determination of residues in soil

Test method	Title	Limit of detection in soil
RRC 83-44	Determination of SC-0224 Anion Residues in Crops and Soils by Liquid Chromatography	CMP and AMPA: 0.06 to 0.1 mg/kg
RRC 85-34	Determination of SC-0224 Anion Residues in Crops Soil, and Water by Liquid Chromatography	CMP and AMPA: 0.05 mg/kg

In the study RRC 85-34 soils were fortified with glyphosate and AMPA between 0.2 and 0.5 mg/kg. Recoveries for AMPA ranged from 79 to 98 % and recoveries for glyphosate ranged from 61 to 111 %. Background concentrations were measured between 0.01 and 0.03 mg/kg which is < 10 % of the fortification amount.

The glyphosate and AMPA are extracted from soil with 0.5 M NH_4OH. The extracts are cleaned up using a cation exchange column. Glyphosate and AMPA are collected separately, converted to fluorescing derivatives with 9-fIuorenylmethyl chloroformate, and determined by HPLC using an anion exchange column and a fluorescence detector.

II. RESULTS AND DISCUSSION

A. DATA

Table 8.1.1.3-94: Summary of residues of (mg/kg) glyphosate and AMPA in soil after application of 6.72 kg/ha Touchdown

Analyte	Storage interval	Residues (mg/kg) Silty clay loam	Sandy loam			St. Johns Fine Sand
		A-23178-2	A-22338-2	A-22338-4	A-22338-7	A-19647-3
CMP	0 Days	1.3	15.7	6.1	5.3	1.6
	1 Year	1.4	7.6	-	-	1.7

	2 Years	0.7	3.6	6.4	6.0	2.0
AMPA	0 Days	2.3	4.5	2.2	1.9	0.7
	1 Year	2.8	6.3	-	-	0.6
	2 Years	2.3	6.9	2.3	2.0	0.8

B. CHARACTERISATION OF RESIDUES

Results for field treated samples reflect the higher variability caused by field application. Glyphosate results decreased between the first and the second year of storage in the silty clay soil, but remain stable in the St. Johns fine sand soil. There is a high variability between the residues of the three subsamples in the sandy loam soil with a decrease for subsample-02, while residues for the other two subsamples are similar over the two year period. Residues of AMPA remain the same for the 0 day, 1 year and 2 year sample analysis.

Some variability is also evident for other soils, slight increases or decreases compared to day 0 data can be seen. All in all, field treated samples thus confirm the storage stability of glyphosate (CMP) and AMPA.

Assessment and conclusion by applicant:

It was shown that AMPA, glyphosate and trimesium residues in soil are stable for two years when stored at -20 °C. The study is considered as supportive information.

Assessment and conclusion by RMS:

The analytical method used in this study is not validated (see section B5).

The study is not acceptable.

B.8.1.1.3.3. Kinetic assessment of field studies

The results of the field studies were kinetically evaluated according to the current EU guidance (FOCUS, 2006, 2014; EFSA 2014) to derive degradation rates for glyphosate and AMPA for comparison with trigger values and as endpoints for input in modelling. For EU sites, the evaluation is presented in [redacted] 2020. For sites outside EU, the evaluation is presented in [redacted] 2020b.

Table 8.1.1.3-95: List of kinetic studies for evaluation of results from field dissipation studies

Annex point	Study	Study type	Substance(s)	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.1.2.2.1/001	[redacted], 2020	Kinetic evaluation	Glyphosate and AMPA	-	Acceptable
CA 7.1.2.2.1/003	[redacted], 2020b	Kinetic evaluation	Glyphosate and AMPA	-	Acceptable

Robinson, 2020 (EU sites)

Please note that for easier reading, RMS comments on the kinetic evaluation provided by the applicant is reported site by site in the study summary. General comments on the acceptability of the study are reported in the final box "Assessment and conclusion by RMS".

The study [redacted] 2020 included kinetic evaluation from results of sites from studies [redacted], 1992a, [redacted], 1992 and [redacted], 1992 that are no longer considered as reliable by RMS. For greater simplicity and easiness of the reading, the methodology part of the summary of [redacted] 2020 was left untouched

but kinetic fittings for these unreliable sites are not presented below. They can be found under appendix 2 for completeness.

Data point:	CA 7.1.2.2.1/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from terrestrial field dissipation studies in Europe
Report No	112148-003
Guidelines followed in study	EFSA (2014): EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662 [37 pp.]. FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics and EFSA $DegT_{50}$ guidance: - Data processing could be reproduced (see RMS comments for details) - Uncertainties regarding normalisation process (see RMS comments for details)
GLP/Officially recognised testing facilities	Not relevant
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes, pending data gaps are addressed (see RMS comments)

I. MATERIALS AND METHODS

The purpose of this evaluation was to conduct a kinetic evaluation for glyphosate and its major soil metabolite aminomethylphosphonic acid (AMPA) using data from field soil dissipation studies in order to: i) derive DT_{50} and DT_{90} values for use in soil exposure calculations and for comparison with trigger values from guidelines, and ii) derive $DegT_{50}$ matrix values for use in environmental exposure models for groundwater and surface water.

Five legacy field dissipation studies, comprised of 10 field trials located in Germany and Switzerland ([redacted], 1992, CA 7.1.2.2.1/013; [redacted], 1992a-d, CA 7.1.2.2.1/008-CA 7.1.2.2.1/011), were evaluated according to the most recent guidance (FOCUS, 2006, 2014; EFSA, 2014). The kinetic evaluation was performed using the model fitting software CAKE 3.3 (CAKE, 2016).

1. Description of the terrestrial field dissipation studies

The five field soil dissipation studies included for kinetic evaluation were conducted at 10 sites in Germany and Switzerland, representing soils and climate typical of Central Europe. Different amounts of glyphosate, formulated as either glyphosate-trimesium or the isopropylamine salt, were applied to bare soil. Soil samples from studies conducted with either formulation of glyphosate were analysed for glyphosate and its metabolite AMPA.

A summary of the trial locations and application data is given in the following table.

Table 8.1.1.3-96: Summary of trial locations and application data in field soil dissipation studies

Study	Trial/ location	Formulation	Crop	Date of Application	Target rate (kg a.s./ha)[1]	Actual rate (kg a.s./ha)[1]
[redacted], 1992, CA 7.1.2.2.1/013	Büchen, Germany	Glyphosate-trimesium	Bare soil	11/04/90	3.31	3.59

	Klein-Zecher, Germany	Glyphosate-trimesium	Bare soil	10/08/90	3.31	3.93
	Unzhurst, Germany	Glyphosate-trimesium	Bare soil	03/05/90	3.3.1	3.31
	Rohrbach, Germany	Glyphosate-trimesium	Bare soil	25/07/90	3.31	3.45
	Herrngiersdorf, Germany	Glyphosate-trimesium	Bare soil	08/05/90	3.31	3.17
	Wang-Inzkofen, Germany	Glyphosate-trimesium	Bare soil	02/07/90	3.31	3.31
[redacted], 1992a, CA 7.1.2.2.1/008	Diegten, Switzerland	Isopropylamine salt	Bare soil	05/09/90	n.r.	3.53
[redacted], 1992b, CA 7.1.2.2.1/009	Egerkingen, Switzerland	Isopropylamine salt	Bare soil	04/09/90	n.r.	3.87
[redacted], 1992c, CA 7.1.2.2.1/010	Bad Krozingen, Germany	Isopropylamine salt	Bare soil	05/09/90	n.r.	3.67
[redacted], 1992d, CA 7.1.2.2.1/011	Menslage, Germany	Isopropylamine salt	Bare soil	07/09/90	n.r.	3.67

n r. = not reported

[1] Converted to glyphosate-equivalent where appropriate

In general, a single treated plot was considered at all trial sites. 20 cores were taken at each sampling time, dissected into soil horizons (up to 30 cm depth) and blended to give a composite sample for each horizon. The duration of sampling varied between 61 and 582 days across the trial sites.

2. Data pre-processing

The data from the legacy field trials required pre-processing in order to generate appropriate input datasets for the kinetic evaluation. The standard procedures recommended by FOCUS (2006, 2014) were applied. Single samples were available for all studies.

The time-zero concentration for the metabolite was set to zero and the initial metabolite amount was converted to parent-equivalent (accounting for the molar weight difference between the compounds) and added to the parent substance.

In all of the studies considered, the LOQ and LOD were indistinguishable; only the 'limit of determination' is reported. Hence, the LOQ and LOD were both assigned the same value and the FOCUS guidance was then applied as follows. Values below LOD were replaced by half the LOD. If the concentrations of the applied substance in soil declined to values below LOD, the curve was cut off after the first value below LOD, unless detections above LOQ were made later in the experiment (FOCUS, 2006, 2014). These corrections were performed along the time course, as well as with depth along the soil horizon, with the exception for 0 DAT where it was assumed that residues only resided in the upper most soil layer.

For each treated plot (trial site) the measured residues (mg/kg) in the different soil layers were converted into residues expressed in kg/ha (considering the layer depth and bulk density) and then summed up. They were then expressed as percentage values of the residue at 0 DAT (so the time zero value is 100 %). Thus, if the maximum concentration occurred after 0 DAT, the respective maximum percentage value was greater than 100 %.

For the four studies of [redacted] (1992), a default value of 1.5 g/cm^3 was assumed for the bulk density. For the study of [redacted] (1992), the horizon-specific bulk density was calculated at each sampling time using the reported soil core surface area, depth and dry weight.

The input values of AMPA were expressed as percentage values of the parent (glyphosate) residue at 0 DAT (correcting for molar weight differences).

Processed residue data, adjusted as described above, are presented in the following tables and were used in the kinetic evaluation.

Table 8.1.1.3-97: Processed residue data (kg/ha and % of DAT 0) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1992)

Time (DAT)	Sum of horizons (0 - 20 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
Büchen				
0	3.25	0.00	100.00	0.00
7	3.15	0.23	96.94	10.61
14	2.76	0.33	84.73	15.51
28	2.17	0.37	66.71	17.37
61	1.12	0.48	34.42	22.26
91	0.89	0.77	27.45	35.83
121	0.39	0.32	12.12	14.93
182	0.44	0.60	13.59	28.24
240	0.31	0.49	9.39	23.09
322	0.26	0.32	8.04	14.76
475	0.26	0.51	7.89	23.81
Klein-Zecher				
0	2.93	0.00	100.00	0.00
7	2.94	0.43	100.10	22.16
14	2.22	0.48	75.69	25.03
28	1.55	0.47	52.74	24.64
61	1.30	0.67	44.16	34.94
91	0.74	0.53	25.28	27.42
119	0.92	0.59	31.26	30.83
201	0.75	0.73	25.55	38.06
244	0.65	0.70	22.15	36.15
298	0.30	0.58	10.36	29.89
479	0.18	0.68	6.09	35.51
567	0.04	0.52	1.42	26.80
Unzhurst				
0	3.47	0.00	100.00	0.00
7	2.59	0.24	74.61	10.63
13	2.47	0.31	71.12	13.72
27	1.92	0.27	55.36	11.91
57	0.71	0.55	20.52	23.92
90	0.53	0.61	15.22	26.91
117	0.35	0.54	9.98	23.63
187	0.25	0.53	7.22	23.04
251	0.24	0.62	7.05	26.98
314	0.22	0.55	6.30	24.21
418	0.15	0.43	4.26	18.74
Rohrbach				
0	2.28	0.00	100.00	0.00
7	2.68	0.34	117.47	22.36
14	2.05	0.46	89.68	30.53
28	1.36	0.44	59.67	29.28
56	0.44	0.66	19.20	43.65
85	0.20	0.63	8.66	41.86
231	0.04	0.56	1.53	37.58
282	-[2]	0.52	-[2]	34.41
418	-[2]	0.28	-[2]	18.60
582	-[2]	0.23	-[2]	15.61
Hermgiersdorf				
0	2.05	0.00	100.00	0.00
6	1.96	0.36	95.89	26.44
13	1.48	0.29	72.53	21.64
28	1.46	0.41	71.60	30.46
58	0.47	0.41	23.08	30.44

Time (DAT)	Sum of horizons (0 - 20 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
90	0.30	0.41	14.61	30.47
125	0.18	0.38	8.81	27.93
168	0.04	0.27	1.96	20.11
330	-[2]	0.28	-[2]	20.91
464	-[2]	0.13	-[2]	9.64
541	-[2]	-[2]	-[2]	-[2]
Wang-Inzkofen				
0	2.97	0.00	100.00	0.00
7	2.11	0.57	71.03	29.03
15	1.44	0.66	48.50	33.71
29	1.37	0.80	46.22	41.12
58	0.69	0.64	23.10	32.67
94	0.40	0.65	13.60	33.39
114	0.36	0.66	12.24	33.96
275	0.24	0.59	7.93	30.06
414	0.14	0.46	4.88	23.55
549	0.04	0.35	1.32	17.82

1 Expressed as Glyphosate equivalent = percentage of Glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
2 Data omitted according to FOCUS (2006, 2014)

Table 8.1.1.3-98: *Processed* residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1992 CA 7.1.2.2.1/008-CA 7.1.2.2.1/011)

Time (DAT)	Sum of horizons (0 - 30 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Diegten				
0	3.61	0.00	100.00	0.00
7	1.66	0.56	45.95	23.51
15	0.88	0.33	24.41	13.97
30	0.45	0.29	12.37	12.07
62	0.49	0.54	13.61	22.94
194	0.36	0.57	10.05	24.21
282	0.13	0.39	3.57	16.31
Egerkingen				
0	2.19	0.00	100.00	0.00
7	0.97	0.19	44.22	13.01
15	0.97	0.37	44.22	25.50
30	0.72	0.47	32.94	32.47
62	0.68	0.51	30.76	35.17
202	0.15	0.34	6.90	23.62
Bad Krozingen				
0	4.26	0.00	100.00	0.00
7	1.42	0.36	33.40	13.02
15	1.23	0.41	28.93	14.79
30	0.67	0.47	15.70	16.61
61	0.60	0.65	14.08	23.31
Menslage				
0	4.20	0.00	100.00	0.00
7	1.99	0.35	47.43	12.71
15	0.89	0.48	21.06	17.50
30	1.03	0.58	24.55	20.87
60	0.77	0.79	18.41	28.53
192	0.36	0.64	8.46	23.15
271	0.44	1.29	10.38	46.89

315	0.20	0.64	4.71	23.20

[1] Expressed as Glyphosate equivalent = percentage of Glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite

3. Normalisation of field degradation half-life values to reference conditions

General approach

Time-step normalisation according to FOCUS (2006, 2014) and Hardy et al. (2003) was conducted in order to derive modelling endpoints at reference conditions (20 °C and pF 2). Daily correction factors for soil temperature (fT) and moisture (fΘ) were calculated for a given reference soil temperature of 20 °C and a reference soil moisture of pF 2.

According to FOCUS, the exponent of the moisture response function was set to 0.7 and the temperature coefficient Q10 was set to 2.58, respectively.

The following constraints were applied to the normalisation procedure:

- no further increase of the degradation rate if soil moisture > reference moisture
- no degradation if soil temperature <0 °C (resulting in a transformed day length of zero)

The obtained correction factors resulted in normalised transformation rates by reducing or increasing day lengths. Processed residue data, in combination with the transformed time course (i.e. under constant temperature and moisture conditions), were used for the evaluation of modelling endpoints according to recommendations for obtaining $DegT_{50}$ matrix values in soil from field dissipation studies for modelling purposes (FOCUS, 2006, 2014; EFSA, 2014). For the time between application and first sampling (0 DAT), no normalisation was considered and application was assumed to occur at time point zero.

Estimation of soil temperature and moisture

Soil temperature and moisture data were not directly available from the trial sites. Therefore, daily values of these variables (mean of top 10 cm) were calculated with the environmental fate model FOCUSPEARL 4.4.4. Site-specific weather and soil data were used as input parameters to the model.

Weather data

In order to estimate the daily soil temperature and moisture, the evapotranspiration process must be defined. The Penman-Monteith approach was selected in FOCUSPEARL v4.4.4 to calculate the potential evapotranspiration. The required meteorological data for this estimation method (maximum and minimum temperature, precipitation, global radiation, average vapour pressure and average wind speed) were obtained from local meteorological stations (where available) and/ or the Monitoring Agricultural ResourceS Unit (MARS) of the EC Joint Research Centre as shown in the following table.

Table 8.1.1.3-99: Availability of weather data

Study	Trial/ location	DWD[1] station	Distance from test site (km)	MARS grid number (25 km grid)
[redacted], 1992, CA 7.1.2.2.1/013	Büchen, Germany	Grambeck (1736): rain, min/ max temp, v.p. Boizenburg (591): wind speed	12.6 10.1	113111 (global radiation)
	Klein-Zecher, Germany	Grambeck (1736): rain, min/ max temp, v.p. Boizenburg (591): wind speed	11.3 22.6	113112 (global radiation)
	Unzhurst, Germany	Rheinau-Freistett (4169): rain, min/ max temp, v.p.	10.2	91104 (global radiation and wind speed)
	Rohrbach, Germany	Bad Bergzabern (377): rain, min/ max temp, v.p.	12.8	94104 (global radiation and wind speed)

Table 8.1.1.3-99: Availability of weather data

Study	Trial/ location	DWD[1] station	Distance from test site (km)	MARS grid number (25 km grid)
	Herrngiersdorf, Germany	Mallersdorf (3147): rain, min/ max temp, v.p.	13.0	92115 (global radiation and wind speed)
	Wang-Inzkofen, Germany	Weihenstephan (5404): rain, min/ max temp, v.p.	17.7	91115 (global radiation and wind speed)
[redacted], 1992a, CA 7.1.2.2.1/008	Diegten, Switzerland	n.a.	-	86103 (rain, min/ max temp, v.p., global radiation and wind speed)
[redacted], 1992b, CA 7.1.2.2.1/009	Egerkingen, Switzerland	n.a.	-	86103 (rain, min/ max temp, v.p., global radiation and wind speed)
[redacted], 1992c, CA 7.1.2.2.1/010	Bad Krozingen, Germany	Schallstadt-Mengen (4419): rain, min/ max temp, v.p. Eshbach (706): wind speed	7.8 4.3	88102 (global radiation)
[redacted], 1992d, CA 7.1.2.2.1/011	Menslage, Germany	Löningen (3044): rain, min/ max temp, v.p.	10.8	109104 (global radiation and wind speed)

n.a. = not available
v.p. = vapour pressure
[1] German Meteorological Office

In accordance with EFSA guidance (2014), the weather stations from which precipitation data were derived were less than 20 km from the actual field site.

In the FOCUSPEARL 4.4.4 model, the weather data for the normalisation included a warm-up period of one year prior to the date of application, thereby accounting for seasonal effects. No irrigation was performed at the trial sites.

Soil profile settings

For the simulations with FOCUSPEARL 4.4.4, soil profiles were created based on the detailed soil properties given in the following tables.

According to FOCUS (2000), the top soil horizon was parameterised with compartments with a layer thickness of 2.5 cm, whereas the subsoil included compartments with a layer thickness of 5 cm. The bulk density was estimated with a continuous pedotransfer function (Bollen et al., 1995). The lower boundary condition of the simulation profiles was set to 'Free Drainage' by default representing common European conditions. The initial groundwater level was set to 300 cm below the ground level. For soil evaporation, the crop factor ('FacEvpSol') and reduction coefficient ('CofRedEvp') were set to the values of 1 (default for bare soils) and 0.79, respectively.

The hydraulic characteristics of the soils were parameterised in FOCUSPEARL according to the 'van Genuchten' parameters (van Genuchten, 1980). The van Genuchten parameters were estimated based on continuous or classified 'HYPRES' pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001).

Table 8.1.1.3-100: Soil characterisation for site Büchen, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 60 cm	60 - 100 cm
Soil texture (USDA)	Loamy sand	Loamy sand	Loamy sand
Sand (%)	80	80	81
Silt (%)	14	12	15
Clay (%)	6	8	4
Organic matter (%)	2.8	2.1	0.8
pH[1]	6.4	6.5	6.7

Bulk density (g/cm3)[2]	1.35	1.40	1.55
Soil hydraulic parameters[3]			
Θres (m^3/m^3)[4]	0.025	0.025	0.025
Θsat (m^3/m^3)	0.4339	0.4185	0.3805
Ksat (m/d)	0.9845	0.4987	0.4261
α (cm^{-1})	0.0535	0.0705	0.0616
λ (-)	-1.2627	-1.6038	-0.0617
n (-)	1.3463	1.3279	1.4228
Θref (pF 2) (m^3/m^3)[5]	0.2480	0.2287	0.1863

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-101: Soil characterisation for site Klein-Zecher, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 60 cm	60 - 100 cm
Soil texture (USDA)	Sandy loam	Sandy loam	Sandy loam
Sand (%)	66	68	62
Silt (%)	21	15	19
Clay (%)	13	17	19
Organic matter (%)	1.9	1.2	0.2
pH[1]	7.0	7.0	7.3
Bulk density (g/cm^3)[2]	1.42	1.50	1.67
Soil hydraulic parameters[3]			
Θres (m^3/m^3)[4]	0.025	0.025	0.01
Θsat (m^3/m^3)	0.4195	0.4000	0.3530
Ksat (m/d)	0.6876	0.3140	0.1431
α (cm-1)	0.0550	0.0752	0.0627
λ (-)	-2.2925	-2.8898	-1.9899
n (-)	1.2651	1.2207	1.1841
Θref (pF 2) (m^3/m^3)[5]	0.2703	0.2617	0.2506

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-102: Soil characterisation for site Unzhurst, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 60 cm	60 - 90 cm	90 - 100 cm[1]
Soil texture (USDA)	Loam	Sandy clay loam	Loam	Loam
Sand (%)	48	53	44	44
Silt (%)	39	31	37	37
Clay (%)	13	16	19	19
Organic matter (%)	1.8	0.6	0.3	0.15
pH[2]	6.7	5.4	5.3	5.3
Bulk density (g/cm3)[3]	1.43	1.58	1.64	1.69
Soil hydraulic parameters[4]				
Θres (m^3/m^3)[5]	0.01	0.01	0.01	0.01
Θsat (m^3/m^3)	0.4211	0.3839	0.3694	0.3560
Ksat (m/d)	0.3360	0.2327	0.1544	0.1071
α (cm^{-1})	0.0335	0.0429	0.0322	0.0315
λ (-)	-1.9296	-2.0472	-1.9183	-1.3176
n (-)	1.2560	1.2135	1.1800	1.1690
Θref (pF 2) (m^3/m^3)[6]	0.2998	0.2765	0.2914	0.2856

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Medium not reported
[3] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[4] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)

[5] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[6] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-103: Soil characterisation for site Rohrbach, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 25 cm	25 - 35 cm	35 - 100 cm
Soil texture (USDA)	Silt loam	Silt loam	Silt loam
Sand (%)	12	13	15
Silt (%)	77	60	70
Clay (%)	11	27	15
Organic matter (%)	1.8	0.5	0.1
pH[1]	8.5	8.5	8.7
Bulk density (g/cm^3)[2]	1.43	1.60	1.71
Soil hydraulic parameters[3]			
Θres (m^3/m^3)[4]	0.01	0.01	0.01
Θsat (m^3/m^3)	0.4171	0.3909	0.3518
Ksat (m/d)	0.0571	0.1057	0.0630
α (cm^{-1})	0.0108	0.0143	0.0083
λ (-)	-0.8235	-2.8613	0.6547
n (-)	1.3017	1.1370	1.2052
Θref (pF 2) (m3/m3)5	0.3527	0.3509	0.3192

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-104: Soil characterisation for site Herrngiersdorf, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 100 cm
Soil texture (USDA)	Clay loam	Silt loam
Sand (%)	23	21
Silt (%)	47	58
Clay (%)	30	21
Organic matter (%)	2.8	0.8
pH[1]	8.0	8.4
Bulk density (g/cm^3)[2]	1.35	1.55
Soil hydraulic parameters[3]		
Θres (m^3/m^3)[4]	0.01	0.01
Θsat (m^3/m^3)	0.4551	0.4017
Ksat (m/d)	0.2175	0.1663
α (cm^{-1})	0.0311	0.0180
λ (-)	-3.5366	-2.4218
n (-)	1.1455	1.1758
Θref (pF 2) (m^3/m^3)[5]	0.3760	0.3424

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-105: Soil characterisation for site Wang-Inzkofen, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 100 cm1
Soil texture (USDA)	Silt loam	Silt loam
Sand (%)	25	25
Silt (%)	51	51
Clay (%)	24	24
Organic matter (%)	2.1	1.05
pH[2]	7.2	7.2

Bulk density (g/cm3)[3]	1.40	1.51
Soil hydraulic parameters[4]		
Θres (m^3/m^3)[5]	0.01	0.01
Θsat (m^3/m^3)	0.4356	0.4129
Ksat (m/d)	0.1929	0.1714
α (cm^{-1})	0.0272	0.0241
λ (-)	-3.1300	-3.0398
n (-)	1.1767	1.1536
Θref (pF 2) (m^3/m^3)[6]	0.3526	0.3478

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Medium not reported
[3] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[4] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[5] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[6] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-106: Soil characterisation for site Diegten, Switzerland ([redacted], 1992a, CA 7.1.2.2.1/008)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Sandy clay	Sandy clay
Sand (%)	47.55[2]	47.55
Silt (%)	13.29[2]	13.29
Clay (%)	39.16[2]	39.16
Organic carbon (%)	1.61	0.81
Organic matter (%)[3]	2.78	1.39
pH (KCl)	7.1	7.1
Bulk density (g/cm3)[4]	1.35	1.47
Soil hydraulic parameters[5]		
Θres (m^3/m^3)[6]	0.01	0.01
Θsat (m^3/m^3)	0.4510	0.4187
Ksat (m/d)	0.7132	0.1165
α (cm^{-1})	0.0597	0.0595
λ (-)	-4.2789	-4.6174
n (-)	1.1347	1.1035
Θref (pF 2) (m^3/m^3)[7]	0.3516	0.3457

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Rescaled such that sum of components = 100 %
[3] OM % = 1.724 × OC % (van Bemmelen factor)
[4] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[5] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[6] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[7] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-107: Soil characterisation for site Egerkingen, Switzerland ([redacted], 1992b, CA 7.1.2.2.1/009)

Soil layer	0 - 30 cm	30 - 100 cm1
Soil texture (USDA)	Clay loam	Clay loam
Sand (%)	34.17[2]	34.17
Silt (%)	28.77[2]	28.77
Clay (%)	37.06[2]	37.06
Organic carbon (%)	1.55	0.78
Organic matter (%)[3]	2.67	1.34
pH (KCl)	7.33	7.33
Bulk density (g/cm3)[4]	1.36	1.48
Soil hydraulic parameters[5]		
Θres (m^3/m^3)[6]	0.01	0.01
Θsat (m^3/m^3)	0.4549	0.4256
Ksat (m/d)	0.3819	0.1107

α (cm^{-1})	0.0438	0.0404
λ (-)	-4.0540	-4.3228
n (-)	1.1267	1.0990
Θref (pF 2) (m^3/m^3)[7]	0.3719	0.3657

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Rescaled such that sum of components = 100 %
[3] OM % = 1.724 × OC % (van Bemmelen factor)
[4] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[5] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[6] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[7] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-108: Soil characterisation for site Bad Krozingen, Germany ([redacted], 1992c, CA 7.1.2.2.1/010)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Sandy loam	Sandy loam
Sand (%)	55.0	55.0
Silt (%)	27.1	27.1
Clay (%)	17.9	17.9
Organic carbon (%)	0.36	0.18
Organic matter (%)[2]	0.62	0.31
pH (KCl)	6.0	6.0
Bulk density (g/cm3)[3]	1.58	1.64
Soil hydraulic parameters[4]		
Θres (m^3/m^3)[5]	0.01	0.01
Θsat (m^3/m^3)	0.3800	0.3660
Ksat (m/d)	0.3949	0.1733
α (cm^{-1})	0.0462	0.0466
λ (-)	-2.4670	-1.9794
n (-)	1.2249	1.1925
Θref (pF 2) (m^3/m^3)[6]	0.2654	0.2684

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] OM % = 1.724 × OC % (van Bemmelen factor)
[3] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[4] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[5] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[6] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-109: Soil characterisation for site Menslage, Germany [redacted], 1992d, CA 7.1.2.2.1/011)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Sand	Sand
Sand (%)	90.69[2]	90.69
Silt (%)	2.10[2]	2.10
Clay (%)	7.21[2]	7.21
Organic carbon (%)	0.25	0.13
Organic matter (%)[3]	0.43	0.22
pH (KCl)	4.73	4.73
Bulk density (g/cm3)[4]	1.61	1.67
Soil hydraulic parameters[5]		
Θres (m^3/m^3)[6]	0.025	0.025
Θsat (m^3/m^3)	0.3370	0.3218
Ksat (m/d)	2.2779	0.5803
α (cm^{-1})	0.0804	0.0947
λ (-)	-0.6077	0.0465
n (-)	1.5662	1.5217
Θref (pF 2) (m^3/m^3)[7]	0.1195	0.1159

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Rescaled such that sum of components = 100 %
[3] OM % = 1.724 × OC % (van Bemmelen factor)
[4] Estimated with a continuous pedotransfer function (Bollen et al., 1995)
[5] Calculated based on continuous HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[6] Calculated based on class HYPRES pedotransfer functions (Wösten et al., 1999, Nemes et al., 2001)
[7] Calculated based on van Genuchten model (van Genuchten, 1980)

4. 10 mm criterion for $DegT_{50}$ matrix evaluation

According to EFSA (2014), for evaluation of $DegT_{50}$ matrix, surface processes like photolysis and volatilisation should be excluded. Therefore, it is recommended for the kinetic evaluation to use data points following at least 10 mm of cumulative precipitation (for SFO kinetics). For this purpose, the first sampling time after 10 mm of cumulative precipitation was defined as day 0, and all later time points were adjusted accordingly.

Table 8.1.1.3-110: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from study [redacted], 1992, CA 7.1.2.2.1/013

Büchen			Klein-Zecher		
DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)
0	0.0	-	0	0.0	-
7	2.0	0.0	7	6.2	0.0
14	4.9	2.9	14	10.7	4.5
28	11.8	9.8	28	20.9	14.7
61	27.5	25.5	61	36.6	30.4
91	46.6	44.6	91	47.4	41.3
121	67.7	65.7	119	53.7	47.5
182	103.7	101.7	201	64.4	58.2
240	120.8	118.8	244	76.7	70.5
322	131.4	129.4	298	94.0	87.8
475	198.8	196.8	479	196.1	190.0
			567	211.1	205.0
Unzhurst			Rohrbach		
DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)
0	0.0	-	0	0.0	-
7	4.5	0.0	7	8.0	-
13	8.0	3.5	14	15.4	-
27	16.5	12.0	28	26.8	-
57	38.6	34.2	56	45.8	0.0
90	68.4	64.0	85	60.8	15.0
117	95.4	91.0	231	88.8	43.0
187	132.9	128.5	282	105.4	59.6
251	146.7	142.2	418	204.0	158.2
314	155.8	151.3	582	246.0	200.2
418	201.7	197.3			
Herrngiersdorf			Wang-Inzkofen		
DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)
0	0.0	-	0	0	-
6	3.7	0.0	7	4.3	0.0
13	8.1	4.3	15	9.8	5.5
28	16.2	12.5	29	20.2	15.9
58	37.9	34.2	58	44.0	39.7
90	63.8	60.1	94	61.9	57.6
125	91.3	87.6	114	70.3	66.0
168	111.4	107.6	275	93.3	89.0
330	136.7	132.9	414	173.2	168.9
464	217.4	213.7	549	216.2	211.9

Table 8.1.1.3-111: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from studies [redacted], 1992, CA 7.1.2.2.1/008-CA 7.1.2.2.1/011

Diegten			Egerkingen		
DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)
0	0	-	0	0	-
7	4.0	-	7	4.1	-
15	9.0	-	15	9.0	-
30	17.4	0.0	30	17.5	0.0
62	31.1	13.7	62	31.4	13.9
194	50.3	32.9	202	53.1	35.6
282	83.8	66.4			
Bad Krozingen			Menslage		
DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)
0	0	-	0	0	-
7	4.4	-	7	3.9	0.0
15	9.6	-	15	7.3	3.5
30	19.0	0.0	30	14.7	10.8
61	34.6	15.6	60	27.1	23.2
			192	53.6	49.7
			271	80.1	76.3
			315	112.2	108.3

In the case of bi-phasic behaviour, kinetic evaluation was performed with the complete data set, and only the slow phase of the bi-phasic decline was considered for estimating half-lives following EFSA (2014).

The number of remaining data points after 10 mm of rainfall per respective trial location are presented in the following table.

Table 8.1.1.3-112: 10 mm rainfall criterion at field trial locations

Study	Trial/ location	Total samples1	10 mm rainfall reached at	No. of samples after 10 mm rainfall
[redacted], 1992	Büchen, Germany	11	3 DAT	10
	Klein-Zecher, Germany	12	4 DAT	11
	Unzhurst, Germany	11	7 DAT	10
	Rohrbach, Germany	10	31 DAT	6
	Herrngiersdorf, Germany	10	3 DAT	9
	Wang-Inzkofen, Germany	10	1 DAT	9
[redacted], 1992a	Diegten, Switzerland	7	18 DAT	4
[redacted], 1992b	Egerkingen, Switzerland	6	19 DAT	3
[redacted], 1992c	Bad Krozingen, Germany	5	17 DAT	2[2]
[redacted], 1992d	Menslage, Germany	8	3 DAT	7

1 Number of samples after performing FOCUS correction of residue data
2 Insufficient data points were remaining to fit the SFO model according to EFSA (2014)

5. Kinetic assessment

Kinetic models

Four kinetic models have been recommended by the FOCUS workgroup for describing the kinetic behaviour of parent substances and their metabolites in soil (FOCUS, 2006, 2014): Single first order

(SFO), First order multi-compartment (FOMC), Double first order in parallel (DFOP) and Hockey stick (HS). In this report, the fitting approaches for trigger and modelling endpoints have been adopted according to FOCUS (FOCUS, 2006, 2014) and EFSA (EFSA, 2014), as appropriate.

Optimisation

The kinetic analyses were conducted using the software package CAKE 3.3. The data were initially fitted with the complete dataset and unconstrained initial concentration (M0) for the parent substance. Iteratively Reweighted Least Squares (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M0) and degradation model parameters k, α, β, g, or tb depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually based on the observed degradation pattern and preliminary model runs.

In pathway fits for derivation of trigger endpoints, the initial amount of metabolite was fixed to 0 % by default, which was in contrast to the pathway fitting for derivation of modelling endpoints. Here, the initial amount of metabolite was not constrained to zero, as several data points from the beginning of the experimental period prior to 10 mm rainfall were cut off. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1 × 10-5 and 100, respectively.

If a pathway fit did not yield visually and/ or statistically reliable results, the kinetic model was further optimised by fixing one or more of the model parameters to either the value derived from a reliable parent-only fit (e.g. M0, k), or to values derived from previous pathway fits with unbound parameters (e.g. ff). A stepwise fixing procedure has been applied in these cases, which is further described in the results chapter for the respective pathway fits.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually based on concentration/ residual - time plots. Generally the residuals should be distributed randomly around the zero line. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

> Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered
>
> Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
>
> Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line

A statistical measure of the quality of a fit is given by the $\chi 2$-test. The $\chi 2$-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. In general, for parent compounds, it is recommended that if the $\chi 2$ error is <15 % then the model has adequately reflected the measured data (FOCUS, 2006, 2014). However, this value should only be considered as a guide and not an absolute cut-off criterion. The guidance can be relaxed for field studies where the residue data can show appreciable scatter. The same also applies for metabolites where the curve fitting is more complex.

Significance of parameters

A single-sided t-test was used to evaluate whether the optimised parameters were significantly different from zero at a chosen significance level of 5 %. In case of metabolite data, a significance level of 10 % or higher may still be acceptable due to the inherent variability that often occurs in these types of data. This is particularly relevant for the degradation rate constants (k) of the SFO, DFOP and HS kinetic models. For the FOMC kinetic model, only the significance of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a 95 % confidence interval on the estimated parameters. As a general principle the confidence interval should be relatively tight and not contain 0 to be considered statistically robust.

Derivation of trigger and modelling endpoints

For derivation of trigger endpoints, the non-normalised dataset was considered, and the kinetic evaluation was conducted according to FOCUS guidance (2006, 2014).

For the parent compound, the best-fit model was accepted for deriving trigger endpoints. For the metabolite, pathway fits were conducted using the best-fit kinetic model for the parent and SFO for the metabolite. In cases where no reliable pathway fit could be established, kinetic endpoints for the parent were derived from the corresponding parent-only fit, and decline fits were conducted for the metabolite (if possible), starting from the maximum observed concentration. The respective day was defined as 0 days after maximum concentration, and later time points were adjusted accordingly.

For derivation of modelling endpoints, the corrected residue data were combined with the normalised day length data. The resulting parent datasets were then evaluated according to EFSA (2014). For the metabolite, if the SFO parent-only fit was accepted after excluding surface processes, the SFO-SFO pathway fit was assessed. If the pathway fit was visually acceptable and resulted in statistically reliable endpoints then the fit was accepted for deriving metabolite endpoints. This is considered appropriate even if the metabolite formation phase was not completely included in the evaluation but the metabolite decline occurred after the parent compound has mostly dissipated, as in this case the metabolite degradation rate can be estimated independently. If no reliable pathway fit for the metabolite could be established, or bi-phasic models were considered for the parent-only fit, further consideration was given to whether a decline fit could be evaluated for the metabolite.

II. RESULTS AND DISCUSSION

Egerkingen

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)
0	0	-	100.00	0.00
7	4.1	-	44.22	13.01
15	9.0	-	44.22	25.50
30	17.5	0.0	32.94	32.47
62	31.4	13.9	30.76	35.17
202	53.1	35.6	6.90	23.62

Table 8.1.1.3-113: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992b, CA 7.1.2.2.1/009) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	81.7	k: 0.0314	26.6	k: 0.0499	k: -0.0095	k: 0.0720	22.1	73.3
FOMC	Poor	99.8	α: 0.3475 β: 1.0927	11.3	-[1]	β: -3.4731	β: 5.6590	6.9	823
DFOP	Good	100.0	k_1: 2.653 k_2: 0.0087 g: 0.5228	5.3	k1: 0.4966 k2: 0.0211	k1: -1183 k2: 0.0008	k1: 1190 k2: 0.0170	1.1	179

Applicant's conclusion

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The FOMC model does not accurately describe the final two data points after 50 DAT well. The DFOP model provides a good visual fit. However, due to the very wide confidence interval for k1, the endpoints are not deemed to be reliable.

Conclusion: No reliable trigger endpoints can be determined for glyphosate

RMS conclusion:

SFO kinetics do not fit well the residues for this soil (bad description of M0, underestimation of the 2 last points) and is not considered suitable for trigger endpoint. Biphasic kinetics provide better fits.

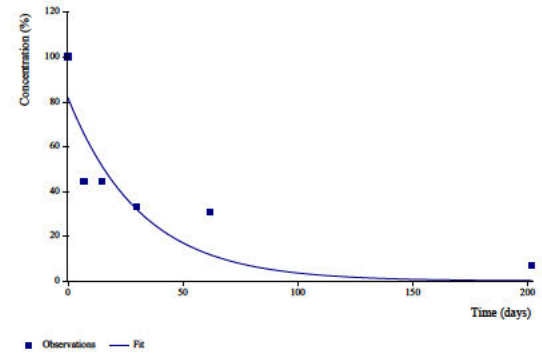
DFOP provides a good visual fit, better than FOMC, with lower chi2-error and a lower extent of residuals.

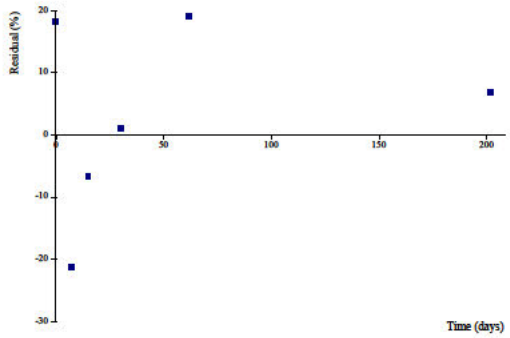
It is noted that t-test for k_1 DFOP fails. It is likely because the fast phase has terminated before sufficient measurements could be taken. In this specific case, RMS considers that this can be accepted.DT_{90} is reached within study period.

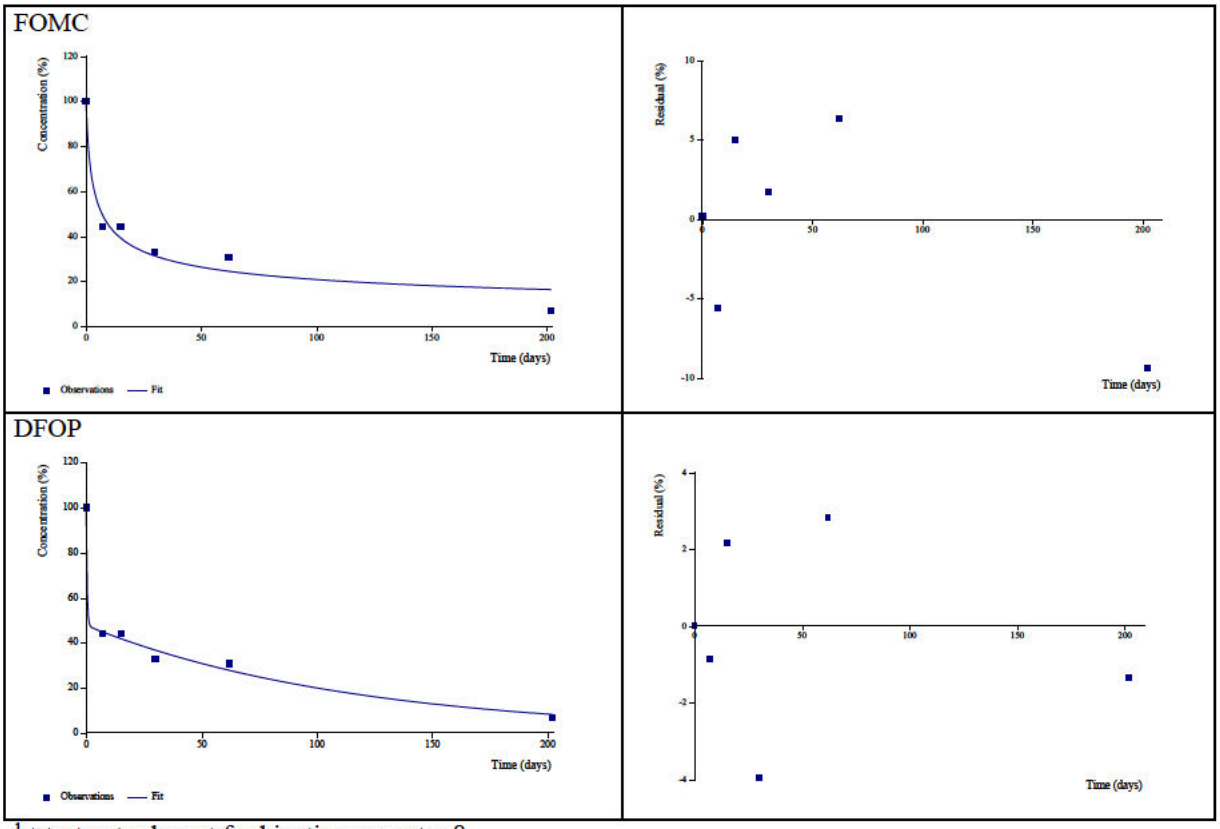
RMS considers that DFOP kinetics are relevant for determining a trigger endpoint for this soil.

DFOP to be used in pathway fit for trigger endpoints.

SFO







[1] t-test not relevant for kinetic parameter β

As the parent-only fit for glyphosate was not acceptable, no pathway fit was tested for soil Egerkingen. For AMPA, no clear decline phase was observed; hence a decline fit was not considered.

RMS conclusion:

In RMS opinion, DFOP kinetics provide an acceptable fit for glyphosate.

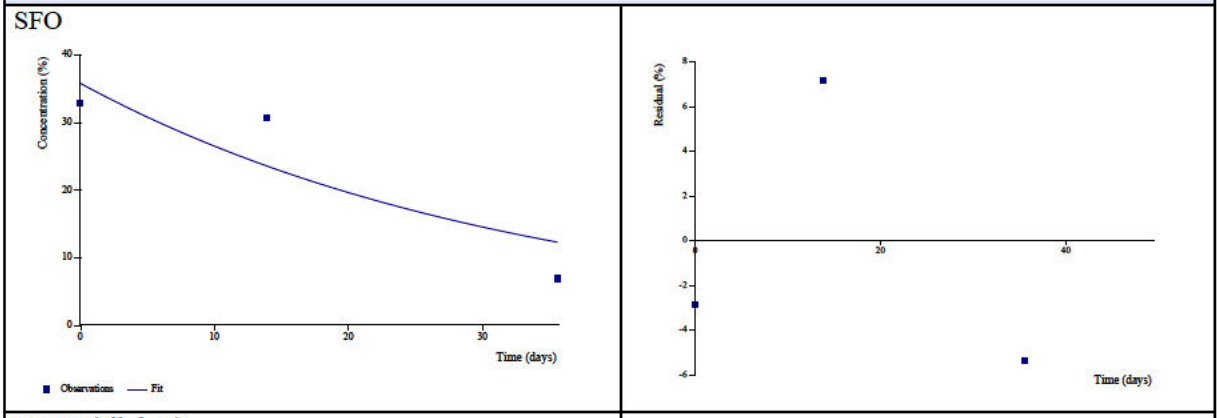
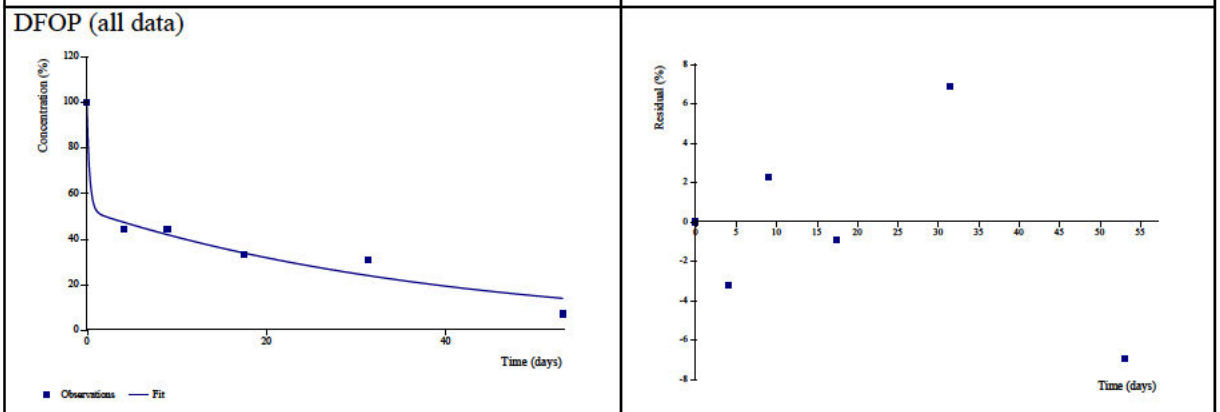
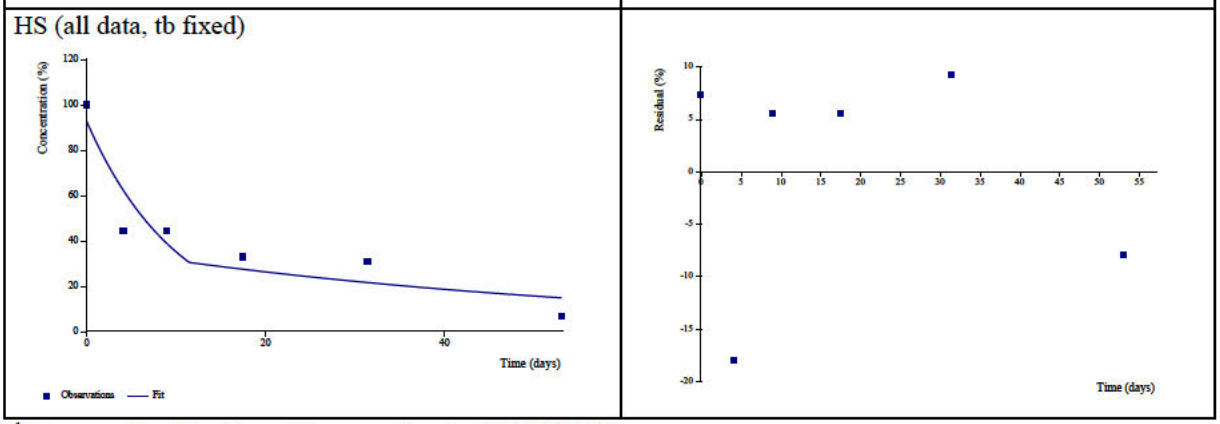
RMS identifies a data gap for the applicant to provide kinetic fittings for AMPA.

Determination of modelling endpoints

Table 8.1.1.3-114: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992b, CA 7.1.2.2.1/009) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	35.8	k: 0.0301	20.4	k: 0.1901	k: -0.2297	k: 0.2900	23.1	76.6
DFOP (full dataset)	Acceptable	100	k_1: 3.273 k_2: 0.0252 g: 0.4759	10.0	k_1: 0.0375 k_2: 0.0457	k_1: -0.8183 k_2: -0.0100	k_1: 7.365 k_2: 0.0600	27.6[2]	-
HS (full dataset, tb fixed)	Poor	92.7	k_1: 0.0971 k_2: 0.0173 t_b: fixed to 11.48	20.1	k_1: 0.0320 k_2: 0.2460	k_1: -0.0106 k_2: -0.0532	k_1: 0.2050 k_2: 0.0880	40.1[2]	-
SFO model: visually, given the scatter in the data, the SFO model describes the degradation of glyphosate adequately. But statistically the parameter k is not significantly different from zero and the confidence interval includes zero. Thus, the DFOP model was alternatively fitted to the whole dataset. DFOP model: the estimated g value is <0.75. The estimated degradation rates are significantly different, but the estimated DFOP breakpoint (6.8 days, normalised) was prior to the time >10 mm rain. (11.48 days, normalised). In accordance with EFSA (2014), the HS model was additionally fitted to the whole dataset.									

Table 8.1.1.3-114: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992b, CA 7.1.2.2.1/009) – modelling endpoints

HS model: in a first model run, the estimated tb was prior to the time >10 mm rain. Therefore, the fitting was repeated with tb fixed to the time when rain was >10 mm (11.48 days, normalised) in accordance with the EFSA (2014). For the repeated fit, the visual fit is poor. Also k2 is not significantly different from zero. Conclusion: No acceptable modelling endpoint could be determined for glyphosate
RMS conclusion: Agrees with the applicant, no reliable modelling endpoint
SFO 
DFOP (all data) 
HS (all data, tb fixed) 

[1] Representing $DegT_{50}$ matrix according to EFSA (2014)
[2] Calculated from the slow-phase (k2) according to EFSA (2014)

As no acceptable modelling endpoints could be determined for glyphosate, no pathway fit was tested for soil Egerkingen. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Bad Krozingen

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
0	0	-	100.00	0.00
7	4.4	-	33.40	13.02
15	9.6	-	28.93	14.79
30	19.0	0.0	15.70	16.61
61	34.6	15.6	14.08	23.31

Table 8.1.1.3-115: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Bad Krozingen of study [redacted] (1992c, CA 7.1.2.2.1/010) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.1	k: 0.1038	22.5	k: 0.0266	k: -0.0027	k: 0.2100	6.7	22.2
FOMC	Good	100.0	α: 0.45 β: 0.7373	5.3	-[1]	β: -2.106	β: 3.5800	2.7	122
DFOP	Good	100.0	k1: 0.4281 k2: 0.0177 g: 0.657	7.5	k1:0.2535 k2: 0.1860	k1: -5.1332 k2: -0.1312	k1: 5.9900 k2: 0.1670	3.1	69.6
HS	Not calculated								

Applicant's conclusion

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The FOMC model provides the best visual fit with the lowest χ2 error.

Conclusion: FOMC to be used in pathway fit for trigger endpoints

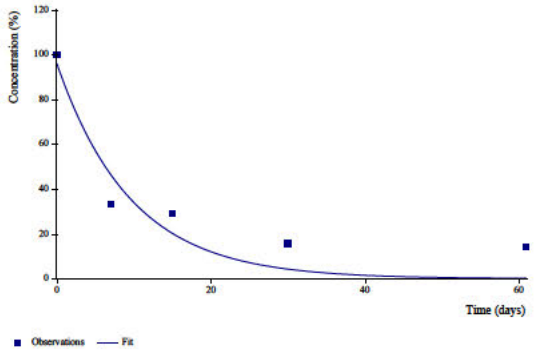
RMS conclusion:

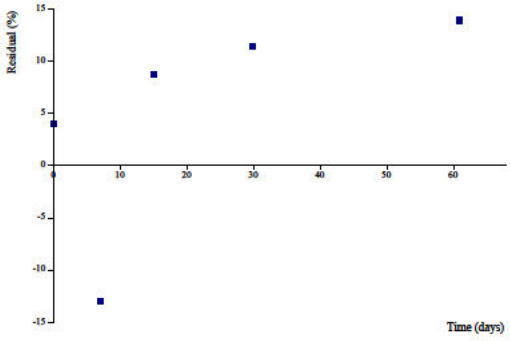
RMS agrees that SFO is not considered suitable.

FOMC is considered as best-fit based on chi2-error, better description overall but mostly for the last point (although the description is quite acceptable for DFOP too). It is noted that the confidence interval of β (FOMC) includes zero, and t test is not acceptable for k_1 and k_2 (DFOP) but this is usually less relevant for trigger endpoints.

Conclusion: FOMC to be used in pathway fit for trigger endpoints.

SFO





FOMC

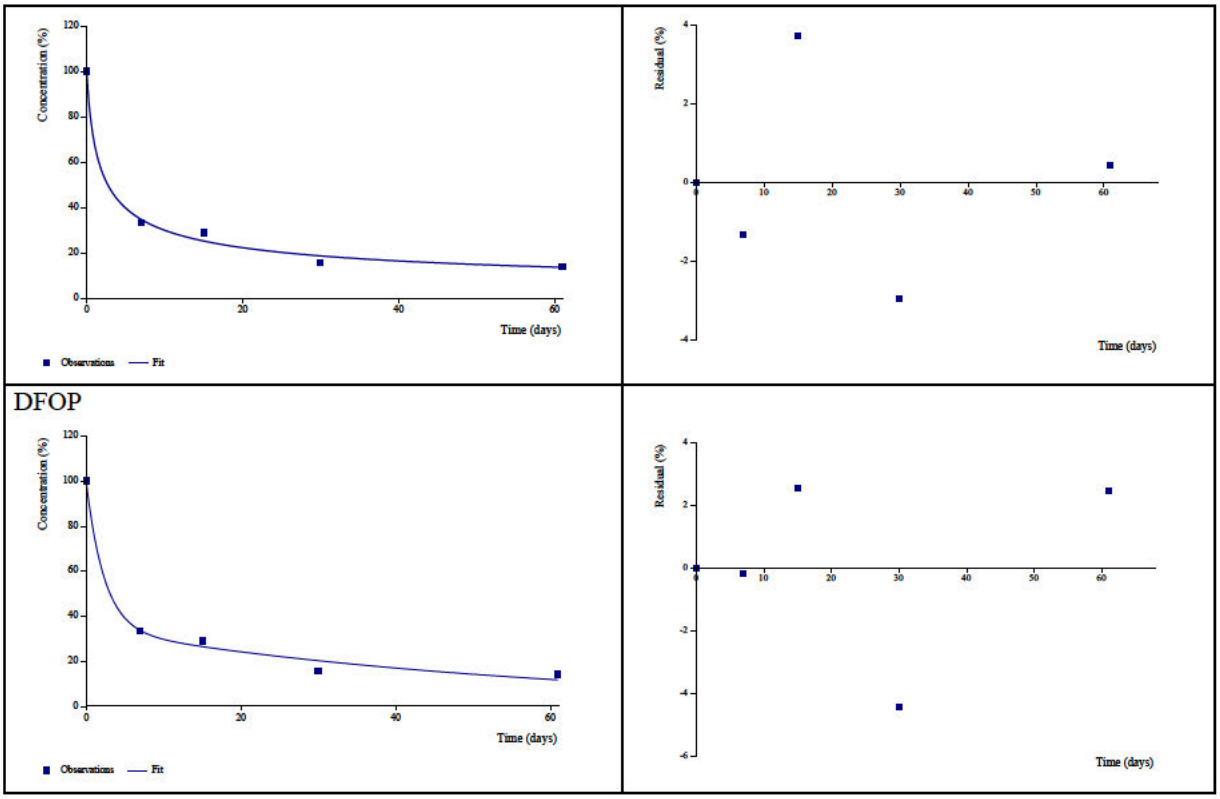


Table 8.1.1.3-116: Kinetic models and goodness-of-fit statistics of pathway fits for soil Bad Krozingen of study [redacted] (1992c, CA 7.1.2.2.1/010) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: FOMC	Good	99.8	α: 0.4726 β: 0.873	5.3	-	β: -1.1730	β: 2.9190	2.9	113
AMPA: SFO	Accep-table	-	k: 6.74×10^{-30}	11.7	k: 0.5	k: -0.0147	k: 0.015	>1000	>1000

Applicant's conclusion

The dissipation of glyphosate is well described by the FOMC model but the confidence interval for parameter β convincingly contains zero. For AMPA, the SFO model provides a visually acceptable fit, but the parameter k is not statistically reliable as no decline phase was observed. A decline fit for AMPA was not performed.

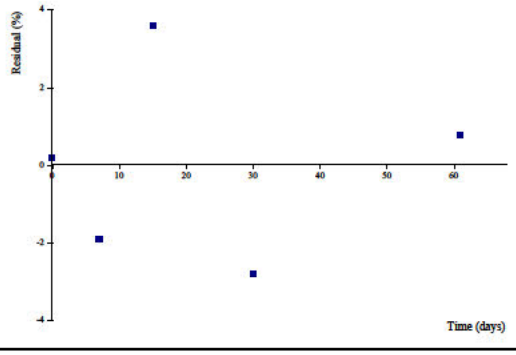
Conclusion: No reliable trigger endpoints for glyphosate or AMPA can be determined

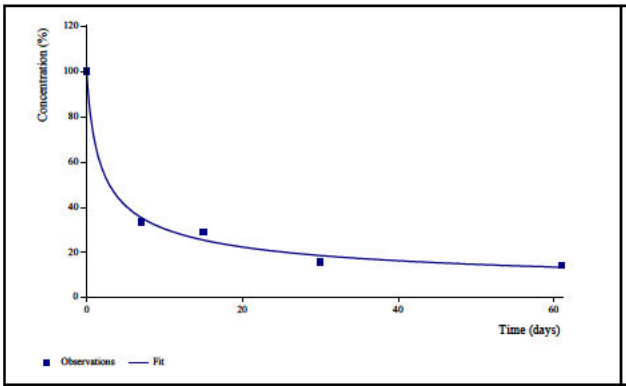
RMS conclusion

The degradation of AMPA is not very well described, with last point underestimated.

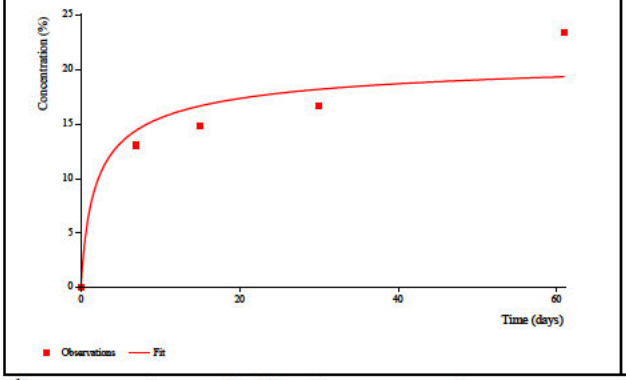
RMS agrees with the applicant, no reliable trigger endpoint for AMPA.

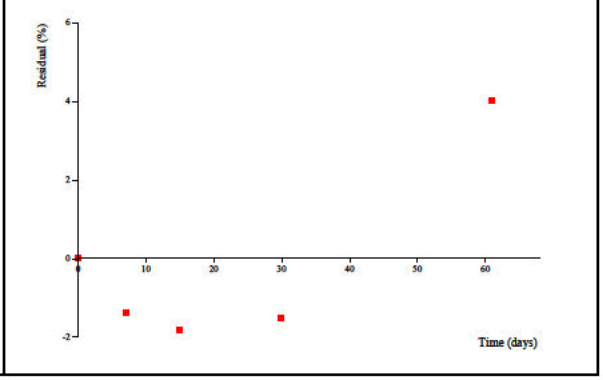
Glyphosate (FOMC)





AMPA (SFO)





[1] t-test not relevant for kinetic parameter β

Determination of modelling endpoints

Table 8.1.1.3-117: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Bad Krozingen of study [redacted] (1992c, CA 7.1.2.2.1/010) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Not calculated								
DFOP (full dataset)	Good	100	k_1: 0.9138 k_2: 0.0340 g: 0.6247	6.7	k_1: 0.3697 k_2: 0.1718	k_1: -25.856 k_2: -0.2249	k_1: 27.683 k_2: 0.2930	20.4[2]	-
HS (full dataset, tb fixed)	Poor	97.1	k_1: 0.1762 k_2: 1.28×10^{-9} t_b: fixed to 11.08	17.2	k_1: 0.0301 k_2: 0.5	k_1: -0.0188 k_2: -0.1767	k_1: 0.3710 k_2: 0.1770	>1000[2]	-

Applicant's conclusion

SFO model: not applied, as after excluding residue data prior to 10 mm rain, only two datapoints remain. Hence, the DFOP model was alternatively fitted to the whole dataset.

DFOP model: the estimated g value is <0.75. However, the estimated degradation rates are not significantly different from zero. In accordance with the EFSA (2014), the HS model was additionally fitted to the whole dataset.

HS model: in a first model run, the estimated t_b was prior to the time >10 mm rain. Therefore, the fitting was repeated with t_b fixed to the time when rain was >10 mm (11.08 days, normalised), in accordance with the EFSA (2014).

For the repeated fit, the visual fit is poor, and k_2 is not significantly different different from zero.

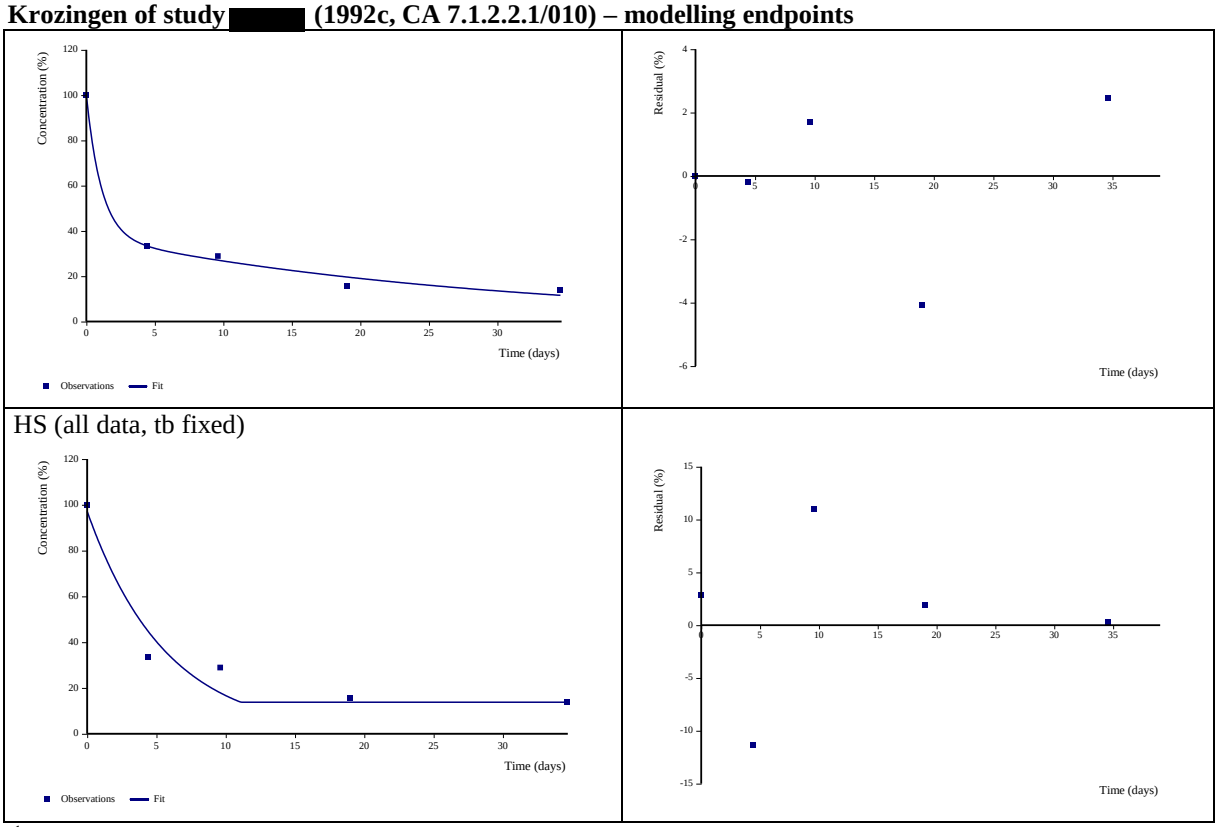
Conclusion: No acceptable modelling endpoint could be determined for glyphosate

RMS conclusion

Agrees with the applicant, no reliable modelling endpoint.

DFOP (all data)	

Table 8.1.1.3-117: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Bad Krozingen of study [redacted] (1992c, CA 7.1.2.2.1/010) – modelling endpoints



[1] Representing $DegT_{50}$ matrix according to EFSA (2014)
[2] Calculated from the slow-phase (k2) according to EFSA (2014)

As no acceptable modelling endpoints could be determined for glyphosate, no pathway fit was tested for soil Bad Krozingen. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Menslage

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
0	0	-	100.00	0.00
7	3.9	0.0	47.43	12.71
15	7.3	3.5	21.06	17.50
30	14.7	10.8	24.55	20.87
60	27.1	23.2	18.41	28.53
192	53.6	49.7	8.46	23.15
271	80.1	76.3	10.38	46.89
315	112.2	108.3	4.71	23.20

Table 8.1.1.3-118: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992d, CA 7.1.2.2.1/011) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.7	k: 0.0856	28.6	k: 0.0053	k: 0.0283	k: 0.1430	8.1	26.9
FOMC	Good	100.1	α: 0.5069 β: 1.598	12.1	-[1]	β: -1.29	β: 4.4870	4.7	149
DFOP	Good	100.4	k1: 0.1781 k2: 0.0041 g: 0.7704	9.4	k1: 0.0036 k2: 0.0315	k1: 0.0800 k2: -0.0004	k1: 0.2760 k2: 0.0090	5.8	201
HS	Not calculated								

Applicant's conclusion

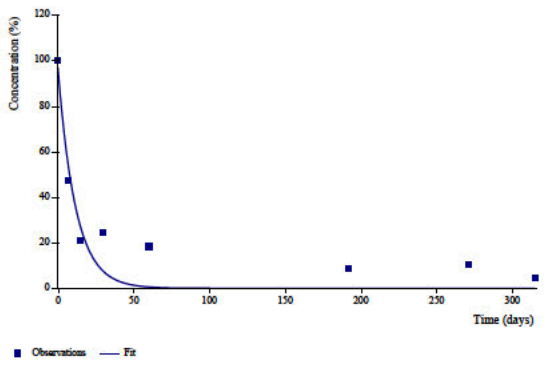
The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best visual fit with the lowest $\chi 2$ error.

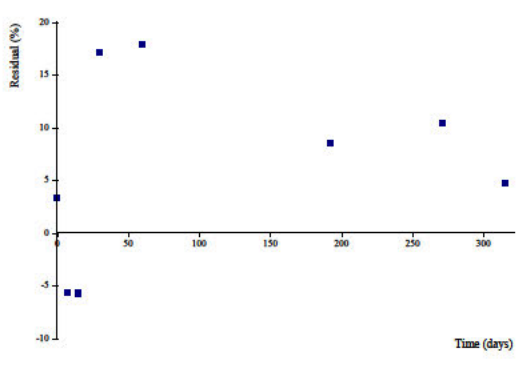
Conclusion: DFOP to be used in pathway fit for trigger endpoints

RMS conclusion

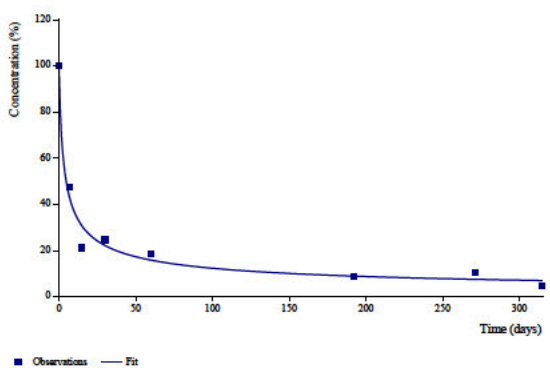
RMS agrees with the applicant, DFOP to be used in pathway fit for trigger endpoints

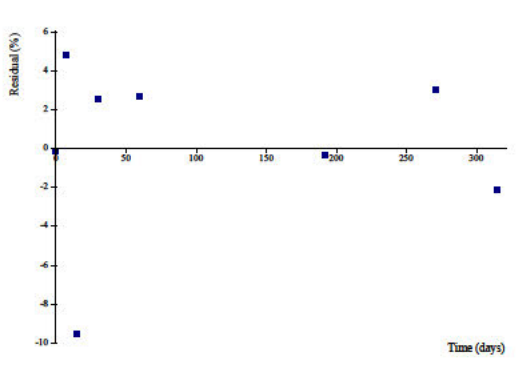
SFO



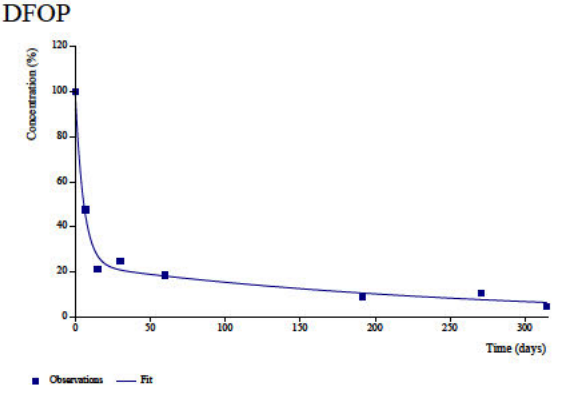


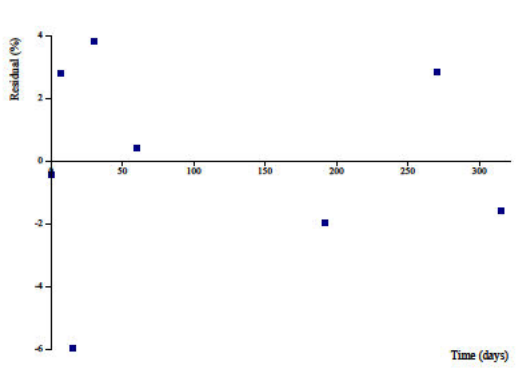
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

Table 8.1.1.3-119: Kinetic models and goodness-of-fit statistics of pathway fits for soil Menslage of study [redacted] (1992d, CA 7.1.2.2.1/011) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	100.3	k1: 0.1771 k2: 0.0042 g: 0.7681	9.4	k1: <0.001. k2: 0.0115	k1: 0.1023 k2: 0.0007	k1: 0.2520 k2: 0.0080	5.8	199
AMPA: SFO	Poor	-	k: 1.56×10-20	26.0	k: 0.5	k: -0.0031	k: 0.0030	>1000	>1000

Applicant's conclusion:

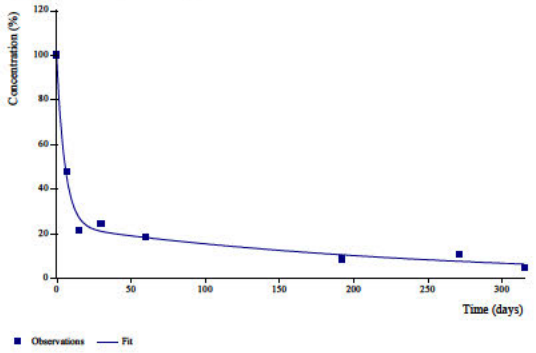
The dissipation of glyphosate is well described by the DFOP model in the pathway fit. For AMPA, the SFO model does not adequately fit the data visually or statistically. A decline fit for AMPA was not performed, as there is no clear decline phase.

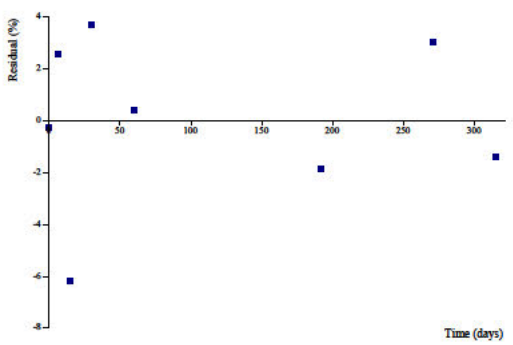
Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate
No reliable trigger endpoints for AMPA can be determined

RMS conclusion:

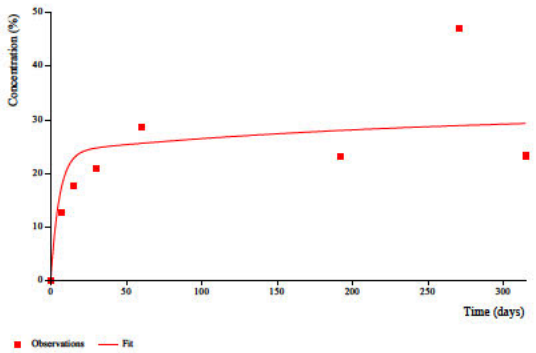
RMS agrees with the applicant, no reliable trigger endpoints for AMPA can be determined.

Glyphosate (DFOP)





AMPA (SFO)



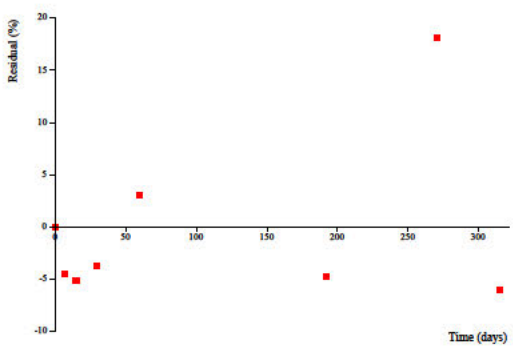


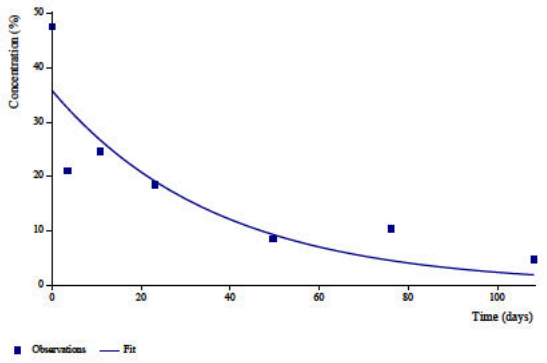
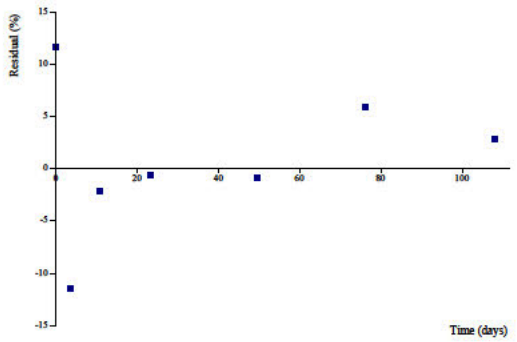
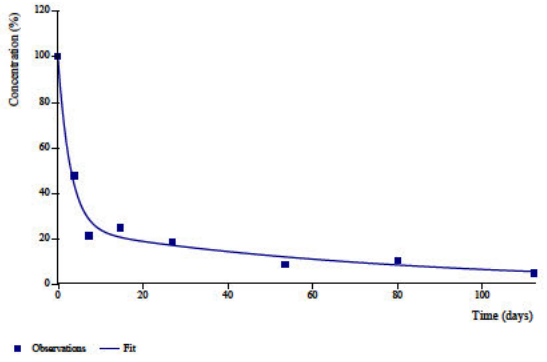
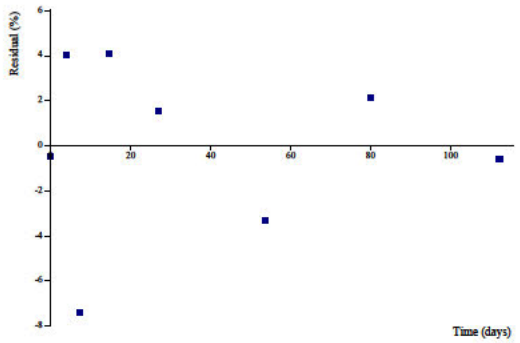
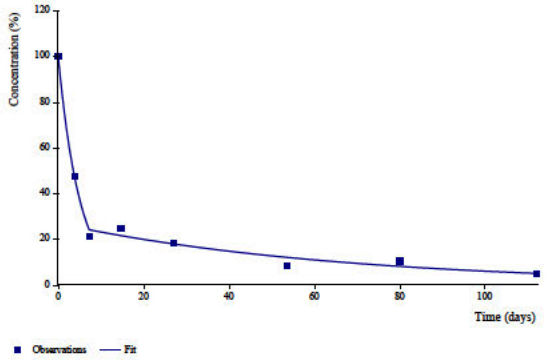
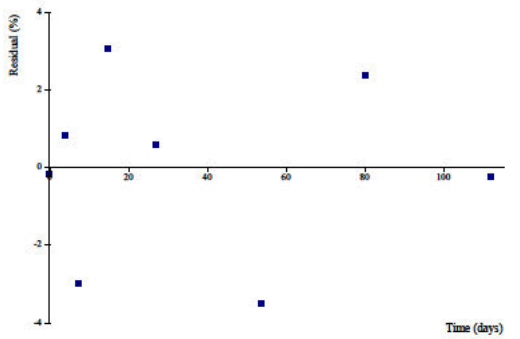
Table 8.1.1.3-120: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992d, CA 7.1.2.2.1/011) – modelling endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Poor	35.7	k: 0.0271	27.7	k: 0.0329	k: -0.0026	k: 0.0570	25.6	85.0
DFOP (full dataset)	Good	100.5	k_1: 0.3380 k_2: 0.0135 g: 0.7581	11.4	k_1: 0.0103 k_2: 0.0650	k_1: 0.0851 k_2: -0.0062	k_1: 0.5910 k_2: 0.0330	51.4[2]	-
HS (full dataset)	Good	100.2	k_1: 0.1961 k_2: 0.0151 t_b: 7.278	6.8	k_1: <0.001 k_2: 0.0086	k_1: 0.1459 k_2: 0.0044	k_1: 0.2460 k_2: 0.0260	46.0[2]	-

Applicant's conclusion:

SFO model: The SFO model does not describe the degradation of glyphosate adequately, especially in the initial phase of decline. Therefore the DFOP model was alternatively fitted to the whole dataset.
DFOP model: the estimated g value is >0.75. In accordance with the EFSA (2014), the HS model was additionally fitted to the whole dataset.
HS model: the estimated tb is after the time >10 mm rain, the visual fit is good with small randomly scattered residuals and k2 is significantly different to zero (at 5 % level).
Conclusion: Slow phase DT_{50} from HS model to be used as modelling endpoint for glyphosate

Table 8.1.1.3-120: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992d, CA 7.1.2.2.1/011) – modelling endpoints

RMS conclusion	
RMS agrees with the applicant, slow phase DT_{50} from HS model to be used as modelling endpoint for glyphosate.	
SFO	
	
DFOP (all data)	
	
HS (all data)	
	

[1] Representing $DegT_{50}$ matrix according to EFSA (2014)
[2] Calculated from the slow-phase (k_2) according to EFSA (2014)

As the SFO parent-only fit for glyphosate was not acceptable, no pathway fit was tested for soil Menslage. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Summary of trigger and modelling endpoints

Table 8.1.1.3-121: Summary of trigger endpoints for glyphosate (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted], 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[1]	0 - 30	40.7	187	6.6	DFOP
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[1]	0 - 30	29.1	364	12.7	DFOP
	Loam (bare soil)	Unzhurst, Germany	6.7[1]	0 - 30	27.0	126	8.5	DFOP
	Silt loam (bare soil)	Rohrbach, Germany	8.5[1]	0 - 30	24.4	81.0	16.0	SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[1]	0 - 30	33.7	112	10.6	SFO
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[1]	0 - 30	15.8	180	9.2	FOMC
[redacted], 1992a, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[2]	0 - 30	6.1	118	5.0	DFOP
[redacted], 1992b, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[2]	0 - 30	-[3]	-[3]	-	-
[redacted], 1992c, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[2]	0 - 30	-[3]	-[3]	-	-
[redacted], 1992d, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[2]	0 - 30	5.8	201	9.4	DFOP

[1] Medium not reported
[2] Measured in KCl
[3] No reliable endpoint could be determined

Table 8.1.1.3-122: Summary of trigger endpoints for AMPA (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted], 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[1]	0 - 30	-[3]	-[3]	-	-
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[1]	0 - 30	521	>1000	13.9	DFOP-SFO
	Loam (bare soil)	Unzhurst, Germany	6.7[1]	0 - 30	634	>1000	11.9	DFOP-SFO
	Silt loam (bare soil)	Rohrbach, Germany	8.5[1]	0 - 30	255	847	15.5	SFO-SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[1]	0 - 30	288	958	11.0	SFO[4]
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[1]	0 - 30	273	907	15.8	FOMC-SFO
[redacted], 1992a, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[2]	0 - 30	-[3]	-[3]	-	-

[redacted], 1992b, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[2]	0 - 30	-[3]	-[3]	-	-
[redacted], 1992c, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[2]	0 - 30	-[3]	-[3]	-	-
[redacted], 1992d, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[2]	0 - 30	-[3]	-[3]		

[1] Medium not reported
[2] Measured in KCl
[3] No reliable endpoint could be determined
[4] Metabolite decline fit

Table 8.1.1.3-123: Summary of modelling endpoints for glyphosate (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH	Depth (cm)	$DegT_{50}$ (d) Norm.[1]	χ^2 error (%)	Kinetic model
[redacted], 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[2]	0 - 30	23.0	12.9	SFO
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[2]	0 - 30	27.9	13.1	SFO
	Loam (bare soil)	Unzhurst, Germany	6.7[2]	0 - 30	25.9	13.4	SFO
	Silt loam (bare soil)	Rohrbach, Germany	8.5[2]	0 - 30	12.7	1.9	SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[2]	0 - 30	21.5	11.4	SFO
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[2]	0 - 30	26.4	11.9	SFO
[redacted], 1992a, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[3]	0 - 30	51.0[4]	6.8	HS
[redacted], 1992b, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[3]	0 - 30	-[5]	-	-
[redacted], 1992c, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[3]	0 - 30	-[5]	-	-
[redacted], 1992d, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[3]	0 - 30	46.0[4]	6.8	HS

[1] $DegT50_{matrix}$ according to EFSA (2014) and FOCUS (2006, 2014)
[2] Medium not reported
[3] Measured in KCl
[4] Calculated from the slow phase: $\ln(2)/k_2$
[5] No reliable endpoint could be determined

Table 8.1.1.3-124: Summary of modelling endpoints for AMPA (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH	Depth (cm)	DT_{50} (d) Norm.[1]	Formation fraction (-).	χ^2 error (%)	Kinetic model
[redacted], 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[2]	0 - 30	-[4]	-[4]	-	-
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[2]	0 - 30	471	0.1984	9.2	SFO-SFO
	Loam (bare soil)	Unzhurst, Germany	6.7[2]	0 - 30	238	0.3192	8.9	SFO-SFO
	Silt loam (bare soil)	Rohrbach, Germany	8.5[2]	0 - 30	119	0.2399	1.2	SFO-SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[2]	0 - 30	90.7	0.2508	7.8	SFO-SFO
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[2]	0 - 30	142	0.2308	7.2	SFO-SFO
[redacted], 1992a, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[3]	0 - 30	-[4]	-[4]	-	-
[redacted], 1992b, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[3]	0 - 30	-[4]	-[4]	-	-
[redacted], 1992c, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[3]	0 - 30	-[4]	-[4]	-	-
[redacted], 1992d, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[3]	0 - 30	-[4]	-[4]	-	-

[1] $DegT50_{matrix}$ according to EFSA (2014)
[2] Medium not reported
[3] Measured in KCl
[4] No reliable endpoint could be determined

Assessment and conclusion by applicant:

The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the study is considered valid and the provided endpoints can be used for risk assessment.

Assessment and conclusion by RMS

For more clarity and consistency in the reporting, RMS comments focus on the sites and studies that were considered reliable to derive endpoints. No further comments are therefore presented for the sites from [redacted] (1992) and [redacted] (1992a).

Data processing

The conversion of measured residues in mg/kg into kg/ha is not detailed in the study report and could not be reproduced by RMS. Therefore, a data gap is set for the applicant to provide more explanations on this conversion and on the parameters used. If available, excel sheets with the detailed calculations should be provided.

Normalisation process:

For Egerkingen site ([redacted] 1992 b), all weather data are taken from the MARS database, whereas in the study reports, at least daily weather data (temperature, precipitation and sunshine hours) from stations located 7 kms from the sites are reported. it should be clarified why data from MARS database

were preferred. A data gap is set for the applicant to justify the use of data from the MARS database instead of data measured at nearest station for normalization of data from Egerkingen site.

For Bad Krozingen site ([redacted] 1992c), it seems that data presented in the study report and data used for normalisation are taken from the same local station Schallstadt-Mengen. However, some major differences are observed between precipitation values presented in [redacted] 1992c study and in [redacted] 2020 study. A data gap is set for the applicant to clarify the differences observed between weather values presented in the studies [redacted], 1992c and [redacted], 2020 for station Schallstadt-Mengen (Bad Krozingen site).

For Menslage site ([redacted] 1992d), the precipitation values considered in [redacted] 2020 for the time-step normalization were taken from "Löningen" site, 10.8 km distant from the field. In [redacted] 1992d precipitation data were taken from "Menslage-Borg" station, 0.2km distant from the site. Data from this closer station are expected to be more representative of the weather conditions during the study. A data gap is set for the applicant to justify the use of data from "Löningen" site for normalization of data in Menslage soil in [redacted], 2020 study, instead of "Menslage-Borg" station mentioned in [redacted], 1992d.

While a default bulk density of 1.5 g/cm^3 was used for data processing for studies of [redacted] (1992b – d), bulk density was estimated from the pedotransfer function from Bollen *et al.* (1995) for the normalization step. A data gap is therefore set for the applicant to justify the use of different approaches in handling the data and rationale behind the choice of a default data or a calculated value.

For studies of [redacted] (1992b – d), as no indication of the soil properties were available for layer depths below 30cm, all properties were inherited from the 0-30 cm layer depth except for organic matter which was considered for 30-100 cm layer depth to be half that of the 0-30 cm layer depth. No rationale is provided for this choice. A data gap is set for the applicant to justify the estimation of organic matter considered for 30-100 cm.

For the soil profile settings, free drainage was used as a lower boundary condition for the PEARL simulations by default representing common European conditions. This assumption may have a serious impact on the model predictions because in case the free drainage option is incorrectly used (*i.e.* in reality there is a shallow(er) groundwater level), the estimated soil moisture in the upper levels may be somewhat dryer than in reality. Therefore, the estimated soil moisture may be an underestimation. This in turn leads to a lower correction factor which will eventually lead to an underestimation of the normalized DT_{50} (i.e. shorter normalised day lengths used than appropriate). A data gap is identified for the applicant to justify the choice of the lower boundary condition (free drainage) for each site.

<u>Kinetic evaluation</u>

As indicated by the EFSA $DegT_{50}$ guidance (2014), no kinetic fittings should be performed for a metabolite formed at more than 5% on a molar basis before 10mm of rainfall has occurred. In the available studies, AMPA is systematically formed at more than 5% before 10 mm of rainfall. As a consequence, in absence of a robust argumentation from the applicant, RMS considers that field modelling endpoint should not be derived for AMPA.

RMS opinion on the selected fits is given in the study summary. For trigger endpoint for AMPA, the assessment in soil Egerkingen should be provided (fit from parent). This is identified as a data gap for the applicant.

In conclusion, the study is considered acceptable, pending the above data gaps are addressed. The current acceptable endpoints derived from this study are summarised below and in B.8.1.1.3.

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Trigger endpoints

Parent	Aerobic conditions – trigger endpoints						
Soil	Location	pH $_{(H2O)}$[a]	Depth (cm)	DT_{50} / DT_{90} (d) actual	Kinetic parameters	St. (χ^2)	Method of calculation
Egerkingen [redacted] (1992b) Clay loam (bare soil)	Germany	7.79	0-30	1.1 / 179	k_1: 2.653 k_2: 0.0087 g: 0.5228	5.3	DFOP
Bad Krozingen [redacted] (1992c) Sandy loam (bare soil)	Germany	6.6	0-30	2.7 / 122	α: 0.45 β: 0.7373	5.3	FOMC
Menslage [redacted] (1992d) Sand (bare soil)	Germany	5.6	0-30	5.8 / 201	k_1: 0.1781 k_2: 0.0041 g: 0.7704	9.4	DFOP

[a)]Measured in KCl in the study, converted to pH_{H2O} considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)

AMPA	Trigger endpoints	Aerobic conditions The precursor from which the f f. was derived was glyphosate						
Soil	Location	pH $_{(H2O)}$[a]	Depth (cm)	DT_{50} (d) actual	DT_{90} (d) actual	St. (χ^2)	f. f. k_f / k_{dp}	Method of calculation
Egerkingen [redacted] (1992b) Clay loam (bare soil)	Germany	7.79	0-30					Data gap for fit from parent

[a)]Measured in KCl in the study, converted to pH_{H2O} considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Modelling endpoints

Parent		Aerobic conditions – modelling endpoints						
Soil	Location.	pH[a)]	Depth (cm)	DT_{50} (d) Norm[b]	Kinetic parameters	DT_{90}(d) Norm[b]	St. (χ^2)	Method of calculation
Menslage [redacted] (1992d) Sand (bare soil)	Germany	5.6	0-30	46.0	k_2: 0.0151	-	6.8	HS – slow phase

[a)]Measured in KCl in the study, converted to pH_{H2O} considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)
[b)]Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7, values are $DegT_{50}$ matrix

No modelling endpoints are determined for AMPA

[redacted], 2020b (USA and Canada sites)

The study [redacted] 2020 included kinetic evaluation from results of sites from studies [redacted], 1989 (a-c) that are no longer considered as reliable by RMS, and from some sites which are not considered representative of European conditions (please refer to [redacted], 2020). For greater simplicity and easiness of the reading, the methodology part of the summary of [redacted] 2020b was left untouched but kinetic fittings for these unreliable sites are not presented below. They can be found under appendix 2 for completeness.

Please note that for easier reading, RMS comments on the kinetic evaluation provided by the applicant is reported site by site in the study summary. General comments on the acceptability of the study are reported in the final box "Assessment and conclusion by RMS".

Data point:	CA 7.1.2.2.1/003
Report author	[redacted]
Report year	2020b
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from terrestrial field dissipation studies in the USA and Canada
Report No	112148-006
Guidelines followed in study	EFSA (2014): EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662 [37 pp.]. FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics and EFSA $DegT_{50}$ guidance: - Data processing could be reproduced (see RMS comments for details) - Uncertainties regarding normalisation process (see RMS comments for details)
GLP/Officially recognised testing facilities	Not relevant
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes, pending data gaps are addressed (see RMS comments)

I. MATERIALS AND METHODS

The purpose of this evaluation was to conduct a kinetic evaluation for glyphosate and its major soil metabolite aminomethylphosphonic acid (AMPA) using data from field soil dissipation studies, in order to: i) derive DT_{50} and DT_{90} values for use in PECsoil calculations and for comparison with trigger values from guidelines, and ii) derive $DegT_{50}$ matrix values for use in environmental exposure models for groundwater and surface water.

Four legacy field dissipation studies were conducted in the United States and Canada ([redacted], 1992, CA 7.1.2.2.1/014; [redacted], 1993a, CA 7.1.2.2.1/006; [redacted], 1993, CA 7.1.2.2.1/005; [redacted], 1989a, CA 7.1.2.2.1/016). In an ecoregion crosswalk assessment of the four studies, the locations of nine field trials were found to be representative for European conditions ([redacted], 2020, CA 7.1.2.2.1/002). Thus, the results of the nine trials were re-evaluated according to the most recent guidance (FOCUS, 2006, 2014; EFSA, 2014). For evaluation of trigger endpoints, all of the nine trials were considered while the evaluation for modelling endpoints was only conducted for four trials where sufficient data for the time-step normalisation procedure was available. The kinetic evaluation was performed using the model fitting software CAKE 3.3.

1. Description of the terrestrial field dissipation studies

The four field soil dissipation studies included for kinetic evaluation were conducted at nine sites in USA and Canada. The locations of the nine field sites were found to be representative for European conditions as reported in an ecoregion crosswalk assessment ([redacted], 2020, CA 7.1.2.2.1/002). Different amounts of glyphosate, formulated as glyphosate-trimesium or the isopropylamine salt, were applied to bare soil. Soil samples from studies conducted with either formulation of glyphosate were analysed for glyphosate and its metabolite.

A summary of the trial locations and application data is given in the following table.

Table 8.1.1.3-125: Summary of trial locations and application data in field soil dissipation studies

Study	Location	Formulation	Crop	Date of Application	Duration of study (d)	Target rate (kg a.s./ha)	Actual rate (kg a.s./ha)
[redacted], CA 7.1.2.2.1/014	Ontario, Canada	Glyphosate-trimesium	Bare soil	30/09/1998	577	5.76	6.41
[redacted], 1993a, CA 7.1.2.2.1/006	Arizona, USA	Isopropylamine salt	Bare soil	16/04/1991	553	7.95[1]	8.08[1]
	California, USA	Isopropylamine salt	Bare soil	18/04/1991	550	7.95[1]	8.83[1]
	Iowa, USA	Isopropylamine salt	Bare soil	06/06/1991	458	7.95[1]	7.94[1]
	Minnesota, USA	Isopropylamine salt	Bare soil	08/07/1991	475	7.95[1]	8.05[1]
	New York, USA	Isopropylamine salt	Bare soil	01/05/1991	546	7.95[1]	7.84[1]
	Ohio, USA	Isopropylamine salt	Bare soil	22/05/1991	545	7.95[1]	8.14[1]
[redacted], 1993, CA 7.1.2.2.1/005	Ontario, Canada	Isopropylamine salt	Bare soil	29/05/1991	537	4.27[2]	4.18[2,3]
[redacted], 1989a, CA 7.1.2.2.1/016	California, USA	Glyphosate-trimesium	Bare soil	07/07/1987	366	4.48	n.a.

n.a. = not available
[1] lb a.e./acre
[2] kg a.e./ha
[3] Mean value; actual application rates for three replicate test plots are 4.21, 4.07 and 4.27 kg a.e./ha

The soil sampling procedure differed between the evaluated studies and a short description is given in the following.

In [redacted] (1992, CA 7.1.2.2.1/014), one trial site in Canada was included in kinetic evaluation. The treated plot was subdivided into four subplots. The zero, one and three day samples were collected up to a soil depth of 10 cm. Seven to eight soil cores per subplot were taken and bulked for analysis which resulted in a total of 30 cores per sampling time. For the subsequent time intervals, soil was sampled to a depth of 30 cm. These soil cores were sectioned into three horizons (0-10 cm, 10-20 cm, 20-30 cm) and soil from each horizon was then bulked in order to obtain a representative sample.

In [redacted] (1993a, CA 7.1.2.2.1/006), six trial sites in the USA were included in kinetic evaluation. For the treated plot at each site, six soil cores were randomly collected to a depth of 121.9 cm (48 inches) from each of the three subplots, sectioned into 15.2 cm (6 inches) depth increments (e.g., 0-15.2 cm, 15.2-30.5 cm, etc.), and composited to afford three representative samples per depth increment per sampling event.

In [redacted] (1993, CA 7.1.2.2.1/005), one trial site in Canada was included in kinetic evaluation. 10 soil cores to a depth of 45 cm were randomly collected from each of the three subplots, sectioned into 15 cm depth increments (e.g., 0-15 cm, 15-30 cm, and 30-45 cm), and composited to afford three representative samples per depth increment per sampling event.

In [redacted] (1989a, CA 7.1.2.2.1/016), one trial site in the USA was included in kinetic evaluation. The sampling procedure for the first month after treatment was as follows: the top 7.6 cm (0-3 inches) of soil were excavated into a sample bag. Five replicates per sampling date were taken with the excavation method. Following the excavation, five cores were also taken up to a soil depth of 121.9 cm (48 inches), sectioned into six increments. Starting with the 1 month sample, the sampling probe was used to collect the samples without excavation of the 0-7.6 cm sample.

2. Data pre-processing

The data from the legacy field trials required pre-processing in order to generate appropriate input datasets for the kinetic evaluation. The standard procedures recommended by FOCUS (2006, 2014) were applied.

The time-zero concentration for the metabolite was set to zero and the initial metabolite amount was added to the parent substance accounting for the molar weight difference between the compounds.

For the two studies by [redacted] (1992, CA 7.1.2.2.1/014) and [redacted] (1989a, CA 7.1.2.2.1/016), the LOQ and LOD were indistinguishable; only the 'limit of determination' is reported. Hence, the LOQ and LOD were both assigned the same value and the FOCUS guidance was then applied as follows. Values below LOD were replaced by half the LOD. If the concentrations of the applied substance in soil declined to values below LOD, the curve was cut off after the first value below LOD.

For the two studies by [redacted] (1993a, CA 7.1.2.2.1/006) and [redacted] (1993, CA 7.1.2.2.1/005), LOD as well as LOQ were reported. Thus, values between LOQ and LOD were set to the measured value. Values below LOD were replaced by half the LOD. If the concentrations of the applied substance in soil declined to values below LOD, the curve was cut off after the first value below LOD, unless detections above LOQ were made later in the experiment (FOCUS, 2006, 2014). These corrections were performed along the time course, as well as with depth along the soil horizon, with the exception for 0 DAT, where it was assumed that residues only resided in the upper most soil layer.

The measured residues (mg/kg) in the different soil layers were converted into residues expressed in kg/ha (considering the layer depth and bulk density) and then summed up. They were then expressed as percentage values of the residue at 0 DAT (so the time zero value is 100 %). Thus, if the maximum concentration occurs after 0 DAT, the respective maximum percentage value is greater than 100 %. As the sampled soil layer depths of studies [redacted] (1993a, CA 7.1.2.2.1/006) and [redacted] (1989a, CA 7.1.2.2.1/016) were given in inches, conversion to cm with the factor 2.54 was performed.

- For the study of [redacted] (1992, CA 7.1.2.2.1/014), the horizon-specific bulk density was calculated at each sampling time using the reported soil core surface area, depth and dry weight.
- For the studies of [redacted] (1993a, CA 7.1.2.2.1/006) and Oppenhuizen & Goure (1993, CA 7.1.2.2.1/005), horizon-specific bulk density was given in the reports.
- For the study of [redacted] (1989a, CA 7.1.2.2.1/016), a default value of 1.5 g/cm3 was assumed for the bulk density.

The input values of AMPA were expressed as percentage values of the parent (glyphosate) residue at 0 DAT (correcting for molar weight differences).

According to FOCUS (2006, 2014), true replicates (and not mean concentration values) at each sampling point should be used for the kinetic evaluation, if available. For the studies [redacted] (1992, CA 7.1.2.2.1/014), [redacted] (1993a, CA 7.1.2.2.1/006) and [redacted] (1993, CA 7.1.2.2.1/005), replicate treated subplots were sampled and analysed. However, either the respective replicate samples were mixed across the subplots resulting in one combined sample [redacted] 1993a, CA 7.1.2.2.1/006), or the replicate results could not be clearly assigned to the individual subplots as this information was not given in the raw data tables ([redacted], 1992, CA 7.1.2.2.1/014; [redacted] 1993, CA 7.1.2.2.1/005). Therefore, the kinetic evaluation was based on mean values.

In the study [redacted] (1989a, CA 7.1.2.2.1/016), four to five samples were taken from a single treated plot. For the soil layers below 7.6 cm (3 inch), samples were mixed to one combined sample. For the uppermost soil layer (0-7.6 cm), the individual samples were analysed separately; in addition, one of the samples was further divided in two subsamples and analysed in duplicate. For kinetic evaluation, the results of the individual samples were averaged to one mean concentration for the uppermost soil layer; the results of duplicate subsample analysis were averaged separately, and the mean value was used for calculating the overall mean concentration. Thus, the evaluation was performed on single residue data per soil layer.

Processed residue data, adjusted as described above, are presented with the kinetic evaluation.

3. Normalisation of field degradation half-life values to reference conditions

Time-step normalisation was conducted for four trials where sufficient data was available. The availability of the weather data for the respective trial sites are summarized in the following table.

Table 8.1.1.3-126: Glyphosate field trial locations and availability of weather data

Study	Trial/ location	Weather station and data availablity	Distance from test site (km)	Data sufficient for normalization?
[redacted], 1992, CA 7.1.2.2.1/014	St. Davids, Ontario, Canada	St. Catherines, Ontario: Daily weather data not available	approx. 5 km	No (no daily weather data available)
[redacted], 1993a, CA 7.1.2.2.1/006	Yuma County, Arizona, USA	Arizona Meteorological Network, Yuma Valley, AZ: rain, irrigation, min/max temp., rel. humidity, soil temp., windspeed, solar rad., ET_0	-[1]	Yes (based on simulated soil temperature and moisture)
	Madera County, California, USA	On-site weather station at Pan-Ag Research Station in Madera, CA: rain, irrigation, min/max temp., rel. humidity, soil temp., windspeed	-[1]	Yes (based on measured soil temperature)
	Des Moines County, Iowa, USA	Danville, Iowa: rain, irrigation, min/max temp., soil temp.	-[1]	Yes (based on measured soil temperature)
	Redwood County, Minnesota, USA	Southwest Experiment Station, University of Minnesota, Lamberton, MN: rain, min/max temp.	-[1]	No (no radiation and soil temperature data available)
	Ontario County, New York, USA	Vegetable Research Farm, New York State Agricultural Experiment Station: rain, min/max temp., rel. humidity, solar rad., windspeed (data gap between 1.11.1991-17.05.1992)	-[1]	Yes (based on simulated soil temperature and moisture)
	Fayette County, Ohio, USA	NOAA Washington Courthouse Station, Division 05, Fayette County: rain, min/max temp., rel. humidity, soil temp., windspeed	-[1]	No (no radiation data, soil temperature data insufficient)
[redacted], 1993, CA 7.1.2.2.1/005	Ayr, Ontario, Canada	Shades Mill Dam, Grand River Conservation Authority weather station: rain, min/max temp.	-[1]	No (no daily weather data available)
[redacted], 1989a CA 7.1.2.2.1/016	Orange Cove, California, USA	WSO Fresno, California: rain, irrigation, min/max temp., windspeed	approx. 40 km	No (available data has poor quality)

n.a. = not available
ET0 = evapotranspiration

For trials Arizona and New York ([redacted], 1993a, CA 7.1.2.2.1/006), detailed weather and soil data were available. Thus, for these two trials, comprehensive normalisation procedure with regard to soil temperature and soil moisture was conducted.

For trials California and Iowa ([redacted], 1993a, CA 7.1.2.2.1/006), the weather data set was incomplete, but soil temperature was reported. As a conservative approach, for these two trials, normalisation was performed for soil temperature, only. The resulting modelling endpoints are worst-case estimates as normalisation for soil moisture would result in lower DT_{50} due to the fact that moisture conversion factors are defined to be below or equal to 1.

General approach

Time-step normalisation according to FOCUS (2006, 2014) and Hardy et al. (2003) was conducted in order to derive modelling endpoints at reference conditions (20 °C and pF 2). Daily correction factors for soil temperature (fT) and moisture (fΘ) were calculated for a given reference soil temperature of 20 °C and a reference soil moisture of pF 2.

According to FOCUS (2000), the exponent of the moisture response function was set to 0.7 and the temperature coefficient Q10 was set to 2.58, respectively.

The following limitations were applied to the normalisation procedure:

- no further increase of the degradation rate if soil moisture > reference moisture
- no degradation if soil temperature < 0 °C (resulting in a transformed day length of zero)

The obtained correction factors result in standardised transformation rates by reducing or increasing day lengths. Processed residue data, in combination with the transformed time course (i.e. under constant temperature and moisture conditions), were used for the evaluation of modelling endpoints according to recommendations for obtaining $DegT_{50}$ matrix values in soil from field dissipation studies for modelling purposes (FOCUS, 2006, 2014; EFSA, 2014). For the time between application and first sampling (0 DAT), no normalisation was considered and application was assumed to occur at time point zero.

Estimation of soil temperature and moisture

Weather data

For trials Arizona and New York, daily values of soil temperature and moisture data (mean of top 10 cm) were simulated with the environmental fate model FOCUSPEARL 4.4.4. Site-specific weather and soil data were used as model input. In accordance with EFSA (2014), the weather stations from which precipitation data were derived were less than 20 km away from the actual trial site.

For trial Arizona, reference evapotranspiration data were available together with minimum and maximum air temperature as well as precipitation (irrigation). Therefore, the 'input' option was selected for the potential evapotranspiration. As measured soil temperature at a depth of 0-10 cm was additionally available for trial Arizona, the data was used in order to verify the simulation results.

For trial New York, data were missing for the parameter 'global radiation' between Dec 1st, 1991 and Dec 5th, 1991. The gap was filled with the average value (i.e. 3138 kJ/m2) of adjacent measurements (i.e. last day before gap: Nov 30th, 1991: 0 kJ/m2; first day after gap: Dec 6th, 1991: 6276 kJ/m2). Further, 'windspeed' data were missing between Nov 1st, 1991 and May 17th, 1992. Due to the large range of this gap it was decided not to use the windspeed data. Therefore, the Makkink approach (windspeed data not required) was selected in FOCUSPEARL v4.4.4 for trial New York to calculate the potential evapotranspiration. The required meteorological data for this estimation method (maximum and minimum air temperature, precipitation (irrigation), global radiation) were obtained from local meteorological stations reported in the study report as shown in the table above.

In the FOCUSPEARL 4.4.4 model, the weather data for the normalisation included a warm-up period of one year prior to the date of application, thereby accounting for seasonal effects.

Soil profile settings

For the simulations with FOCUSPEARL 4.4.4, soil profiles were created for the trials Arizona and New York based on the detailed soil properties given in the following tables.

The soil was parameterised with 26 compartments which differed in thickness. Six numerical compartments were applied to the top soil (0 – 15 cm; converted from inch to cm) with a layer thickness of 2.5 cm each. Five numerical compartments were applied to the following 15 – 30 cm (converted from inch to cm) with a layer thickness of 3 cm each. The subsequent soil depth (30 – 105 cm; converted from inch to cm) was parameterised with 15 compartments with a layer thickness of 5 cm each. The lower boundary condition of the simulation profiles was set to 'Free Drainage' by default representing common European conditions. The initial groundwater level was set to 300 cm below the ground level. For soil evaporation, the crop factor ('FacEvpSol') and reduction coefficient ('CofRedEvp') were set to the values of 1 (default for bare soils) and 0.79, respectively.

The hydraulic characteristics of the soils were parameterised in FOCUSPEARL according to the 'van Genuchten' parameters (van Genuchten, 1980). The van Genuchten parameters were estimated based on continuous 'ROSETTA' pedotransfer functions (Schaap et al., 2001).

Table 8.1.1.3-127: Soil characterisation for site Arizona, USA ([redacted], 1993a, CA 7.1.2.2.1/006)

Soil layer (cm)[1]	0 - 15	15 - 30	30 - 45	45 - 60	60 - 75	75 - 90	90 - 105
Soil texture (USDA)	clay loam	clay loam	clay loam	loam	sandy loam	sandy loam	loamy sand
Sand (%)	37.3	27.3	25.3	41.3	53.3	69.3	83.3
Silt (%)	29.2	39.2	38.0	32.0	38.0	24.0	12.0
Clay (%)	33.5	33.5	36.7	26.7	8.7	6.7	4.7
Organic matter (%)	0.9	1.0	0.7	0.4	1.0	0.1	1.3
pH[2]	8.0	8.2	8.2	8.4	8.3	8.3	8.4
Bulk density (g/cm^3)[3]	1.14	1.14	1.11	1.15	1.28	1.28	1.19
Soil hydraulic parameters[4]							
θ_{res} (m^3/m^3)	0.0896	0.0909	0.0953	0.0795	0.0411	0.0398	0.0441
θ_{sat} (m^3/m^3)	0.5114	0.5116	0.5298	0.4891	0.4011	0.4265	0.4720
K_{sat} (m/d)	0.4089	0.4155	0.5024	0.3821	0.6332	1.2077	3.1132
α (cm^{-1})	0.0137	0.0109	0.0125	0.0113	0.0137	0.0313	0.0436
λ (-)	-0.5144	-0.3022	-0.4626	-0.2338	-0.2693	-0.8081	-0.7461
n (-)	1.4325	1.4692	1.4349	1.4852	1.4876	1.4670	1.6893
θ_{ref} (pF 2) (m^3/m^3)[5]	0.4067	0.4210	0.4295	0.3964	0.3045	0.2547	0.1942

[1] Converted from inch; in order to harmonize input in PEARL, 6 inch was assumed to equal 15 cm for each soil layer. Conversion differences were regarded as negligible.
[2] Buffer medium unknown
[3] Measured values derived from study report
[4] Calculated based on continuous ROSETTA pedotransfer functions (Schaap *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 8.1.1.3-128: Soil characterisation for site New York, USA ([redacted], 1993a, CA 7.1.2.2.1/006)

Soil layer (cm)[1]	0 - 15	15 - 30	30 - 45	45 - 60	60 - 75	75 - 90	90 - 105
Soil texture (USDA)	sand clay loam	clay loam	clay loam	clay	clay loam	loam	clay loam
Sand (%)	53.3	25.3	21.3	25.3	29.3	33.3	33.3
Silt (%)	24.0	42.0	46.0	32.0	38.0	40.0	39.2
Clay (%)	22.7	32.7	32.7	42.7	32.7	26.7	27.5
Organic matter (%)	2.1	0.8	0.1	0.3	0.2	0.1	0.2
pH[2]	5.8	6.4	7.3	7.3	7.5	7.6	7.8
Bulk density (g/cm^3)[3]	1.14	1.09	1.17	1.12	1.15	1.15	1.24
Soil hydraulic parameters[4]							
θ_{res} (m^3/m^3)	0.0719	0.0916	0.0903	0.1003	0.0894	0.0807	0.0797
θ_{sat} (m^3/m^3)	0.4899	0.5249	0.5038	0.5401	0.5059	0.4862	0.4649
K_{sat} (m/d)	0.6273	0.5555	0.3556	0.5096	0.3900	0.4013	0.2401
α (cm^{-1})	0.0151	0.0103	0.0094	0.0161	0.0109	0.0088	0.0092
λ (-)	-0.4003	-0.2157	-0.1775	-0.8453	-0.2940	-0.0434	-0.1549
n (-)	1.4475	1.4809	1.4976	1.3758	1.4729	1.5318	1.5202
θ_{ref} (pF 2) (m^3/m^3)[5]	0.3753	0.4353	0.4239	0.4283	0.4160	0.4103	0.3898

[1] Converted from inch; in order to harmonize input in PEARL, 6 inch was assumed to equal 15 cm for each soil layer. Conversion differences were regarded as negligible.
[2] Buffer medium unknown

[3] Measured values derived from study report
[4] Calculated based on continuous ROSETTA pedotransfer functions (Schaap *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Correction factors for soil temperature and moisture

For trials Arizona and New York, daily correction factors for soil temperature and soil moisture were calculated based on the results of the simulations in FOCUSPEARL 4.4.4 (mean of top 10 cm).

For trials California and Iowa, reported soil temperature data were used directly for calculation of daily correction factors for soil temperature.

For trial California, soil temperature data were missing between April 18th, 1991 and May 31st, 1991 due to a malfunction of the machine. The gap was filled with the reference soil temperature of 20 °C, resulting in a correction factor of 1 (i.e. no normalisation). This is regarded as a conservative approach as daily mean soil temperatures for this time period are usually below 20 °C. Comprehensive soil temperature data was available in the study report starting before the application date for trial California. Thus, average soil temperatures were calculated from available data before and after the gap. This resulted in calculated average soil temperatures of 13.8 °C for the time period April 1st to April 18th and average soil temperatures of 19.8 °C for the following month of the gap. The United States Department of Agriculture (USDA) which published soil series descriptions and classifications from across the United States found that the mean annual soil temperature in the trial area of the California trial ranges from 15.5 to 18.3 °C (60 to 65 degrees F). This finding can be regarded as a further confirmation of the appropriateness of the selected temperature for the missing time period. Another small gap in soil temperature data was detected on July 16th, 1992 which was filled with the average value (i.e. 27.5 °C) of adjacent measurements (i.e. July 15th, 1992: 26.7 °C and July 17th, 1992: 28.3 °C).

For trial Iowa, soil temperature data were missing between August 1st, 1992 and August 9th, 1992. The gap was filled with the average value (i.e. 22.5 °C) of adjacent measurements (i.e. July 31st, 1992: 20 °C and August 10th, 1992: 25 °C).

4. 10 mm criterion for $DegT_{50}$ matrix evaluations

According to EFSA (2014), for evaluation of the $DegT_{50}$ matrix, surface processes like photolysis and volatilisation should be excluded. Therefore, it is recommended for the kinetic evaluation to use data points following at least 10 mm of cumulative precipitation (for SFO kinetics). For this purpose, the first sampling time after 10 mm of cumulative precipitation was defined as day 0, and all later time points were adjusted accordingly. The resulting normalised field sampling times, as well as eliminated sampling intervals (EFSA, 2014) are presented with the kinetic assessment.

Table 8.1.1.3-129: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from study [redacted], 1993a, CA 7.1.2.2.1/006

Arizona			California		
DAT (d)	t_{norm} (d)	t_{norm} (d) (>10 mm rainfall)	DAT (d)	t_{norm} (d)	t_{norm} (d) (>10 mm rainfall)
0	0.0	-	0	0.0	-
1	0.7	-	1	1.0	-
7	5.6	0.0	7	7.0	0.0
14	12.5	6.9	14	14.0	7.0
21	20.0	14.4	21	21.0	14.0
28	27.9	22.3	29	29.0	22.0
64	85.4	79.8	61	61.8	54.8
92	149.5	143.9	91	101.1	94.1
122	225.3	219.7	123	146.6	139.6
184	373.5	367.9	183	209.1	202.1
364	486.8	481.2	365	267.6	260.6
462	666.4	660.8	456	391.6	384.6
553	882.2	876.6	550	514.5	507.5
Iowa			**New York**		
DAT (d)	t_{norm} (d)	t_{norm} (d)	DAT (d)	t_{norm} (d)	t_{norm} (d)

		(>10 mm rainfall)			(>10 mm rainfall)
0	0.0	-	0	0.0	-
1	0.9	-	1	0.6	-
7	7.7	0.0	7	2.7	0.0
14	16.3	8.5	14	6.9	4.3
21	24.8	17.1	21	11.6	8.9
29	43.2	35.4	30	21.3	18.6
62	89.2	81.4	61	48.1	45.5
92	126.9	119.2	90	79.1	76.4
123	155.7	148.0	120	107.7	105.0
190	183.0	175.3	180	141.7	139.0
366	243.4	235.6	362	162.3	159.6
458	339.6	331.8	453	222.0	219.3
			546	280.6	277.9

Normalisation of day lengths was applied to the four trials Arizona, California, Iowa and New York only. Normalised day lengths were determined using the correction factors for soil temperature (applicable for all four trials) and/ or moisture (applicable for trials Arizona and New York only) as described above. The number of remaining data points after 10 mm of rainfall per respective trial location are presented in the following table.

Table 8.1.1.3-130: 10 mm rainfall criterion at field trial locations

Study	Trial/ location	Total samples[1]	10 mm rainfall reached at	No. of samples after 10 mm rainfall
[redacted] (1993a, CA 7.1.2.2.1/006)	Arizona, USA	13	7 DAT	11
	California, USA	13	5 DAT	11
	Iowa, USA	12	4 DAT	10
	New York, USA	13	2 DAT	11

[1] Number of samples after FOCUS correction of residue data

5. Kinetic assessment

Kinetic models

Three kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first order (SFO), first order multi-compartment (FOMC = Gustafson and Holden model) and double first order in parallel (DFOP) (FOCUS; 2006, 2014).

Optimisation

The kinetic analyses were conducted using the software package CAKE 3.3. The data were initially fitted with the complete dataset and unconstrained initial concentration (M0) for the parent substance. Iteratively Reweighted Least Squares (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M0) and degradation model parameters k, α, β or g depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually based on the observed degradation pattern and preliminary model runs.

In pathway fits for derivation of trigger endpoints, the initial amount of metabolite was fixed to 0 % by default which is in contrast to the pathway fitting for derivation of modelling endpoints. Here, the initial amount of metabolite was not constrained to zero as several data points from the beginning of the experimental period prior to 10 mm rainfall were cut off. Decline fits of the metabolite were treated similarly to parent as described above. In pathway fits for derivation of modelling endpoints, the initial amount of metabolite was not constrained. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1 × 10-5 and 100, respectively.

If a kinetic fit did not yield visually and/ or statistically reliable results, the kinetic model was further optimised by fixing one or more of the model parameters to either the measured value (e.g. M0) or to estimated values derived from a reliable parent-only fit (e.g. k). A stepwise fixing procedure has been applied in these cases, which is further described in the results chapter for the respective pathway fits.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually based on concentration/residual - time plots. Generally the residuals should be distributed randomly around the zero line. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line.
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered.
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered.

A statistical measure of the quality of a fit is given by the $\chi 2$-test. The $\chi 2$-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. In general, for parent compounds, it is recommended that if the $\chi 2$ error is <15 % then the model has adequately reflected the measured data (FOCUS, 2006, 2014). However, this value should only be considered as a guide and not an absolute cut-off criterion. The guidance can be relaxed for field studies where the residue data can show appreciable scatter. The same also applies for metabolites where the curve fitting is more complex.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised degradation rate constants (k) of the SFO and DFOP kinetic models were significantly different from zero at a chosen significance level of 5 %. For the FOMC kinetic model, only the confidence interval of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test and confidence interval were used as supporting information for the decision making process. The CAKE software also reports a 95 % confidence interval on the estimated parameters. It should be relatively tight and not contain 0 to be considered statistically robust.

Derivation of trigger and modelling endpoints

For derivation of trigger endpoints, the non-normalised dataset was considered and the kinetic evaluation was conducted with CAKE 3.3 according to FOCUS guidance (2006, 2014); the corresponding trigger DT_{50} and DT_{90} values are reported.

For the parent compound, the best-fit model was accepted for deriving trigger endpoints. For the metabolite, pathway fits were conducted using the best-fit kinetic model for the parent and SFO for the metabolite. In cases where no reliable pathway fit could be established, kinetic endpoints for the parent were derived from the corresponding parent-only fit, and decline fits were conducted for the metabolite (if possible), starting from the maximum observed concentration. The respective day was defined as 0 days after maximum concentration, and later time points were adjusted accordingly.

For derivation of modelling endpoints, the corrected residue data were combined with the normalised day length data that were obtained as described above. The resulting datasets were then evaluated according to FOCUS (2006, 2014). The DT_{50} calculated from SFO model was preferably selected as modelling endpoints.

II. RESULTS AND DISCUSSION

Ontario ([redacted] 1993) – only trigger endpoints derived

Table 8.1.1.3-131: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1993, CA 7.1.2.2.1/005)

Time (DAT)	Sum of horizons (0 - 45 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Ontario				
0	1.69[2]	0.00[3]	100.00	0.00
1	1.08	0.24	63.73	21.64
7	1.00	0.26	59.01	23.25
14	0.82	0.28	48.39	25.23
28	0.31	0.22	18.42	19.48
57	0.12	0.20	7.21	18.04
86	0.10	0.32	6.15	28.64
129	0.06	0.21	3.56	18.94
177	0.07	0.29	3.91	26.31
364	0.07	0.24	4.15	21.82
537	0.01	0.03	0.59	2.69

LOD = 0.01 mg/kg for glyphosate, 0.03 mg/kg for AMPA
LOQ = 0.05 mg/kg for glyphosate and AMPA
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0

Table 8.1.1.3-132: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Initial fitting									
SFO	Poor	84.6	k: 0.0479	21.4	k: <0.05	-	-	14.5	48.0
FOMC	Poor	86.4	α: 2.3540 β: 37.69	21.7	-[1]	β: -87.35	β: 162.7	12.9	62.5
DFOP	Good	85.4	k1: 0.0551 k2: 0.0017 g: 0.9420	22.3	k1<0.05 k2: 0.427	-	-	13.7	54.4

Applicant's conclusion

None of the applied kinetic models accurately describe the residue data of glyphosate in an initial fitting as M0 was clearly underestimated. Thus, the fitting was repeated with M0 fixed to the measured initial residue value (100 %).

RMS conclusion

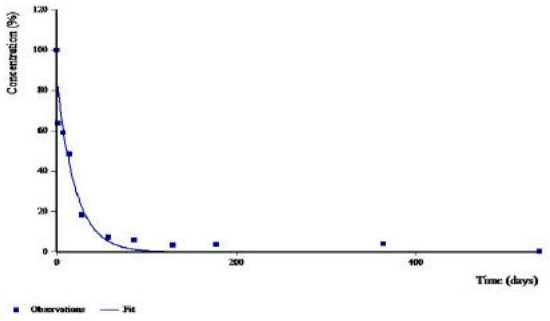
Agrees with the applicant that M0 is underestimated. However as indicated below, the fits with M0 fixed are not considered better than the fits with free M0.

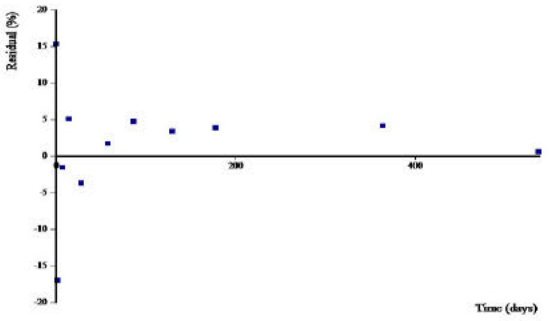
DFOP is the visual best fit for this soil but its k_2 is statistically unreliable, which is most likely due to the very low degradation at the end of the study. The g parameter is very close to 1 which indicates that most of the degradation will be described by k_1.

RMS considers that DFOP kinetics with free M0 parameter should be considered for persistence endpoint.

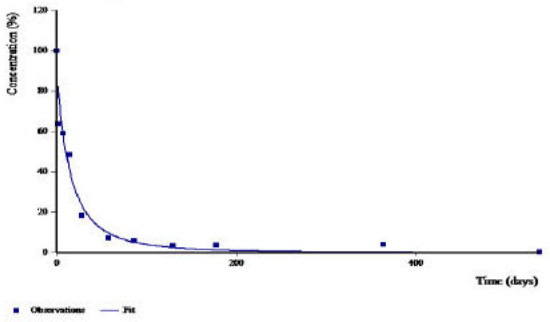
Table 8.1.1.3-132: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

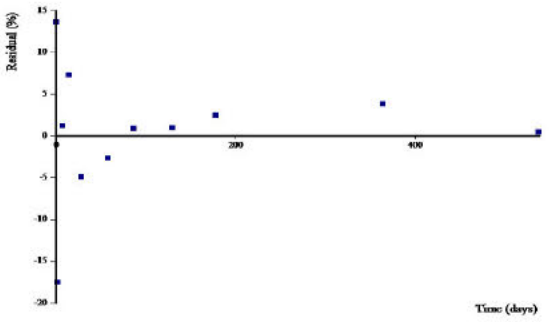
Initial fitting: SFO



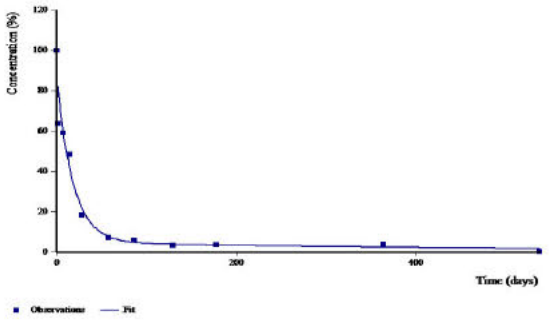


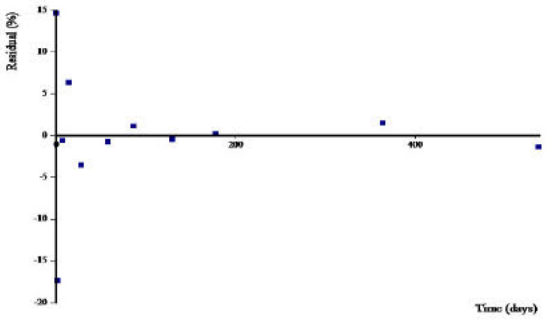
Initial fitting: FOMC





Initial fitting: DFOP





Repeated fitting: parent M0 fixed to measured initial concentration

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	fixed to 100.0	k: 0.0625	37.3	k: <0.001	-	-	11.1	36.9
FOMC	Accep-table	fixed to 100.0	α: 1.044 β: 8.7520	34.7	-	β: -10.03	β: 27.53	8.3	70.6
DFOP	Accep-table	fixed to 100.0	k1: 17.2 k2: 0.0363 g: 0.2939	16.4	k1: 0.486 k2: <0.001	-	-	9.5	53.9

Applicant's conclusion

The visual fit improved for all models. The DFOP model provides the best visual fit and the lowest $\chi 2$ error. Thus, DFOP is selected as the best-fit model for parent-only fit.

Conclusion: DFOP with fixed M0 (100 %) to be used in pathway fit for trigger endpoints.

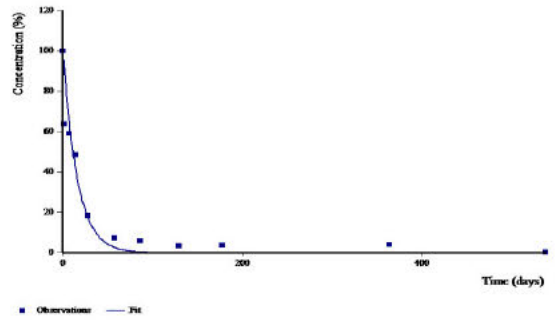
RMS conclusion

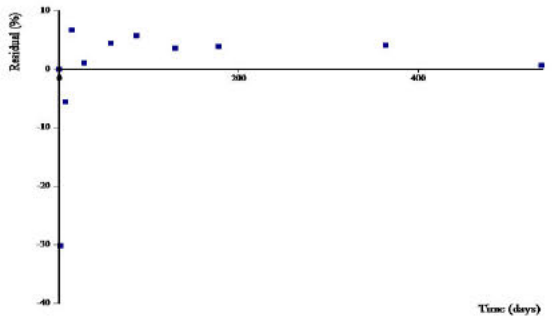
For SFO and FOMC with M0 fixed, RMS does not agree that the overall degradation of glyphosate is better described than with M0 free. $\chi 2$ error (37.3 and 34.7% for SFO and FOMC) is higher than for M0 free (21.4 and 21.7%).

Table 8.1.1.3-132: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

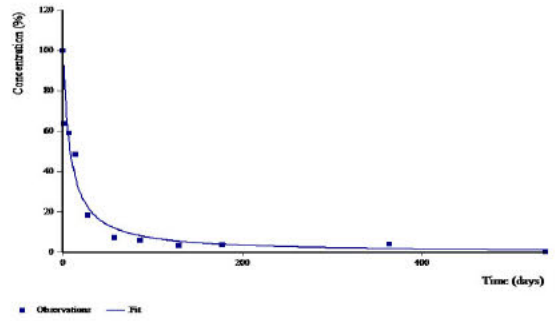
For DFOP, χ2 error is slightly better with fixed M0 (16.4%) compared to M0 free (22.3%). However, the 5 last data points are underestimated.
RMS considers that the fit with M0 free should be relied on.

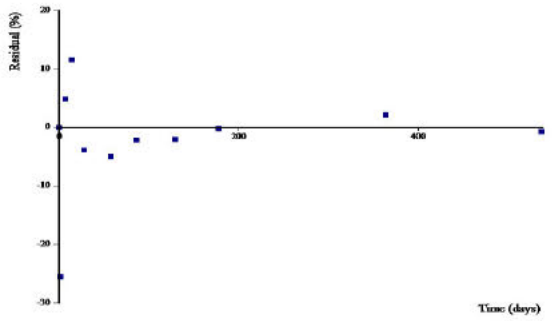
Repeated fitting: SFO



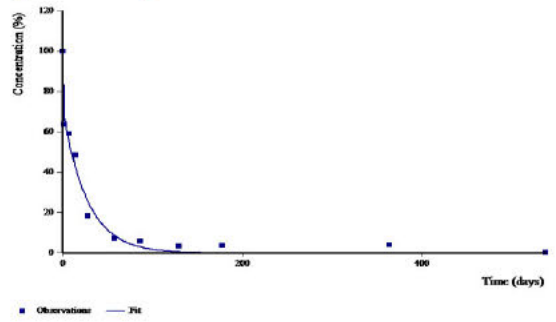


Repeated fitting: FOMC





Repeated fitting: DFOP



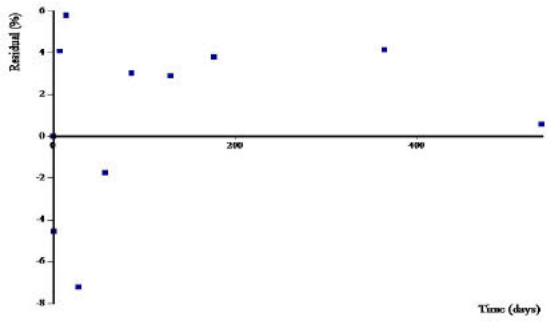
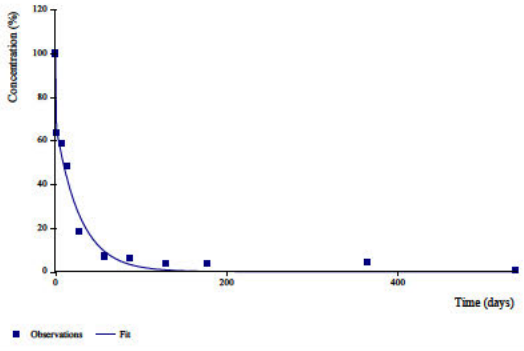
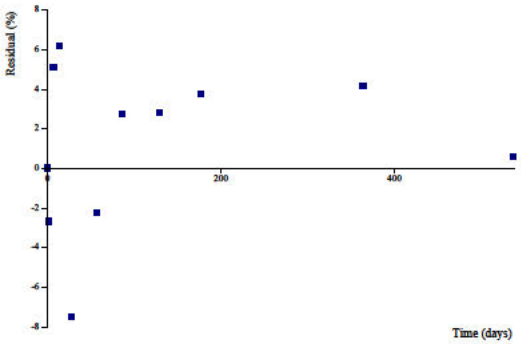
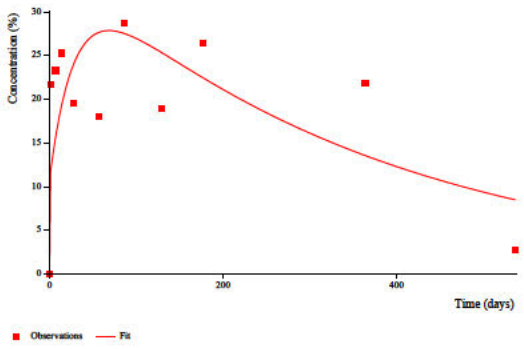
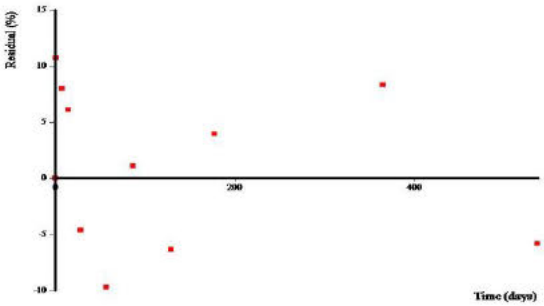


Table 8.1.1.3-133: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 100.0	fixed to k1: 17.2 k2: 0.0363 g: 0.2939	14.4	-	-	-	9.5	53.9	-
AMPA: SFO	Poor	-	k: 0.0027	27.3	k: 0.020	k: 0.0001	k: 0.005	256	850	0.342 (±0.052)

Applicant's conclusion
In an initial fitting, an internal error in CAKE 3.3 led to a mismatch of the plots of metabolite fit and the corresponding residuals (data not shown). Thus, the fitting was repeated with the initial parameters for the

Table 8.1.1.3-133: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
parent fixed to results from parent-only fit. The resulting visual fit for AMPA is poor due to the large scattering of the residue data.										
Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate No trigger endpoints can be derived for AMPA										
RMS conclusion As mentioned above, RMS does not agree with the selection of DFOP with fixed M0 for parent. However, considering that data for AMPA are really scattered, no further fitting is deemed necessary. **RMS agrees with the applicant, no trigger endpoint can be derived for AMPA**										
Glyphosate: DFOP										
AMPA: SFO										

Normalization not performed.

California ([redacted] 1993a) – Trigger & modelling endpoints derived

Table 8.1.1.3-134: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, California soil)

Time (DAT)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Sum of horizons (0 - 60 cm)			
			Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0.0	-	4.29[2]	0.00[3]	100.00	0.00
1	1.0	-	4.45	0.36	103.76	12.65
7	7.0	0.0	2.85	0.73	66.41	26.07
14	14.0	7.0	2.45	0.85	57.06	30.02
21	21.0	14.0	1.29	0.73	30.10	26.07
29	29.0	22.0	1.49	0.82	34.76	29.23
61	61.8	54.8	1.16	0.80	26.99	28.44
91	101.1	94.1	0.40	0.60	9.35	21.33
123	146.6	139.6	0.13	0.58	3.12	20.58

Time (DAT)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Sum of horizons (0 - 60 cm)			
			Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
183	209.1	202.1	0.20	0.51	4.67	18.18
365	267.6	260.6	0.11	0.67	2.60	23.70
456	391.6	384.6	0.11	0.91	2.60	32.42
550	514.5	507.5	0.07	0.82	1.56	29.26

LOD = 0.02 mg/kg for glyphosate, 0.04 mg/kg for AMPA
LOQ = 0.05 mg/kg for glyphosate and AMPA
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0)

Table 8.1.1.3-135: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	99.3	k: 0.0400	18.1	k: <0.001	-	-	17.3	57.5
FOMC	Good	104.4	α: 1.196 β: 17.25	12.7	-[1]	β: -2.377	β: 36.88	13.5	101
DFOP	Accep-table	104.7	k1: 0.1124 k2: 0.0148 g: 0.5490	12.7	k1: 0.045 k2: 0.023	-	-	13.0	102

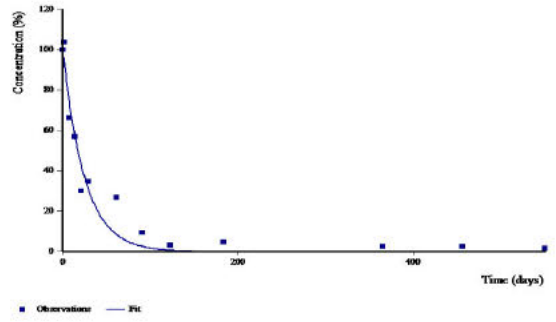
Applicant's conclusion
The SFO model provides an acceptable visual and statistically reliable fit. The bi-phasic models further improve the visual fit. The FOMC model provides the best visual fit during the whole study period. Thus, FOMC is selected as the best-fit model for parent-only fit.
Conclusion: FOMC to be used in pathway fit for trigger endpoints.

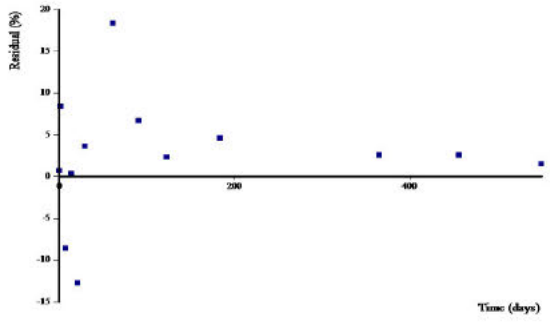
RMS conclusion

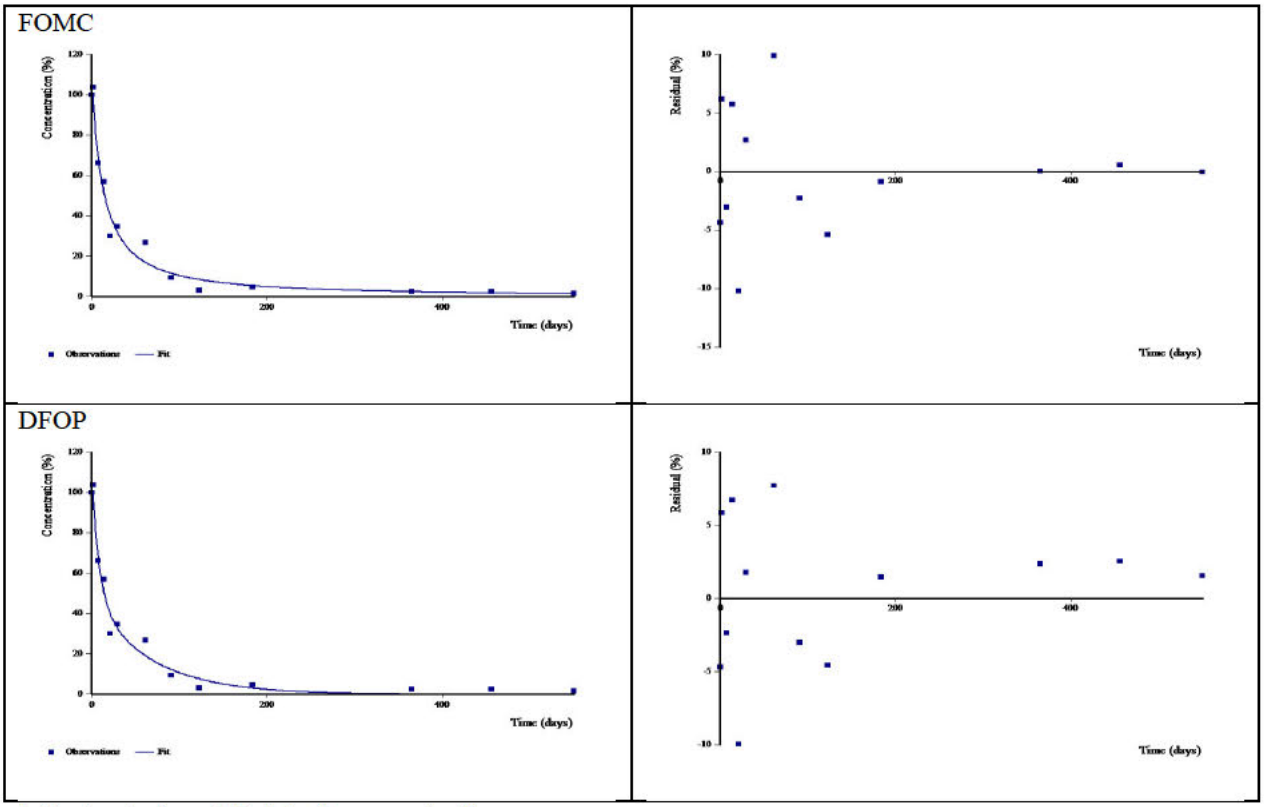
Considering the visual and kinetic fittings, RMS is of the opinion that while FOMC and DFOP both provide good fits, DFOP should be considered as the best fit considering the fact that as there are less degrees of freedom for the DFOP fit, the same Chi-square value for both models means that DFOP fit the data better. Additionally, all parameters are statistically reliable for DFOP kinetics (contrary to FOMC kinetics for this soil).

DFOP to be used in pathway fit for trigger endpoints.

SFO







1 t-test not relevant for kinetic parameter β

Table 8.1.1.3-136: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	106.2	α: 1.029 β: 12.74	13.0	-	β: -0.0823	β: 25.57	12.3	107	-
AMPA: SFO	Poor	-	k: 0.0006	26.8	k: 0.193	-	-	>1000	>1000	0.323 (±0.055)

Applicant's conclusion

The dissipation of glyphosate is well described by the pathway fit. The formation and decline of AMPA is not acceptably described as the residue data of the metabolite are greatly scattered.

Conclusion: Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate

No trigger endpoints can be derived for AMPA

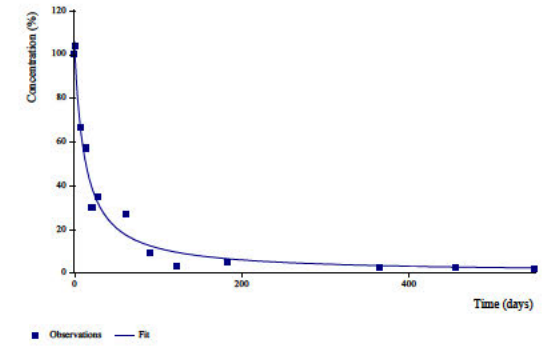
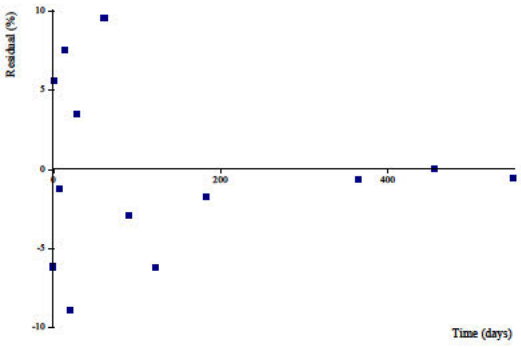
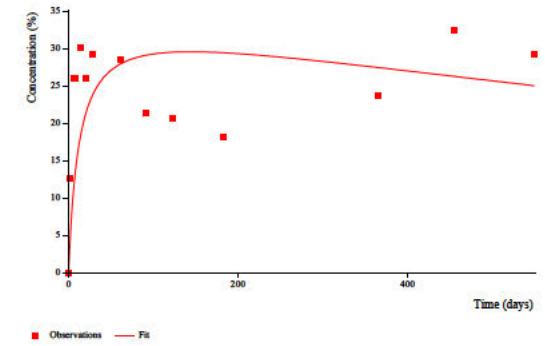
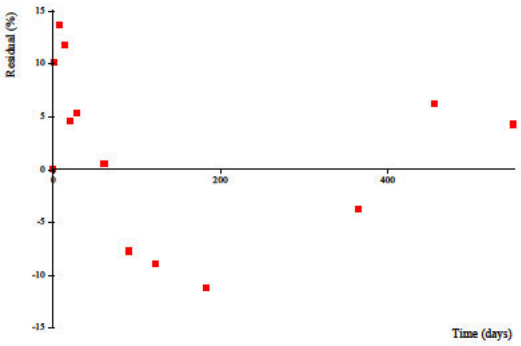
RMS conclusion

RMS considered that DFOP should be used for pathway fit for glyphosate. However, considering the scatter of the data for AMPA, RMS agrees with the applicant that no reliable fit can be derived for AMPA.

A fit with DFOP-SFO is not deemed necessary in this case.

Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate

Table 8.1.1.3-136: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: FOMC										
AMPA: SFO										

Modelling endpoint

Table 8.1.1.3-137: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5% level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	60.5	k: 0.0213	22.0	k: <0.05	-	-	32.6	108

Applicant's conclusion

The SFO model provides a visually acceptable and statistically reliable fit to describe the degradation of glyphosate.

Conclusion: SFO fit to be used for deriving modelling endpoints for glyphosate.

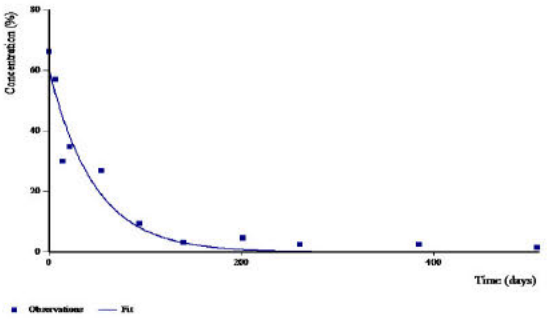
RMS conclusion

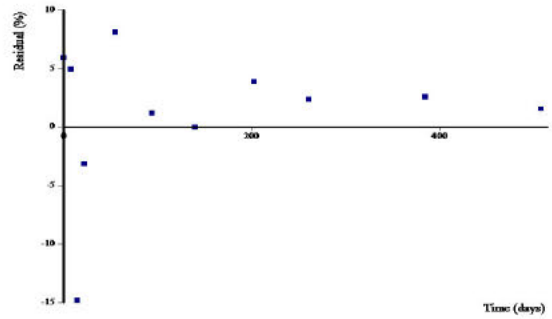
RMS agrees with the applicant, SFO fit to be used for deriving modelling endpoints for glyphosate.

It is observed that the last data points are underestimated with SFO kinetics. However glyphosate has already reached 90% degradation at this time, no significant impact is foreseen in this case and further following of the EFSA $DegT_{50}$ Guidance flowchart for legacy studies solely for the underestimation of the two timepoints > DT_{90} is not required

Table 8.1.1.3-137: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

SFO





As no formation or decline phase of AMPA was observed, it was neither possible to perform a pathway fit with the combined residue data of glyphosate and AMPA nor to perform a metabolite decline fit for trial California. Thus, no modelling endpoints were derived for AMPA.

RMS conclusion

Applicant's argumentation for not fitting modelling endpoint for AMPA is not sufficient as the lack of a decline phase should not – as an only justification – prevent the residues to be fitted.

However, as indicated by the EFSA $DegT_{50}$ guidance (2014), no kinetic fittings should be performed for a metabolite formed at more than 5% on a molar basis before 10mm of rainfall has occurred. No kinetic fittings should therefore be performed for AMPA in this case.

New York ([redacted] 1993a) – Trigger & modelling endpoints derived

Table 8.1.1.3-138: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, New York soil)

Time (DAT)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Sum of horizons (0 - 60 cm)			
			Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0.0	-	5.10[2]	0.00[3]	100.00	0.00
1	0.6	-	4.43	0.19	86.82	5.66
7	2.7	0.0	3.20	0.29	62.64	8.77
14	6.9	4.3	8.01	0.88	156.96	26.33
21	11.6	8.9	7.87	0.76	154.24	22.77
30	21.3	18.6	4.77	0.88	93.52	26.33
61	48.1	45.5	2.88	0.54	56.49	16.06
90	79.1	76.4	3.61	1.01	70.74	30.02
120	107.7	105.0	2.10	0.68	41.17	20.16
180	141.7	139.0	2.41	0.85	47.29	25.34
362	162.3	159.6	0.95	0.38	18.70	11.36
453	222.0	219.3	0.97	0.67	19.00	20.04
546	280.6	277.9	1.09	0.74	21.44	22.21

LOD = 0.02 mg/kg for glyphosate, 0.04 mg/kg for AMPA
LOQ = 0.05 mg/kg for glyphosate and AMPA
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0

Table 8.1.1.3-139: Kinetic models and goodness-of-fit statistics of parent-only fits for trial New York of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	109.5	k: 0.0049	31.2	k: 0.018	-	-	142	471
FOMC	Poor	111.1	α: 3.0020 β: 511.2	32.1	-[1]	β: -4768	β: 5790	133	590
DFOP	Poor	111.8	k1: 0.0068 k2: 0.0000 g: 0.8729	33.2	k1: 0.369 k2: 0.500	-	-	125	>1000

Applicant's conclusion

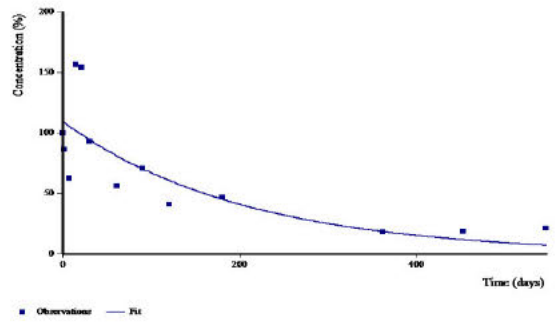
None of the applied kinetic models adequately describe the residue data of glyphosate. The visual fits are poor due to the large scattering of the residue data, and the resulting residuals are large.

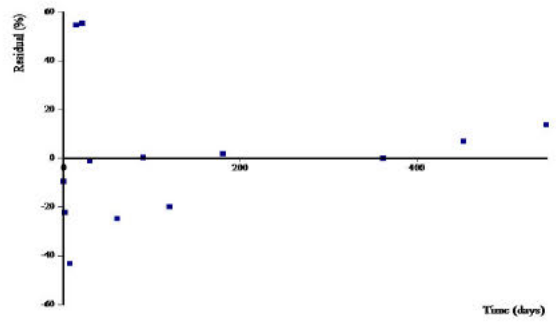
Conclusion: No trigger endpoints can be derived for glyphosate

RMS conclusion

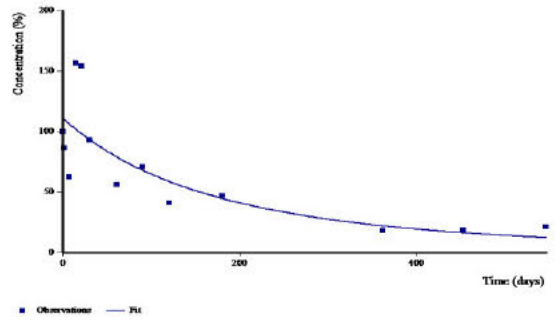
RMS agrees with the applicant, no trigger endpoint can be derived for glyphosate.

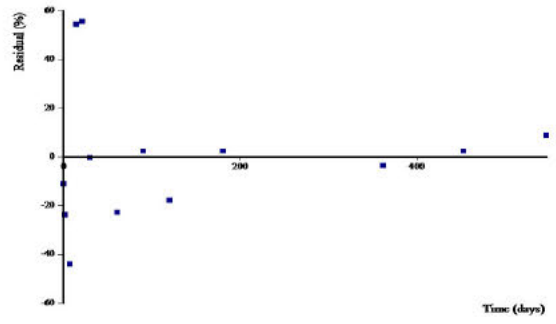
SFO



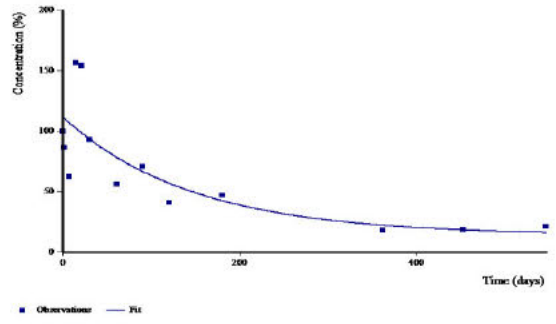


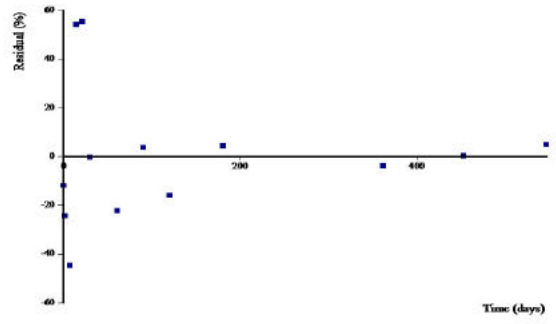
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

As for glyphosate, none of the tested models provided an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA trial New York. As no clear decline phase was visible for AMPA, a metabolite decline fit was not performed and no trigger endpoints were derived for AMPA.

Modelling endpoints

Table 8.1.1.3-140: Kinetic models and goodness-of-fit statistics of parent-only fits for trial New York of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5% level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	120.6	k: 0.0087	32.3	k: <0.05	-[1]	-[1]	79.5	264

Applicant's conclusion

Due to the large scattering of the residue data, the clearly overestimated M0 value and the resulting high $\chi 2$ error, the SFO fit is not acceptable.

Conclusion: No modelling endpoints can be derived for glyphosate.

RMS conclusion

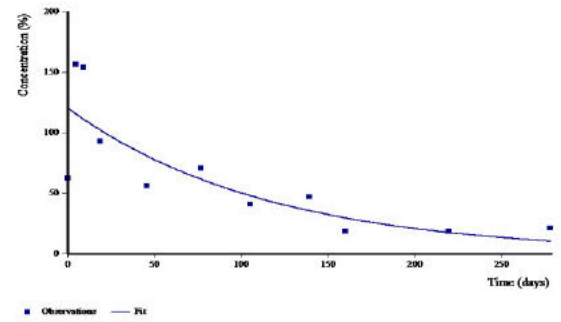
The applicant considers that the SFO fit is not acceptable due to the large scattering of the residue data, the clearly overestimated M0 value and the resulting high $\chi 2$ error level.

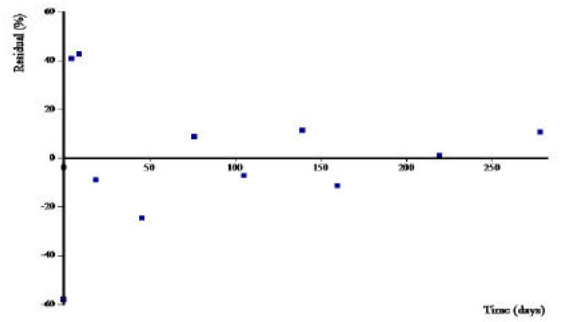
RMS notes that field dissipation studies inherently show more data scatter. In addition, the M0 point is artificially created by discarding the data points before 10 mm of rain occurred therefore considering that the fits result in the overestimation of an artificial M0 might not be relevant.

It is agreed that the Chi-square value of 32.3% is rather high with this kinetic fittings, but the flowchart for deriving $DegT_{50}$ endpoints should be further followed.

RMS identifies a data gap for the applicant to provide further kinetic fittings for glyphosate.

SFO





Ohio ([redacted] 1993a) – Only trigger endpoints derived

Table 8.1.1.3-141: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, Ohio)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	4.24[2]	0.00[3]	100.00	0.00
1	3.08	0.62	72.78	22.24
7	1.12	0.97	26.49	34.89
14	0.90	0.82	21.34	29.44
21	1.22	1.04	28.85	37.30
30	1.04	0.95	24.50	34.29
61	0.22	0.65	5.13	23.45
90	0.15	0.47	3.55	16.82
121	0.12	0.43	2.75	15.62
177	0.07	0.38	1.57	13.81

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
365	0.05	0.23	1.17	8.39
455	0.05	0.18	1.17	6.58
545	0.02	0.15	0.40	5.38

LOD = 0.02 mg/kg for glyphosate, 0.04 mg/kg for AMPA
LOQ = 0.05 mg/kg for glyphosate and AMPA
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0

Table 8.1.1.3-142: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ohio of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

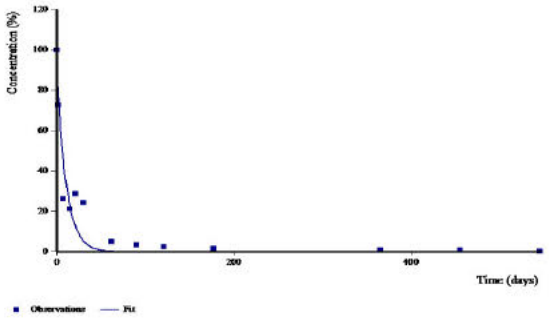
Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	86.9	k: 0.0918	35.9	k: <0.001	-	-	7.6	25.1
FOMC	Accep-table	100.0	α: 0.6327 β: 1.4950	19.1	-	β: -0.3040	β: 3.293	3.0	55.4
DFOP	Good	100.6	k1: 0.5430 k2: 0.0194 g: 0.6704	13.3	k1: 0.001 k2: 0.002	-	-	2.4	61.5

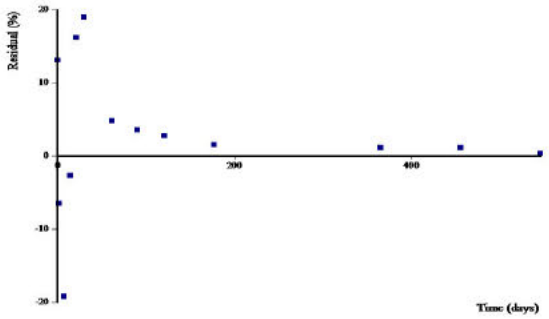
Applicant's conclusion
Dissipation of glyphosate was best described by bi-phasic models. SFO model does not properly estimate the dissipation. The DFOP model provides the best visual fit and the lowest $\chi 2$ error. Thus, DFOP is selected as the best-fit model for parent-only fit.
Conclusion: DFOP to be used in pathway fits for trigger endpoints.

RMS conclusion

RMS agrees with the applicant, DFOP to be used in pathway fits for trigger endpoints

SFO





FOMC

Table 8.1.1.3-142: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ohio of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

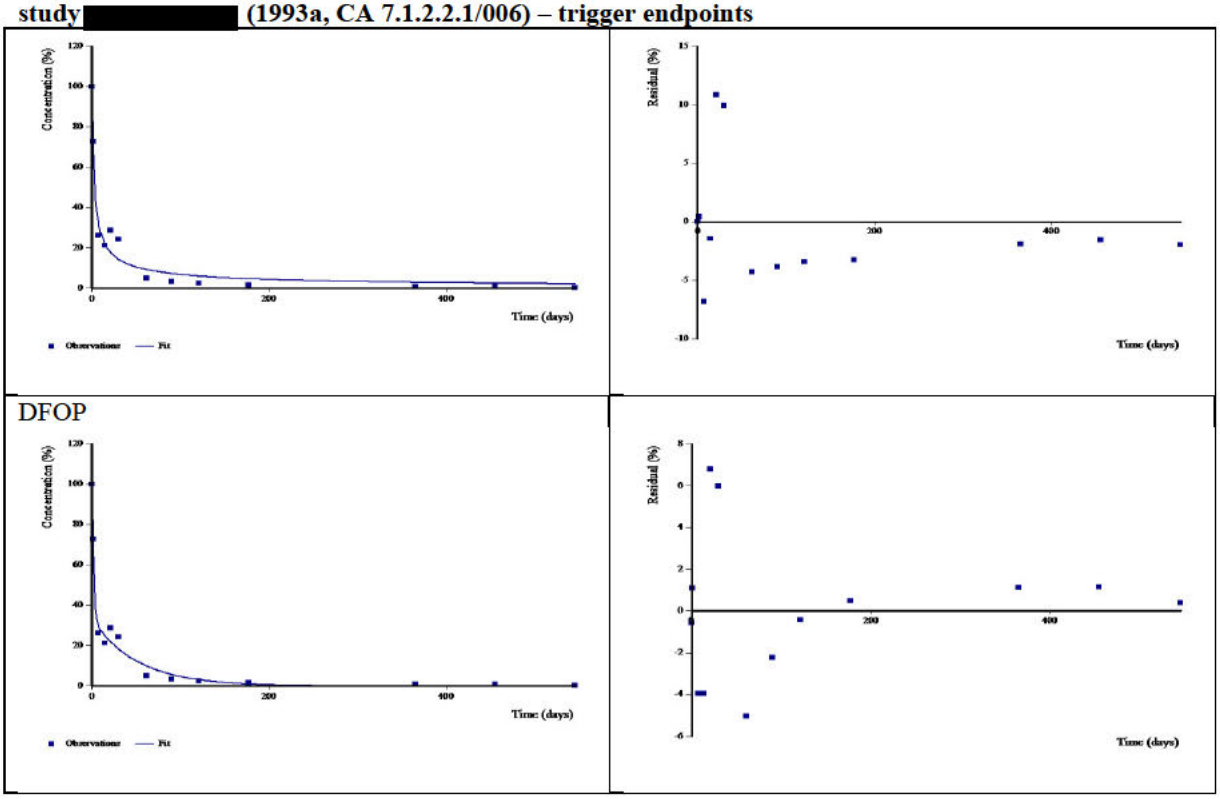


Table 8.1.1.3-143: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ohio of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	101.7	k1: 0.5996 k2: 0.0187 g: 0.6764	13.5	k1: <0.001 k2: <0.001	-	-	2.1	62.8	-
AMPA: SFO	Acceptable	-	k: 0.0107	17.5	k: <0.001	-	-	65.0	216	0.510 (±0.055)

Applicant's conclusion

Dissipation of glyphosate is well described. The formation and decline of AMPA are acceptably described by the fit even though later data points are underestimated.

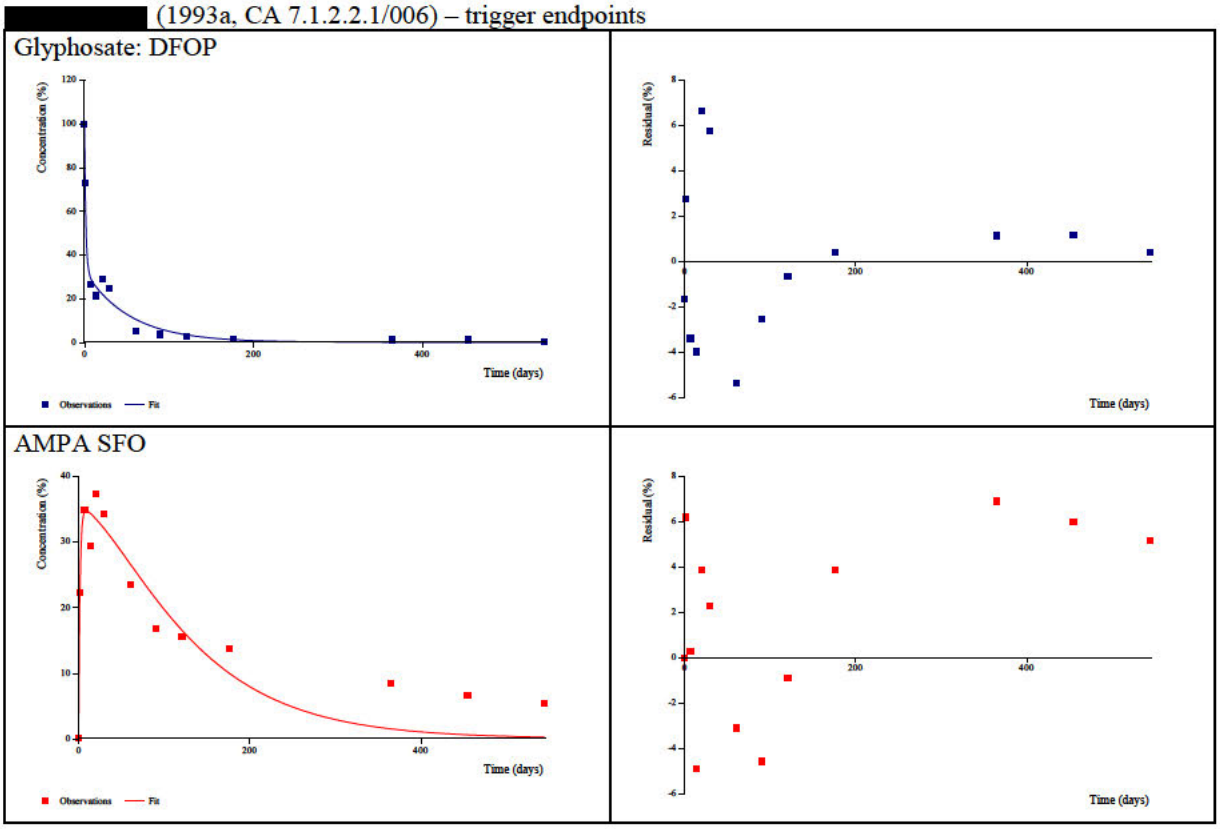
Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

RMS conclusion

RMS does not agree that AMPA is well described. The last data points for AMPA are significantly underestimated.

A decline fit from the maximum observed should be provided by the applicant. This is identified as a data gap

Table 8.1.1.3-143: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ohio of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints



Normalization not performed.

Summary of trigger and modelling endpoints

Table 8.1.1.3-144: Summary of trigger endpoints for glyphosate (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	2.4	49.2	15.9	FOMC
[redacted] (1993a, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	-[2]	-[2]	-	-
	Loamy sand	California, USA	6.3	0 – 60	13.5	101	12.7	FOMC
	Silty clay loam	Iowa, USA	6.0	0 – 60	147	>1000	14.6	FOMC
	Loam	Minnesota, USA	6.5	0 – 60	-[2]	-[2]	-	-
	Sandy clay loam	New York, USA	5.8	0 – 60	-[2]	-[2]	-	-
	Loam	Ohio, USA	7.8	0 – 60	2.1	62.8	13.5	DFOP
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	9.5	53.9	16.4	DFOP
[redacted] (1989a, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	5.4	35.3	5.1	FOMC

Table 8.1.1.3-144: Summary of trigger endpoints for glyphosate (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model

[1] Medium unknown
[2] No reliable endpoint could be determined

Table 8.1.1.3-145: Summary of trigger endpoints for AMPA (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	155	514	16.5	FOMC-SFO
[redacted] (1993a, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	97.6	630	15.3	DFOP[3]
	Loamy sand	California, USA	6.3	0 – 60	-[2]	-[2]	-	-
	Silty clay loam	Iowa, USA	6.0	0 – 60	-[2]	-[2]	-	-
	Loam	Minnesota, USA	6.5	0 – 60	302	>1000	10.3	SFO[3]
	Sandy clay loam	New York, USA	5.8	0 – 60	-[2]	-[2]	-	-
	Loam	Ohio, USA	7.8	0 – 60	65.0	216	17.5	DFOP-SFO
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	-[2]	-[2]	-	-
[redacted] (1989a, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	111	370	15.4	FOMC-SFO

[1] Medium unknown
[2] No reliable endpoint could be determined
[3] Decline fit

Table 8.1.1.3-146: Summary of modelling endpoints for glyphosate (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	$DegT_{50}$ (d) Norm.[2]	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	-[3]	-	-
[redacted] (1993a, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	-[3]	-	-
	Loamy sand	California, USA	6.3	0 – 60	32.6	22.0	SFO
	Silty clay loam	Iowa, USA	6.0	0 – 60	182	15.9	SFO
	Loam	Minnesota, USA	6.5	0 – 60	-[3]	-	-
	Sandy clay loam	New York, USA	5.8	0 – 60	-[3]	-	-
	Loam	Ohio, USA	7.8	0 – 60	-[3]	-	-

Table 8.1.1.3-145: Summary of trigger endpoints for AMPA (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	-[3]	-	-	
[redacted] (1989a, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	-[3]	-	-	

[1] Medium unknown
[2] $DegT50_{matrix}$ according to EFSA (2014)
[3] No reliable endpoint could be determined

Table 8.1.1.3-147: Summary of modelling endpoints for AMPA (grey rows correspond to soils that were discarded by RMS)

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	$DegT_{50}$ (d) Norm.[2]	ff (-)	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	-[3]	-[3]	-	-
[redacted] (1993a, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	303	-	21.1	SFO[4]
	Loamy sand	California, USA	6.3	0 – 60	-[3]	-[3]	-	-
	Silty clay loam	Iowa, USA	6.0	0 – 60	-[3]	-[3]	-	-
	Loam	Minnesota, USA	6.5	0 – 60	-[3]	-[3]	-	-
	Sandy clay loam	New York, USA	5.8	0 – 60	-[3]	-[3]	-	-
	Loam	Ohio, USA	7.8	0 – 60	-[3]	-[3]	-	-
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	-[3]	-[3]	-	-
[redacted] (1989a, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	-[3]	-[3]	-	-

[1] Medium unknown
[2] $DegT50_{matrix}$ according to EFSA (2014)
[3] No reliable endpoint could be determined
[4] Decline fit

Assessment and conclusion by applicant:

The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the study is considered valid and the provided endpoints can be used for risk assessment.

Assessment and conclusion by RMS:

For more clarity and consistency in the reporting, RMS comments focus on the sites and studies that were considered reliable to derive endpoints. These are: Ontario from [redacted] (1993a), California, New York and Ohio from [redacted] (1993).

Data processing

The conversion of measured residues in mg/kg into kg/ha is not detailed in the study report and could not be reproduced by RMS. Therefore, a data gap is set for the applicant to provide more explanations on this conversion and on the parameters used. If available, excel sheets with the detailed calculations should be provided.

The study [redacted] (1993a, CA 7.1.2.2.1/006) reports that for California, the residues of glyphosate (and consequently AMPA) at DAT0 showed evidence of contamination at lower depths. The study author indicates that it was attributed to contamination during sampling, supposedly due to the fact that no pre-excavation of the top soil was performed prior to core sampling (as it has been done on the other soils). RMS considers the argument as valid but notes that, in this case, the whole of the residues measured at DAT0 for glyphosate and AMPA should be considered to be extracted in the first layer; and converted with the bulk density of this layer only. The applicant should confirm that this approach has been used. A data gap is set for the applicant to clarify the approach used for processing of the data at T0 and relevant bulk density used in California site ([redacted], 1993a).

For some sites, replicate values were not used. The applicant argued that replicate samples were mixed across the subplots resulting in one combined sample ([redacted], 1993a, CA 7.1.2.2.1/006), or the replicate results could not be clearly assigned to the individual subplots as this information was not given in the raw data tables ([redacted], 1993, CA 7.1.2.2.1/005). However, since results for 3 replicates are available in the study report, RMS considers that the kinetic evaluation should be done on the replicates values. Updated kinetics considering individual replicates should be provided for sites from [redacted], 1993a, CA 7.1.2.2.1/006 and [redacted], 1993, CA 7.1.2.2.1/005. A data gap is set for the applicant to update kinetics for the four sites from [redacted], 1993a, CA 7.1.2.2.1/006 and [redacted], 1993, CA 7.1.2.2.1/005 considering replicate values.

Normalisation process

No attempt of normalisation for temperature and moisture was performed for Ohio ([redacted] 1993a) and Ontario ([redacted] 1993) sites since weather data from these sites were not available or were of insufficient quality. In addition, it is noted that daily weather data from local stations are available in the study report for Ontario ([redacted] 1993). It seems that it was not investigated whether data from weather stations located close to the trial sites are available (as done in [redacted] 2020 for the European sites). If relevant data are available, normalisation procedure should be followed. A data gap is set for the applicant to provide a normalisation of data from sites Ohio [redacted] 1993a) and Ontario [redacted] 1993), if reliable data can be obtained from available weather stations, and to provide a kinetic assessment to derive modelling endpoints.

As mentioned under [redacted] 2020 study, free drainage was used as a lower boundary condition for the PEARL simulations by default representing common European conditions. This assumption may have a serious impact on the model predictions because in case the free drainage option is incorrectly used (*i.e.* in reality there is a shallow(er) groundwater level), the estimated soil moisture in the upper levels may be somewhat dryer then in reality. Therefore, the estimated soil moisture may be an underestimation. This in turn leads to a lower correction factor which will eventually lead to an underestimation of the normalized DT_{50} (i.e. shorter normalised day lengths used than appropriate). A data gap is identified for the applicant to justify the choice of the lower boundary condition (free drainage) for each site.

Kinetic assessment

The kinetic evaluation is generally well performed, according to FOCUS and EFSA guidance.

For New York site ([redacted] 1993a), the applicant is requested to provide further kinetic fitting for glyphosate according to EFSA $DegT_{50}$ flowchart. This is identified as a data gap.

For Ohio site ([redacted] 1993a), the applicant is requested to provide a decline fit from maximum occurrence of AMPA, in order to derive trigger endpoint for AMPA. This is identified as a data gap.

In conclusion, the study is considered acceptable, pending the above data gaps are addressed. The current acceptable endpoints derived from this study are summarised below and in B.8.1.1.3.

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Trigger endpoints

Parent	Aerobic conditions – trigger endpoints						
Soil	Location	pH[a]	Depth (cm)	DT_{50} / DT_{90} (d) actual	Kinetic parameters	St. (χ^2)	Method of calculation
Ontario [redacted] (1993) Loamy sand (bare soil)	Canada	6.8	0-45	13.7 / 54.4	k_1: 0.0551 k_2: 0.0017 g: 0.9420	22.3	DFOP
California [redacted] (1993a) Loamy sand (bare soil)	USA	6.3	0-121.9	13.0 / 102	k_1: 0.1124 k_2: 0.0148 g: 0.5490	12.7	DFOP
Ohio [redacted] (1993a) Loam (bare soil)	USA	7.8	0-121.9	2.4 / 61.5	k_1: 0.5430 k_2: 0.0194 g: 0.6704	13.3	DFOP

[a]medium not given – value from the 0-15 cm depth layer

AMPA	Trigger endpoints	Aerobic conditions Metabolite dosed or the precursor from which the f f. was derived was glyphosate						
Soil	Location	pH $_{(H2O)}$[a]	Depth (cm)	DT_{50} (d) actual	DT_{90} (d) actual	St. (χ^2)	f. f. k_f / k_{dp}	Method of calculation
Ohio [redacted] (1993a) Loam (bare soil)	USA	7.8	0-121.9					Data gap for decline fit

[a] medium not given – value from the 0-15 cm depth layer

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Modelling endpoints

Parent		Aerobic conditions – modelling endpoints						
Soil	Location.	pH[a]	Depth (cm)	DT_{50} (d) Norm[b].	Kinetic parameters	DT_{90}(d) actual	St. (χ^2)	Method of calculation
California [redacted] (1993a) Loamy sand (bare soil)	USA	6.3	0-121.9	32.6	k: 0.0213	108	22.0	SFO
New York [redacted] (1993a) Sandy clay loam (bare soil)	USA	5.8	0-121.9					Data gap, further fits required (following EFSA $DegT_{50}$ flowchart)

[a] medium not given – value from the 0-15 cm depth layer

[b]Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7, values are $DegT_{50}$ matrix

No modelling endpoints are determined for AMPA.

B.8.1.1.3.4. Relevant articles from literature search

Within the Literature Review Report performed for glyphosate on peer reviewed publications (2010-2020), three publications were identified that could provide information potentially relevant to this data point.

Table 8.1.1.3-148: Field dissipation - relevant articles from literature search

Study	Study type	Substance(s)	Status
Passeport, E., *et al 2014*	Behaviour in field	Glyphosate	Reliable with restrictions
Todorovic, G. *et al. 2014*	Dissipation in field	Glyphosate	Reliable with restrictions
Tush D. *et al. 2018*	Adsorption Dissipation in field	Glyphosate	Reliable with restrictions

Passeport et al 2014

Data point:	CA 7.1.2.2.1/026
Report author	Passeport, E., *et al.*
Report year	2014
Report title	Dynamics and mitigation of six pesticides in a "Wet" forest buffer zone
Document No	DOI 10.1007/s11356-013-1724-8 E-ISSN 1614-7499
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Pesticide pollution is one of the main current threats on water quality. This paper presents the potential and functioning principles of a "Wet" forest buffer zone for reducing concentrations and loads of glyphosate, isoproturon, metazachlor, azoxystrobin, epoxiconazole, and cyproconazole. A tracer injection experiment was conducted in the field in a forest buffer zone at Bray (France). A fine time-scale sampling enabled to illustrate that interactions between pesticides and forest buffer substrates (soil and organic rich litter layer), had a retarding effect on molecule transfer. Low concentrations were observed for all pesticides at the forest buffer outlet thus demonstrating the efficiency of "Wet" forest buffer zone for pesticide dissipation. Pesticide masses injected in the forest buffer inlet directly determined concentration peaks observed at the outlet. Rapid and partially reversible adsorption was likely the major process affecting pesticide transfer for short retention times (a few hours to a few days). Remobilization of metazachlor, isoproturon, desmethylisoproturon, and AMPA was observed when non-contaminated water flows passed through the forest buffer. Our data suggest that pesticide sorption properties alone could not explain the complex reaction mechanisms that affected pesticide transfer in the forest buffer. Nevertheless, the thick layer of organic matter litter on the top of the forest soil was a key parameter, which enhanced partially reversible sorption of pesticide, thus retarded their transfer, decreased concentration peaks, and likely increased degradation of the pesticides. Consequently, to limit pesticide pollution transported by surface water, the use of already existing forest areas as buffer zones should be equally considered as the most commonly implemented grass buffer strips.

Materials and Methods

The forest buffer zone is located at the outlet of a tile drained agricultural watershed at Bray (France).

Chemicals

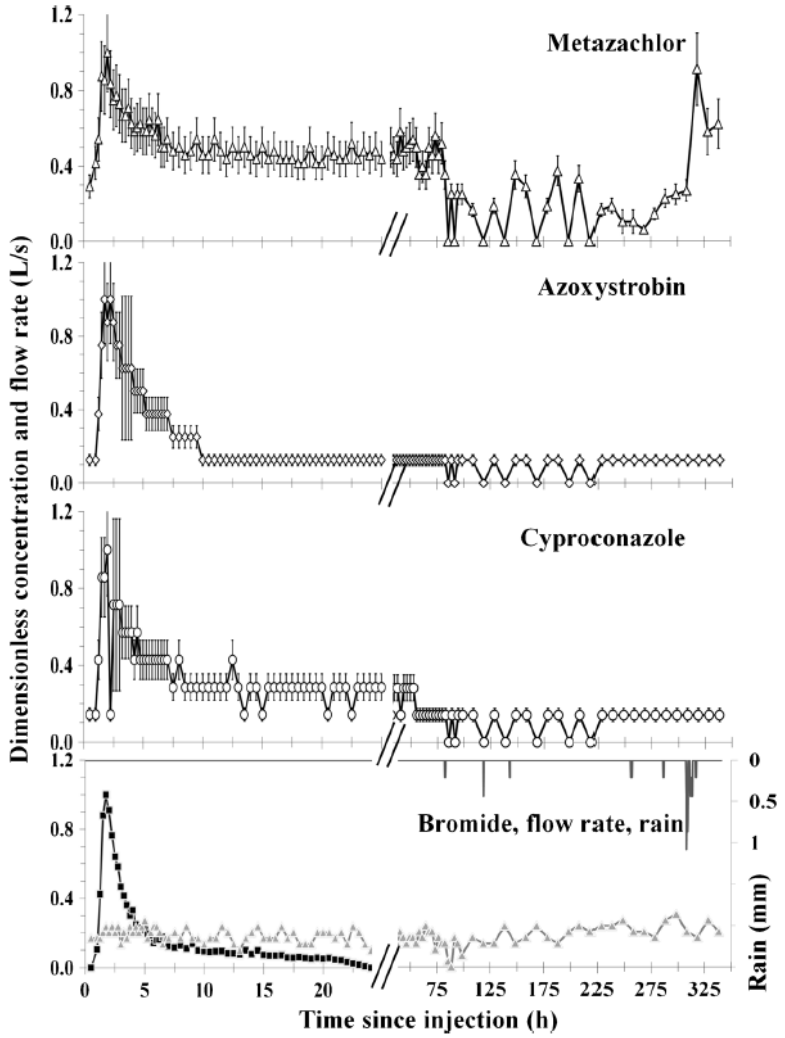
An injection solution was prepared with six pesticides and potassium bromide as a conservative tracer.

Pesticides were provided by farmers and diluted in deionized water before injection. Commercial solutions that were used are indicated into parentheses: three herbicides, glyphosate (Glyphogan), isoproturon (Isoproturon), and metazachlor (Novall), and three fungicides, azoxystrobin (Priori Xtra), cyproconazole (Amistar Xtra), and epoxiconazole (Opus) were selected for their contrasting properties and wide use in agriculture.

Tracer Experiment

The forest buffer tracer experiment took place for a period of 14 days, from 19 February 2009, 10:50 to 5 March 2009, 13:20 in a reduced portion of the forest buffer, using watershed outlet flows as incoming flows into the forest buffer. The experimental plot was delimited with soil border levees leading to a 54 m^2 surface area (36 m×1.5 m). Only one significant rainfall event occurred on 308.5 h after the start of the experiment, with a cumulative rainfall depth of 9.94 mm, measured with the on-site tipping bucket rain gauge (R01 3030A Danae, Précis Mécanique, Bezons, France). Water temperature was 5.9±3.7 °C during the course of the experiment, and was close to or greater than monthly averages. The inlet flow rate was 0.32±0.08 L/s. At the outlet, a flow restriction helped manually measuring flow rates by frequently timing the filling of a container with a known volume. Water from the watershed was allowed to flow through the forest buffer experimental plot on 18 February 2009 at 15:50, in order to saturate the soil and ensure a permanent flow rate for the next day injection. Two peristaltic pumps (Eijkelkamp 12 V SDEC Reignac-sur-Indre, France) were used to ensure a 0.30 L/s injection flow rate during 78 s. Grab water samples or samples collected by means of a time-dependent automated sampler (ISCO 3700 Neotek, Trappes, France) were taken at the outlet of the experimental plot. The sampling frequency was modified along the course of the experiment: every 15 min for the first 7 h, every 30 min until 28.5 h after the start of the experiment, then every 3 h until 94 h since injection, and every 10 h from days 4 to 10 following the start of the experiment. Finally, five grab water samples were taken at forest buffer inlet to control pesticides' background concentrations coming from the artificially drained watershed.

Figure 8.1.1.3-2: Flowrate at the forest outlet (gray triangle, bottom panel, in liter per second), and dimensionless (C/C_{max}) concentration pattern during the first 24 h (left panels) and the next 350 h (right panels) after injection, for molecules that exhibited the clearest transfer pattern: metazachlor (white triangles), azoxystrobin (white diamonds), cyproconazole (white circles), and bromide (black squares).



The double slash bars (//) indicate a change in time step.
C concentration at time t;
C_{max} peak concentration measured 2 h (metazachlor, azoxystrobin, and cyproconazole) and 1.8 h (bromide) after injection.
No rainfall event occurred during the first 24 h; rain beyond 24 h (bottom-most right-hand side panel) is plotted on the right hand vertical axis, in reverse order.
Error bars correspond to dimensionless expanded uncertainties, i.e., expanded uncertainties on concentrations (U, coverage factor = 2), divided by C_{max}

Analytical method

Water sample analysis

Subsamples were taken from water samples, filtered and analyzed for bromide with ion chromatography and an IonPac AS9-HC column. The limit of quantification (LQ) was 1 mg/L. Metazachlor, cyproconazole, epoxiconazole, azoxystrobin, isoproturon and two of its metabolites, desmethylisoproturon and 1-(4-isopropylphenyl)urea, were extracted by solid-phase extraction on pre-filtered samples, and then analyzed by high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (LCMS-MS). Limits of quantification were 0.02 µg/L for these seven

pesticides and metabolites. Glyphosate and its main metabolite, AMPA, were first derivatized with 9-fluorenylmethyl chloroformate (FMOC) before LC-MS-MS analysis (LQ=0.1 µg/L for both glyphosate and AMPA).

Litter and soil sampling and analysis

Litter, and soil grab samples were taken in the forest experimental plot at the end of the tracer experiment. Another litter and soil samples were collected outside the experimental plot to compare with those collected inside the experimental plot. All samples were frozen before pesticide analysis. Glyphosate and AMPA were extracted by ultrasonic waves in water, then derivatized with FMOC and analyzed by LCMS-MS, whereas extraction for the other molecules from soil samples was carried out with ultrasonic waves in acetone. Extracts were analyzed by LC-MS-MS. Litter samples were treated with an internal procedure developed by the laboratory (Institut Pasteur de Lille). Limits of quantification were 0.01 mg/kg dry weight for each compound.

Data analysis

The hydraulic retention time was calculated based on the bromide conservative tracer using the moment theory on residence time distribution (see Passeport *et al.* (2010), Kadlec and Wallace (2008)).

Statistical analyses

Pearson correlation coefficients were determined with the R software to detect possible correlations among pesticide concentrations, injected masses, and pesticide physico-chemical properties.

Table 8.1.1.3-149: Forest buffer inlet concentrations

Molecule	Pesticide inlet concentrations (µg/L)				
	Time from injection (h)				
	LQ[a]	−0.58	6.75	24.17	339
Glyphosate	0.1	n.d.[b]	<LQ	<LQ	n.d.
AMPA	0.1	0.30	<LQ	<LQ	0.30
Isoproturon	0.02	1.60	1.20	1.30	1.40
Desmethylisoproturon	0.02	0.12	0.11	0.10	0.11
1-(4-isopropylphenyl)urea	0.02	<LQ	<LQ	<LQ	n.d.
Metazachlor	0.02	0.29	0.30	0.25	0.19
Epoxiconazole	0.02	<LQ	0.02	n.d.	n.d.
Azoxystrobin	0.02	<LQ	<LQ	n.d.	n.d.
Cyproconazole	0.02	<LQ	<LQ	n.d.	n.d.

[a] Limit of quantification
[b] *n.d.* is "not detected"

Results

Hydrology

Water ran off through the forest buffer experimental plot as a shallow sheet flow with an average outlet flow rate of 0.18± 0.11 L/s (average ± expanded uncertainty for 95 % confidence interval). Bromide started to be detected 1 h after injection and reached a concentration peak 1.8 h after injection. Bromide recovery rate and hydraulic residence time were 74 % and 6.3 h, respectively.

Inlet water quality

During the experiment, watershed tile-drain flows continuously entered the experimental plot at a controlled flow rate of 0.3 L/s. We determined that some of the studied pesticides also entered the experimental plot via watershed flows during the course of the experiment. Non-negligible concentrations of isoproturon, desmethylisoproturon, glyphosate, AMPA and metazachlor were measured. Epoxiconazole was detected once (6.8 h after injection) but with a concentration at the limit of quantification. The most recent applications of glyphosate and metazachlor on the Bray watershed were approximately 16 months before the start of the experiment.

Table 8.1.1.3-150: Tracer experiment dynamics characteristics and mass recovery rates

Molecule	Peak conc ± U(C) (µg/L)	Peak conc time (h after injection)	Percent recovery (%)	Time for conc reaching < LQ (h after injection)
Bromide	1750	1.75	74	24.0
Glyphosate	0.05±0.03	NA[a]	NA	NA
AMPA	0.30±0.08	1.75	NA	NA
Isoproturon	1.70±0.41	2.50	NA	NA
Desmethylisoproturon	0.14±0.03	NA	NA	NA
1-(4-isopropylphenyl)urea	0.02±0.01	NA	NA	NA
Metazachlor	0.48±0.10	2.00	NA	NA
Epoxiconazole	0.04±0.01	2.75	NA	NA
Azoxystrobin	0.08±0.02	2.00	22	10.0
Cyproconazole	0.07±0.02	2.00	45	13.5

[a] NA means Not Available, when peak concentration ("Peak Conc") time could not be clearly identified and mass balances could not be reasonably calculated due to a large portion of the concentration dataset below limits of quantifications

Pesticide dynamics description

Apart from isoproturon, concentrations were lower than 0.50 µg/L for AMPA and metazachlor, and did not exceed 0.15 µg/L for the other pesticides (glyphosate, azoxystrobin, epoxiconazole, cyproconazole, desmethylisoproturon, and 1-(4-isopropylphenyl)urea). Only injections of metazachlor, azoxystrobin and cyproconazole resulted in a clear transfer pattern at the forest plot outlet. Two hours after injection, these pesticides exhibited concentration peaks of 0.48 ± 0.10, 0.08 ± 0.02, and 0.07 ± 0.02 µg/L for metazachlor, azoxystrobin, and cyproconazole, respectively. These concentration peaks were observed closely after that of the conservative tracer, which was recorded 1.8 h after injection. For glyphosate, AMPA, epoxiconazole, and 1-(4-isopropylphenyl)urea, concentrations at the forest plot outlet were so low that only a qualitative assessment of the data can reasonably be performed. In addition, high background concentration levels of isoproturon and desmethylisoproturon hindered an accurate quantitative analysis of the data for these two molecules. In all water samples, glyphosate concentrations were below the LQ and those for AMPA never exceeded 0.30 ± 0.08 µg/L. No temporal variation was observed for these molecules, besides two small AMPA concentration rises, one after injection (between 1.8 and 3.8 h) and a second one after the rainfall event (between 318.5 and 328.5 h). Concentration peaks for the injected molecules were significantly correlated (p value=1.75×10^{-5}) with background concentrations, highlighting the strong influence that this artifact exerted on the results. The second strongest correlation (despite not significant at a α=5 % significance level) was between pesticide concentration peaks and injected masses. With this small dataset, no statistically significant correlations were found between the ratios and the pesticide sorption properties.

Discussion

Hydrology

The ratio between outlet and inlet flow rates (0.61), and the bromide recovery rate (74 %) are suggestive of some water losses outside the experimental plot, via infiltration, possibly due to poor soil levee compaction, earthworm burrows, and tree roots.

Forest buffer efficiency for pesticide removal

A key conclusion of our study relies on the fact that, for most pesticides, very low concentrations were measured at the forest outlet, thus demonstrating the efficiency of such buffer zones for pesticide removal.

Sorption as part of a complex set of removal processes

The high sorption coefficients of glyphosate, AMPA and epoxiconazole may partly explain their low concentrations measured at the forest outlet. Contrary to glyphosate and AMPA, epoxiconazole was detected on dead leaves at the forest plot inlet and middle zones 14 days after injection even after large

rainfall events. This supports a possible strong adsorption of epoxiconazole onto the forest litter. Because epoxiconazole was not detected in the soil below the litter layer, it is likely that the litter layer acted as a key sorption material that prevents strongly sorbing pesticides from leaching to deep soil horizons.

Degradation and remobilization of pesticides

Due to the moderately long half-lives of their parent molecules, glyphosate and isoproturon, the detection of AMPA and desmethylisoproturon at the beginning of the experiment can hardly be attributed to the injected parent molecules. It should be noted that AMPA, isoproturon, and desmethylisoproturon were detected at the forest plot inlet indicating that these molecules were also transferred to the experimental plot from the tile-drain watershed. Glyphosate and isoproturon were applied previously on the agricultural watershed and may have been partially degraded in the catchment and forest buffer soils thus generating these metabolites.

"Dry" vs. "Wet" buffer zone

In this study, the "Wet" forest buffer soil had a high clay content thus limiting downward infiltration. Even if water losses via infiltration might occur, it could not explain alone the observed pesticide removal. It is a fundamental difference with "Dry" buffer zones like grass areas, where infiltration plays a crucial role. The second major difference between grass and forest buffer zones lies in the presence of thick litter layer rich in organic matter in the latter. The litter provides many sorption sites for pesticides and is biologically active, thereby biodegrading retained pesticides. Consequently, when buffer zone soil is saturated, pesticide sorption and degradation should more easily occur in forested areas than in grass areas, provided that the contaminated water runs off through the litter layer as a shallow and slow water flow.

Conclusions

The objective of this experiment was to demonstrate at the field scale the potential of forest buffer zones to reduce the concentrations and loads of pesticides presenting a wide range of physico-chemical properties. Very low concentrations were measured at the forest outlet thus suggesting a potential of the forest buffer to effectively reduce the pollution with pesticides. Understanding processes, which govern the removal of pesticides through the forest buffer was beyond the scope of this study. However, the fine sampling frequency used in this study helped to provide some explanations about the observed dynamics of pesticide transfer through the forest buffer zone. At short time-scales (lower than a month), retention processes are suspected to dominate. Our results highlighted the dual role of organic matter. On the one hand, organic substrates enabled rapid adsorption of pesticides transported in highly contaminated flows. On the other hand, when fresher (i.e., less contaminated) flows crossed the forest buffer, previously adsorbed pesticides were shown to desorb thus being released back to the water column. Organic matter also plays an indirect role in this process as it supports growth of microbial populations. Any forested area adequately located in the landscape could be used as an efficient buffer zone for reducing pesticide pollution. Indeed, even old wood that were not necessarily well maintained could be good candidates for buffering pesticide contaminated flows provided a thick litter layer has had time to accumulate over time. At a short time scale (here approx. 350 h), highly organic material would therefore mainly act as a retarding factor that temporarily affect pesticide dynamics. For extended periods of water retention, degradation reactions leading to metabolites are likely to occur, however, more research is needed to confirm the extent of pesticide degradation that could be achieved. The results of this study are suggestive of a high potential of "Wet" forest buffer zone for the reduction of downstream pesticide concentrations and loads. Further research should investigate the efficiency of forest buffers for pesticide removal (1) under various climatic conditions, and for a wide range of forest buffer (2) sizes and shapes, and (3) locations in the watershed (headstream vs. downstream). Such results are needed to better understand pesticide fate and the role of the litter layer, and to establish guidelines to design forest buffer zones and incorporate them in land management strategies.

Assessment and conclusion by applicant:

The article describes the mitigation of glyphosate among other pesticides by a wet forest buffer zone in France. Not all required parameters are reported to check validity of the study (e.g. information on test substance, analytical method, characterization of soil).

The article is classified as reliable with restrictions.

Assessment and conclusion by RMS:

As mentioned by the applicant, further details are needed to ensure the validity of the study. This article provides information on the reduction of concentrations from forest buffer zones. The fluxes and concentrations of tested pesticides were reduced when transferred through the buffer zones. This article can therefore not be used in the risk assessment but still provides interesting information on the efficacy of forest buffer zone. However it does not provide information on data requirement "7.1.2.2.1 Soil dissipation studies".

Todorovic et al 2014

Data point:	CA 7.1.2.2.1/027
Report author	Todorovic, G. *et al.*
Report year	2014
Report title	Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils
Document No	DOI 10.2478/intag-2013-0031
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Erosion processes can strongly influence the dissipation of glyphosate and aminomethylphosphonic acid applied with Roundup Max in agricultural soils; in addition, the soil structure state shortly before erosive precipitations fall can be a key parameter for the distribution of glyphosate and its metabolite. Field rain simulation experiments showed that severe erosion processes immediately after application of Roundup Max can lead to serious unexpected glyphosate loss even in soils with a high presumed adsorption like the Cambisols, if their structure is unfavourable. In one of the no-tillage-plot of the Cambisol, up to 47% of the applied glyphosate amount was dissipated with surface run-off. Moreover, at the Chernozem site with high erosion risk and lower adsorption potential, glyphosate could be found in collected percolation water transported far outside the 2x2 m experimental plots. Traces of glyphosate were found also outside the treated agricultural fields.

Materials and Methods

The experiments were carried out where following soil tillage systems were compared in 3 field replications:

– conventional tillage (CT) with plough with and without cover crop during winter period;

– no-tillage (NT) with cover crop during winter period.

The investigated soils were a Chernozem from loess at Pixendorf and a sandy stagnic Cambisol from tertiary carbonate free sediments at Kirchberg, Austria. In order to investigate the influence of erosion

and tillage on glyphosate and AMPA, two rain simulation experiments were conducted in 3 field replications (1, 2, 3) within the CT and NT plots. For this, Roundup Max was applied onto rain simulation soil plots according to the common agricultural practice (180 mg glyphosate/m^2). In both sites, the vegetation cover degree was typically higher in the NT-plots (80-100% of weed cover) than in the CT-plots (only few yield residues of maize) and the application was carried out in sunny and not windy weather shortly before starting the rain simulation experiment (worst case scenario). The average slope in both sites was 12-15% at the Cambisol and 10% at the Chernozem. Both sites are known as rather erodible. The soil surface of the Chernozem immediately before the rain simulation was crumby; in turn, the cambisol had a crusted, dry, and cracky surface. The rain simulator was designed as a portable equipment, the spray pattern was generated by full jet nozzles, the rain fall intensity was controlled with intermittent spraying.

During 60 min of rain simulation with 30 mm, run-off fractions were collected at different time intervals at the Chernozem and averagely at the Cambisol and cooled in boxes. In the laboratory, the run-off samples were immediately centrifuged to separate the liquid from the solid phase. Immediately after the rain simulation, soil samples were collected within the simulation soil plots of 2x2 m at different depths (0-2, 2-5, 5-10, 10-15, and 15-20 cm at the Chernozem and at 0-2 and 2-5 cm at the Cambisol). Glyphosate and AMPA were analyzed according to Rampazzo *et al.*, 2013. All physical and chemical analyses on soil samples were carried out according to the standard methods.

Table 8.1.1.3-151: Fe-oxide distribution in the investigated soils

Site	Soil type (WRB)	Depth (cm)	Fe_o (mg kg^{-1})	Fe_d (mg kg^{-1})	Fe_o/Fe_d
Pixendorf	Chernozem	0-5	983	7 970	0.12
		5-20	1 040	8 378	0.12
Kirchberg	Cambisol	0-5	3 422	14 843	0.23
		5-20	3 726	15 032	0.25

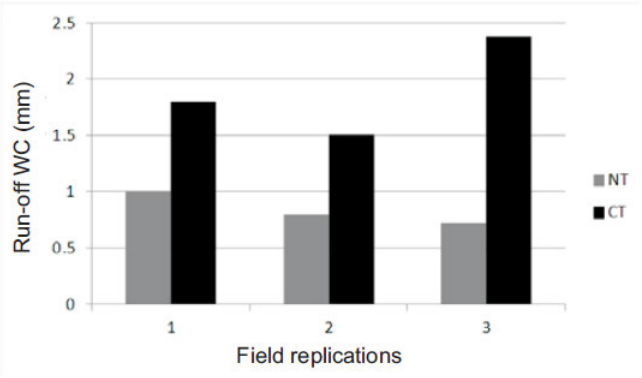
Fe_o – amorphous (weakly crystallized) Fe-oxides, oxalate-soluble; Fe_d – well crystallized Fe-oxides, dithionite-soluble.

Results and Discussion

The Chernozem shows the development from loess with typical silty texture (topsoil 0-20 cm, 12 % clay, 65 % silt, 23% sand, pH 7.3, 15 % $CaCO_3$ and 3 % OM), whereas the Cambisol is a loamy sandy soil (topsoil 0-20 cm, 14 % clay, 33 % silt, 53 % sand, pH 5.7, no $CaCO_3$ and 3 % OM). The Chernozem exhibited a low content and the Cambisol a high content of Fe oxides and therefore the expected sorption capacity for glyphosate and AMPA was theoretically higher at the Cambisol.

Figure 8.1.1.3-3 shows the amount of total (liquid and solid) run-off after the rain simulation experiments on the Chernozem. Before glyphosate and AMPA were analyzed, a separation of the solid and liquid run-off phase in the laboratory was carried out. The CT-plots produced the highest run-off amounts because of their lower protecting weed cover, causing a splash of the surface by the erosive precipitation with consequent loss of infiltrability. On the other hand, the amount of runoff at the Chernozem was 10 times lower than the Cambisol because of its crumby structure with a better infiltration rate during the rainfall simulation, whereas the soil surface of the Cambisol was compacted and crusty. The different amounts of run-off between the 3 field replications of the Chernozem were due to the inhomogeneity of the field conditions. Consequently, the total amount of glyphosate washed out of the plots by liquid run-off at the Chernozem was much higher in the CT-plots than in the NT-plots.

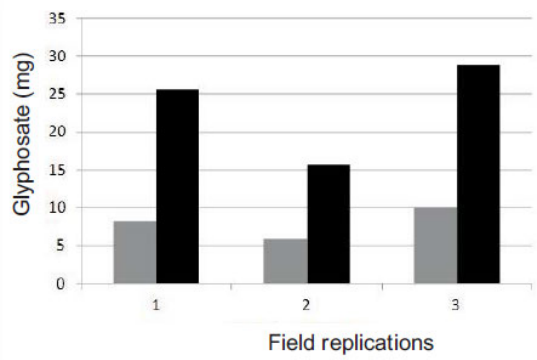
Figure 8.1.1.3-3: Chernozem: total run-off of the conventional tillage (CT) and no-tillage (NT) plots in the 3 field replications, WC – water column



A fractionation of the time-dependent glyphosate contents in run-off-fractions of the Chernozem at time intervals of 15 min is shown below. As it was expected, the first fraction showed the highest contents in both variables CT and NT and then decreasing with time. The CT-plots showed again higher glyphosate contents than the NT-plots, which instead showed higher glyphosate concentration (less dilution) at the same time .

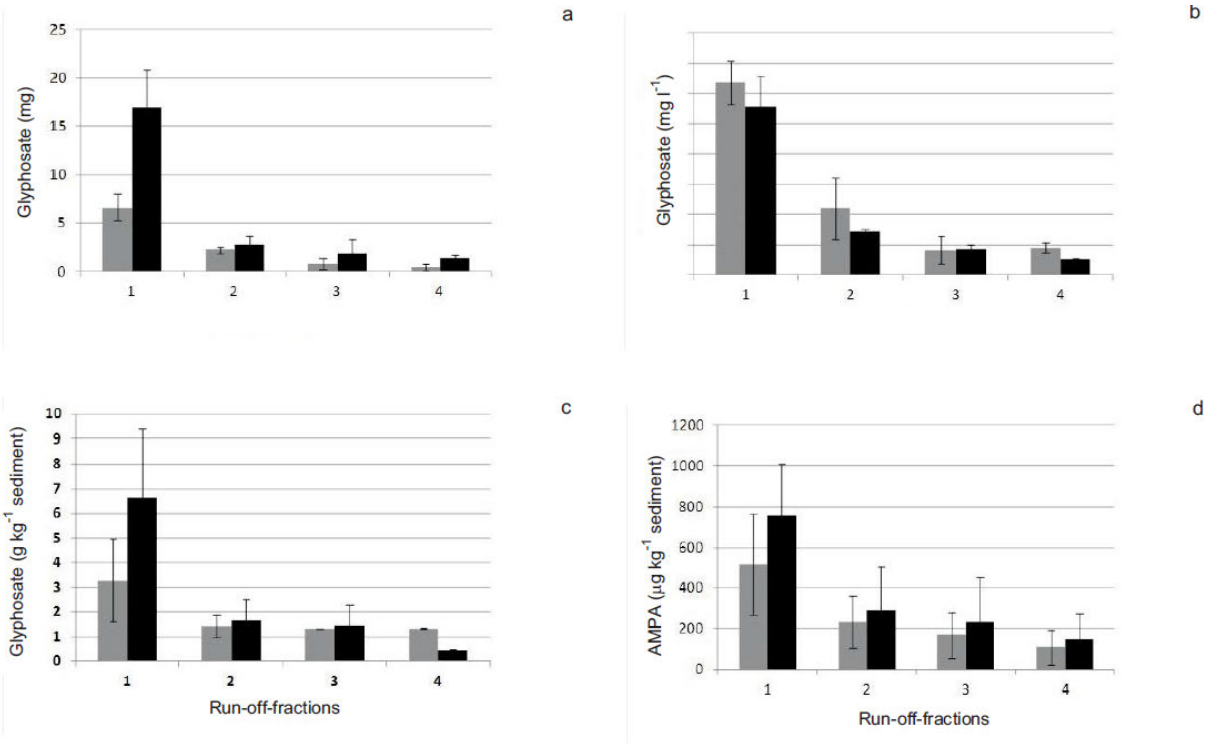
According to Gjettermann *et al.*, 2011, desorption kinetics are important for evaluating the significance of dissolved and particle-facilitated transport of glyphosate. Consequently, the separation from water and solid phases should be done within a short time of minutes. We managed to do this within 30 min from field sampling. The contents of glyphosate and AMPA in the solid phase of run-off in the Chernozem are shown below. The glyphosate contents retained by the run-off sediment is an analogue to that in the total and fractionated runoff, where the first collected fraction of run-off sediment contains the highest amounts of glyphosate which then generally decreases in the following fractions and the CT-plots shows higher amounts than the NT-plots. Analogous is the distribution of AMPA in the sediment. Since the loss of glyphosate by run-off was higher in the CT-plots, the amount of glyphosate and AMPA adsorbed by the Chernozem immediately after the rain simulation experiments was consequently higher in the NT-plots. Moreover, there is a clear depth function of the adsorption of glyphosate and AMPA through the soil immediately after Roundup Max application and rainfall simulation at the Chernozem. The glyphosate and AMPA contents clearly decreased with soil depth.

Figure 8.1.1.3-4: Chernozem: total amounts of glyphosate in liquid run-off at the 3 field replication plots.



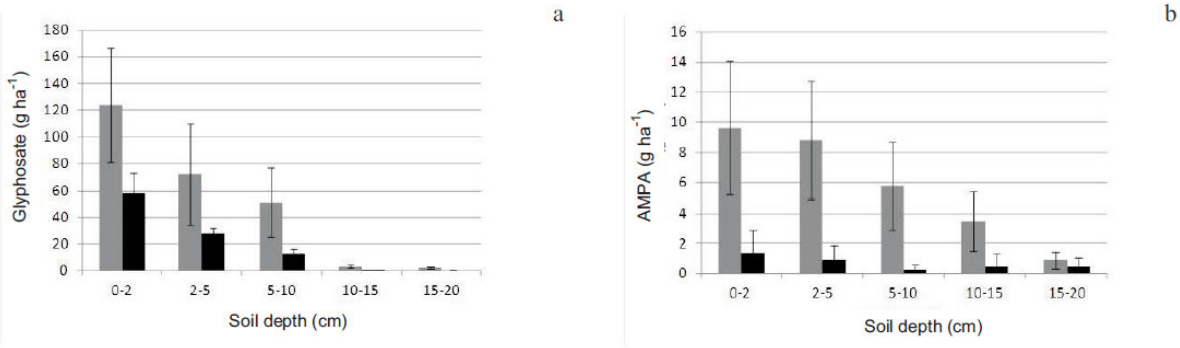
conventional tillage (CT); no-tillage (NT)

Figure 8.1.1.3-5: Chernozem: a – glyphosate amount, b – glyphosate concentrations in liquid, and c – glyphosate contents, d –AMPA contents in the solid phase of run-off-fractions at 15-min intervals (average of 3 field replications).



conventional tillage (CT); no-tillage (NT)

Figure 8.1.1.3-6: Chernozem: a – glyphosate contents, and b – AMPA contents in the soil within the rain simulation plots (average value from the 3 field replications).



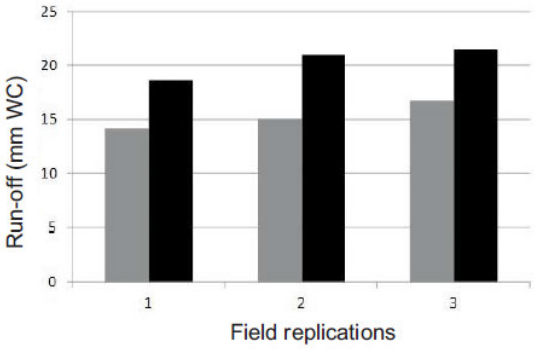
conventional tillage (CT); no-tillage (NT)

The Chernozem had a favourable crumby structure in the NT-plots, with no cracks, no preferential flow, and optimal conditions for water retention in the upper soil layers at the moment of the rainfall simulation experiment, so that more than 50 % of the adsorbed glyphosate was retained in the first 5 cm of the soil. The fact that AMPA could already be detected 1 h after the Roundup Max application underlines the quick glyphosate degradation in soil, as reported by Mamy *et al.* (2005) as well. The total (liquid and solid) amount of surface run-off in the Cambisol is shown below. The Cambisol had a dry, crusty, and very deeply cracky soil surface of the CT-plots before starting the rainfall simulation and therefore the first amount of the precipitation quickly infiltrated in the cracks, but very soon a splash process and loss of infiltration took place due to the fine sandy texture and low surface protection by weeds. This led to a higher surface run-off of the CT-plots than the NT-plots. Consequently, figures show that the contents and concentrations of glyphosate in the liquid run-off of the NT-plots of the Cambisol were much higher than in the CT-plots. In the dry and cracky soil surface of the CT-plots, it took some time before run-off started and glyphosate could easily enter deeper into the soil; on the other

hand, the NT-plots had a nearly 100% weed cover, as reported also by Locke and Bryson (1997); consequently, this might buffer potential effects of glyphosate in the soil (Locke *et al.*, 2008).

In this study, most of the applied glyphosate adhered to the photosynthetically active plant organs (stem and leaves) immediately after application; consequently, glyphosate was literally washed out of the 2x2 m simulation plots with runoff and had less time to infiltrate the soil surface. Based on the high content of pedogenical Fe-oxides (15 000 mg Fe_d/kg), high soil adsorption of glyphosate was expected for the Cambisol. The surprisingly high loss of glyphosate by surface run-off (in one of the 3 field replications about 47% of the applied glyphosate) measured in this study confirmed the crucial effect of soil structure and preferential flow on the dissipation of glyphosate after heavy erosive precipitations, which were also be observed by other scientists. The contents of glyphosate and AMPA in the solid phase of run-off at the Cambisol are shown. The concentrations of glyphosate and AMPA in the solid phase of run-off at the Cambisol are similarly distributed to the corresponding aqueous fractions of run-off; they are mostly higher in the NT-plots than in the CT-plots. Figure 8.1.1.3-10s show the content of glyphosate and AMPA adsorbed by the soil immediately after the rain simulation experiments at the Cambisol. Immediately after the rain simulation experiment, a very clear distribution in the soil appears: glyphosate and AMPA are first adsorbed in the upper 0-2 cm of the soil and only a small amount reaches the next soil depth of 2-5 cm. In general, the NT-plots show a clearly lower content of glyphosate and AMPA as compared to the CT-plots. This is explained by the respectively higher glyphosate contents in run-off of NT-plots. The soil losses of the Chernozem and Cambisol through erosion processes are shown.

Figure 8.1.1.3-7: Cambisol: total run-off of the CT- and NT-plots in the 3 field replications, mm WC – mm water column.



At both sites, the soil loss from the CT-plots, measured as sediment in the surface run-off, was higher than from the NT-plots because of the much lower vegetation cover before the simulation experiment, splash, and reduction of infiltration. The loss of the Cambisol soil was 10 times higher than that of the Chernozem. The reason for this is that the two experimented soils had a completely different soil structure and surface conditions before starting the rain simulation. The Chernozem had a very friable, crumby, permeable structure after the wheat yield. The Cambisol stood right after the corn yield, the soil surface was crusty and less permeable, except for shrinking cracks which swelled during the experiment.

Figure 8.1.1.3-8: Cambisol: total amounts of glyphosate in liquid run-off at the 3 field replication plots.

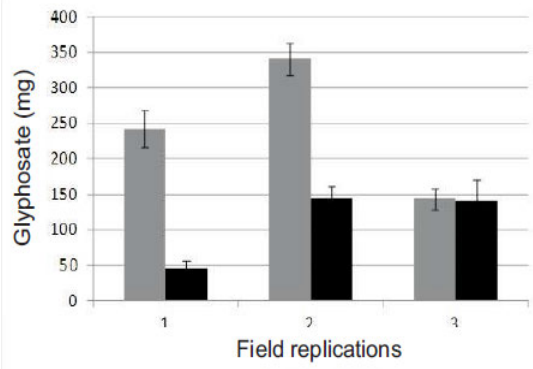
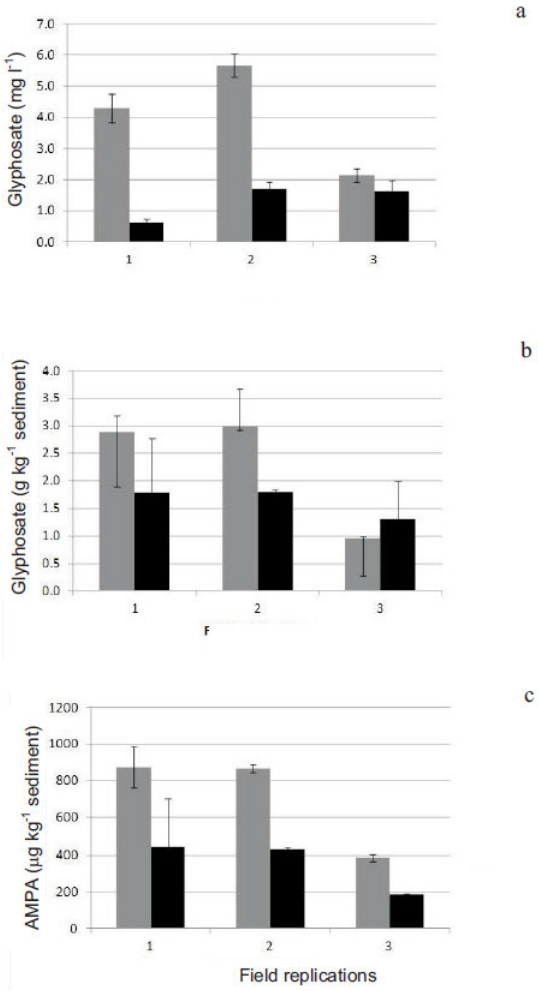


Figure 8.1.1.3-9: Cambisol: a – glyphosate concentrations in liquid, b – glyphosate, and c –AMPA contents in the solid phase of run-off at the 3 field replications (average of the 60 min rain simulation).



The Chernozem at Pixendorf and surroundings is generally known as a location with high erosion risk because of the high silt amount (> 60 mass %) and especially with corn crop, where deep gully erosion forms. The erosion rills discharge downslope to an artificial run-off retention basin at the footslope of the experimental field. This basin can run over and flow downwards on different paths and is collected through further toeslope retention basins. Water samples from both retention basins were analyzed and

Figure 8.1.1.3-10: Cambisol: a – glyphosate and b – AMPA contents in the soil within the rain simulation plots (average value from the 3 field replications).

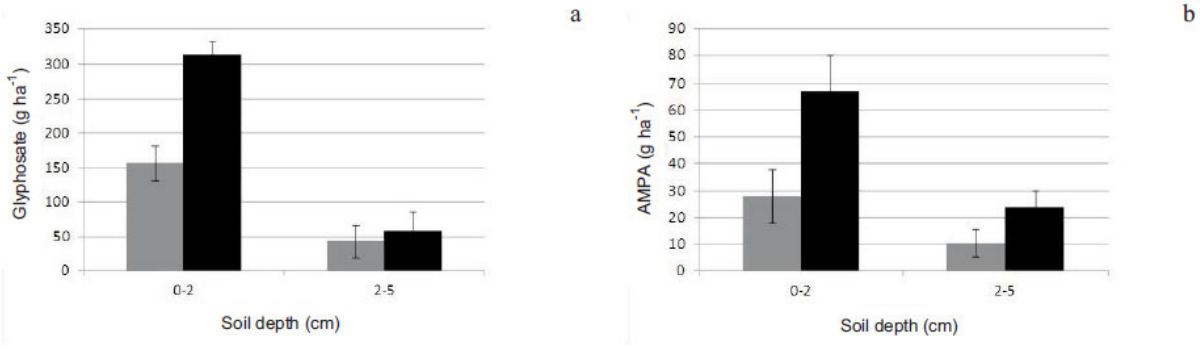


Figure 8.1.1.3-11: Total soil loss of the investigated soils after the rain simulation experiments (averages of 3 field replications).

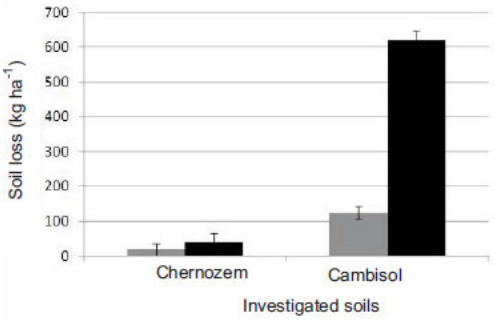


Figure 8.1.1.3-12: Glyphosate concentrations in natural run-off retention basins outside the experimental fields

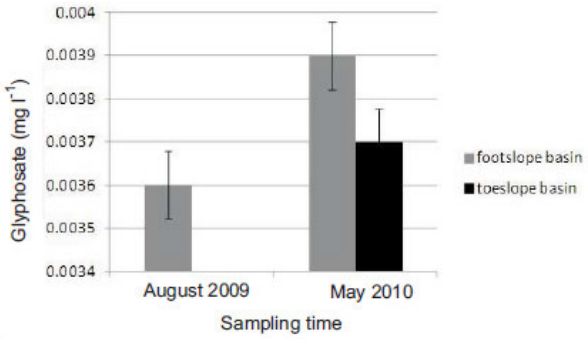
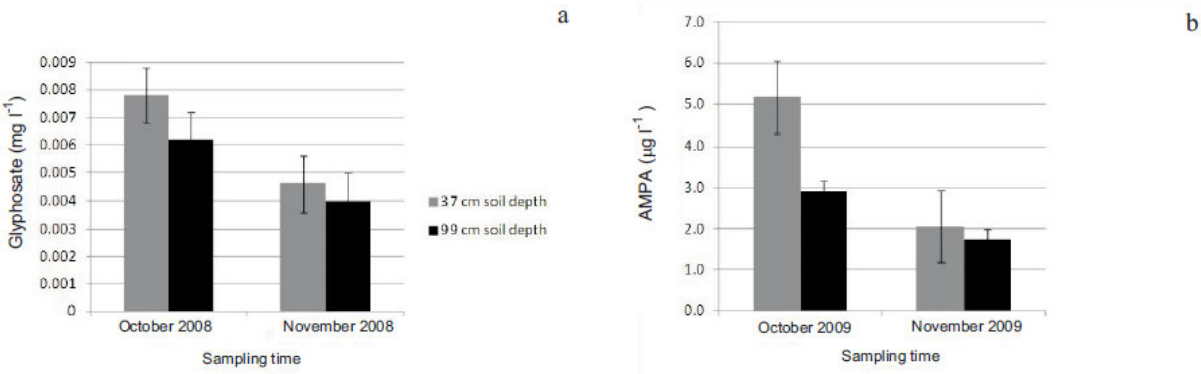


Figure 8.1.1.3-13: Concentrations of: a – glyphosate, and b – AMPA in percolation water at 2 different times and soil depths



Conclusions

1. The rain simulation experiments clearly showed that even in a potentially high glyphosate adsorbing soil like the Cambisol, erosion and surface run-off can lead to severe glyphosate loss if the soil structure state *eg* compaction degree, crusting, infiltrability, pore size distribution, in the case of erosive precipitations shortly after Roundup Max application, is unfavourable. In this study, in one of the NT-plot repetitions, up to 47% of the applied glyphosate amount were dispersed with run-off.

2. Traces of glyphosate in collected percolation soil water at Pixendorf, probably from previous conventional field application of Roundup Max, confirmed the general low glyphosate adsorption capacity of Chernozems from Loess and the risk of transport towards groundwater.

3. Analysis of water from run-off retention basins in the landscape in the surroundings of the investigated Chernozem confirmed that through high erosion processes, especially in maize crop, glyphosate is partly transported outside the treated agricultural fields.

Assessment and conclusion by applicant:

The article describes the runoff behavior of glyphosate and AMPA in two field experiments in two different European agricultural soils with artificial rainfall. No details on the description of the analytical method and of statistical analysis are provided.

In addition, water samples from percolation water and from two run-off retention basins were analyzed for glyphosate and AMPA but no details on experimental design, sampling or analytical method are given.

The article is therefore classified as reliable with restrictions for the runoff experiment while the results for percolation water and the run-off retention basins are considered not reliable.

Assessment and conclusion by RMS:

This article focuses on the impact on the agricultural management of the soil on the runoff of glyphosate and AMPA in soil. As mentioned by the applicant, further information would be needed to ensure its validity. It provides confirmation that the soil management and rainfall impacts the dissipation of glyphosate and its transfer. This article is informative. However it does not provide information on data requirement "7.1.2.2.1 Soil dissipation studies".

Tush et al. 2018

Data point:	CA 7.1.1.3
Report author	Tush D. *et al.*
Report year	2018
Report title	Dissipation of polyoxyethylene tallow amine (POEA) and glyphosate in an agricultural field and their co-occurrence on streambed sediments
Document No	The Science of the total environment (2018), Vol. 636, pp. 212-219
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The environmental fate of polyoxyethylene tallow amine (POEA), an additive in glyphosate herbicide formulations, has not been studied. This study examined the dissipation of POEA; glyphosate; and aminomethylphosphonic acid (AMPA), a degradation product of glyphosate, in the top 45 cm of soil from an agricultural field where glyphosate was applied. The concentration of these compounds was

also analyzed in bed sediment samples from watersheds in agricultural and urban areas from six states (Georgia, Hawaii, Iowa, Mississippi, North Carolina, South Carolina). The field studies show that POEA, glyphosate, and AMPA persist on the soil from planting season to planting season but dissipate over time with little migration into deeper soil. POEA, glyphosate, and AMPA were found on the bed sediment samples in urban and agricultural watersheds.

Materials and methods

Chemicals

POE 15 tallow amine and POE 5 tallow amine technical mixtures (ChemService Inc.,West Chester, PA) were used as the POEA standards. Glyphosate and AMPA standards were also obtained as powders from Chem Service Inc. Isotopically labeled standards of glyphosate ($^{13}C_2$, ^{15}N) and AMPA (^{13}C, ^{15}N, D_2) were obtained from Cambridge Isotope Laboratories (Woburn, MA) for use as internal standards. The test soil (characterized by the Kansas State Research and Extension Soil Testing Lab: loam, 3.2% organic matter) was collected near Fourmile Creek in Iowa from a pasture that was not used for crop production to the best of our knowledge.

Field dissipation study

Field soil samples were collected from an active agricultural field (silt loam, 5.7 % OM, low potential for erosion and slow run-off) to which glyphosate had been applied over the period of a year as an add-on to the National Water Quality Assessment Program (NAWQA). The samples were obtained from an active tile-drained agricultural field from the Leary Weber Ditch Basin in Sugar Creek watershed in Indiana were used to examine the transport of glyphosate and AMPA in 2004–2005. The study site was planted in corn in 2003 and rotated into Roundup Ready soybeans in 2004. Soil core samples were collected in April 2004 before glyphosate formulation application, in May 2004 soon after the first glyphosate formulation application, in July 2004 before the second glyphosate formulation application, in October 2004 after harvest, and in April 2005 after winter. Soil core samples were collected from three random locations on the field to a depth of 45 cm during each sampling period using a stainless-steel manual corer. Each soil core was then divided into three depth intervals of 0–15, 15–30, and 30–45 cm and placed into baked wide-mouth glass jars. The samples were then shipped overnight on ice before being stored at−20 °C until thawed for sample processing. Daily rainfall (totals) were measured on-site.

Streambed sediment samples

Streambed sediment samples were collected from 2006 to 2014 in Georgia, North Carolina, and South Carolina, Hawaii, Iowa and Mississippi. Samples were collected using a stainless-steel scoop into baked wide-mouth glass jars and shipped overnight on ice. Sediment samples were subsequently frozen at −20 °C until thawed for processing. After thawing, the samples were placed on aluminum foil and homogenized. A 5 g aliquot was taken by subsampling 10 different portions of the sample spread out on the foil for analysis of glyphosate and AMPA. Two 1 g aliquots were taken by subsampling 3 or more locations in the jar after the samples were homogenized and used for POEA analysis (one unspiked and one spiked for use in the standard addition calculation).

Preparation of standard solutions

A stock solution of POEA was made before each experiment at an approximate concentration of 10 mg/mL. Approximately 0.1 g of POEA was diluted to 10 mL with acetonitrile in a volumetric flask. The POEA solution used for spiking was made by serial dilutions from the stock solution in 2-mL vials using acetonitrile. The POEA stock solution and dilutions were disposed of after each experiment.

Stock solutions of glyphosate and AMPA (1 mg/mL) were made in acetonitrile from neat standards and stored at 4 °C in high density polyethylene bottles. A standard mix solution of glyphosate and AMPA (1 ng/µL each) was prepared in Type I water from the stock solutions. An internal standard mix of labeled glyphosate and labeled AMPA (1 ng/µL each) was made in Type I water.

Generation of spiked test soil for POEA extraction and quantitation

Two different aliquots of the test soil (Fourmile Creek, IA) were treated with POEA. A mass of water sufficient to saturate the test soil was added to a Pyrex beaker and spiked with POEA. An aliquot of the

test soil was then added to the spiked water and thoroughly stirred to disperse the POEA evenly through the soil. One of the test soils was spiked with POE 15 tallow amine (81 ng/g) and the other spiked with POE 5 tallow amine (56 ng/g). The test soils were left for an hour before being divided into 1 g aliquots (based on the dry weight of the test soil) for extraction and analysis.

Sample analysis
POEA
POEA was extracted and analyzed from sediment samples, which included accelerated solvent extraction (ASE) followed by ultra-high performance liquid chromatography (UPLC)/time of flight mass spectrometry (TOF-MS). All samples were held frozen until analysis in 2015 and aliquots were air dried. For the test soil and for the field soil samples, four separate aliquots were extracted by ASE. The first aliquot was not spiked and each remaining aliquot was individually spiked with a different, increasing amount of POE 15 tallow amine solution in acetonitrile when added to the ASE cell. For the sediment samples, two separate aliquots were extracted by ASE. The first aliquot was not spiked and the second was spiked with POE 15 tallow amine when added to the ASE cell. The ASE cells were then left open for ~15 min to allow the acetonitrile to evaporate. The samples were then extracted, analyzed, and quantitated using standard addition. The concentration of POEA was calculated based on the sum of the areas of all detected homologs. Two assumptions are made for the quantitation of POEA; that each homolog gives the same molar response as every other homolog and that the molar spike concentration is a known quantity based on the mass added and the average molecular mass of the distribution.

Glyphosate and AMPA
Glyphosate and AMPA were extracted from solid samples by adding a 5 g sample aliquot to a 50-mL polypropylene centrifuge tube with a screw top cap and adding 25 mL of 0.5 M potassium hydroxide. A stable isotope-labeled glyphosate and AMPA solution (100 µL at 1 ng/µL each) was added to each centrifuge tube. To the spiked samples, a standard of glyphosate and AMPA (100 µL at 1 ng/µL each) was added to each centrifuge tube. Standard curves were generated by adding 100 µL of the stable isotope-labeled solution and the appropriate amount of standards to 50-mL polypropylene centrifuge tubes containing 25 mL of 0.5 M potassium hydroxide. All the samples and standard were placed on a shaker table for 45 min. The samples were subsequently centrifuged for 10 min at 5000 ×g. A 5-mL aliquot of the centrifuged supernatant then was pipetted into a 19-mL polystyrene round bottom test tube with a screw top. The supernatant aliquots were then derivatized with 9-fluorenylmethoxycarbonyl chloride (FMOC) by adding 2 mL of a 5 mM FMOC solution and then incubating for 24 h in a 40 °C enclosed water bath.

After derivatization, the reaction then was quenched by adding 800 µL of a 2% phosphoric acid solution. The pH then was adjusted to 6 using 0.5 M hydrochloric acid and then adjusted to 9 using a 5% sodium borate solution. A 1-mL aliquot of the sample then was pipetted into 2-mL clear glass autosampler vials and stored in the dark at 4 °C until analysis.

Samples were analyzed using an Acquity H-class Bio UPLC (Waters Corp., Milford, MA) with a Triple Quad 5500 system (AB Sciex, Framingham, MA) in positive electrospray ionization (ESI) mode. Glyphosate and AMPA derivatives were separated by injecting 100 µL of sample and using 5 mM aqueous ammonium acetate and acetonitrile gradient separation on a Waters Acquity BEH column (2 × 50 mm, 1.7 µm packing) at 40 °C. Two multiple reaction monitoring (MRM) transitions were measured for each analyte. Identification was based on the retention time and the ion ratio of the two transitions. Quantitation was conducted using a linear 1/x weighted external standard curve.

Results
POEA extraction and analysis methods
The method of standard addition was examined to obtain some quantitative understanding of the concentration of POEA in agricultural soil and streambed sediment relative to glyphosate and AMPA. The lack of stable isotope-labeled standards and the potential for matrix effect disparities in using an external standard curve made the method of standard additions the best option for quantitation. The recovery of POE 15 tallow amine was 36% ± 3%. The recovery data indicate that the slight difference

between adding the initial aqueous spike to the whole soil sample and adding the subsequent standard addition spikes in organic solvent on the soil sample aliquot in the extraction cell has a substantial effect on the degree to which POEA is adsorbed and that some fraction of POEA is not readily recoverable from the soil with this method.

The test soils are initially spiked with POEA and saturated with water to simulate the aging of POEA on an environmental sample, whereas the standard addition spikes were added on the sample in the ASE cell. Concentrations of POEA determined by standard addition underestimated the spiked concentration by a factor of nearly threefold. The standard addition spike may be more easily extractable because it only comes in contact with a small part of the soil, it has less contact time with the soil before extraction, or differences in the interaction due to the different polarities of the solvents. The data also show that POE 5 tallow amine had a recovery of 29% ± 4%. The apparent recovery of POE 5 tallow amine assumed to be caused by larger adsorption constant of POEA homologs, but the assumption that every homolog generates the same instrument response cannot be ruled out without further experimentation.

The data comparing single point standard addition to multipoint standard addition is shown in the following table and the average result for POE 15 tallow amine shows no difference. As would be expected, using fewer points in the standard addition calculation increases the standard deviation (3% and 4% up to 8% in both cases).

Table 8.1.1.3-152: Comparison of single and multipoint standard additions. Additions were 81 ng and 56 ng for POE 15 and POE 5 tallow amine respectively on 1.0 g of test soil. Number of replicates = 3.

Number of additions	POE 15 tallow amine			POE 5 tallow amine		
	Average (µg/kg)	Standard deviation	Percent of actual	Average (µg/kg)	Standard deviation	Percent of actual
3	30	3	36%	16	4	29%
1	29	8	36%	15	8	27%

Dissipation of POEA, glyphosate, and AMPA

The results of the POEA analysis (not corrected for extraction recovery) are shown in table below and are compared with the glyphosate and AMPA results for the 0–15 cm segment. The 4/15/2004 sample contains POEA and was collected before the first glyphosate formulation application of the year. Because no glyphosate formulation application was recorded in 2003, the POEA on these samples was from a prior application, likely in 2002 when the field was planted in soybeans, or from another source (e.g. drift from neighboring fields). This indicates the possibility that POEA is persistent from year to year. There is a large increase in POEA concentration in the 0 to 15 cm interval of the soil after application of a glyphosate formulation, indicating that the glyphosate formulation applied contained POEA. This increase in POEA concentration following the formulation application is followed by decreasing concentrations through 10/21/2004. Some of the decrease in POEA concentration can be attributed to the loss of the C_{18}u homologs relative to the ratio of the homologs in the formulation, but this would not account for full extent of the loss. The remaining losses are caused either by the overall degradation of POEA, an increase in non-extractable POEA, or the transport of POEA away from the field. POEA appear to migrate downward to the 15–45 cm core intervals, but the concentrations in the deeper soil are much lower than in the 0–15 cm interval throughout the course of the study. It is unclear why the concentration of POEA appears to increase from 10/21/2005 sample to the 4/19/2005 sample as there was no recorded glyphosate formulation treatment to the field. If there is an actual increase in concentration of POEA on the field over that time some possibilities for this increase include: an application of some other treatment that also contains POEA (perhaps even unintentional, i.e. a POEA-contaminated application tank), drift from a neighboring field, or because of sample inhomogeneity. Otherwise, the 10/21/2005 sample is an anomaly, likely caused by sample inhomogeneity. Actual POEA concentrations in the soil were likely greater than those shown in tables below because analytical recovery was 36% as determined in the laboratory. The sample collected on 5/24/2004 may have had POEA in excess of 1 mg/kg in the shallow soil if a correction is applied for the low method recovery.

The distribution of homologs in the 0–15 cm segments of the soil core samples for the first four sample dates are shown in the figures. The $C_{18}u$ homologs of POEA in the soil core samples are lower in relative concentration than in the POEA technical mixtures. There appears to be a slight shift in the distribution to lower masses (i.e. few total ethoxylate groups) on the more aged POEA. The homolog distribution for the 5/24/2004 sample has almost a bimodal appearance for the C16s and C18s moieties with POEA having been added more recently.

Glyphosate follows a similar trend to that of POEA. There is some residual glyphosate remaining in the soil prior to the recorded applications, <20 µg/kg. There is a large increase in glyphosate concentration in the soil following the applications, and then a decrease in concentration throughout the remainder of the study. Interestingly the concentration of glyphosate is less than the concentration of POEA in the 0–15 cm soil layer in the first post application sample and yet the mass of POEA is probably 30% or less than the mass of glyphosate in the formulation. This indicates that more of the POEA is adsorbed to the soil than glyphosate and that the loss of POEA is slower than glyphosate. Unlike POEA, there is no increase in concentration of glyphosate on the 0–15 cm segment of the 4/19/2005 sample, although there is a slight increase in the 15–30 cm segment. This suggests that the increase of POEA on the 0–15 cm segment of the 4/19/2005 sample was not caused by an undocumented glyphosate formulation application.

As with both POEA and glyphosate, there is AMPA in the soil from previous glyphosate formulation applications. The concentration of AMPA in the 0 to 15 cm soil layer remains unchanged after the applications of glyphosate, but increases in the 7/7/2004 sample. The increase of AMPA concentration is delayed from the application relative to both POEA and glyphosate because AMPA is not applied directly but is a degradation product of glyphosate.

Table 8.1.1.3-153: Concentration of POEA, glyphosate, and AMPA in soil.

Date	POEA (µg/kg)			Glyphosate (µg/kg)			AMPA (µg/kg)		
	Depth 0–15 cm	Depth 15–30 cm	Depth 30–45 cm	Depth 0–15 cm	Depth 15–30 cm	Depth 30–45 cm	Depth 0–15 cm	Depth 15–30 cm	Depth 30–45 cm
4/15/2004	98	NA	NA	18	NA	NA	110	NA	NA
5/24/2004	420	25	5.5	220	12	11	110	12	8.5
7/7/2004	320	6.7	3.7	110	34	13	180	24	15
10/21/2004	77	6.2	6.9	36	<1.0	<1.0	120	3.0	<1.0
4/19/2005	230	12	6.3	4.7	1.4	<1.0	41	8.9	5.7

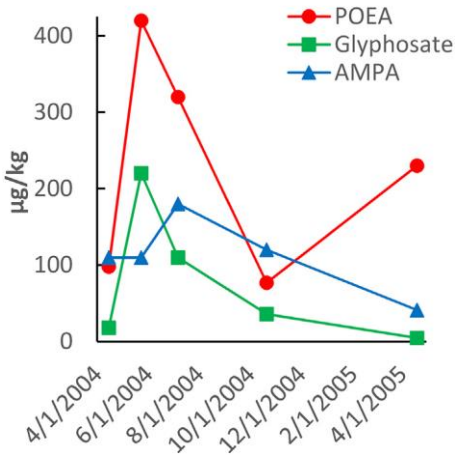


Figure 8.1.1.3-14: Soil concentration of POEA, Glyphosate, and AMPA on the 0–15 cm segment.

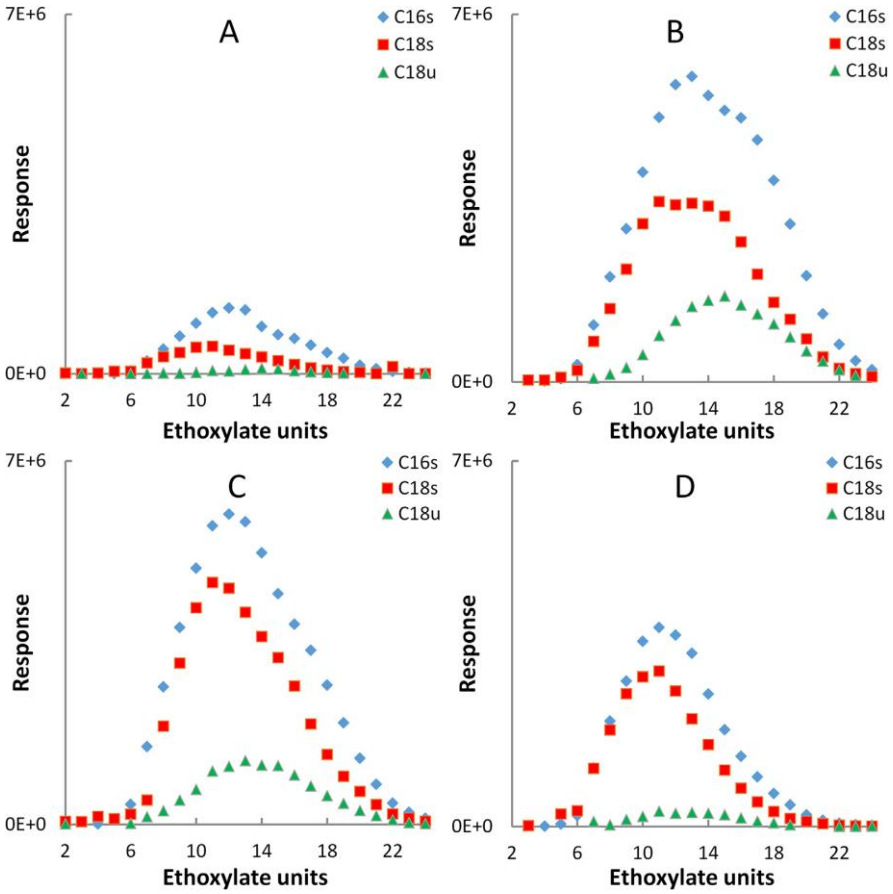


Figure 8.1.1.3-15: Distribution of POEA homologs on soil core samples (0–15 cm depth). (A) 4/15/2004, 98 µg/kg total POEA; (B) 5/24/2004, 420 µg/kg total POEA; (C) 7/7/2004, 320 µg/kg total POEA; (D) 10/21/2004, 77 µg/kg total POEA. Figure shows instrument response, not homolog concentration.

Co-occurrence of POEA and glyphosate on streambed sediment

A set of samples for which glyphosate and/or AMPA had been detected were chosen to assess whether POEA occurs on bed sediment in streams and rivers from areas where glyphosate is applied. The data show that each sample analyzed contained quantifiable concentrations of POEA, along with glyphosate and/or AMPA. These results suggest that in areas where glyphosate is used in agriculture or urban settings, POEA will likely be found on the streambed sediments.

Representative distributions of POEA found on streambed sediment sample 11 (Bogue Phalia, MS) and the same sample spiked with POE 15 are shown in figures below. The homolog distributions on sediment are similar to those found on aged agricultural soil samples; the $C_{18}u$ homologs are much lower than would be expected based on the distribution found in the technical mixtures. The process that $C_{18}u$ homologs are degraded remains an unanswered question, but the loss of the $C_{18}u$ homologs is shown on both agricultural field soil and in the streambed sediments. It is unclear if the $C_{18}u$ homologs degrade before they are transported to the stream and deposited in streambed sediments or if the $C_{18}u$ homologs can be transported from the field to the streambed sediments and then continue to be degraded. It is also not known whether the occurrence of POEA on streambed sediment originated from POEA-contaminated soil particles that are transported from the field that subsequently settle into the streambed sediment or the dissolved transport of POEA followed by redistribution into suspended and streambed sediment. The shift in the number of ethoxylate groups can be seen in the $C_{18}u$ moiety group. In the unspiked sample, the most abundant is the EO11 moiety, but the EO15 is most abundant after spiking. The concentrations of glyphosate and/or AMPA are generally higher than the concentration of POEA on the bed sediments, even if it is assumed that only 1/3 of the sediment-adsorbed POEA was extracted, whereas the concentration of POEA was greater than glyphosate and AMPA in the soil samples. This suggests that both glyphosate and AMPA are more readily transported from the field than POEA.

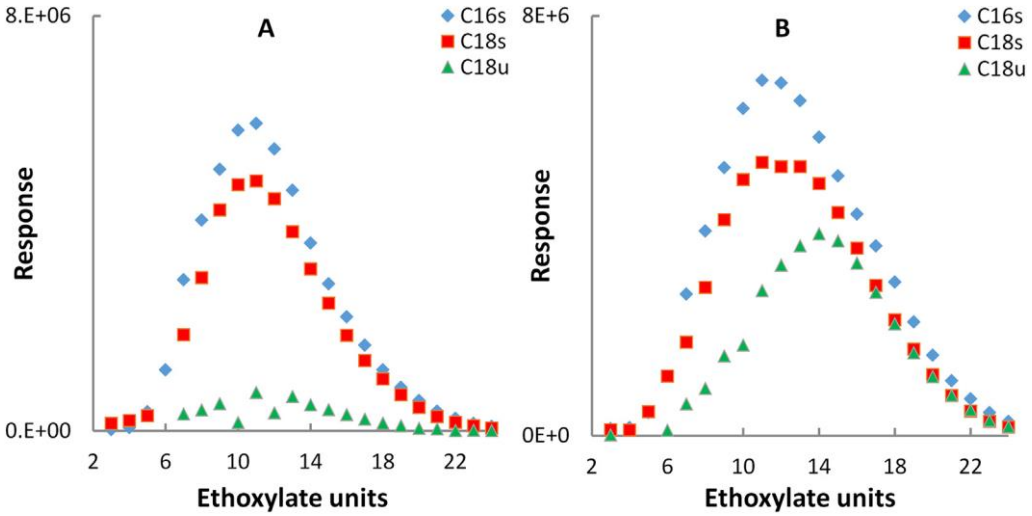


Figure 8.1.1.3-16: Distribution of POEA homologs on bed sediment samples. (A) Sample #11, 150 µg/kg total POEA, unspiked; (B) sample 11, spiked with POE 15 tallow amine. Figure shows instrument response, not homolog concentration.

Table 8.1.1.3-154: Summary of POEA, glyphosate, and AMPA concentrations in bed sediment from selected sites.

	State	River	Collection date	POEA (µg/kg)	Glyphosate (µg/kg)	AMPA (µg/kg)	Land use
1	GA	Big Creek	6/9/2014	1.3	240	160	Urban
2	GA	Nancy Creek	6/5/2014	25	210	150	Urban
3	GA	N. Fork Peachtree Creek	6/12/2014	9.8	480	480	Urban
4	GA	Sope Creek	6/4/2014	28	490	300	Urban
5	HI	Kapehu Stream	1/21/2014	13	500	630	Agricultural
6	IA	Beaver Creek	6/7/2006	3.1	1.5	<1.0	Agricultural
7	IA	South Fork Iowa River	11/13/2006	11	8.4	<1.0	Agricultural
8	IA	South Fork Iowa River	9/18/2007	8.4	9.0	4.6	Agricultural
9	IA	South Fork Iowa River	9/18/2007	4.2	5.6	7.4	Agricultural
10	MS	Bogue Phalia	5/16/2007	160	700	710	Agricultural
11	MS	Bogue Phalia	5/16/2007	150	1300	370	Agricultural
12	MS	Tommie Bayou	6/24/2008	110	370	620	Agricultural
13	NC	Ellerbe Creek	6/8/2014	12	50	52	Urban
14	NC	Little Hope Creek	6/10/2014	79	4800	130	Urban
15	SC	Enoree River	6/8/2014	10	72	71	Urban
16	SC	Wildcat Creek	6/11/2014	44	68	92	Urban

Conclusion

The field dissipation study shows that POEA persists on the soil from year to year with some degradation and may actually persist for 2 years or more after application. Concentrations of both glyphosate and AMPA decrease more rapidly than POEA on the field because glyphosate and AMPA are more readily transported off the field and degrade faster. These results suggest that POEA is likely a widespread contaminant on agricultural soils where glyphosate formulations are applied.

The examination of streambed sediments suggest that POEA could also be a widespread contaminant in the bed sediments of streams that drain areas where glyphosate formulations are applied. This potential widespread contamination of POEA may have existed since the mid 1990's when glyphosate resistant crops were released. POEA homologs with saturated tallow moieties are more persistent in the environment than those with unsaturated tallow moieties. There is some evidence of the degradation of the ethoxylate chains (i.e. lower number of ethoxylate units over time), but it appears to be a slow process.

POEA, glyphosate, and AMPA typically co-occur not only on the soils from agricultural fields but also in the bed sediments in streams draining areas where glyphosate was applied. The concentrations of POEA over time were higher than those of glyphosate and AMPA on the field soils, but lower in the streambed sediments. That POEA, glyphosate, and AMPA persist and are transported together in the environment is a novel discovery.

Assessment and conclusion by applicant:

Study was conducted in the US but provides data on POEA, glyphosate, and AMPA adsorption and dissipation in top 45 cm of soil and in stream bed sediments. Conclusions useful in qualitative rather than quantitative way.

Assessment and conclusion by RMS:

Adsorption and dissipation of glyphosate in soil were studied in this article. Information on substance used (purity) is not indicated, the stability of the test item is not provided, nor the mass balance or efficacy of the extraction method. The soil used has a 5.7% OM content, which is above OECD recommendations for dissipation. Additionally no raw data are available.

The article provides supportive information on the dissipation and adsorption of glyphosate but no reliable endpoints can be derived for use in risk assessment.

B.8.1.1.3.5. Soil accumulation studies

Field accumulation studies have not been performed. Potential accumulation of glyphosate and AMPA is addressed by calculations (see Vol. 3 CP).

B.8.1.1.4. *RMS' summary on rate of degradation in soil*

B.8.1.1.4.1. Laboratory studies

The rates of degradation of glyphosate and its metabolite AMPA were evaluated following the recommendations of the FOCUS Kinetic guidance. A summary of laboratory trigger and modelling endpoints is presented in the following tables.

The rate of degradation of glyphosate in standard dark aerobic laboratory studies has been determined in 10 different soils at 20/25°C. The degradation of glyphosate is mostly better described by biphasic kinetics. The trigger DT_{50} and DT_{90} values of glyphosate range from 0.7 to 78.9 days and from 14.9 to 1660 days, respectively.

Modelling DT_{50} values for glyphosate for modelling in parent-only fits range from 2.2 to 161.1 days (pooling SFO kinetics, FOMC DT_{90}/3.32 and slow phase DFOP DT_{50} as recommended in FOCUS kinetics guidance). Corresponding modelling DT_{90} (used for assessment of pH dependence) range from 7.2 to 378.4 days.

Modelling DT_{50} values for glyphosate for modelling in a pathway fit were based on DFOP kinetics, also when <10% parent remains, because FOMC kinetics (leading to DT_{50}=DT_{90}/3.32) cannot be applied in a linked model run with a metabolite. In the pathway fit, normalized modelling DT_{50} ranged between 0.1 and 10 days for fast-phase and between 2.4 and 161.1 days for slow-phase. Normalised modelling DT_{90} values (used for the assessment of pH dependence) range between 6.4 and 378.4 days.

Based on the available modelling values, pH dependence cannot be excluded, with higher persistence with decrease of soil pH (see detailed evaluation in B 8.1.1.4.3 below).

The rates of degradation of metabolite AMPA are mostly issued from parent-applied studies and were also investigated in three soils under dark aerobic laboratory conditions in AMPA-applied studies. Degradation of AMPA followed single-first-order degradation. The trigger DT_{50} and DT_{90} values of AMPA range from 28.6 to 1040 days and from 95 to 3450 days, respectively. Modelling DT_{50} were in the range of 13-1040 days, with formation fraction of 0.196-0.480 (mean 0.29) from glyphosate. Based on the available values, pH dependence cannot be excluded, with higher persistence with decrease of soil pH (see detailed evaluation in B 8.1.1.4.3 below).

Under anaerobic laboratory conditions glyphosate does not degrade significantly.

Summary on trigger endpoints

Glyphosate - trigger

Rate of degradation in soil (aerobic) laboratory studies glyphosate (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.1.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.1)

Parent	Dark aerobic conditions – Trigger endpoints					
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50} /DT_{90} (d)	Kinetic parameters	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	8.8/57.3	k_1: 0.2138 k_2: 0.03023 g: 0.4345	2.9	DFOP
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	2.3 / 14.9	α: 1.414 β: 3.635	4.2	FOMC
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	3.9 / 38.7	k_1: 0.3125 k_2: 0.03172 g: 0.6584	5.0	DFOP
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	78.9 / 588	k_1: 0.05856 k_2: 0.003146 g: 0.3644	3.4	DFOP
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	0.7 / 16.2	k_1: 2.306 k_2: 0.08875 g: 0.58	8.2	DFOP
[redacted] (1995): Arrow Sandy loam	6.4[a]	20 / 40	37.8 / 1660	α: 0.4539 β: 10.47	2.3	FOMC
[redacted] (1993): Les Evouettes Silt loam	6.1[b]	20 / 40	11.5 / 358	α: 0.51 β: 3.96	5.9	FOMC
[redacted] (1993): Speyer 2.2 Sand	6.0[b]	20 / 40	2.0 / 151	k_1: 8.104 k_2: 0.01078 g: 0.4893	8.6	DFOP
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[b]	20 / 40	6.2 / 20.4	k: 0.1127	8.0	SFO
[redacted] (1992):	6.9	20 / 40	9.0/ 63.7	k1:0.3685	9.7	DFOP

Speyer 2.1, dose group A Sand				k2: 0.02889 g: 0.3702		

[a] Calculated with equation reported in EFSA guidance 2017[9]: $pH_{H2O}=0.982pH_{CaCl2} + 0.648$.
[b] Medium not reported, H_2O assumed

AMPA - trigger

Rate of degradation in soil (aerobic) laboratory studies AMPA (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.1.2 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.1)

AMPA	Trigger endpoints Dark aerobic conditions Metabolite dosed or the precursor from which the f f. was derived was glyphosate						
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50}/ DT_{90} (d)	f. f. k_f / k_{dp}	Kinetic parameters	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	112 / 373	0.1955	k: 0.006181	7.6	SFO
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	28.6 / 95.1	0.3000	k: 0.02421	3.5	SFO
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	88.2 / 293	0.2004	k: 0.007863	6.2	SFO
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	1000 / 3320	0.2618	k: 0.00069	9.2	SFO
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	96.4 / 320	0.2793	k: 0.007187	10.1	SFO
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[a]	20 / 40	79.2 / 263	0.3406	k: 0.008753	8.2	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	200 / 666	0.4796	k: 0.003459	3.2	SFO
[redacted] (2017): Warsop Loamy sand	4.71	20 / pF 2	326 / 1080	-	k: 0.002128	1.3	SFO
[redacted], 2020: 18-Acres Sandy clay loam	5.5	20 / pF 2	1040 / 3450	-	k: 0.000666	3.0	SFO
[redacted], 2020: Brierlow, Silt loam	5.7	20 / pF 2	1000 / 3320	-	k: 0.000693	3.2	SFO

[a] Medium not reported, H_2O assumed

Summary on modelling endpoints

Glyphosate - modelling

9 EFSA (European Food Safety Authority), 2017. EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2017;15(10):4982, 115 pp. https://doi.org/10.2903/j.efsa.2017.4982

Two tables are provided regarding modelling endpoints for glyphosate. The first-one provides endpoints derived from parent-only fits and could be used for modelling of the parent only.

The second one provides endpoints from pathway fits (Glyphosate → AMPA) and should be used when AMPA is included in modelling.

Rate of degradation in soil (aerobic) laboratory studies glyphosate (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.1.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.1)

Parent		Dark aerobic conditions – **Modelling endpoints based on parent-only fits**						
Soil	pH (H_2O)	t. °C / % MWHC	Actual DT_{50} /DT_{90} (d)	Modelling DT_{50} (not normalized)[a]	DT_{50} (d) 20 °C pF2/10kPa[b]	DT_{90}[e] (d) 20 °C pF2/10kPa[a]	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	9.0/60	18.1	9.9	32.0	4.0	FOMC
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	2.3/15	4.5	2.2	7.2	4.2	FOMC
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	4.0/37	11.1	5.1	17.0	4.5	FOMC
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	76.3/523	192.6	109.8	298.1	2.6	DFOP
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	1.0/20.1	6.1	6.5	21.7	8.6	FOMC
[redacted] (1995): Arrow Sandy loam	6.4[c]	20 / 40	37.4/440	187.3	161.1	378.4	3.6	DFOP
[redacted] (1993): Les Evouettes Silt loam	6.1[d]	20 / 40	11.5/358	107.8	71.2	236.3	5.9	FOMC
[redacted] (1993): Speyer 2.2 Sand	6.0[d]	20 / 40	2.0/151	64.3	44.4	104.2	8.6	DFOP
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[d]	20 / 40	6.1/20.3	6.1	3.2	10.8	8.0	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	6.0/165	49.7	49.7	165.0	6.8	FOMC
pH dependence					Yes, glyphosate is more persistent with decreasing pH			

[a] DT_{90}/3.32 for FOMC kinetics; ln(2)/k2 value for DFOP kinetics

[b] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7

[c] Calculated with equation reported in EFSA guidance 2017[4]: $pH_{H2O}=0.982pH_{CaCl2} + 0.648$.

[d] Medium not reported, H_2O assumed

[e] Modelling DT_{90} also reported since it is used to assess pH-dependency

Parent	Dark aerobic conditions – **Modelling endpoints based on pathway fits** (glyphosate → AMPA)							
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50} /DT_{90} (d)	Kinetic parameters	Fast | Slow DT_{50} (d) 20 °C pF2/10kPa[a]	DT $_{90}$[d] (d) 20 °C pF2/10kPa[a]	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	8.8 / 57.3	k_1: 0.2138 k_2: 0.03023 g: 0.4345	1.8 | 12.6	31.5	2.9	DFOP
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	2.3 / 13.4	k_1: 0.9889 k_2: 0.1375 g: 0.3704	0.3 | 2.4	6.4	4.8	DFOP
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	3.9 / 38.7	k_1: 0.3125 k_2: 0.03172 g: 0.6584	1.0 | 10.1	17.8	5.0	DFOP
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	78.6 / 588	k_1: 0.05856 k_2: 0.003146 g: 0.3644	6.7 | 125.6	335.2	3.4	DFOP
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	0.7 / 16.2	k_1: 2.306 k_2: 0.08875 g: 0.58	0.3 | 8.4	17.5	8.2	DFOP
[redacted] (1995): Arrow Sandy loam	6.4[b]	20 / 40	37.4 / 440	k_1: 0.0595 k_2: 0.0037 g: 0.4852	10.0 | 161.1	378.4	4.7	DFOP
[redacted] (1993): Les Evouettes Silt loam	6.1[c]	20 / 40	9.8 / 192	k_1: 0.2084 k_2: 0.008013 g: 0.5339	2.2 | 57.1	126.7	6.3	DFOP
[redacted] (1993): Speyer 2.2 Sand	6.0[c]	20 / 40	2.0 / 151	k_1: 8.104 k_2: 0.01078 g: 0.4893	0.1 | 44.4	104.2	8.6	DFOP
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[c]	20 / 40	6.2 / 20.4	k: 0.1127	3.3	10.8	8.0	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	9.0 / 63.7	k_1:0.3685 k_2: 0.02889 g: 0.3702	1.9 | 24.0	63.7	9.7	DFOP
pH dependence					Yes, glyphosate is more persistent with decreasing pH			

[a] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[b] Calculated with equation reported in EFSA guidance 2017[10]: pH_{H2O}=0.982pH_{CaCl2} + 0.648.
[c] Medium not reported, H_2O assumed
[d] Modelling DT_{90} also reported since it is used to assess pH-dependency

AMPA - modelling

Rate of degradation in soil (aerobic) laboratory studies AMPA (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.1.2 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.1)

10 EFSA (European Food Safety Authority), 2017. EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2017;15(10):4982, 115 pp. https://doi.org/10.2903/j.efsa.2017.4982

AMPA	Modelling endpoints Dark aerobic conditions Metabolite dosed or the precursor from which the f f. was derived was glyphosate						
Soil	pH (H_2O)	t. °C / % MWHC	DT_{50}/ DT_{90} (d)	f. f. k_f / k_{dp}	DT_{50} (d) 20 °C pF2/10kPa[b)]	St. (χ^2)	Method of calculation
[redacted] (2010): Gartenacker Loam	7.1	20 / 50% pF2.5	112 / 373	0.1955	61.6	7.6	SFO
[redacted] (2010): Drusenheim Loam	7.4	20 / 50% pF2.5	28.6 / 95.1	0.3000	13.4	3.9	SFO
[redacted] (2010): Pappelacker Loamy sand	7.0	20 / 50% pF2.5	88.2 / 293	0.2004	40.6	6.2	SFO
[redacted] (2010): 18-Acres Sandy clay loam	5.7	20 / 50% pF2.5	1000 / 3320	0.2618	570	9.2	SFO
[redacted] (1996): Soil B Sandy loam	6.7	25 / 75 % FC	96.4 / 320	0.2793	104	10.1	SFO
[redacted] (1993): Speyer 2.3 Loamy sand	6.9[a]	20 / 40	79.2 / 263	0.3406	42	8.2	SFO
[redacted] (1992): Speyer 2.1, dose group A Sand	6.9	20 / 40	200 / 666	0.4796	200	3.2	SFO
[redacted] (2017): Warsop Loamy sand	4.71	20 / pF 2	326 / 1080	-	326	1.6	SFO
[redacted], 2020: 18-Acres Sandy clay loam	5.5	20 / pF 2	1040 / 3450	-	1040	3.0	SFO
[redacted], 2020: Brierlow, Silt loam	5.7	20 / pF 2	1000 / 3320	-	1000	3.2	SFO
Mean value (n=7)				0.29			
pH dependence					Yes, AMPA is more persistent with decreasing pH		

[a] Medium not reported, H_2O assumed

B.8.1.1.4.2. Field studies

The rates of degradation of glyphosate and its metabolite AMPA were evaluated following the recommendations of the FOCUS Kinetic guidance and EFSA $DegT_{50}$ guidance.

Information on the dissipation of glyphosate in soil under field conditions was investigated in several dissipation trials, conducted in Europe, USA and Canada. An Ecoregion Crosswalk exercise was performed to evaluate the representativeness of sites from outside EU for European conditions. A data gap has been set for the applicant to provide a comparison of actual field sites properties instead of default root ecoregions.

Several data gaps were also identified regarding the kinetic analysis of the field data. Based on the currently available data, reliable endpoints could be obtained for a limited number of sites.

For glyphosate, reliable field $DissT_{50}$ (trigger endpoints) were obtained from a total of six sites. Degradation is biphasic. $DissT_{50}$ and $DissT_{90}$ range between 1.1-13.7 days and 54.4-201 days, respectively. RMS notes that no field dissipation study was performed in Southern Europe. However

one site in California is considered as representative of Southern Europe conditions (from the ecocrosswalk region comparison with ENASGIPS).

Reliable modelling $DegT_{50}$ were obtained from 2 sites only (32.6-46 days). pH dependence cannot be assessed due to the limited dataset. With only two field modelling DT_{50} considered reliable at this time of the assessment, normalized field data are pooled with laboratory values, following the EFSA $DegT_{50}$ guidance (2014).

Metabolite AMPA was analysed in the available field dissipation studies and occurred at a maximum occurrence of 46.9 %. No reliable trigger endpoints could be derived at this time (data gap on the kinetic fittings are identified on two soils). RMS highlights that for AMPA the two field DT_{50} would only cover a pH of 7.8. Since AMPA was shown to be more persistent in laboratory under acidic conditions, this range of pH investigated in field would not be sufficient. In any case, a data gap for additional field data is identified.

All of the field studies are "legacy studies" as qualified by the EFSA $DegT_{50}$ guidance. No modelling DT_{50} could be derived for AMPA since it occurred at more than 5% before 10 mm rain.

The field endpoints are summarised below.

Glyphosate

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Trigger endpoints

Parent	Aerobic conditions – trigger endpoints						
Soil	Location	pH	Depth (cm)	DT_{50} / DT_{90} (d) actual	Kinetic parameters	St. (χ^2)	Method of calculation
Egerkingen [redacted] (1992b) Clay loam (bare soil)	Switzerland	7.79 [a]	0-30	1.1 / 179	k_1: 2.653 k_2: 0.0087 g: 0.5228	5.3	DFOP
Bad Krozingen [redacted] (1992c) Sandy loam (bare soil)	Germany	6.6 [a]	0-30	2.7 / 122	α: 0.45 β: 0.7373	5.3	FOMC
Menslage [redacted] (1992d) Sand (bare soil)	Germany	5.6 [a]	0-30	5.8 / 201	k_1: 0.1781 k_2: 0.0041 g: 0.7704	9.4	DFOP
Ontario [redacted] (1993) Loamy sand (bare soil)	Canada	6.8[b]	0-45	13.7 / 54.4	k_1: 0.0551 k_2: 0.0017 g: 0.9420	22.3	DFOP
California [redacted] (1993a) Loamy sand (bare soil)	USA	6.3 [b]	0-121.9	13.0 / 102	k_1: 0.1124 k_2: 0.0148 g: 0.5490	12.7	DFOP
Ohio [redacted] (1993a) Loam (bare soil)	USA	7.8 [b]	0-121.9	2.4 / 61.5	k_1: 0.5430 k_2: 0.0194 g: 0.6704	13.3	DFOP

[a)]Measured in KCl in the study, converted to pH_{H2O} considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)
[b)]medium not given – value from the 0-15 cm depth layer

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Modelling endpoints

Parent		Aerobic conditions – modelling endpoints						
Soil	Location.	pH	Depth (cm)	DT_{50} (d) Norm[b].	Kinetic parameters	DT_{90}(d) Norm[b].	St. (χ^2)	Method of calculation
Menslage [redacted] (1992d) Sand (bare soil)	Germany	5.6 [a]	0-30	46.0	k_2: 0.0151	-	6.8	HS – slow phase
California [redacted] (1993a) Loamy sand (bare soil)	USA	6.3 [c]	0-121.9	32.6	k: 0.0213	108	22.0	SFO
New York [redacted] (1993a) Sandy clay loam (bare soil)	USA	5.8	0-121.9					Data gap, further fits required (following EFSA $DegT_{50}$ flowchart)

Please note that data gap are identified for further normalization of field values – see assessment under point 8.1.1.3.3

[a)] Measured in KCl in the study, converted to pH_{H2O} considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)

[b)] Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7, values are $DegT_{50}$ matrix

[c)] medium not given – value from the 0-15 cm depth layer

AMPA

Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1) - Trigger endpoints

AMPA	Trigger endpoints	Aerobic conditions Metabolite dosed or the precursor from which the f f. was derived was glyphosate						
Soil	Location	pH (H2O)	Depth (cm)	DT_{50} (d) actual	DT_{90} (d) actual	St. (χ^2)	f. f. k_f / k_{dp}	Method of calculation
Egerkingen [redacted] (1992b) Clay loam (bare soil)	Germany	7.79 [a]	0-30					Data gap for fit from parent
Ohio [redacted] (1993a) Loam (bare soil)	USA	7.8 [b]	0-121.9					Data gap for decline fit

[a)] Measured in KCl in the study, converted to pH_{H2O} considering the formula pH_{H2O} = 0:860pH_{KCl} + 1.482 presented in the EFSA guidance for predicting environmental concentration in soil (2017)

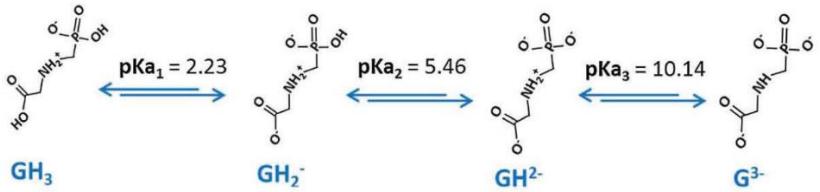
[b] medium not given – value from the 0-15 cm depth layer

No acceptable modelling field data are available for AMPA.

B.8.1.1.4.3. Glyphosate: Assessment of pH dependency and pool of laboratory and field modelling endpoints according to the EFSA $DegT_{50}$, 2014

Glyphosate comprises of one basic amino function and three ionizable acidic sites. It has a number of pKa (2.23, 5.46 and 10.14 according to Dollinger *et al.* 2015 – see point B 8.1.2; values consistent with validated pKa of 2.34 and 5.73 reported for glyphosate acid in the LoEP Phys-Chem section) and therefore exists as multiple species depending on pH, as presented below (Dollinger *et al.* 2015). At typical soil pH 5-9, the main species are GH_2^- and GH^{2-}, corresponding to net negative charges of one and two, respectively.

Speciation of glyphosate through the entire soil pH range (Dollinger *et al* (2015))



pH dependency - Laboratory values

The pH dependency of laboratory degradation rates of glyphosate was further investigated.

For laboratory endpoints, pH_{H2O} is reported in the above tables for all soils, with pH values measured in $CaCl_2$ or KCl converted in pH_{H2O} using the equation reported in EFSA guidance 2017.

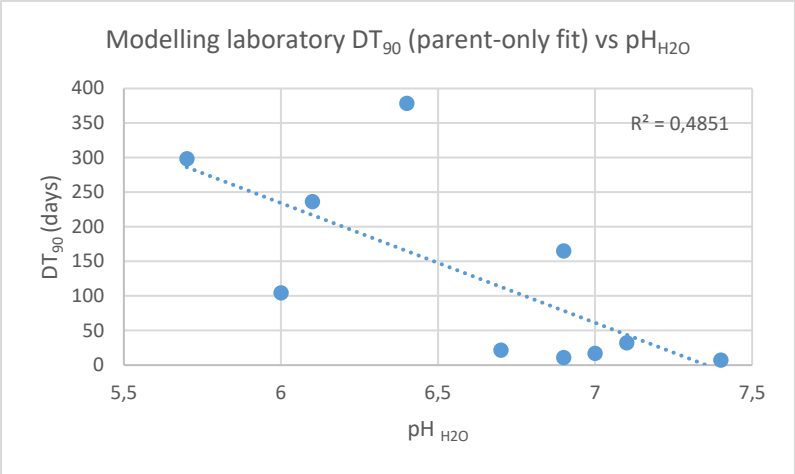
The matrix was not mentioned in 3 soils (Les Evouettes from [redacted] 1993; Speyer 2.2 and Speyer 2.3 from [redacted] 1993), it was assumed that the pH had been measured in H_2O. This is of minor impact since the objective is to check whether a pH-dependence can be observed, but there is no need to establish a true relation describing the pH dependent degradation (as this cannot be used in the FOCUS models). Additionally, considering the data and pH range of these three soils, if soil pH had been measured in another matrix than H_2O, it is not expected that any significant impact would be observed.

There is no clear guidance for assessing the pH dependency. From experience from previous evaluations, RMS notes that this assessment is generally performed based on modelling endpoints. In RMS opinion, this makes sense since the outcome of this check impacts the values to be selected for modelling (for soil exposure, pH-dependency is less relevant since the maximum trigger values are selected anyway). In addition, the use of normalised values ensures that no influence of moisture or temperature on the data would intervene. Finally, in case laboratory and field values are mixed, the use of normalised modelling endpoints allows comparing similar values.

In this case, since modelling endpoints are almost all derived from biphasic kinetics, RMS proposes to consider the normalised modelling DT_{90} for each soil for testing the relation with pH as DT_{90} better reflects the overall degradation of glyphosate in case of biphasic kinetic. RMS notes that usually the DT_{50} values are used for assessing the pH dependency of a compound, but in the case of biphasic kinetics, the time at which 50% of the compound is degraded is not as meaningful as for single order kinetics, since it does not take into account the slower degradation of the compound. The use of a DT_{90} allows a better appreciation of the overall degradation as 90% of the compound will have degraded at that time. In RMS opinion, the comparison based on DFOP slow phase may biaised the analysis since DT_{50} would be overestimated.

Modelling endpoints from parent-only fits

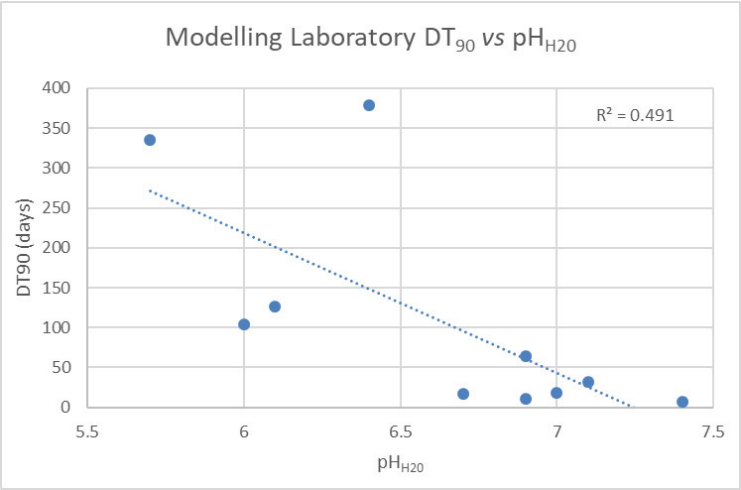
The following figure presents the relation between modelling normalized DT_{90} from parent-only fits and pH_{H20}.



RMS notes that there is no linear relation, but visually there is a trend for higher persistence at pH below 6.7. The Kendall test was applied to the dataset and indicates that there is a correlation between the degradation of glyphosate and the pH of the soils. The calculated Kendall tau was -0.539 with a t-test of 0.039, the pH dependency of normalized DT_{50} of glyphosate (parent-only endpoints) with soil pH(H_2O) is considered as significant.

Modelling endpoints from pathway fit

The following figure presents the relation between normalised laboratory modelling DT_{90} and pH_{H20}.



RMS notes that there is no linear relation, but visually there is a trend for higher persistence at pH below 6.7. The Kendall test was applied to the dataset and indicates that there is a correlation between the degradation of glyphosate and the pH of the soils. The calculated Kendall tau was -0.539 with a t-test of 0.039, the pH dependency of normalized DT_{90} of glyphosate with soil pH(H_2O) is considered as significant. It is noted that it gives the same results as presented above for endpoints from parent-only fits.

Based on the above evidence both with parent-only and pathway fit endpoints, pH-dependence should be taken into account for glyphosate in the exposure calculations.

pH dependency - field values

Field modelling endpoints are available on two soils only, which does not allow to check the relation between field degradation rates and pH.

Pool of laboratory and field modelling endpoints

Following the EFSA $DegT_{50}$ guidance (2014), a comparison of the acceptable field and laboratory derived $DegT_{50matrix}$ should be done for selection of endpoint to be used for modelling. It is however also indicated that this procedure may not be appropriate for substances for which the degradation is dependant of the pH of the soil.

For sake of being thorough, RMS still attempted to follow the EFSA $DegT_{50}$ flowchart.

Geomean laboratory modelling DT_{50} value for glyphosate was not determined due to pH-dependence, however it can be concluded that it would be < 240 days:

- For endpoints derived from parent-only fits: all modelling endpoints are < 240 days;
- For endpoints derived from pathway fits: all slow DFOP DT_{50} are below 240 days; all DT_{90} are below 240*3.32=797 days).

Therefore, next step is to test whether the field $DegT_{50}$ matrix are equivalent to the laboratory DT_{50} values. RMS notes that the 2 reliable field values were obtained from soils with pH_{H20}<6.7.

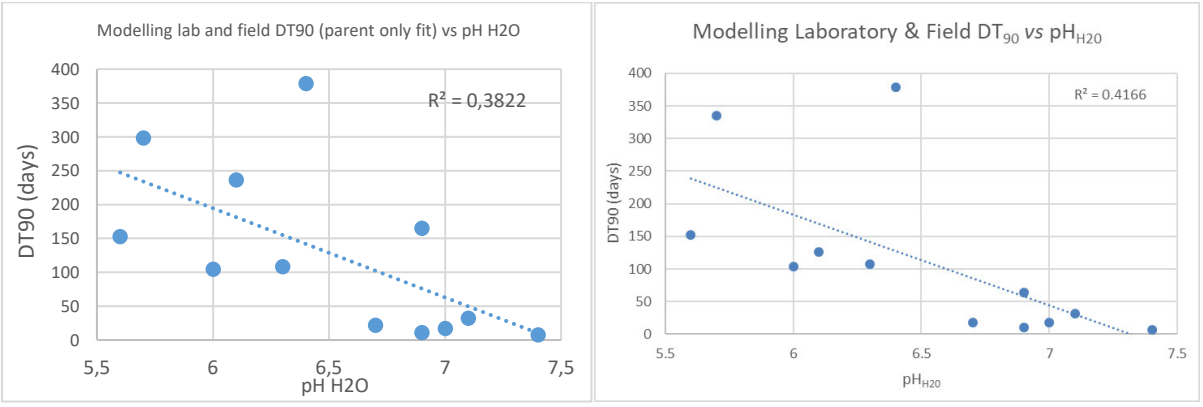
As previously, the comparison is based on DT_{90} values, in order to account for biphasic degradation. For endpoints from parent-only fits, field studies show equal $DegT_{90}$ to laboratory studies when considering the complete laboratory dataset; and they also show equal $DegT_{90}$ when only considering soils with pH_{H2O} < 6.7.

For endpoints from pathway fits, When comparing laboratory DT_{90} values and field DT_{90} values in the EFSA $DegT_{50}$ endpoint selector, it can be concluded that:

- field studies show equal $DegT_{90}$ to laboratory studies when considering the complete laboratory dataset;
- field studies show shorter $DegT_{90}$ than laboratory studies when considering only laboratory DT_{90} values for soils with pH_{H20} < 6.7.

Therefore, in any case, and since there are only 2 field DT_{50} values, the recommendation is then to pool laboratory and field values in order to select the endpoint to be used in modelling.

Therefore, a final check was performed on pH dependency when including the additional two field soils. The following figures present the relation between normalised laboratory and field modelling endpoints and pH_{H20} (left: laboratory modelling endpoints from parent-only fits pooled with field modelling endpoints; right: laboratory modelling endpoints from pathway fits pooled with field modelling endpoints).



Once again, RMS notes that there is no linear relation, but visually there is a trend for higher persistence at pH below 6.7. The Kendall test was applied to the dataset and indicates that there is a correlation between the degradation rates of glyphosate and the pH of the soils. The calculated Kendall tau was:

- -0.473 with a t-test of 0.039 for the endpoints derived from the parent-only dataset, the pH dependency of normalized DT_{90} of glyphosate with soil pH(H_2O) from lab and field values is considered as significant;
- -0.565 with a t-test of 0.013 for the endpoints derived from the pathway fit dataset, the pH dependency of normalized DT_{90} of glyphosate with soil pH(H_2O) from lab and field values is considered as significant.

Based on the above evidence, pH-dependence should be taken into account in the exposure calculations. The choice of the modelling endpoints is discussed in Vol. 3 CP.

B.8.1.1.4.4. AMPA: Assessment of pH dependency

A pKa of 5.4 for AMPA is available from the adsorption literature section.

pH-dependency – laboratory values

The degradation of AMPA follows a single order kinetic in laboratory soils. Modelling DT_{50} were further analyzed to check whether they are pH-dependent. For laboratory endpoints, pH_{H2O} is reported in the above tables for all soils, with pH values measured in $CaCl_2$ or KCl converted in pH_{H2O} using the equation reported in EFSA guidance 2017.

The matrix was not mentioned in Speyer 2.3 from [redacted] (1993). As performed for glyphosate, the pH was assumed to have been measured in water.

The following figure presents the relation between normalised laboratory modelling DT_{50} and pH_{H20}.

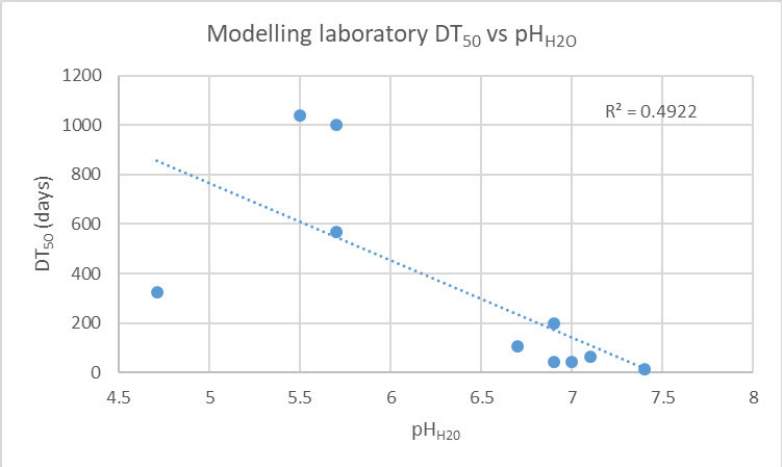


Figure 8.1.1.4-1: normalized DT_{50} for AMPA according to pH value of the lab soil data

RMS notes that visually, there is a trend for higher persistence at pH below 6.7. The Kendall test was applied to the dataset and indicates that there is a correlation between the degradation of AMPA and the pH of the soils. The Kendall tau was calculated to be -0.705 with a t-test of 0.007, the pH dependency of normalized DT_{90} of AMPA with soil pH(H_2O) is considered as significant.

Based on the above evidence, pH-dependence should be taken into account in the exposure calculations.

pH dependency - field values

As presented under point B 8.1.2.2 above, there are currently no normalized degradation rates for AMPA from field studies.

B.8.1.2. Adsorption and desorption in soil

B.8.1.2.1. *Adsorption and desorption of the active substance*

B.8.1.2.1.1. Laboratory studies

The adsorption and desorption behaviour in soil of glyphosate (PMG) was investigated in various soils in 11 batch equilibrium studies. Nine studies are existing ones and were previously evaluated in DAR (2001) or in RAR (2015). Two new studies were submitted by the task force in this renewal dossier.

Table 8.1.2.1-1: List of existing and new batch adsorption studies on glyphosate

Annex point	Study	**Previous evaluation in RAR (2015) / DAR (2001)**	**Status in RAR (2021)**
CA 7.1.3.1.1/001	[redacted], 2020a	New study	Acceptable
CA 7.1.3.1.1/002	[redacted], 2020b (Addendum to [redacted] 2020a)		
CA 7.1.3.1.1/030	[redacted], 2020c (Additional report to [redacted] 2020a)		
CA 7.1.3.1.1/004	[redacted], 1996	Accepted in RAR (2015)	Not acceptable
CA 7.1.3.1.1/005	[redacted], 1996	Accepted in RAR (2015)	Not acceptable
CA 7.1.3.1.1/007	[redacted], 1993	Accepted in RAR (2015)	Not acceptable
CA 7.1.3.1.1/008	[redacted], 1992	Considered as new since it was not mentioned in RAR (2015) nor in DAR (2001)	Not acceptable
CA 7.1.3.1.1/009	[redacted], 1992	Accepted in RAR (2015)	Not acceptable
CA 7.1.3.1.1/011	[redacted], 1986	Accepted in RAR (2015)	Not acceptable
CA 7.1.3.1.1/003	[redacted], 2001	Accepted in RAR (2015)	Not acceptable
CA 7.1.3.1.1/006	[redacted], 1994	Not mentioned in RAR (2015) but not accepted in DAR (2001)	Not acceptable
CA 7.1.3.1.1/010	[redacted], 1991	Not mentioned in RAR (2015) but not accepted in DAR (2001)	Not acceptable
CA 7.1.3.1.1/013	[redacted], 1978	Not mentioned in RAR (2015) but not accepted in DAR (2001)	Not acceptable

[redacted], 2020 a & b & c

Data point:	CA 7.1.3.1.1/001+ 002+030
Report author	[redacted].
Report year	2020
Report title	CA 7.1.3.1.1/001: Glyphosate – Adsorption/Desorption of [^{14}C]Glyphosate in Ten Soils – Final Report CA 7.1.3.1.1/002: Glyphosate – Adsorption/Desorption of [^{14}C]Glyphosate in Ten Soils – 1st amendment to Final Report CA 7.1.3.1.1/30: Glyphosate – Adsorption/Desorption of [14C]Glyphosate in Ten Soils, Experiments Supporting IES Study 20190441
Report No	20190441 and 20200276
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Parental mass balance below 90% on some samples - Adsorption percentage < 20% for some soils at the highest concentration - K_D*(soil/solution) ratio < 0.3 for some soils at the highest concentration
GLP/Officially recognised testing facilities	Yes
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[glycine-1-^{14}C]Glyphosate	
Batch No.	MXM 20013
Specific activity	5.81 MBq/mg
Radiochemical purity	>98 %

2. Test Soils

The soils were sampled from the upper 20 cm soil layers. None of the plots has been treated with pesticides for at least four years. The soils were air-dried at ambient temperature and sieved through a 2-mm sieve. For the definitive test, all soils were sterilised by X-ray irradiation before use to prevent degradation. A description of the soils used is summarised in the tables below.

Table 8.1.2.1-2: Physico-chemical properties of test soils

Parameter		Results				
Soil		1	2	3	4	5
		Speyer 2.2	RefeSol 01-A-05	18 Acres	M-SL-PF (Mutchler)	Speyer 2.3
Horizon (cm)		0-20	0-20	0-20	0-20	0-20
Geographic Location						
City		Hanhofen	Schmallenberg	Berkshire	Grand Forks	Offenbach
State		Rhineland-Palatinate	North Rhine-Westphalia	South East England	North Dakota	Hesse
Country		Germany	Germany	UK	USA	Germany
Textural Class (USDA)		Sandy loam	Loamy sand	Sandy Clay loam[1]	Sandy clay loam	Sandy loam
Sand	(5 µm – 2 mm) (%)	78.3	76.6	56	62	59.6
Silt	(2 µm – 5 µm) (%)	13.7	17.7	24	17	33.6
Clay	(< 2 µm) (%)	8.0	5.7	20	21	6.8
pH	- in 0.01 M $CaCl_2$	5.6	5.33	6.2	6.1	5.9
	- in water	5.21	6.11	6.11	6.44	7.02
Organic Carbon		1.71	0.80	1.9	1.9	0.67
Organic Matter		2.95	1.38	3.3	3.3	1.16
Cation Exchange Capacity (meq/100 g)		9.2	7.601	14.3	16.9	7.6

USDA: United States Department of Agriculture

[1] *RMS correction, the soil was erroneously reported as loamy sand*

Parameter	Results				
Soil	6	7	8	9	10
	RefeSol 02-A-06	Gartenacker	Speyer 6S	Speyer 5M	LAD-SL-PF (Pavillion)
Horizon (cm)	0-20	0-20	0-20	0-20	0-20
Geographic Location					
City	Schmallenberg	Vouvry	Siebeldingen	Mechtersheim	Fremont
State	North Rhine-Westphalia	Wallis	Rhineland-Palatinate	Rhineland-Palatinate	Wyoming
Country	Germany	Switzerland	Germany	Germany	USA
Textural Class (USDA)	Silt loam	Loam	Clay	Sandy loam	Sandy loam
Sand (5 µm – 2 mm) (%)	4.1	46	24.1	57.8	76

Silt (2 µm – 5 µm) (%)		80.1	46	35.1	30.9	11
Clay (< 2 µm) (%)		15.8	8	40.8	11.3	13
pH	- in 0.01 M CaCl2	6.19	7.1	7.2	7.4	8.1
	- in water	6.98	7.16	7.32	7.56	8.11
Organic Carbon		0.92	2.1	1.78	0.92	0.87
Organic Matter		1.59	3.6	3.07	1.59	1.50
Cation Exchange Capacity (meq/100 g)		5.911	8.4	25.7	13.3	17.6

USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Sealed Teflon tubes were used as test systems. The experiments were performed with duplicate soil samples. All experiments were performed at 20 ± 2 °C in the dark. Tubes were shaken to keep the soil in homogeneous suspension.

Soil samples were pre-equilibrated with at least 90 % of the target volume of 0.01 M $CaCl_2$ for approx. 16 hours at 20 ± 2 °C prior to application of the test item.

Preliminary tests:

In the preliminary tests, the optimal soil-to-solution ratio, the appropriate adsorption equilibration time and adsorption of test item to test vessel surface in absence of soil was determined. The preliminary phase also included tests on extractability from soil and the stability of glyphosate in the presence of sterilised or non-sterilised soil for various contact times.

Test 1:

Adsorption tests at a soil-to-solution ratio of 1:25 (i.e. 1 g soil to 25 mL aqueous phase), at a test item concentration of 0.497 µg/mL using 9 non-sterilised soils (soil 3 not used as it was not yet available), and an equilibrium time of 24 hours. Test 1 served as starting point to observe the adsorption behaviour of glyphosate to different types of soil. Aliquots of the supernatants only were submitted to LSC and HPLC analysis.

The amount of radioactivity adsorbed to soil ranged from 63.2% to 95.8% (mean values) for the nine soils investigated. For all soils the portion of radioactivity adsorbed was considered as very high (i.e. out of the range indicated by the guideline of 20 to 80% AR). Consequently, the soil-to-solution ratio was increased for the following tests.

Results from HPLC are not presented in the report. Clarifications provided by the applicant indicate that glyphosate represented 100% of the radioactivity present in the supernatant (% ROI), except for M-SL-PF (soil 4) (HPLC not performed since radioactivity in the supernatant was too low) and for Speyer 6S (soil 8) (glyphosate represented 86.0% ROI, mean of replicates).

Test 2:

Adsorption tests at a soil-to-solution ratio of 1:50 (i.e. 1 g soil to 50 mL aqueous phase) with ten soils, all non-sterilised, at a test concentration of 0.325 µg/mL and a contact time of 22 hours. Aliquots of the supernatants and soil extracts were submitted to LSC and HPLC analysis.

For the four soils RefeSol 01-A (soil 2), Gartenacker (soil 7), Speyer 5M (soil 9) and LAD-SL-PF (soil 10) the total recovery ranged from 92.6% to 95.2% AR. For soil Speyer 2.2 (soil 1) the mean recovery was 90.4% for one replicate being above and the other below 90% AR. For the other soils the mean recovery was below 90%, i.e. ranging from 76.5% to 88.9%. Furthermore, the amount of radioactivity recovered in the supernatant of samples of soil 18 Acres (soil 3) and M-SL-PF (soil 4) was extremely low (i.e. 0.8 and 1.3% AR, respectively).

As a consequence of results of test 2, investigations for soils Speyer 2.2 (soil 1), 18 Acres (soil 3), M-SL-PF (soil 4), Speyer 2.3 (soil 5), RefeSol 02-A (soil 6) and Speyer 6S (soil 8) were continued at higher soil to solution ratios.

For the remaining three soils Gartenacker (soil 7), Speyer 5M (soil 9) and LAD-SL-PF (soil 10)**,** a ratio 1:50 was considered acceptable for the definitive phase, being close to the 'ideal distribution' of 50% between soil and water.

HPLC chromatography of the combined soil extracts demonstrated insignificant degradation of Glyphosate (portion of test item expressed as mean percentage ROI was 93.5% in minimum). The situation was similar for the supernatant (mean ROI 98.5% in minimum) with the exception for soil Speyer 6S (soil 8) where Glyphosate was detected at mean 87.3% ROI. Due to the low radioactivity in supernatants of soil samples of soils 18 Acres (soil 3) and M-SL-PF (soil 4), no HPLC analysis was performed.

Test 3:

The third test of the preliminary phase adsorption test was performed with soils 1, 3, 4, 5, 6 and 8 at two soil-to-solution ratios (1:100 and 1:200). All soils were sterilised. For 1:100 ratio, test was performed at a concentration of 0.475 µg/mL for an equilibrium time of 22 hours. For 1:200 ratio, test was performed at a concentration of 0.511 µg/mL for an equilibrium time of 24 hours. . Aliquots of the supernatants and soil extracts were submitted to LSC and HPLC analysis.

For soils 5, 6 and 8, the mean recovery of radioactivity was > 90% AR for both soil-to-solution ratios. The higher radioactivity in supernatants at the 1:200 ratio allowed for an adequate analysis of radioactivity in the water phase. It was also close to the 'ideal distribution' of 50% between soil and water. The ratio was therefore selected in the definitive phase for the three soils Speyer 2.3 (soil 5), RefeSol 02-A (soil 6) and Speyer 6S (soil 8).

Total recoveries of radioactivity were, with one exception, still below, but closer to 90% AR for soils Speyer 2.2 (soil 1), 18 Acres (soil 3) and M-SL-PF (soil 4).

HPLC chromatography of soil extracts showed no significant degradation of the test item (mean percentage ROI 96.1% in minimum). In supernatant solutions degradation of Glyphosate was observed only for soil M-SL-PF (soil 4) with mean percentage ROI of 82.5% and 82.8% test item at the ratios 1:100 and 1:200, respectively. Glyphosate represented 100% ROI in the supernatant for the other soils.

The results of test 3 indicated that conditions of adsorption had to be optimised for the three soils Speyer 2.2 (soil 1), 18 Acres (soil 3) and M-SL-PF (soil 4). From comparison of total recoveries for two soil-to-solution ratios and the associated analytical results for soil M-SL-PF (soil 4), it became obvious that a further increase of the soil-to-solution ratio would not significantly improve the situation. Conclusively, it was decided to further investigate adsorption by reduction of the contact time for the three soils Speyer 2.2 (soil 1), 18 Acres (soil 3) and M-SL-PF (soil 4).

Test 4:

The fourth test was performed with soils 1, 3 and 4 at a soil-to-solution ratio of 1:200. The test was performed using sterilised soils at a test item concentration of 0.505 µg/mL and reduced equilibrium times of 2 and 4 hours. Aliquots of the supernatants and soil extracts were submitted to LSC and HPLC analysis.

The total recovery of radioactivity was more than 90% AR after 2 and 4 hours of adsorption for the three soils. The radioactivity in supernatants was decreasing with time for the three soils.

After 2 and 4 hours, HPLC chromatography of soil extracts and supernatants showed no significant degradation of the test item (mean percentage ROI 100% in each phase; not determined in supernatant for 18 Acres after 2 hours).

Test 5:

In order to investigate whether results of Test 1 to 4 were also applicable for a lower test concentration of 0.057 mg/L, a last pre-test was done. The fifth test was performed with all ten soils at soil-to-solution ratios of 1:195 for soils 1-6 and 8 and about 1:50 for soils 7, 9 and 10. The test was performed using sterilised soils at a test item concentration of 0.057 µg/mL. An equilibrium time of 4 hours was selected. Aliquots of the soil extracts were submitted to LSC and HPLC analysis. HPLC analysis of the supernatant was not performed since no degradation had been observed in previous tests.

Results of Test 5 are presented below. The total mass balances of radioactivity ranged from 88.5% to 98.9% AR for the ten soils. This test did not include the determination of non-extractable radioactivity (NER) in extracted soil.

Table 8.1.2.1-3: Preliminary phase, Test 5: Radioactivity in supernatant and extracted from soil, soil-to-solution ratio of 1:200 or 1:50, sterile soil, 4 hours adsorption

Soil	Mass applied [µg]	Mass in supernatant [µg]	Super-natant [% AR]	Extracted mass [µg]	Adsorbed to soil (extracted) [% AR]	Total mass [% AR]	Parental mass balance [% AR]*
Speyer 2.2							
Replicate A	11.08	5.24	47.3	5.02	45.3	92.6	92.6
Replicate B	11.08	4.80	43.3	5.22	47.1	90.5	90.5
Mean	11.08	5.02	45.3	5.12	46.2	91.5	91.5
RefeSol 01-A							
Replicate A	11.08	6.50	58.7	3.48	31.4	90.1	89.8
Replicate B	11.08	6.50	58.7	3.13	28.2	86.9	86.6
Mean	11.08	6.50	58.7	3.30	29.8	88.5	88.2
18 Acres							
Replicate A	11.08	1.75	15.8	8.24	74.3	90.1	90.1
Replicate B	11.08	1.75	15.8	8.10	73.1	88.9	88.8
Mean	11.08	1.75	15.8	8.17	73.7	89.5	89.5
M-SL-PF							
Replicate A	11.08	3.01	27.2	6.96	62.8	89.9	90.1
Replicate B	11.08	2.44	22.0	7.26	65.5	87.6	87.7
Mean	11.08	2.73	24.6	7.11	64.1	88.8	88.9
Speyer 2.3							
Replicate A	11.08	6.87	62.0	3.57	32.2	94.2	94.4
Replicate B	11.08	6.55	59.1	4.04	36.4	95.5	91.6
Mean	11.08	6.71	60.6	3.80	34.3	94.9	93.0
RefeSol 02-A							
Replicate A	11.08	4.21	37.9	5.86	52.8	90.8	90.5
Replicate B	11.08	4.34	39.2	5.72	53.0	92.2	91.9
Mean	11.08	4.27	38.6	5.79	52.9	91.5	91.2
Gartenacker							
Replicate A	11.08	4.98	44.9	5.47	49.3	94.2	94.7
Replicate B	11.08	4.86	43.9	5.71	51.6	95.5	95.9
Mean	11.08	4.92	44.4	5.59	50.4	94.8	95.3
Speyer 6S							
Replicate A	11.08	5.71	51.5	5.03	45.4	96.9	97.2
Replicate B	11.08	5.86	52.8	5.04	45.5	98.3	98.6
Mean	11.08	5.78	52.2	5.04	45.4	97.6	97.9
Speyer 5M							
Replicate A	11.08	5.50	49.7	5.24	47.3	97.0	96.9

Replicate B	11.08	5.69	51.3	5.25	47.4	98.7	98.6
Mean	11.08	5.60	50.5	5.25	47.4	97.9	97.7
LAD-SL-PF							
Replicate A	11.08	6.13	55.3	4.77	43.1	98.4	99.0
Replicate B	11.08	6.22	56.2	4.79	43.2	99.4	99.9
Mean	11.08	6.18	55.7	4.78	43.1	98.9	99.5

Percent values are given in percent of initial applied radioactivity (AR).
Test concentration 0.057 mg/L.
* As provided by the applicant following RMS request. Parental mass balance is based on: radioactivity measured by LSC in supernatant (it is assumed that all radioactivity corresponds to glyphosate) + radioactivity identified as glyphosate in soil extracts based on HPLC.

Percentages of glyphosate in the soil extract after 4 hours of adsorption from this test 5 was 98.4-100% ROI.

Conclusion from preliminary tests:

The conditions as used in Test 5 were considered suitable for the definitive phase as regards the soil-to-solution ratio. An adsorption time of 4 hours was selected for the definitive phase. This can be regarded as conservative since the resulting Koc values are lower - for the sake of best stability of the test item Glyphosate given under the conditions of the test, i.e. to reduce the potential for degradation in a best possible way.

The preliminary tests also showed that adsorption of glyphosate on to the vessels was insignificant, and glyphosate was stable in CaCl2 solution.

Definitive tests:

The definitive phase was performed with sterilised soils. The adsorption step was carried out using pre-equilibrated samples at a soil-to-solution ratio of 1:200 (for soils 1-6 and 8) or 1:50 (for soils 7, 9 and 10). Glyphosate was applied at nominal concentrations of 5.00, 1.61, 0.50, 0.16 and 0.05 mg/L in aqueous 0.01 M $CaCl_2$ solution. The definitive adsorption step was carried out for an equilibrium time of 4 hours in the dark at 20 ± 2 °C under continuous agitation. The radioactivity was determined by LSC for aqueous supernatants and for soil extracts. Furthermore, radioactivity in extracted soil samples was determined by combustion/LSC. No desorption steps were performed.

A series of control samples without soil, but containing five test item concentration each (duplicates) were subjected to precisely the same steps as the test samples in order to check for the stability of the test item in $CaCl_2$ solution.

Additionally, test item stability was investigated in aqueous supernatants ($CaCl_2$) and soil extracts for the medium concentration 0. 5 mg/L for all soils and for all concentrations for soils 2, 3 and 4 by HPLC analysis. The aqueous supernatants of the lowest test concentration were also analysed for test item by TLC.

In the additional study (Report 20200276), aqueous supernatants and soil extracts of soil 1 and of soils 5 to 10 originating from the definitive phase of IES study 20190441 were analysed by HPLC, for the 2 highest concentrations (5 and 1.6 mg/L).

2. Analytical Procedures

After the adsorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity content was analysed by liquid scintillation counting (LSC).

Within the preliminary stability tests soil samples were extracted three times at ambient temperature with 0.25 M ammonium hydroxide/0.1 M monopotassium phosphate following the adsorption phase. The extracts were combined for analysis. Aqueous $CaCl_2$ solutions and combined soil extracts were analysed by HPLC/radiodetection. Extracted soil samples were dried, combusted and analysed by LSC to determine non-extractable radioactivity.

Within the definitive phase soil samples were extracted following the adsorption step as described for the preliminary tests for the test concentration of 0.50 mg/L of all soils. Additionally, soils of all test

concentrations were extracted for soils 2, 3 and 4. Aqueous CaCl2 solutions and combined soil extracts were analysed by HPLC/radiodetection (at all concentrations for soils 2, 3 and 4, and only at the medium concentration for other soils). The aqueous supernatants of the lowest test concentration were also analysed for test item by TLC. In the additional study (Report 20200276), aqueous supernatants and soil extracts of soil 1 and of soils 5 to 10 originating from the definitive phase of IES study 20190441 were additionaly analysed by HPLC, for the 2 highest concentrations (5 and 1.6 mg/L).

The following LOQ and LOD were determined for the analytical methods:

Table 8.1.2.1-4: Estimated LOD and LOQ for the analytical methods

Method	Phase	LOD	LOQ
LSC	Aqueous phase	0.063 µg/L (0.13% AR for the lowest test concentration)	0.125 µg/L (0.26% AR for the lowest test concentration)
	Soil extract	4.01 µg/kg (0.04% AR for the lowest test concentration)	8.03 µg/kg (0.08% AR for the lowest test concentration)
	Non extractables	0.47 µg/kg (0.05% AR for the lowest test concentration)	0.95 µg/kg (0.010% AR for the lowest test concentration)
HPLC	Aqueous phase	4.21 µg/L (0.81% AR for the middle test concentration)	8.42 µg/L (1.8% AR for the middle test concentration)
	Soil extract	4.35 µg/L (0.25% AR for the middle test concentration)	8.69 µg/L (0.51% AR for the middle test concentration of 0.5 mg/L_)
TLC	Aqueous phase	0.07 µg/L (0.15% AR for the lowest test concentration)	0.14 µg/L (0.30% AR for the lowest test concentration)

3. Calculations

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation, based on measured concentrations in the aqueous phases and soil extracts determined by LSC. The measured concentrations by LSC of soil 4 were corrected by results from the chromatographic analysis in order to take into account the slight extent of degradation observed. For the other soils, Glyphosate was considered stable under the test conditions with no need for correction of the adsorption results.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Material balance was determined during the definitive phase. Since it was below 90% on some samples (Speyer 2.2 at concentrations 0.5 and 0.16 mg/L; RefeSol 01-A at 0.5 and 0.05 mg/L and Speyer 6S at 0.05 mg/L), a repetition of these tests was done in the additional report 20200276. These repeated tests are considered to replace those in the initial study.

For the definitive phase, mean material balances (including non extractable residues) ranged from 92.9 to 99.6 % of applied radioactivity (% AR) for soil 1, from 90.7 to 98.7 % AR for soil 2, from 97.5 to 103.9 % AR for soil 3, from 90.0 to 97.7 % AR for soil 4, from 92.8 to 99.1 % AR for soil 5, from 93.2 to 98.6 % AR for soil 6, from 92.6 to 97.5 % AR for soil 7, from 91.7 to 102.0 % AR for soil 8, from 98.0 to 101.2 % AR for soil 9 and from 96.5 to 101.6 % AR for soil 10.

B. STABILITY OF TEST ITEM

The test item was stable in aqueous 0.01 M CaCl2 solution, i.e. in absence of soil and did not show adsorption to the surface of the test vessels. After incubation for 4 hours the test item was detected with ≥97.9 % AR.

Stability of the test item in the definitive phase was checked by HPLC (or TLC when necessary) at all concentrations for soils 2, 3 and 4 and at 0.5, 1.6 and 5 mg/L concentrations for other soils. As previously

indicated, no significant degradation (% ROI ≥ 90%) was observed in all soils, except soil 4. As a consequence, for determination of adsorption values, correction by HPLC results of the amount of radioactivity in supernatant and in soil extracts determined by LSC was done for soil 4 only.

Table 8.1.2.1-5: Definitive phase – Percentage of [^{14}C]Glyphosate (% ROI) in supernatant and soil extracts after 4 hours adsorption

Soil	Test concentration (mg/L)	Radioactivity in supernatant after adsorption [% ROI]	Radioactivity in soil extracts after adsorption [% ROI]
1 Speyer 2.2	5	98.03	97.67
		97.88	96.10
	1.6	100	100
		100	100
	0.5	98.46	98.21
		98.30	98.64
2 RefeSol 01-A	5	98.79	99.03
		97.87	100
	1.6	98.18	100
		98.43	100
	0.5	96.13	100
		95.49	100
	0.16	97.34	100
		96.22	100
	0.05	100	100
		100	100
3 18 Acres	5	97.91	99.08
		98.49	99.13
	1.6	100	100
		97.42	100
	0.5	96.91	99.01
		96.86	98.84
	0.16	93.82	100
		95.08	99.15
	0.05	90.2*	98.7*
		96.7*	99.2*
4 M-SL-PF	5	96.80	96.93
		96.62	97.26
	1.6	94.11	97.61
		93.08	98.62
	0.5	91.96	98.47
		91.62	98.39
	0.16	92.05	98.40
		92.48	98.52
	0.05	89.0*	98.4*
		82.2*	99.1*
5 Speyer 2.3	5.0	98.23	98.71
		98.26	100
	1.6	100	100
		100	100
	0.5	98.09	98.87
		98.42	100
6 RefeSol 02-A	5.0	100	98.73
		98.43	100.00
	1.6	100	100
		100	100
	0.5	98.42	99.03
		97.61	99.02
7 Gartenacker	5.0	98.53	100
		96.94	98.86
	1.6	97.15	98.26
		100	100

	0.5	97.2	99.11
		97.45	99.55
8 Speyer 6S	5.0	98.01	100
		98.38	100
	1.6	95.65	100
		97.01	100
	0.5	98.3	100
		97.26	100
9 Speyer 5M	5.0	98.41	98.48
		98.16	98.30
	1.6	100	100
		100	100
	0.5	98	99.46
		97.97	100
10 LAD-SL-PF	5.0	98.01	100
		97.96	100
	1.6	97.34	100
		97.90	100
	0.5	98.36	99.01
		98.17	99.61

* Concentration in the supernatant was too low to be determined by HPLC. TLC results are presented.

C. FINDINGS

For the definitive tests, the percentage of radioactivity adsorbed to the soil ranged from 11.0 to 52.9 % in soil 1, in soil 2 from 14.0 to 45.5 %, in soil 3 from 28.6 to 80.6 %, in soil 4 from 25.4 to 76.6 %, in soil 5 from 14.6 to 34.3 %, in soil 6 from 19.6 to 55.2 %, in soil 7 from 21.3 to 47.6 %, in soil 8 from 17.8 to 46.0 %, in soil 9 from 19.9 to 44.3 % and in soil 10 from 20.3 to 44.1 %.

Table 8.1.2.1-6: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values, expressed in % AR))

Soil	**Test Concentration [mg/L]**				
	5.00	**1.61**	**0.50**	**0.16**	**0.05**
1 (Speyer 2.2)	11.0	19.6	39.4	51.8	52.9
2 (RefeSol 01-A-05)	14.0	24.1	30.5	31.3	45.5
3 (18 Acres)	28.6	47.0	64.2	74.5	80.6
4 (M-SL-PF (Mutchler))	25.4	44.0	60.9	76.6	74.9
5 (Speyer 2.3)	14.6	19.8	23.4	32.6	34.3
6 (RefeSol 02-A-06)	19.6	30.4	38.0	50.1	55.2
7 (Gartenacker)	21.3	29.5	34.2	42.4	47.6
8 (Speyer 6S)	17.8	23.6	31.3	37.7	46.0
9 (Speyer 5M)	19.9	26.9	32.3	38.9	44.3
10 (LAD-SL-PF (Pavillion))	20.3	28.6	31.7	40.6	44.1

Tables for total mass balance and for measured concentrations in the aqueous phase and extracts used for the Freundlich evaluation are presented below. The linearised Freundlich isotherms and the corresponding plot of the residuals are shown in the figures below.

Legend:
maq (ads): Radioactivity in supernatant after adsorption
ms (ads): Radioactivity in soil extract after adsorption (without residue water)
NER (ads): Radioactivity irreversibly bound to soil
Values are given in percent of applied radioactivity (AR).

Table 8.1.2.1-7: Definitive phase – Adsorption - soil 1 (Speyer 2.2): Mass balance of radioactivity

Soil 1 Test concentration	Mass applied [µg]	m_{aq} (ads) [% AR]	m_s (ads) [% AR]	NER (ads) [% AR]	Total recovery [% AR]
5 mg/L					
Sample A	1007.9	82.2	11.5	0.4	94.1
Sample B		85.1	10.5	0.4	96.0
1.6 mg/L					

Sample A	311.8	71.6	22.0	0.8	94.3
Sample B		75.8	17.3	0.6	93.7
0.5 mg/L*					
Sample A	107.72	57.1	39.2	1.5	97.8
Sample B		57.3	39.6	1.3	98.2
0.16 mg/L*					
Sample A	33.45	45.6	52.5	2.2	100.3
Sample B		45.7	51.1	2.3	99.0
0.05 mg/L					
Sample A	9.67	36.6	52.4	2.5	91.5
Sample B		38.4	53.5	2.3	94.2

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil
* Results from additional study 20200276

Table 8.1.2.1-8: Definitive phase – Adsorption - soil 2 (RefeSol 01-A): Mass balance of radioactivity

Soil 2 Test concentration	Mass applied [µg]	m_{aq} (ads) [% AR]	m_s (ads) [% AR]	NER (ads) [% AR]	Total recovery [% AR]
5 mg/L					
Sample A	1007.9	80.8	14.9	0.6	96.3
Sample B		83.9	13.0	0.4	97.4
1.6 mg/L					
Sample A	311.8	71.5	24.5	0.6	96.6
Sample B		72.3	23.7	0.6	96.6
0.5 mg/L*					
Sample A	107.72	64.7	29.2	0.9	94.9
Sample B		66.0	31.7	1.0	98.7
0.16 mg/L					
Sample A	32.21	59.3	29.8	1.0	90.1
Sample B		57.6	32.7	1.0	91.3
0.05 mg/L*					
Sample A	9.92	53.6	45.8	0.9	100.4
Sample B		51.4	45.3	2.0	98.7

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil
* Results from additional study 20200276

Table 8.1.2.1-9: Concentrations of glyphosate used to determine adsorption isotherms in soils 1 and 2

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)*	Concentration in soil (µg/g)[a]
1 (Speyer 2.2)	0.05	0.018	5.064
	0.05	0.019	5.174
	0.17 [b]	0.076	17.549
	0.17 [b]	0.076	17.076
	0.54 [b]	0.307	42.251
	0.54 [b]	0.309	42.644
	1.56	1.116	68.567
	1.56	1.181	53.886
	5.04	4.143	116.317
	5.04	4.289	105.553
2 (RefeSol 01-A-05)	0.05 [b]	0.027	4.547
	0.05 [b]	0.025	4.489
	0.16	0.096	9.605
	0.16	0.093	10.531
	0.54 [b]	0.348	31.491
	0.54[b]	0.355	34.151
	1.56	1.115	76.506
	1.56	1.127	73.890
	5.04	4.072	149.933
	5.04	4.229	131.448

[a] Based on LSC analysis of supernatant and soil extracts;
[b] Results from additional study 20200276

Table 8.1.2.1-10: Definitive phase – Adsorption - soil 3 (18 Acres): Mass balance of radioactivity

Soil 3 Test concentration	Mass applied [µg]	m_{aq} (ads) [% AR]	m_s (ads) [% AR]	NER (ads) [% AR]	Total recovery [% AR]
5 mg/L					
Sample A	1007.9	66.5	27.8	2.4	96.7
Sample B		66.1	29.5	2.5	98.2
1.6 mg/L					
Sample A	311.8	48.5	46.9	4.3	99.7
Sample B		48.7	47.2	4.2	100.1
0.5 mg/L					
Sample A	104.41	28.3	62.2	5.8	96.3
Sample B		27.9	66.3	5.8	100.0
0.16 mg/L					
Sample A	32.21	18.7	74.7	7.2	100.7
Sample B		18.6	74.2	7.1	100.0
0.05 mg/L					
Sample A	9.67	14.2	78.0	7.9	100.1
Sample B		12.4	83.1	8.4	103.9

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil

Table 8.1.2.1-11: Definitive phase – Adsorption - soil 4 (M-SL-PF Mutchler): Mass balance of radioactivity

Soil 4 Test concentration	Mass applied [µg]	maq (ads) [% AR]	ms (ads) [% AR]	NER (ads) [% AR]	Total recovery [% AR]
5 mg/L					
Sample A	1007.9	67.6	25.7	2.5	95.8
Sample B		68.1	25.1	2.6	95.8
1.6 mg/L					
Sample A	311.8	50.2	43.9	4.1	98.3
Sample B		48.4	44.1	4.6	97.1
0.5 mg/L					
Sample A	104.41	30.4	60.8	4.9	96.1
Sample B		29.8	61.0	4.9	95.7
0.16 mg/L					
Sample A	32.21	17.1	75.5	4.3	96.9
Sample B		16.3	77.7	4.6	98.6
0.05 mg/L					
Sample A	9.67	15.2	76.5	3.6	95.3
Sample B		13.1	73.4	3.5	90.0

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil

Table 8.1.2.1-12: Concentrations of glyphosate used to determine adsorption isotherms in soils 3 and 4

Soil	**Initial concentration of test solution (µg/mL)**	**Concentration of supernatant (µg/mL)***	**Concentration in soil (µg/g)***
3 (18 Acres)	0.05	0.007	7.548
	0.05	0.007	8.036
	0.16	0.030	24.068
	0.16	0.030	23.912
	0.52	0.148	64.938
	0.52	0.146	69.220
	1.56	0.756	146.253
	1.56	0.760	147.034
	5.04	3.353	279.970
	5.04	3.334	297.557
4 (M-SL-PF (Mutchler))	0.05	0.006	7.398
	0.05	0.005	7.101

	0.16	0.025	23.937
	0.16	0.024	24.629
	0.52	0.146	62.507
	0.52	0.143	62.683
	1.56	0.733	133.712
	1.56	0.706	134.284
	5.04	3.293	251.260
	5.04	3.319	245.717

* Based on LSC analysis of supernatant and soil extracts for soil 3 / Based on LSC analysis of supernatant and soil extracts, corrected by HPLC results for soil 4.

Table 8.1.2.1-13: Definitive phase – Adsorption - soil 5 (Speyer 2.3): Mass balance of radioactivity

Soil 5 Test concentration	Mass applied [µg]	m_{aq} (ads) [% AR]	m_s (ads) [% AR]	NER (ads) [% AR]	Total recovery [% AR]
5 mg/L					
Sample A	1007.9	82.2	14.3	1.1	97.6
Sample B		81.7	14.9	1.2	97.7
1.6 mg/L					
Sample A	311.8	78.1	19.8	1.5	99.5
Sample B		77.2	19.8	1.6	98.7
0.5 mg/L					
Sample A	104.41	64.5	23.8	1.9	90.2
Sample B		70.5	23.0	1.8	95.3
0.16 mg/L					
Sample A	32.21	62.0	32.7	2.5	97.2
Sample B		61.1	32.6	2.7	96.3
5 mg/L					
Sample A	9.67	57.2	36.9	3.6	97.6
Sample B		60.8	31.7	3.1	95.5

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil

Table 8.1.2.1-14: Definitive phase – Adsorption - soil 6 (RefeSol 02-A): Mass balance of radioactivity

Soil 6 Test concentration	Mass applied [µg]	m_{aq} (ads) [% AR]	m_s (ads) [% AR]	NER (ads) [% AR]	Total recovery [% AR]
5 mg/L					
Sample A	1007.9	76.0	18.6	1.5	96.1
Sample B		75.6	20.7	1.7	98.0
1.6 mg/L					
Sample A	311.8	65.1	30.0	2.6	97.7
Sample B		66.1	30.8	2.5	99.5
0.5 mg/L					
Sample A	104.41	55.2	37.4	3.2	95.7
Sample B		53.0	38.6	3.4	95.0
0.16 mg/L					
Sample A	32.21	41.9	49.4	4.5	95.9
Sample B		39.2	50.8	5.3	95.3
0.05 mg/L					
Sample A	9.67	31.7	59.2	6.2	97.2
Sample B		36.0	51.2	6.0	93.2

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil

Table 8.1.2.1-15: Concentrations of glyphosate used to determine adsorption isotherms in soils 5 and 6

Soil	**Initial concentration of test solution (µg/mL)**	**Concentration of supernatant (µg/mL)***	**Concentration in soil (µg/g)***
5 (Speyer 2.3)	0.05	0.028	3.567
	0.05	0.029	3.065
	0.16	0.100	10.533
	0.16	0.098	10.487

	0.52	0.337	24.846
	0.52	0.368	24.014
	1.56	1.218	61.789
	1.56	1.204	61.849
	5.04	4.144	143.860
	5.04	4.116	149.739
6 (RefeSol 02-A-06)	0.05	0.015	5.729
	0.05	0.017	4.950
	0.16	0.068	15.924
	0.16	0.063	16.373
	0.52	0.288	39.047
	0.52	0.277	40.346
	1.56	1.015	93.436
	1.56	1.031	96.129
	5.04	3.829	187.024
	5.04	3.811	208.159

* Based on LSC analysis of supernatant and soil extracts

Table 8.1.2.1-16: Definitive phase – Adsorption - soil 7 (Gartenacker): Mass balance of radioactivity)

Soil 7	Mass applied	m_{aq} (ads)	m_s (ads)	NER (ads)	Total recovery
Test concentration	[µg]	[% AR]	[% AR]	[% AR]	[% AR]
5 mg/L					
Sample A	1007.9	72.8	20.7	1.7	95.1
Sample B		72.7	22.0	1.9	96.6
1.6 mg/L					
Sample A	311.8	65.5	29.5	2.7	97.7
Sample B		64.6	29.4	2.7	96.7
0.5 mg/L					
Sample A	104.41	55.8	33.4	3.3	92.6
Sample B		54.7	34.9	3.0	92.6
0.16 mg/L					
Sample A	32.21	51.5	42.2	3.9	97.6
Sample B		51.0	42.5	4.0	97.5
0.05 mg/L					
Sample A	9.67	47.2	50.0	4.6	101.7
Sample B		46.5	45.3	4.7	96.6

Five test concentrations, soil-to-solution ratio: 1:50, 4 hours contact time with sterile soil

Table 8.1.2.1-17: Definitive phase – Adsorption - soil 8 (Speyer 6S): Mass balance of radioactivity

Soil 8	Mass applied	m_{aq} (ads)	m_s (ads)	NER (ads)	Total recovery
Test concentration	[µg]	[% AR]	[% AR]	[% AR]	[% AR]
5 mg/L					
Sample A	1007.9	75.7	17.9	1.5	95.0
Sample B		75.6	17.8	1.4	94.8
1.6 mg/L					
Sample A	311.8	67.6	25.1	1.4	94.1
Sample B		70.7	22.1	2.1	94.8
0.5 mg/L					
Sample A	104.41	58.0	31.4	2.3	91.7
Sample B		58.2	31.3	2.3	91.8
0.16 mg/L					
Sample A	32.21	54.1	39.0	2.7	95.7
Sample B		55.3	36.5	2.7	94.4
0.05 mg/L*					
Sample A	9.92	54.5	45.6	2.2	102.3
Sample B		53.3	46.3	2.1	101.7

Five test concentrations, soil-to-solution ratio: 1:200, 4 hours contact time with sterile soil

* Results from additional study 20200276

Table 8.1.2.1-18: Concentrations of glyphosate used to determine adsorption isotherms in soils 7 and 8

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)*	Concentration in soil (µg/g)[a]
7 (Gartenacker)	0.05	0.023	1.208
	0.05	0.023	1.095
	0.16	0.083	3.399
	0.16	0.082	3.423
	0.52	0.291	8.730
	0.52	0.285	9.107
	1.56	1.022	23.000
	1.56	1.007	22.928
	5.04	3.669	52.055
	5.04	3.666	55.525
8 (Speyer 6S)	0.05[b]	0.027	4.526
	0.05 [b]	0.026	4.592
	0.16	0.087	12.557
	0.16	0.089	11.743
	0.52	0.303	32.738
	0.52	0.304	32.705
	1.56	1.055	78.147
	1.56	1.102	68.835
	5.04	3.814	180.204
	5.04	3.809	179.612

[a] Based on LSC analysis of supernatant and soil extracts
[b] Results from additional study 20200276

Table 8.1.2.1-19: Definitive phase – Adsorption soil 9 (Speyer 5M): Mass balance of radioactivity

Soil 9	Mass applied	m_{aq} (ads)	m_s (ads)	NER (ads)	Total recovery
Test concentration	[µg]	[% AR]	[% AR]	[% AR]	[% AR]
5 mg/L					
Sample A	1007.9	76.1	19.7	1.8	97.7
Sample B		76.3	20.1	1.9	98.2
1.6 mg/L					
Sample A	311.8	70.3	26.8	2.2	99.3
Sample B		69.7	26.9	2.2	98.8
0.5 mg/L					
Sample A	104.41	63.5	32.4	2.3	98.3
Sample B		63.7	32.1	2.5	98.3
0.16 mg/L					
Sample A	32.21	57.9	39.0	3.2	100.1
Sample B		58.7	38.8	2.8	100.3
5 mg/L					
Sample A	9.67	52.5	44.9	3.8	101.1
Sample B		53.7	43.8	3.7	101.2

Five test concentrations, soil-to-solution ratio: 1:50, 4 hours contact time with sterile soil

Table 8.1.2.1-20: Definitive phase – Adsorption - soil 10 (LAD-SL-PF (Pavillion)): Mass balance of radioactivity

Soil 10	Mass applied	m_{aq} (ads)	m_s (ads)	NER (ads)	Total recovery
Test concentration	[µg]	[% AR]	[% AR]	[% AR]	[% AR]
5 mg/L					
Sample A	1007.9	77.2	20.7	2.0	99.9
Sample B		76.4	20.0	1.6	98.0
1.6 mg/L					
Sample A	311.8	70.2	29.0	2.2	101.4
Sample B		71.1	28.3	1.9	101.3
0.5 mg/L					

Sample A	104.41	62.0	32.6	2.3	96.9
Sample B		63.2	30.9	2.0	96.1
0.16 mg/L					
Sample A	32.21	59.2	39.5	2.6	101.3
Sample B		57.6	41.6	2.7	101.9
5 mg/L					
Sample A	9.67	55.3	43.8	3.1	102.2
Sample B		54.2	44.5	3.0	101.6

Five test concentrations, soil-to-solution ratio: 1:50, 4 hours contact time with sterile soil

Table 8.1.2.1-21: Concentrations of glyphosate used to determine adsorption isotherms in soils 9 and 10

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)*	Concentration in soil (µg/g)*
9 (Speyer 5M)	0.05	0.025	1.085
	0.05	0.026	1.058
	0.16	0.093	3.139
	0.16	0.095	3.123
	0.52	0.332	8.468
	0.52	0.333	8.378
	1.56	1.096	20.928
	1.56	1.086	21.008
	5.04	3.837	49.753
	5.04	3.845	50.542
10 (LAD-SL-PF (Pavilion))	0.05	0.027	1.059
	0.05	0.026	1.075
	0.16	0.095	3.183
	0.16	0.093	3.352
	0.52	0.324	6.151
	0.52	0.330	5.848
	1.56	1.094	22.597
	1.56	1.109	22.057
	5.04	3.893	52.106
	5.04	3.852	50.350

* Based on LSC analysis of supernatant and soil extracts

The adsorption coefficients $K_{F(ads)}$ of glyphosate ranged from 18.11 to 166.35 mL/g for all soils. The Freundlich exponents 1/n were in the range of 0.546 to 0.777. The corresponding, calculated $K_{F, OC}$(ads) values varied from 1031 to 9615 mL/g. For details see table below.

Table 8.1.2.1-22: [14C]Glyphosate: Freundlich adsorption parameters in soils at 20 °C

Soil	Adsorption			
	$K_{F(ads)}$	1/n	R^2	$K_{F, OC}$(ads)
1 (Speyer 2.2)	59.4434	0.546	0.9578	3476.22
2 (RefeSol 01-A-05)	59.8046	0.704	0.9890	7475.57
3 (18 Acres)	166.3529	0.579	0.9870	8755.42
4 (M-SL-PF (Mutchler))	152.4533	0.546	0.9810	8023.86
5 (Speyer 2.3)	52.8781	0.751	0.9955	7892.25
6 (RefeSol 02-A-06)	88.4624	0.658	0.9941	9615.48
7 (Gartenacker)	21.6447	0.757	0.9977	1030.70
8 (Speyer 6S)	70.5279	0.736	0.9965	3962.24
9 (Speyer 5M)	18.8542	0.770	0.9989	2049.37
10 (LAD-SL-PF (Pavillion))	18.1119	0.777	0.9902	2081.83

III. CONCLUSIONS

The Freundlich adsorption coefficients KF(ads) of glyphosate as investigated for ten soils ranged from 18.11 to 166.35. The corresponding values normalised for organic carbon content of soil $K_{F, OC}$(ads) varied between 1030 and 9615.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline. In the initial report (CA 7.1.3.1.1/001) total material balance were below 90 % for some replicates at single test concentrations of three soils. The respective tests were repeated in CA 7.1.3.1.1/030 with the respective material balances being above 90%. The results reported in CA 7.1.3.1.1/030 are considered to replace those in study 20190441.

The study is considered acceptable to address this data point.

All relevant quality checks following OECD 106 Evaluators Checklist were performed. Mass balances of radioactivity were from 94.9 to 99.0 % (5 mg/L) and percentage adsorption was from 14.9 to 89.6 % in the definitive test. Estimated KFE/KF values were rather variable from 1.1 to 3.6, dependent on soil and test concentration. The validity of the analytical method was confirmed over the entire range of concentrations measured (LOQ = 0.26 % AR and at least two orders of magnitude lower than lowest test concentration).

K_D x soil/solution ratios were between 0.1 and 7.1 in all soils. The graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots.

The R^2 of the standard linear regressions ranged from 0.982 to 0.999.

Glyphosate: Evaluation of result according to EU OECD 106 Evaluators Checklist

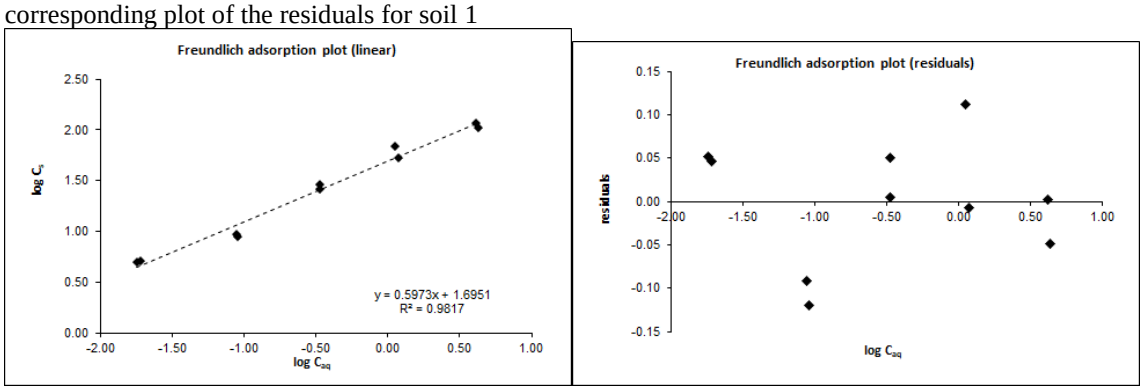
		Soil				
	Units	1	2	3	4	5
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:200	1:200	1:200	1:200	1:200
Mass balance (at 5 mg/L)	% AR	95.0	96.8	97.5	95.8	97.6
Adsorbed percentage	%	14.9-62.5	16.1-50.0	33.5-85.4	34.2-89.6	17.8-41.7
K_D x (soil:solution ratio)		0.1-1.4	0.2-0.9	0.4-5.7	0.4-7.1	0.2-0.6
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	59.428 (47.852-73.805)	59.795 52.619-67.949	166.375 (141.732-195.302)	152.395 (127.581-182.036)	52.888 (48.681-57.458)
$^{ads}1/n$ (95 % confidence interval)	-	0.547 (0.452-0.642)	0.704 (0.643-0.765)	0.580 (0.525-0.635)	0.542 (0.484-0.601)	0.751 (0.711-0.791)
$^{ads}R^2$	-	0.957	0.989	0.987	0.983	0.996
$^{ads}K_{F,OC}$	L/kg OC	3475	7474	8757	8021	7894
K_{FE} / K_F (5 mg/L)	-	1.42/1.55	1.23/1.29	1.18/1.18	1.24/1.25~~1~~	1.25/1.24

		Soil				
	Units	6	7	8	9	10
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:200	1:50	1:200	1:50	1:50

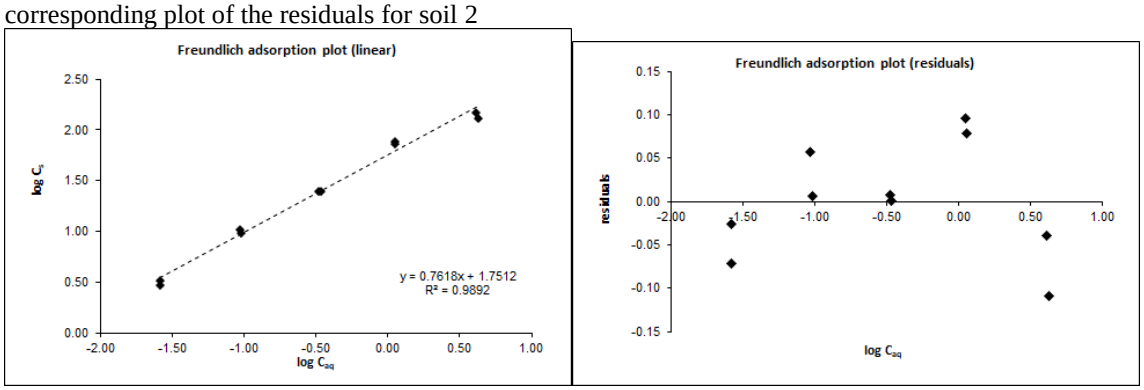
Mass balance (at 5 mg/L)	% AR	97.0	95.9	94.9	98.0	99.0
Adsorbed percentage	%	24.0-68.8	27.2-52.1	24.3-48.0	23.7-47.9	22.8-45.8
K_D x (soil:solution ratio)		0.2-1.9	0.3-1.1	0.2-0.9	0.3-0.9	0.3-0.8
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	88.447 (80.480-97.201)	21.644 (20.230-23.157)	70.533 (65.517-75.934)	18.851 (18.047-19.691)	18.111 (15.868-20.670)
$^{ads}1/n$ (95 % confidence interval)	-	0.656 (0.616-0.696)	0.758 (0.727-0.789)	0.735 (0.700-0.770)	0.769 (0.748-0.790)	0.777 (0.714-0.840)
$^{ads}R^2$	-	0.994	0.997	0.997	0.999	0.990
$^{ads}K_{F,OC}$	L/kg OC	9614	1031	3963	2049	2082
K_{FE} / K_F (5 mg/L)	-	1.24/1.23	1.28/1.28	1.37/1.37	1.20/1.20	1.15/1.14

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

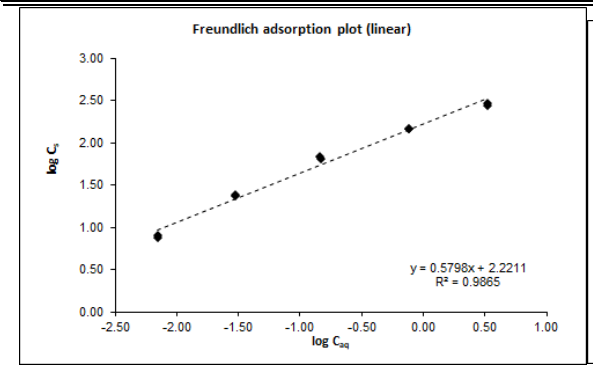
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 1

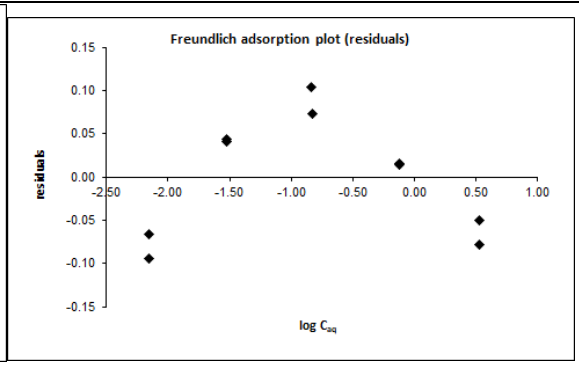


Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 2

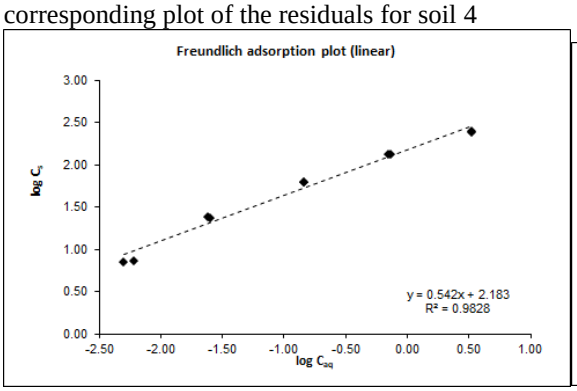


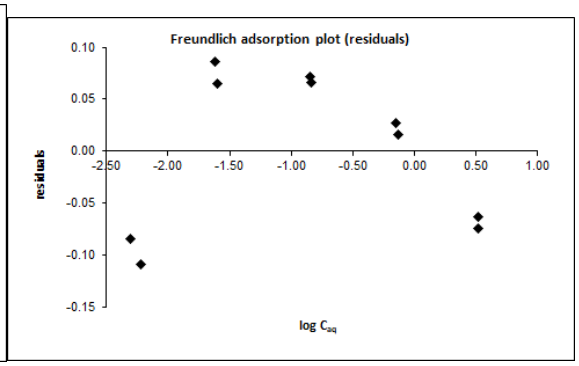
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 3



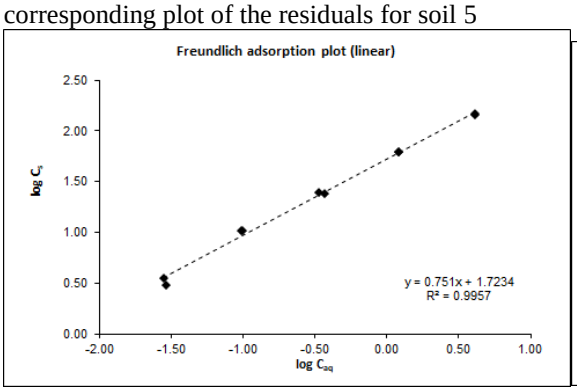


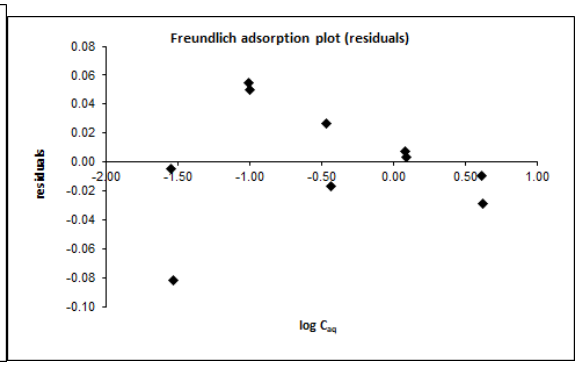
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 4



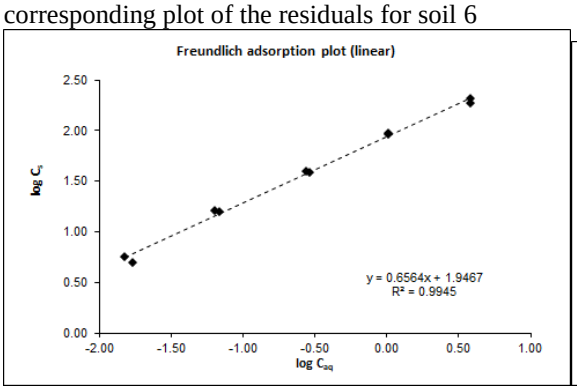


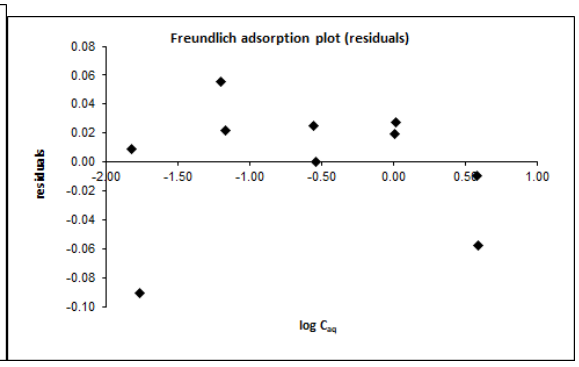
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 5



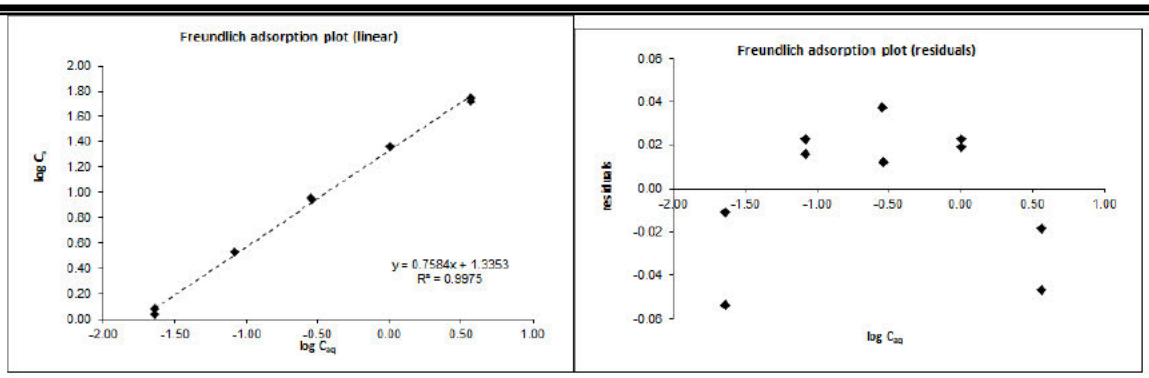


Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 6

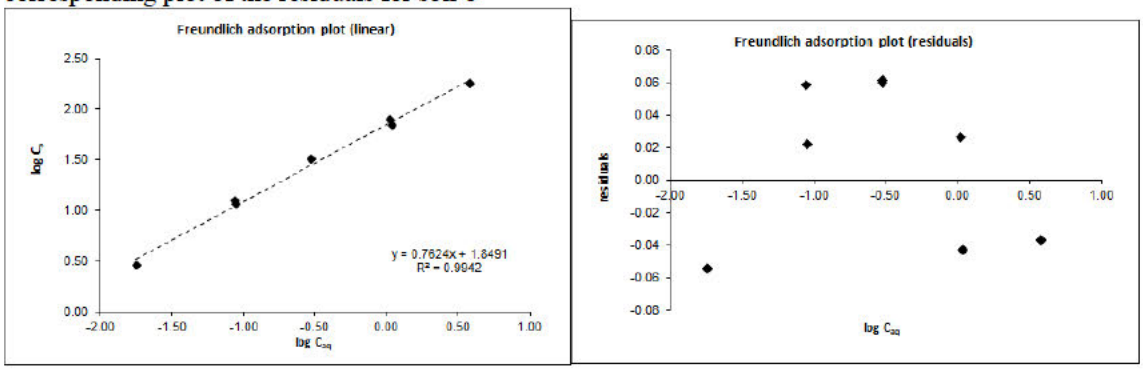




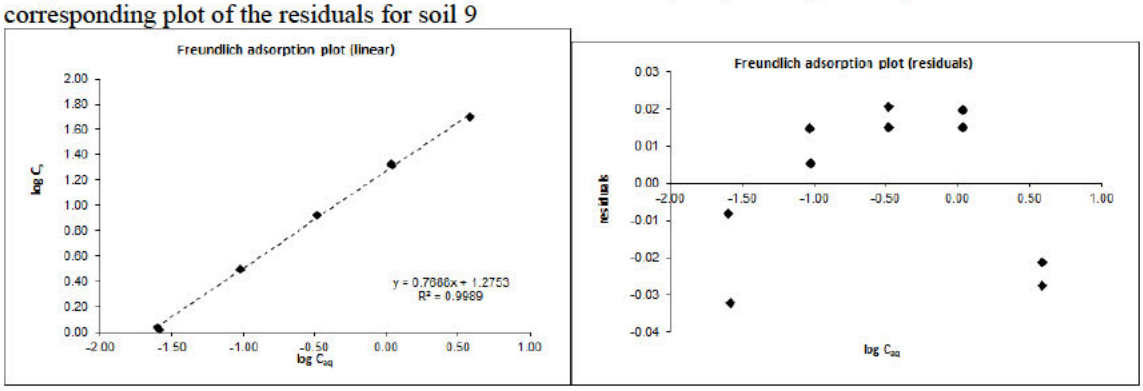
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 7



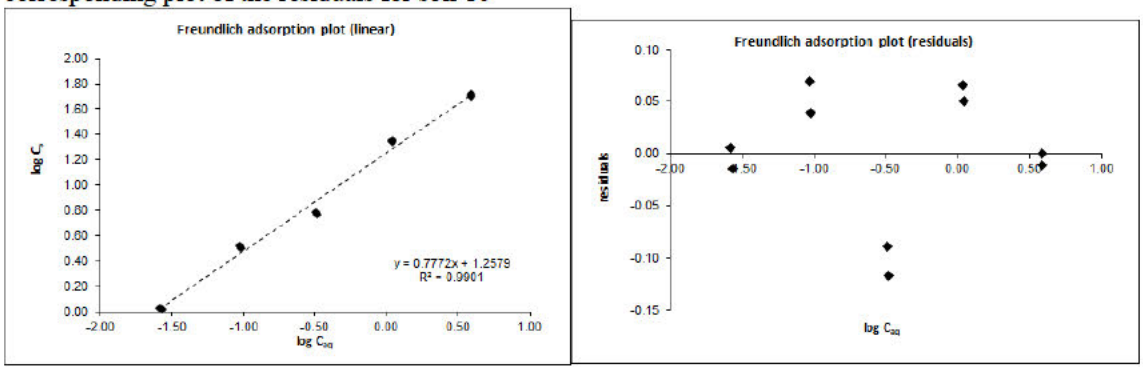
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 8



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 9



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 10



Assessment and conclusion by RMS:

The study summary was amended based on the additional information provided by the applicant further to RMS request and considering both amendments of the study report. Further clarifications provided are also discussed below.

1/ Regarding the results of the preliminary tests and the selected design for the definitive test, RMS noted the following points:

- Five preliminary tests were performed in order to determine the best soil/solution ratios and to check test item stability. While for soils 5-10, stability of glyphosate and appropriate mass balance were reported to be obtained with 22-24h equilibrium, the definitive phase was then performed with an equilibrium time of 4h for all soils, without further justification.
- No test was performed to determine the best equilibrium time for each soil, and it is wondered whether that equilibrium would be reached with an equilibrium time of 4 hours only.
- For soils 1, 3 and 4, the applicant states that either stability of glyphosate or appropriate mass balance could not be obtained in preliminary tests 1-3. It was then decided to reduce the equilibrium time. An alternative approach could have been to use the direct method, without reducing the equilibrium time.

The applicant was requested to better justify the approach used for the definitive test. Applicant stated that the main issue was the stability of the substance, the complete answer is reproduced below:

"Stability is expressed in terms of 'parental mass balance' of the test item. Test substance stability is demonstrated when >90 % of applied radioactivity (AR) were recovered for the portion of radioactivity representing the test item in a given soil adsorption sample after a certain contract time with soil at the given test concentration.

Investigations therefore started to determine stability expressed as 'parental mass balance' under the various conditions in preliminary tests. Recovery of radioactivity included radioactivity determination in the 0.01 M aqueous calcium chloride solution and in combined soil extracts. Combination of values each determined for total radioactivity (% AR) with those of the relative proportion from chromatographic analytical results for each of the two phases (% ROI) to finally result in 'parental mass balances'.

This concept can cause additional challenges for chemicals with very strong adsorption accompanied by degradation - when trying to optimize the experimental conditions for the various parameters like soil-to-solution-ratio, use of sterile vs non-sterile soil, test concentration, contact time with soil to also demonstrate stability. There was extensive testing of the variables when the study was performed.

Strong adsorption resulted in the amount of soil per sample to be reduced to the technically feasible limit for a soil-to-water ratio of 1-to-200. Strong sorption reduced in parallel the concentration of test item glyphosate dissolved in the aqueous phase, i.e. 0.01 M aqueous calcium chloride solution, significantly that was available for chromatographic analysis to allow for confirmation of stability. In turn, prolonged contact times to soil like 24 hours resulted in ageing of adsorbed residues paralleled by microbial decomposition to form non-extractable residues. In this context, the decline in extraction efficiency of test item from soil is an indication for degradation. All factors contributed to a fast decline of ^{14}C-test item detectable by chromatography in aqueous solution and soil extracts with time.

Conclusively and in order to fulfil best the requirements of the Guideline and the Guidance, the contact time with soil was lowered to 4 hours, allowing for sufficient extractability of radioactive residues from soil and analysis for test item stability in aqueous supernatants and soil extracts. The results of chromatographic analysis from preliminary tests 1 to 3 (see also in detail tabulated below) indicated in terms of % ROI that radioactivity in supernatants and soil extracts consisted regularly to 95 to 100 % of the test item. Dependent on conditions, exceptions were observed for Soils 4 and 8. For Soil 8, this could be overcome by change of conditions and the use of sterilised soil in the definitive test while % ROI was still below 90 % ROI for Soil 4. For the definitive test and for Soil 4, values of total radioactivity in supernatants and soil extracts were corrected for the portion of radioactivity attributable to the test item. For the other soils, no correction was performed with radioactivity in supernatants and soil extracts being represented quantitatively by the test item.

When it comes to 'parental mass balances', these were variable and all near the 90 % AR for the various soils. Though stability and reversibility of adsorption under the conditions of the test was given for most soils, a more conservative approach than use of the indirect approach was taken for evaluation of results of the definitive tests to result in a 'hybrid situation' for this study. Based on chromatographic results the radioactivity in supernatants and soil extracts was set as quantitative, i.e. all being represented by the test item (except for Soil 4). However, distribution coefficients were calculated only on the basis of radioactivity in supernatants and soil extracts without consideration of non-extractable radioactive residues. The approach is thus more conservative than a 'pure' indirect method approach and justified on the basis of stability demonstrated in supernatants and soil extracts. Again, the lower portion of radioactivity represented by test item was considered for Soil 4.

Given the overall time pressure in conduct of the study, conditions for conduct of the definitive test could not all be 'fully' optimised and reflected by preliminary tests. This is to some extent also indicated by a lack in material balances for some samples/soils in IES report 20190441 that were later amended by IES report 20200276.

Though adsorption conditions of the test technically being 'near to optimum', all soils but one fulfilled the stability criterion to finally apply the direct method according to OECD 106. For Soil 4, the stability criterion of 90 % AR in terms of parental mass balance was failed slightly (87.1-90.7 % AR). The low proportion of degradation observed was thus considered in the calculation of adsorption distribution values Kd.

Considering the characteristics of the test item, the test conditions had to be adapted accordingly for fulfilment of the various conditions set by the EFSA Evaluators Checklist for use of a 'modified indirect method approach' not considering NER in distribution coefficient calculation as explained above. The resulting experimental conditions for the definitive test were a compromise to comply best with all the requirements set by the OECD Guideline and the EFSA Checklist.

Being a compromise in view of the adsorption and degradation characteristics of the compound, the technical limitations do not allow to reach the ideal of adsorption equilibrium – this would also apply in case the direct method would be applied and runtimes of 24 hours.

When considering that prolonged contact times are rather unlikely to result in more optimal values of adsorption due to increasing degradation, this approach resulting in lower adsorption values is again regarded as conservative and justified.

Conditions for the definitive tests including procedures of handling and processing of samples was reported in document IES report 20190441. For some soils and samples with low overall mass balances of radioactivity, this data was amended by IES report 20200276 - affecting also parental mass balances used in the EFSA Evaluators Spreadsheet as quality criterion.

Investigations as summarized in the amendment confirmed that adsorption result quality was influenced only by low parental mass balances – from formal and strict application of the criteria set in the EFSA Evaluators Checklist and its spreadsheet. Low parental mass balances had been caused simply by low total mass balances of radioactivity.

The two IES reports underline that stability was given for nine out of ten soils under conditions of the definitive test. In combination with adsorption of more than 20 % AR clearly observed, the conduct and evaluation according to the indirect method was thus formally justified. For the nine soils including Soils 1 and 3, this procedure was supported by analysis in preliminary tests, but also by analytical results in definitive tests. Soil 4 was the sole exception showing some degradation - as considered in calculation of adsorption distribution coefficients in the definitive tests accordingly.

Nevertheless, a conservative approach was taken in evaluation of data to derive the adsorption distribution coefficients as indicated earlier in this document."

The justification provided by the applicant regarding the design used in the definitive test, conducted with reduced equilibrium time, is considered appropriate. For all soils except soil 4, the method used in the definitive test is a kind of hybrid method between indirect and direct method: LSC measurements are done in both soil extracts and supernatants, and calculations are based on these

measurements (assuming that all measured radioactivity corresponds to glyphosate). This is strictly equivalent to direct method only if parental mass balance is 100%. For soil 4, the standard direct method was used (calculations based on HPLC measurements in both soil extracts and supernatant).

2/ In the study reports, for preliminary tests and definitive tests, results from HPLC measurements in supernatant and soil extracts are expressed in % ROI in each compartment and not in % AR. This does not enable to check easily that parental mass balance (sum of % AR of glyphosate in supernatant and in soil extracts, bound residues excluded) is ≥90% AR. The applicant was requested to address this point.

Only the parental mass balance in preliminary test 5 was provided by the applicant to RMS (and presented under tables above). It can be seen that mean parental mass balance is above 90%, except for RefeSol 01-A (soil 2), 18 Acres (soil 3) and M-SL-PF (soil 4). This explains that HPLC measurements in both soil extracts and supernatant were performed at all concentrations in the definitive test in these soils.

Although requested by RMS, parental mass balance for the definitive test, for concentrations at which HPLC measurements are available, was not provided. They were calculated by RMS, from information available in the reports and are presented below (please note that calculations are based on rounded values presented in the report).

Parental mass balance during the definitive test – RMS calculations

Soil	Test concentration (mg/L)	Amount of glyphosate after adsorption - HPLC results (% ROI) - From study reports		Total radioactivity after adsorption - LSC results (% AR) - From study reports		Amount of glyphosate after adsorption (% AR) – RMS calculation		Parental mass balance (% AR) – RMS calculation	Mean parental mass balance (% AR) – RMS calculation
		Supernatant	Soil extracts	Supernatant	Soil extracts	Supernatant	Soil extracts		
Soil 1 Speyer 2.2	5	98.03	97.67	82.2	11.5	80.6	11.2	91.8	92.6
		97.88	96.1	85.1	10.5	83.3	10.1	93.4	
	1.6	100	100	71.6	22	71.6	22.0	93.6	93.4
		100	100	75.8	17.3	75.8	17.3	93.1	
	0.5	98.46	98.21	57.1	39.2	56.2	38.5	94.7	95.1
		98.3	98.64	57.3	39.6	56.3	39.1	95.4	
Soil 2 RefeSol 01-A	5	98.79	99.03	80.8	14.9	79.8	14.8	94.6	94.8
		97.87	100	83.9	13	82.1	13.0	95.1	
	1.6	98.18	100	71.5	24.5	70.2	24.5	94.7	94.8
		98.43	100	72.3	23.7	71.2	23.7	94.9	
	0.5	96.13	100	64.7	29.2	62.2	29.2	91.4	93.1
		95.49	100	66	31.7	63.0	31.7	94.7	
	0.16	97.34	100	59.3	29.8	57.7	29.8	**87.5**	**87.8**
		96.22	100	57.6	32.7	55.4	32.7	**88.1**	
	0.05	100	100	53.6	45.8	53.6	45.8	99.4	98.1
		100	100	51.4	45.3	51.4	45.3	96.7	
Soil 3 18 Acres	5	97.91	99.08	66.5	27.8	65.1	27.5	92.7	93.5
		98.49	99.13	66.1	29.5	65.1	29.2	94.3	
	1.6	100	100	48.5	46.9	48.5	46.9	95.4	95.0
		97.42	100	48.7	47.2	47.4	47.2	94.6	
	0.5	96.91	99.01	28.3	62.2	27.4	61.6	**89.0**	90.8
		96.86	98.84	27.9	66.3	27.0	65.5	92.6	
	0.16	93.82	100	18.7	74.7	17.5	74.7	92.2	91.7

		95.08	99.15	18.6	74.2	17.7	73.6	91.3	
	0.05	90.2	98.7	14.2	78	12.8	77.0	**89.8**	92.1
		96.7	99.2	12.4	83.1	12.0	82.4	94.4	
Soil 4 M-SL-PF	5	96.8	96.93	67.6	25.7	65.4	24.9	90.3	90.3
		96.62	97.26	68.1	25.1	65.8	24.4	90.2	
	1.6	94.11	97.61	50.2	43.9	47.2	42.9	90.1	**89.3**
		93.08	98.62	48.4	44.1	45.1	43.5	**88.5**	
	0.5	91.96	98.47	30.4	60.8	28.0	59.9	**87.8**	**87.6**
		91.62	98.39	29.8	61	27.3	60.0	**87.3**	
	0.16	92.05	98.4	17.1	75.5	15.7	74.3	90.0	90.8
		92.48	98.52	16.3	77.7	15.1	76.6	91.6	
	0.05	89	98.4	15.2	76.5	13.5	75.3	**88.8**	**86.2**
		82.2	99.1	13.1	73.4	10.8	72.7	**83.5**	
Soil 5 Speyer 2.3	5	98.23	98.71	82.2	14.3	80.7	14.1	94.9	95.0
		98.26	100	81.7	14.9	80.3	14.9	95.2	
	1.6	100	100	78.1	19.8	78.1	19.8	97.9	97.5
		100	100	77.2	19.8	77.2	19.8	97.0	
	0.5	98.09	98.87	64.5	23.8	63.3	23.5	**86.8**	**89.6**
		98.42	100	70.5	23	69.4	23.0	92.4	
Soil 6 RefeSol 02-A	5	100	98.73	76	18.6	76.0	18.4	94.4	94.7
		98.43	100	75.6	20.7	74.4	20.7	95.1	
	1.6	100	100	65.1	30	65.1	30.0	95.1	96.0
		100	100	66.1	30.8	66.1	30.8	96.9	
	0.5	98.42	99.03	55.2	37.4	54.3	37.0	91.4	90.7
		97.61	99.02	53	38.6	51.7	38.2	90.0	
Soil 7 Gartenacker	5	98.53	100	72.8	20.7	71.7	20.7	92.4	92.3
		96.94	98.86	72.7	22	70.5	21.7	92.2	
	1.6	97.15	98.26	65.5	29.5	63.6	29.0	92.6	93.3
		100	100	64.6	29.4	64.6	29.4	94.0	
	0.5	97.2	99.11	55.8	33.4	54.2	33.1	**87.3**	**87.7**
		97.45	99.55	54.7	34.9	53.3	34.7	**88.0**	
Soil 8 Speyer 6S	5	98.01	100	75.7	17.9	74.2	17.9	92.1	92.1
		98.38	100	75.6	17.8	74.4	17.8	92.2	
	1.6	95.65	100	67.6	25.1	64.7	25.1	**89.8**	90.2
		97.01	100	70.7	22.1	68.6	22.1	90.7	
	0.5	98.3	100	58	31.4	57.0	31.4	**88.4**	**88.2**
		97.26	100	58.2	31.3	56.6	31.3	**87.9**	
Soil 9 Speyer 5M	5	98.41	98.48	76.1	19.7	74.9	19.4	94.3	94.5
		98.16	98.3	76.3	20.1	74.9	19.8	94.7	
	1.6	100	100	70.3	26.8	70.3	26.8	97.1	96.9
		100	100	69.7	26.9	69.7	26.9	96.6	
	0.5	98	99.46	63.5	32.4	62.2	32.2	94.5	94.5
		97.97	100	63.7	32.1	62.4	32.1	94.5	
Soil 10 LAD-SL-PF	5	98.01	100	77.2	20.7	75.7	20.7	96.4	95.6
		97.96	100	76.4	20	74.8	20.0	94.8	

	1.6	97.34	100	70.2	29	68.3	29.0	97.3	97.6
		97.9	100	71.1	28.3	69.6	28.3	97.9	
	0.5	98.36	99.01	62	32.6	61.0	32.3	93.3	93.0
		98.17	99.61	63.2	30.9	62.0	30.8	92.8	

Note: Calculations based on rounded values

Mean parental mass balances are generally above 90% AR with some few exceptions (in bold) at which they remain very close to 90% AR (when excluding soil 4, all values are ≥87.7 % AR). RMS considers that stability was globally demonstrated. For M-SL-PF (soil 4), parental mass balance is below 90% for 3 concentrations, then final calculations are based on direct method (HPLC results).

3/ RMS notes that HPLC analysis for all concentrations in both aqueous supernatant and soil extracts are available for soils 2, 3 and 4. While HPLC results were used to derive the adsorption endpoints for soil 4 (since some degradation was observed), they were not used for soils 2 and 3. The applicant argued that *"Correction of values was performed for Soil 4 only since stability had been demonstrated for the other soils"*.

Although RMS agrees that stability of glyphosate has been demonstrated for soils 2 and 3 in the definitive test (with the exception of soil 2 at concentration of 0.16 mg/L, with a mean parental mass balance of 87.8% AR), RMS is still of the opinion that since HPLC results are available for both supernatant and soil extracts at all concentrations, the use of HPLC results would provide more accurate adsorption endpoints. However it can be agreed that no significant impact on the overall adsorption endpoint is expected in this case.

Regarding the OECD checklist tables completed by the applicant, RMS notes that indirect method is mentioned in the tables, however both the measured concentrations in supernatant and soil extracts were directly entered in the excel tool. This is considered appropriate.

The mass balance reported in the table for the highest concentration correspond to total recovery (including bound residues) and not to parental mass balance as recommended. RMS notes that different values are reported in the excel tool, but the origin of these values remain unclear to RMS. However, these values have an impact only for the estimation of K_{FE}/K_F, which is only relevant for indirect method. In this case, RMS considers that results of this ratio indicated in the applicant's tables should not be considered further.

The LOQ for LSC in aqueous phase is at least 2 orders of magnitude below the lowest nominal concentration tested. All LSC measured values in soil extracts and water are above the LOQ. As already discussed, parental mass balance was acceptable in most cases. The adsorbed percentage is below 20% on few occasions (soil 1, 2 and 5 at the highest concentration only). K_D x (soil:solution ratio) is sometimes below 0.3 for the highest concentration. However considering that calculations are based on measurements in both soil extracts and supernatant, and parental mass balance at the highest concentration is systematically above 90%, RMS considers that results can be considered as reliable. The study is overall well performed and the study design has been justified.

The study is considered acceptable.

[redacted], *1996*

Data point:	CA 7.1.3.1.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: adsorption and desorption properties in 5 soils
Report No	RJ2152B
Guidelines followed in study	OECD Guideline 106 US EPA 163-1

Deviations from current test guideline	From OECD Guideline 106 (January 2000): • Preliminary test only investigated the equilibrium time • Soil to solution ratio not optimal for 4 of 5 soils (very high adsorption percentage) • The concentrations used do not cover 2 orders of magnitude • Total recovery < 90% for some samples • Results of parental mass balance not reported in detail but indications that test item was not stable (degradation >10 % AR in supernatant and soil extracts for respective fraction reported)
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C]glyphosate (PMG label)

Lot No.	Not provided
Specific activity	1.67 GBq/mmol
Radiochemical purity	95 %

2. Test Soils

The soils were sieved to a particle size of ≤2 mm and were air-dried prior to use. The soils were gamma irradiated with between 25 and 40 kGy before application. A description of the soils used is presented in the table below.

Table 8.1.2.1-23 Physico-chemical properties of test soils

Parameter	Results				
Soil Designation	Lilly Field	Visalia	Wisborough Green	Champaign	18 Acres
City	Churt	Visalia	Wisborough Green	Champaign	Warfield, Bracknell
State	Surrey	California	Sussex	Illinois	Berkshire
Country	England	United States	England	United States	England
Textural Class (USDA)	Sand	Sandy loam	Silty clay loam	Silty clay loam	Sandy loam
Sand (50 µm – 2 mm) (%)	92	69	8	12	58
Silt (2 µm – 50 µm) (%)	4	18	60	52	23
Clay (< 2 µm) (%)	4	13	32	36	19
pH in soil:water (1:2)	5.7	8.4	5.7	6.2	7.4
Organic Carbon (%) 1	0.29	0.58	2.27	2.15	1.80
Organic Matter (%)	0.5	1.0	3.9	3.7	3.1
Cation Exchange Capacity (meq/100 g)	1.8	7.3	11.9	28.3	14.4
Water Holding Capacity					
at 1/3 bar (%)	3.1	10.4	30.9	22.7	17.1
at 15 bar (%)	1.1	4.8	19.8	13.5	10.4

1 calculated using the conversion factor as follows: % organic carbon = % organic matter / 1.72
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes with self-sealing caps were used as test systems. The experiments were performed in duplicate.

In preliminary tests, the appropriate adsorption and desorption equilibration times were determined.

For the definitive phase, the adsorption step was carried out using sterile air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:20. Glyphosate was applied at nominal concentrations of 2.0, 1.0, 0.2, 0.1, and 0.05 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 4 hours at 20 ± 2 °C under continuous agitation.

The desorption phase was performed by supplying pre-absorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution. The resultant samples were re-equilibrated for 21 hours at 20 ± 5 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and desorption step, the aqueous supernatant was separated from the soil by centrifugation and radioactivity in the supernatants was determined by liquid scintillation counting (LSC).

In the mass balance test, two additional samples at each concentration for Visalia soil and two additional samples at 0.2 mg/L for the other soils were analysed by LSC after the adsorption step. After transferring the supernatant, the wet soil was extracted with phosphate buffer followed by two acetone washes. Radioactivity in the extracts was quantified by LSC. Soil samples were combusted followed by quantitation using LSC. Aliquots of aqueous supernatants and soil extracts were analysed by thin layer chromatography (TLC) and radiodetection for degradates.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation using the indirect method, based on total recovery in supernatant.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Material balances were established for all concentrations tested for soil Visalia only. For all other soils material balances were established for one test concentration (0.2 µg/mL) only. After adsorption/desorption recovery of radioactivity ranged from 84 to 105 % for soil Visalia. For the remaining soils material balances (duplicates of 0.2 µg/mL samples) were 97 and 98 % for soil Champaign, 87 to 95 % for soil Wisborough Green, 96 to 97 % for soil 18 Acres soil and 88 to 93 % for soil Lilly Field.

B. STABILITY OF TEST ITEM

The TLC analysis of aqueous and soil extracts showed a single major metabolite aminomethylphosphonic acid (AMPA) in addition to parent glyphosate. Only relative amounts of glyphosate and AMPA in aqueous supernatant and soil extracts were reported. Results show that recovery of parent glyphosate was always <90 % of the radioactivity in aqueous adsorption supernatant and soil extract. The only exception was aqueous adsorption supernatant of the 1.0 µg/mL sample of soil Visalia with 94 % relative glyphosate recovery. However, glyphosate in the soil extract of this sample amounted to 67 % only with 9.9 % AMPA formed.

C. FINDINGS

The percentage of glyphosate adsorbed onto the soil ranged from 78 to 93 % (mean 87 %) in soil Lilly Field from 31 to 54 % (mean 44 %), in soil Visalia from 97 to 98 % (mean 97 %), in soil Wisborough Green, in soil Champaign from 97 to 98 % (mean 98 %) and in soil 18 Acres from 85 to 94 % (mean 91 %).

The adsorption coefficients K_F(ads) of glyphosate calculated based on the Freundlich isotherms of the four test soils ranged from 9.4 to 700 mL/g. The Freundlich exponents 1/n were in the range of 0.72 to 0.94, demonstrating a decrease in adsorption with increasing rate of application, there was however no saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC}$(ads) values ranged from 1600 to 33000 mL/g.

The desorption coefficients corrected for organic carbon, $K_{F, OC}$(des), ranged from 3000 to 56000 mL/g.

Table 8.1.2.1-24: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values)

Soil	Test Concentration [mg/L]

	2.0	**1.0**	**0.2**	**0.1**	**0.05**
Lilly Field	78	85	90	93	92
Visalia	31	31	53	51	54
Wisborough Green	97	97	97	97	98
Champaign	97	98	98	98	98
18 Acres	85	88	93	94	94

Table 8.1.2.1-25: [^{14}C]Glyphosate: Adsorption and desorption parameters in soil at 20 °C

Soil	**Adsorption**				**Desorption**
	K_F	**1/n**	**R^2**	**$K_{F, OC}$**	**$K_{F, OC}$**
Lilly Field	64	0.75	0.99	22000	50000
Visalia	9.4	0.72	0.99	1600	3000
Wisborough Green	470	0.93	1.00	21000	21000
Champaign	700	0.94	0.98	33000	56000
18 Acres	90	0.76	1.00	5000	6600

III. CONCLUSIONS

Glyphosate was strongly adsorbed in the five soils tested. The adsorption coefficients K_F(ads) of glyphosate calculated based on the Freundlich isotherms of the four test soils ranged from 9.4 to 700 mL/g. The Freundlich exponents 1/n were in the range of 0.72 to 0.94, demonstrating a decrease in adsorption with increasing rate of application, there was however no saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC}$(ads) values ranged from 1600 to 33000 mL/g.

The desorption coefficients corrected for organic carbon, $K_{F, OC}$(des), ranged from 3000 to 56000 mL/g.

Assessment and conclusion by applicant:

The assessment of data in the test was performed using the indirect method to calculate adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, the established PMB was insufficient with regard to test item stability to fulfil this criterion. Although, only relative amounts of glyphosate and AMPA in aqueous supernatants and soil extracts are reported it can be stated that degradation of test item glyphosate was >10 % since the relative recoveries were with only one single exception below 90 %.

The data of the study are therefore considered as supportive information. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

A further evaluation of results according to the EU OECD 106 Evaluators Checklist is presented for information.

Glyphosate: Results of evaluation of data according to EU OECD 106 Evaluators Checklist

	Units	Lillyfield	Visalia	Wisborough Green	Champaign	18 Acres [3]
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw mL	1:20	1:20	1:20	1:20	1:20
Parental mass balance (at highest conc.) (trigger 90%)	%	<90 [1]	<90 [1]	<90 [1]	<90[1]	<90 [1]
Adsorbed percentage	%	76.5-92.3	27.0-52.8	96.4-97.4	97.2-98.3	84.3-94.2
K_D x (soil:solution ratio) (>0.3 si indirect: >0.1 si direct)		3.5-12.3	0.4-1.2	28.3-38.9	35.9-58.6	5.7-16.8
$^{ads}K_F$ (95% confidence interval)	L/kg dw	64.547 (39.825-104.618)	9.426 (6.376-13.936)	470.551 (251.947-878.828)	708.663 (227.856-2204.044)	89.272 (74.195-107.414)

$^{ads}1/n$ (95% confidence interval)	-	0.746 (0.619-0.873)	0.725 (0.562-0.888)	0.935 (0.808-1.061)	0.938 (0.725-1.151)	0.762 (0.717-0.807)
$^{ads}R^2$	-	0.992	0.985	0.995	0.985	0.999
$^{ads}K_{F,OC}$	L/kg OC	21516	1571	20459	33746	4960
K_{FE} / K_F	-	- [2]	- [2]	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

[1] Values of parental mass balance not reported. However, degradation of glyphosate reported to be >10 %.

[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to a missing results of the parental mass balance providing the f-factor necessary for the calculations.

[3] Typo in Table 11 p.44 for concentration in aq. solution resulting in different results if used in the checklist as reported. Correct value should be 0.0156 µg/L instead of 0.156 µg/L.

Figure 8.1.2.1-1: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Lillyfield

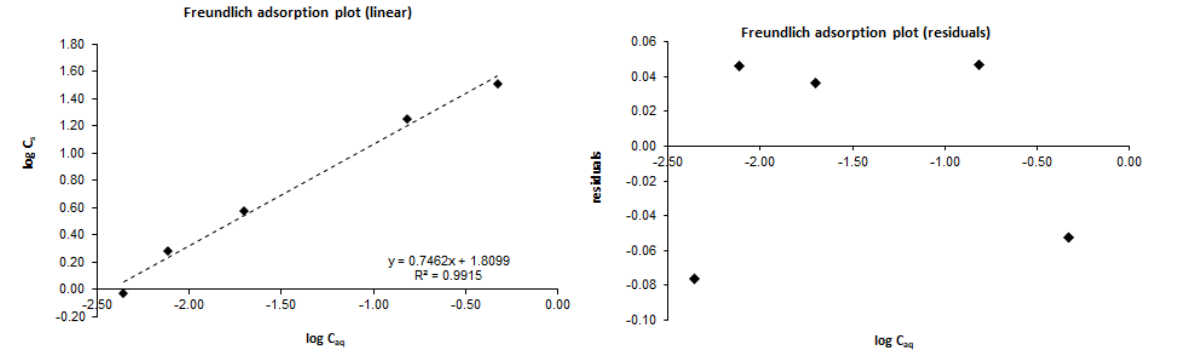


Figure 8.1.2.1-2: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Visalia

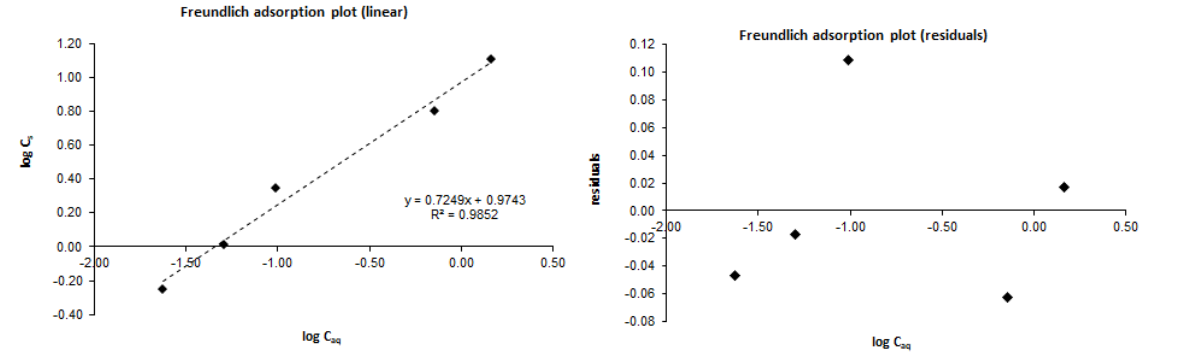


Figure 8.1.2.1-3: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Wisborough Green

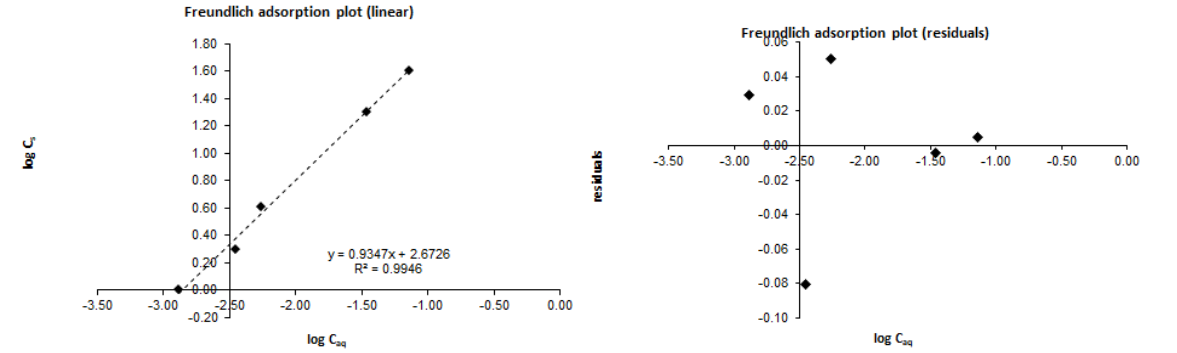


Figure 8.1.2.1-4: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Champaign

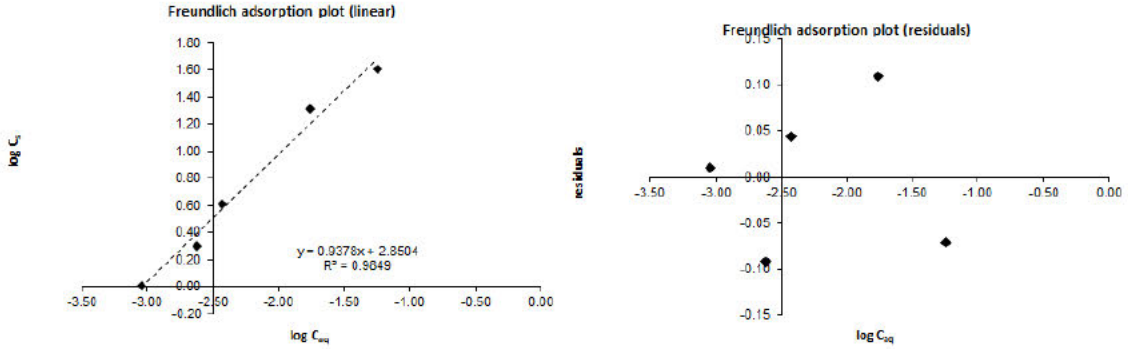
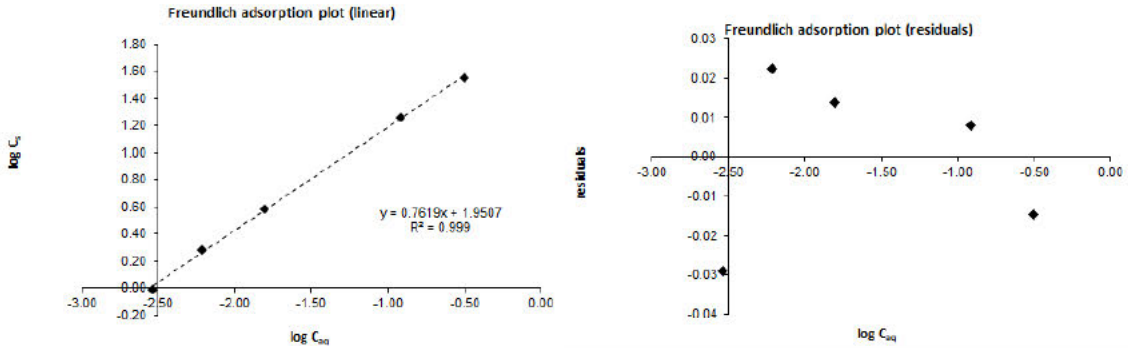


Figure 8.1.2.1-5: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 18 Acres



Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. Preliminary test was limited to the investigation of appropriate equilibrium time, soil to solution ratio used in the definitive phase is not considered optimal for 4 of the 5 soils (very high adsorption percentage measured in Lilly Field, Wisborough Green, Champaign and 18 Acres), the range of concentrations in the definitive test do not cover 2 orders of magnitude, total recovery is below 90% in some samples, and results of parental mass balance are not reported in details.

The stability of glyphosate was checked during the preliminary study, performed only with Visalia soil. LSC and TLC analysis were performed in supernatant and soil extracts and glyphosate was found to degrade, forming up to 15% AR AMPA in aqueous and soil phases.

For definitive test, an equilibration time of 4h was considered and the soil and aqueous phases were sterilized. TLC analysis of both liquid and soil phases was performed at all concentrations for Visalia soil and only at 0.2 mg/L for other soils. As indicated by the applicant, parental mass balance was not directly reported, however, the TLC analysis presented in the study indicate that glyphosate was not stable in these conditions either, with up to 33% AR AMPA formed during the adsorption phase, as shown below.

Table 13 : Percentage of Glyphosate, AMPA and Entrained Baseline Material From Aqueous and Soil Phase Extracts, as Determined by Thin Layer Chromatography and Phosphorimage Analysis

Soil	Nominal Rate of Application (µg mL^{-1})	Phase (Aqueous or Soil)	Step (Adsorption or Desorption)	% Extract as Glyphosate Acid	% Extract as AMPA	% of Extract as Baseline Entrained Material
'Visalia'*	0.1	Aqueous	Adsorption	56	33	6.0
	0.2			86	9.6	0.8
	1.0			94**	5.3	0.8
	2.0			83	12	0.6
"	0.05	"	Desorption	88	8.0	1.8
	0.1			75**	24	1.0
	0.2			74**	26	0.0
	1.0			79**	20	0.5
	2.0			72	14	1.4
'Champaign'	0.2	"	Adsorption	79	5.3	8.3
"	0.2	"	Desorption	79	12	3.0
'Wisborough Green'	0.2	"	Adsorption	82	4.4	3.7
"	0.2	"	Desorption	74	8.5	4.2
'18 Acres'	0.2	"	Adsorption	77	6.3	1.3
"	0.2	"	Desorption	78	8.1	1.3
'Lilly Field'	0.2	"	Adsorption	83	5.3	2.6
"	0.2	"	Desorption	90	4.5	1.2
'Visalia'	0.05	Soil	Adsorption	87**	4.0	8.6
	0.1			80	7.3	9.5
	0.2			65	11	1.7
	1.0			67	9.9	6.4
	2.0			77	9.4	1.1
"	0.05	"	Desorption	74	7.1	15
	0.1			75**	5.8	20
	0.2			77	12	0.0
	1.0			71	11	0.6
	2.0			71	27	2.1
'Champaign'	0.2	"	Adsorption	62	14	13
"	0.2	"	Desorption	77	13	2.4
'Wisborough Green'	0.2	"	Adsorption	57	13	4.3
"	0.2	"	Desorption	64	14	4.9
'18 Acres'	0.2	"	Adsorption	71	8.6	2.6
"	0.2	"	Desorption	78	6.5	3.1
'Lilly Field'	0.2	"	Adsorption	68	8.1	6.0
"	0.2	"	Desorption	66	9.8	5.2

* Radioactivity in lowest application rate too low for quantification purposes
** Values adjusted due to high background values achieved

Additionally, chromatography of the soil extracts led to some radioactivity entrained on the baseline of the plates. This radioactivity could be glyphosate or AMPA according to the study author.

Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was glyphosate, results cannot be considered as reliable.

The applicant indicates that the raw data of the study possibly could provide additional information to derive K_D value for the concentration tested in the parental mass balance test by applying the direct method. This is not considered needed since no robust value could be obtained considering that inadequate soil to solution ratio was used (except for Visalia soil) and sufficient Freundlich reliable values are available in other studies.

The study is not acceptable.

[redacted], *1996*

Data point:	CA 7.1.3.1.1/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate: determination of adsorption and desorption properties based on the OECD method 106
Report No	95-111-1020
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-equilibration of soils - Only 4 concentrations used - Soil to solution ratio not optimal (very high adsorption percentage)

	- Parental mass balance not established (no HPLC analysis of the soil extracts), but indications that it is not stable in the supernatant of Speyer 2.3 soil
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (PMG label)	
Lot No.	D1
Specific activity	11.7 MBq/mg (316 µCi/mg)
Radiochemical purity	99.6 %

2. Test Soils

The soils were collected prior to study start (depth of ± 20 cm) and sieved to a particle size of 2 mm. The soils were air-dried, the moisture content was adjusted, and soils were conditioned in aqueous 0.01 M CaCl2 solution before application. A description of the soils used is in the table below.

Table 8.1.2.1-26: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	**Speyer 2.1**	**Speyer 2.2**	**Speyer 2.3**
Country	Germany	Germany	Germany
Textural Class (DIN 4220)	Sand	Loamy sand	Loamy sand
Sand (> 63 µm) (%)	88.4	81.2	60.9
Silt (2 µm – 63 µm) (%)	9.8	13.4	29.6
Clay (< 2 µm) (%)	1.9	5.5	9.5
pH in $CaCl_2$	5.9	5.6	6.4
Organic Carbon (%)	0.62	2.32	1.22
Organic Matter (%) [1]	1.07	3.99	2.10
Cation Exchange Capacity (meq/100 g)	5.0	10.9	10.2
Water Holding Capacity maximum (g/100 g dry soil)	31	48	39

[1] calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72
DIN: Deutsches Institut für Normung

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes with Teflon® screw caps were used as test systems. The experiments were performed in triplicate.

For the preliminary phase, tests on glyphosate adsorption to the surface of the test vessels at all test concentrations and the appropriate adsorption equilibration times at the highest test concentration (5 mg/L) were performed. Two additional samples per soil were prepared at the highest test concentration (5 mg/L) for the material balance test.

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M CaCl2 solution with a soil-to-solution ratio of 1:5 (5 g soil/ 25 mL solution). 14C-Glyphosate was applied at nominal concentrations of 4.66, 0.98, 0.19, and 0.04 mg/L in aqueous 0.01 M CaCl2 solution. The adsorption step was carried out for 5 hours in the dark at 20 ± 1 °C under continuous agitation.

In each desorption phase, the supernatant was removed and fresh aqueous 0.01 M CaCl2 solution was added to the tubes. The resultant samples were re-equilibrated for 24 hours at 20 ± 1 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and each desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of radioactivity in the supernatants was analysed by LSC.

In the mass balance test, two additional samples per soil were analysed by LSC after the adsorption step. After transferring the supernatant, the wet soil was extracted three times with phosphoric acid in CaCl2. Radioactivity in the extracts was quantified by LSC. Unextractable radioactivity in the soil samples was determined by combustion followed by quantitation using LSC.

After the adsorption step and each desorption step, aliquots of the supernatant of the 4.66 mg/L test solution were analysed by high-performance liquid chromatography (HPLC) for degradates.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation using the indirect method, based on total recovery in supernatant.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances after 5 hours of equilibration were 95.3 % of applied radioactivity (% AR) for soil Speyer 2.1, 96.0 % AR for soil Speyer 2.2 and 95.8 % AR for soil Speyer 2.3.

B. STABILITY OF TEST ITEM

HPLC analysis of the supernatant of the 4.66 mg/L test solutions showed that after the first desorption step 65.9 % of the radioactivity present in the Speyer 2.3 sample consisted of degradates (mainly AMPA). After the second desorption step, 71.8 % of the radioactivity present in the Speyer 2.3 sample consisted of degradates. No degradates were found for the Speyer 2.1 and Speyer 2.2 soils. Results of the chromatographic analyses of aqueous supernatants and soil extracts after the adsorption step were not reported.

C. FINDINGS

The percentage of glyphosate adsorbed onto the soil ranged from 84.3 to 92.9 % for soil Speyer 2.1, from 93.7 to 97.3 % for soil Speyer 2.2 and from 87.6 to 94.7 % for soil Speyer 2.3. Results are presented in the table below:

Table 8.1.2.1-27: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values)

	Test Concentration [mg/L]			
	4.66	**0.98**	**0.19**	**0.04**
Speyer 2.1	84.3	89.9	91.9	92.9
Speyer 2.2	93.7	96.0	96.9	97.3
Speyer 2.3	87.6	92.2	93.8	94.7

The adsorption coefficients KF(ads) of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 29.52 to 71.72 mL/g. The corresponding, calculated $K_{F, OC}$(ads) values varied between 3091 and 4762 mL/g. Results are presented in the table below:

Table 8.1.2.1-28: [^{14}C]Glyphosate: Adsorption parameters in soil at 20 °C

Soil	**Adsorption**			
	$K_{F(ads)}$ [mL/g]	**1/n**	**R^2**	**$K_{F(ads), OC}$ [mL/g]**
Speyer 2.1	29.52	0.843	0.997	4762
Speyer 2.2	71.72	0.840	0.997	3091
Speyer 2.3	37.72	0.837	0.997	3092

The desorption coefficients KF(des) of glyphosate after the first desorption calculated based on the Freundlich isotherms of the three test soils ranged from 39.59 to 118.07 mL/g. The corresponding, calculated $K_{F, OC}$(des) values varied between 3245 and 8178 mL/g.

The desorption coefficients KF(des) of glyphosate after the second desorption calculated based on the Freundlich isotherms of the three test soils ranged from 51.72 to 123.6 mL/g. The corresponding, calculated $K_{F, OC}$(des) values varied between 4240 and 9401 mL/g. Results are presented in the table below:

Table 8.1.2.1-29: [^{14}C]Glyphosate: Desorption parameters in soil at 20 °C

Soil	First desorption				Second desorption			
	$K_{F\ (des)}$ [mL/g]	**1/n**	**R^2**	**$K_{F, OC(des)}$ [mL/g]**	**$K_{F\ (des)}$ [mL/g]**	**1/n**	**R^2**	**$K_{F, OC(des)}$ [mL/g]**
Speyer 2.1	50.70	0.910	0.999	8178	58.29	0.883	0.999	9401
Speyer 2.2	118.07	0.878	0.999	5089	123.6	0.844	0.999	5327
Speyer 2.3	39.59	0.872	0.999	3245	51.72	0.899	0.999	4240

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 29.52 to 71.72 mL/g. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3091 and 4762 mL/g.

Assessment and conclusion by applicant:

The assessment of data in the test was performed using the indirect method to calculate adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, the data reported do not allow for the conclusion that the test substance was stable.

The data of the study are therefore considered as supportive information. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

Though the study does not fulfil the requirements as set out in the EU OECD 106 Evaluators Checklist, the results of the study were summarised formally below.

Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Speyer 2.1	Speyer 2.2 [3]	Speyer 2.3
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	- [1]	- [1]	- [1]
Adsorbed percentage	%	84.8-93.3	94.1-97.7	88.3-95.1
K_D x (soil:solution ratio)		5.6-14.0	15.9-42.7	7.5-19.7
$^{ads}K_F$ (95% confidence interval)	L/kg dw	29.608 (26.468-33.119)	71.570 (62.714-81.676)	37.733 (33.615-42.355)
$^{ads}1/n$ (95% confidence interval)	-	0.845 (0.815-0.874)	0.839 (0.811-0.867)	0.838 (0.809-0.866)
$^{ads}R^2$	-	0.998	0.998	0.998
$^{ads}K_{F,OC}$	L/kg OC	4775	3085	3093
K_{FE} / K_F	-	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

[1] Values of parental mass balance not reported. No information on NER. After 2nd desorption step >80% remain adsorbed.

[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to a missing results of the parental mass balance providing the f-factor necessary for the calculations.

[3] Typo in Table 4 p.39 of original study for concentration in aq. solution of third replicate of lowest test concentration resulting in different results if used in the checklist as reported. Correct value should be 0.00100 µg/L instead of 0.00010 µg/L

Figure 8.1.2.1-6: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.1

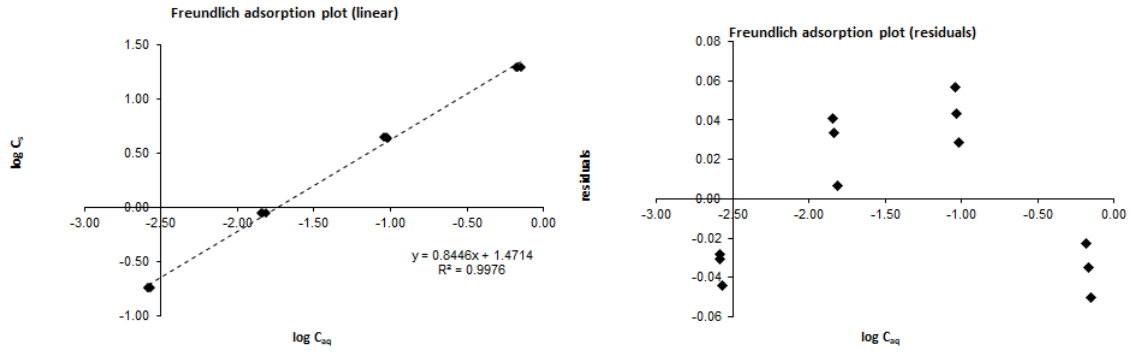


Figure 8.1.2.1-7: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.2

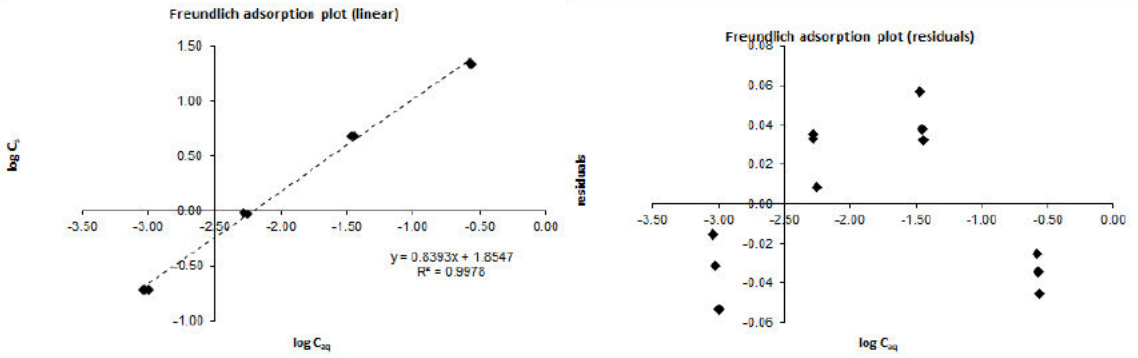
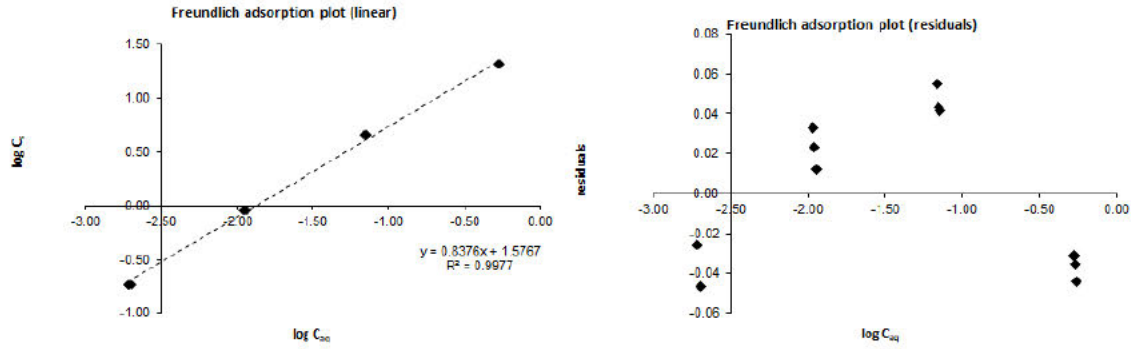


Figure 8.1.2.1-8: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.3



Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. There was no pre-equilibration of soils, only 4 concentrations were tested instead of 5, selected soil to solution ratio is not considered optimal (very high adsorption percentage measured) and parental mass balance was not established.

The information provided in the study report are not sufficient to confirm the stability of the substance. While HPLC analysis in the supernatants of Speyer 2.1 and 2.2 showed that 100% of the radioactivity was determined as glyphosate, glyphosate and two degradates were observed in the supernatant of soil Speyer 2.3. No analysis of any soil extracts was performed and so the parental mass balance is not available for any of the soils. It is also noted that the overall mass balance, including bound residues, were close to 90% AR, indicating that it is likely that parental mass balance is below 90%.

Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was glyphosate, results cannot be considered as reliable.

The applicant indicates that the raw data of the study possibly could provide additional information to derive K_D value for the concentration tested in the parental mass balance test by applying the direct method. This is not considered needed since no robust value could be obtained considering that inadequate soil to solution ratio was used.

This study is not considered acceptable.

[redacted], *1993*

Data point: CA 7.1.3.1.1/007

Report author	[redacted]
Report year	1993
Report title	Glyphosate isopropylaminesalt adsorption/desorption
Report No	PR93/017
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-tests to determine optimal soil:solution ratio and equilibration time - No pre-equilibration of soils - Test conducted at a single concentration - Soil to solution ratio not optimal (very high adsorption percentage) - Parental mass balance after the adsorption phase not available and low recovery of test item after the desorption phase
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Glyphosate isopropylamine salt (non-labelled)	
Lot No.	10819
Chemical purity	98 %

2. Test Soils

The characteristics of test soils is summarised in the table below.

Table 8.1.2.1-30: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	2.1 (#14292)	2.3 (#3101)	F3, 341 (F331)
Country	Germany	Germany	Germany
Textural Class (USDA)	Sand	Loamy sand	Sandy loam
(630 µm – 2 mm) (%)	4.5 ± 0.6	3.2 ± 0.6	1.1 ± 0.2
(200 µm – 630 µm) (%)	62.9 ± 2.4	32.5 ± 3.2	13.5 ± 0.3
(63 µm – 200 µm) (%)	20.0 ± 2.8	28.4 ± 2.9	25.2 ± 0.7
(20 µm – 63 µm) (%)	4.7 ± 2.0	16.4 ± 3.3	30.3 ± 0.5
(6 µm – 20 µm) (%)	2.5 ± 0.7	7.5 ± 1.2	10.0 ± 0.4
(2 µm – 6 µm) (%)	1.9 ± 0.8	3.9 ± 0.5	4.7 ± 0.3
(< 2 µm) (%)	3.5 ± 1.6	8.3 ± 1.4	15.2 ± 0.4
pH in water	5.9	6.3	7.3
Organic Carbon	0.70 ± 0.07	1.34 ± 0.14	1.20 ± 0.07
Organic Matter [1]	1.20	2.30	2.06
Cation Exchange Capacity (mval/100 g)	4.9 ± 0.8	9.5 ± 0.9	13 ± 0.0
Water Holding Capacity maximum (g/100 g soil DW)	26.1	34.9 ± 1.6	45.7

[1] calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

No preliminary tests were performed. For the definitive phase, the adsorption step was carried out at a soil-to-solution ratio of 1:5 (2 g soil/10 mL solution). Test item was applied at a nominal concentration

of 5 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 16 hours under continuous agitation. In parallel control samples were prepared without soil and test solution only.

For each of the two successive desorption steps, fresh aqueous 0.01 M $CaCl_2$ solution was added to the pre-adsorbed soil samples the tubes. The resultant samples were re-equilibrated for 16 hours under continuous agitation followed by centrifugation.

2. Analytical Procedures

The aqueous supernatant after adsorption was separated by centrifugation and the glyphosate isopropylamine salt residues in the supernatant were analysed by gas chromatography (GC). The aqueous supernatant after each desorption step was separated by centrifugation. HPLC-clean up of the supernatant was performed to collect two fractions (fraction 1: AMPA, fraction 2: Glyphosate) and fractions were analysed by gas chromatography (GC). The limit of detection in tap water (method not validated for 0.01 M $CaCl_2$) was 0.0.2 µg/L for glyphosate and 0.06 µg/L for AMPA.

To determine the recovery of the test item, glyphosate isopropylamine salt was extracted from the soil with water and phosphoric acid following the desorption phase. Soil extracts were analysed by GC. For soil the limit of detection was 20 µg/kg for glyphosate and AMPA.

Adsorption coefficients of glyphosate were calculated by the indirect method.

II. RESULTS AND DISCUSSION

A. STABILITY OF TEST ITEM

Total recoveries of test item in aqueous adsorption and desorption supernatants and soil extract following the desorption phase were 74.8 and 75.2 % for soil 2.1, 62.0 and 63.0 % for soil 2.3, and 32.6 and 34.4 % for soil F3, 341. The reason for parental mass balances ≤75 % was the formation of non-extractable residues following various extraction steps.

B. FINDINGS

Most of the glyphosate isopropylaminesalt (89.9 to 94.6 %) was adsorbed to the soil and 6.3 to 7.4 % was desorbed following two desorption cycles.

Table 8.1.2.1-31: Glyphosate isopropylamine salt: Recovery in supernatant

Soil	Replicate	Percentage[1]	
		Adsorption	Desorption
2.1	1	9.3	7.4
	2	5.6	6.9
2.3	1	7.0	7.4
	2	5.4	6.3
F3, 341	1	10.1	6.3
	2	7.2	6.8

[1] Mean values expressed as percentage of applied glyphosate

The adsorption coefficients $K_{D(ads)}$ of glyphosate isopropylamine salt calculated on the three test soils ranged from 54.4 to 76.5 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 4533 to 9486 mL/g. Results are presented in the table below:

Table 8.1.2.1-32: Glyphosate isopropylamine salt: Adsorption parameters in soil

Soil	Adsorption	
	$K_{D(ads)}$ [mL/g]	$K_{D, OC(ads)}$ [mL/g]
2.1	66.4	9486
2.3	76.5	5709
F3, 341	54.4	4533

III. CONCLUSIONS

The adsorption coefficients $K_{D(ads)}$ of glyphosate isopropylamine salt calculated on the three test soils ranged from 54.4 to 76.5 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 4533 to 9486 mL/g.

Assessment and conclusion by applicant:

The test was performed using the indirect method for determination of adsorption to soil since the concentration of the test item was determined in aqueous adsorption supernatant only and not in the soil phase (i.e. soil extracts). Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, no sufficient parental mass balance was established since the recovery of the test item was investigated following the desorption phase. Soil extraction and analysis of extracts for test item following the adsorption step was not performed.

The data are therefore regarded as supporting. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

Though the study does not fulfil the requirements as set out in the EU Evaluators Checklist, the results of the study were summarised formally in the table below. No graphical and statistical evaluation according to Freundlich and the EU Evaluators Checklist is possible.

Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Soil 2.1	Soil 2.2	Soil 2.3
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	75.0 [1]	62.5 [1]	33.5 [1]
Adsorbed percentage	%	90.8-94.4	93.0-94.6	90.0-92.8
K_D x (soil:solution ratio)		9.9-16.9	13.3-17.5	9.0-12.9
$^{ads}K_F$ [2] (95% confidence interval)	L/kg dw	66.8	77.0	54.7
$^{ads}1/n$ (95% confidence interval)	-	- [3]	- [3]	- [3]
$^{ads}R^2$	-	- [3]	- [3]	- [3]
$^{ads}K_{F,OC}$ [2]	L/kg OC	9545	5747	4560
K_{FE} / K_F	-	1.4	1.7	>3.5

Note: Values derived from the EU OECD 106 evaluators checklist may vary from those in the study reports due to rounding errors.

[1] Large amounts of NER formed.

[2] Only K_D because of one test concentration only

[3] Not applicable because of one test concentration only

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. No preliminary study was performed to determine the adequate soil:solution ratio and equilibration time, soils were not pre-equilibrated,only one single concentration was used, and the soil to solution ratio used is not considered optimal (very high adsorption percentage measured), and the parental mass balance available after the desorption step indicates that the test item is not stable.

Some chromatograms for soil 2.3 were provided in the study report as examples. HPLC analysis of the supernatant of soil 2.3 after 16 hours equilibrium time clearly shows the presence of glyphosate but also of a degradation product formed in higher amounts than glyphosate.

The applicant indicates that the raw data of the study possibly could provide additional information to derive K_D for the concentration tested in the parental mass balance test by applying the direct method. This is not considered needed since no robust value could be obtained considering that inadequate soil to solution ratio was used.

The study is not considered acceptable.

[redacted], *1992*

Data point:	CA 7.1.3.1.1/009
Report author	[redacted]
Report year	1992
Report title	(14C)-Glyphosate : Adsorption/desorption in soil
Report No	7180
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision N: Chemistry: Environmental Fate Section 163-1 (October, 1982)
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-equilibration of soils - No preliminary test to determine the optimal soil to solution ratio - Soil to solution not optimal for 5 of the 6 soils (very high adsorption percentage) - Test conducted at a single concentration - No parental mass balance established (no chromatographic analysis of soil) but indication that test item is not stable in the supernatant
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C]glyphosate (PMG label)

Lot No.	CFQ.6228 and CFQ.6647
Specific activity	11.2 MBq/mg (304 µCi/mg) and 11.1 MBq/mg (299 µCi/mg)
Radiochemical purity	99.2 % and 99.4 %

2. Test Soils

The soils were stored covered under outdoor conditions. Soils were moistened with deionised water and the excess water was allowed to evaporate. The soils were kept moist by the addition of deionised water before application. The characterisation of test soils used is summarised in the table below.

Table 8.1.2.1-33: Physico-chemical properties of test soils and sediment

Parameter	**Results**					
Soil Designation	**Greenan sand**	**Auchincruive**	**Headley Hall**	**Californian sandy soil**	**Les Evouettes II**	**Darnconner sediment**
Geographic Location						
City	-	-	Leeds	-	-	-
State	-	-	West Yorkshire	-	-	-
Country	Scotland	Scotland	England	United States	Switzerland	Scotland
Textural Class (USDA)	Sand	Sandy loam	Sandy clay loam	Loamy sand	Silt loam	Loam
Sand (%)	95	75	47	83	38.0	39
Silt (%)	2	12	21	11	50.7	40
Clay (%)	3	13	32	6	11.3	21
pH						
- in water	5.7	7.1	7.8	8.3	6.1	7.1
- in KCl	4.7	6.1	7.1	7.6	5.3	6.0
Organic Carbon	0.8	1.6	1.4	0.6	1.4	3.0
Organic Matter (%) [1]	1.38	2.75	2.41	1.03	2.41	5.16
Cation Exchange Capacity (meq/100 g))	5.0	12.0	13.0	7.0	15.5	17.0

WHC at 33KPa (%)	8.1	18.1	23.5	11.9	29.7	NA
Bulk Density (disturbed) (g/mL)	1.38	1.05	1.09	1.44	0.88	1.14

[1] calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72
USDA: United States Department of Agriculture; NA: Not applicable

B. STUDY DESIGN

1. Experimental Conditions

Culture glass tubes with screw caps were used as test vessels. The experiments were performed in duplicate.

The preliminary phase consisted of tests on adsorption of glyphosate to the test vessels, test item stability and the appropriate adsorption and desorption equilibration times.

For the definitive phase, the adsorption test was carried out at a soil-to-solution ratio of 1:5. Soils and sediment were sterilised by gamma irradiation (25 KGy) prior to application. Glyphosate was applied at a nominal concentration of 5 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 16 hours under continuous agitation.

For each desorption step, fresh aqueous 0.01 M $CaCl_2$ solution was added to pre-adsorbed soil samples and the resultant samples were re-equilibrated for 16 hours under continuous agitation.

2. Analytical Procedures

After the adsorption step and each desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of radioactivity in the supernatants was analysed by liquid scintillation counting (LSC).

Triplicate aliquots of soil samples were combusted followed by quantitation using radioassay.

Aliquots of Headley Hall, Californian sandy soil, and Les Evouettes II supernatants were analysed by thin layer chromatography (TLC) for glyphosate and degradation products.

Adsorption coefficients of glyphosate were calculated by analysis of the adsorption data by the indirect method.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Following 48 h of equilibration, mean material balances were 89.74% AR for Greenan sand, 88.02 % AR for Auchincruive sandy loam, 92.99 % AR for Headley Hall sandy clay loam, 92.19 % AR for the Californian loamy sand, 94.60 % AR for Les Evouettes II silt loam and 96.75 % AR for Darnconner loam sediment.

B. STABILITY OF TEST ITEM

Stability of glyphosate was investigated in aqueous $CaCl_2$ supernatants following the adsorption step only. Headley Hall, Californian sandy soil, and Les Evouettes II supernatants were analysed and in all cases the majority of the sample radioactivity (82 to 94 %) co-chromatographed with the glyphosate standard.

C. FINDINGS

In all soil/sediment types with the exception of Californian sandy soil, >89 % AR was adsorbed after 16 hours equilibration and <6 % was desorbed after two 16 hour desorption steps. In Californian sandy soil, approximately 50 % AR was adsorbed after 16 hours and approximately 19 % was desorbed after two 16 hour desorption steps. Results are presented in the table below:

Table 8.1.2.1-34: Percentage of applied radioactivity present in supernatant after the adsorption phase and in the soil residues after both desorption phase (combustion)

Soil	% AR in the supernatant – adsorption phase	% AR in soil (soil combustion after adsorption and 2 desorption phases)
Greenan sand	1.81	86.77

Auchincruive	0.58	86.98
Headley Hall	7.35	79.25
Californian sandy soil	39.51	31.59
Les Evouettes II	8.77	97.37
Darnconner sediment	0.93	92.20

The adsorption coefficients $K_{D(ads)}$ of glyphosate calculated on the five test soils and one sediment ranged from 5 to 811 mL/g and the corresponding $K_{DOC(ads)}$ values ranged from 884 to 50660 mL/g. Results are presented in the table below:

Table 8.1.2.1-35: [^{14}C]Glyphosate: Adsorption coefficients in soil and sediment at a single test concentration of 5 mg/L

Soil/Sediment	**Adsorption**	
	K_D(ads) [mL/g]	**$K_{D, OC}$(ads) [mL/g]**
Greenan sand	263	32838
Auchincruive	811	50660
Headley Hall	50	3598
Californian sandy soil	5	884
Les Evouettes II	48	3404
Darnconner sediment	510	17010

III. CONCLUSIONS

The adsorption coefficients $K_{D(ads)}$ of glyphosate calculated on the five test soils and one sediment ranged from 5 to 811 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 884 to 50660 mL/g.

Assessment and conclusion by applicant:

The test was performed using the indirect method for determination of adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, no PMB was determined in this test to fulfil this criterion.

The results of the study are thus considered as supportive information. Though the study does not fulfil actual data requirements, the results of the study were summarised formally in the table below. No graphical and statistical evaluation according to Freundlich and the EU Evaluators Checklist is possible. The test was performed at one test concentration only.

Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Greenan sand	Auchin-cruive	Headley Hall	Californian sandy soil	Les Evouettes II	Darnconner sediment
Adsorption method	-	indirect	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	- 1	- 1	- 1	- 1	- 1	- 1
Adsorbed percentage	%	98.1-98.2	99.3-99.4	90.9-91.1	52.4-53.2	90.5-90.7	98.6-99.3
K_D x (soil:solution ratio)		51.1-54.0	150.5-171.4	10.0-10.3	1.1	9.5-9.7	68.4-134.1
Ads K_F [1] (95% confidence interval)	L/kg dw	262.6	804.8	50.7	5.6	48.0	506.5
ads1/n (95% confidence interval)	-	- 3	- 3	- 3	- 3	- 3	- 3
Ads R^2	-	- 3	- 3	- 3	- 3	- 3	- 3
Ads $K_{F,OC}$ [2]	L/kg OC	32821	50301	3621	931	3431	16882
K_{FE} / K_F	-	- 4	- 4	- 4	- 4	- 4	- 4

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
1 PMB was not established. Only aq. supernatants analysed by chromatographic methods (≥ 30% NER).
2 Only KD because of one test concentration only
3 not applicable because of one test concentration only
4 The check for systemic errors (expressed as KFE / KF) could not be performed due to missing parental mass balance providing the f-factor necessary for the calculations.

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. Soils were not pre-equilibrated, no preliminary test to determine the optimal soil to solution ratio was performed, the selected soil to solution ratio is not optimal for 5 of the 6 soils (very high adsorption percentage measured), only one single concentration was used and no parental mass balance was established.

The total recovery of radioactivity ranges from 84.47 to 101.51 % AR, it is therefore expected that parental mass balance is < 90% in some samples. The available TLC analysis in supernatants indicate that the test item is not stable in some samples. No chromatographic analysis of the test item in soil was performed.

Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was glyphosate, results cannot be considered as reliable.

RMS also notes that the radioactivity present in the supernatants after the adsorption phase is very low for Greenan sand soil (1.81%AR), Auchncruive soil (0.58%AR) and Darnconner sediment (0.93% AR). It is not clear from the study report whether the analytical method is appropriate to ensure accurate measurements.

The study is not considered acceptable.

[redacted], *1986*

Data point:	CA 7.1.3.1.1/011
Report author	[redacted]
Report year	1986
Report title	Australian notification base testing requirements for N- (Phosphonomethyl) Iminodiacetic Acid (Glyphosate Intermediate), Part II: Adsorption/Desorption Data.
Report No	MSL-5393
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-equilibration of soils - Only 4 concentrations used - No preliminary test to determine optimal soil to solution ratio - Soil to solution ratio not optimal (very high adsorption percentage) - Total mass balance < 90% (approx. 70 % AR) - No parental mass balance established (chromatographic analysis of adsorption supernatants only), but glyphosate reported to be not stable in supernatant (≤59 % test item)
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (label position not reported)

Lot No.	C-927.3A
Specific activity	8.79 mCi/mmol
Radiochemical purity	98.0 %

2. Test Soils

The soils were sieved to a particle size of ≤425 µm. The soils were air-dried before application. A description of the soils used is in the table below.

Table 8.1.2.1-36: Physico-chemical properties of test soils

Parameter	**Results**		
Soil Designation	**Drummer**	**Dupo**	**Spinks**
Geographic Location			
City	Decatur	St. Charles	East Lansing
State	Illinois	Missouri	Michigan
Country	USA	USA	USA
Textural Class	Silty clam loam	Silt loam	Loamy sand
Sand [%]	16.0	18.0	74.0
Silt [%]	56.4	68.0	22.4
Clay [%]	27.6	14.0	3.6
pH in water	6.5	7.4	5.2
Organic Carbon [%]	1.45	0.87	1.10
Organic Matter [%] [1]	2.49	1.50	1.89
Cation Exchange Capacity [meq/100 g]	20.2	8.7	5.8

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72

B. STUDY DESIGN

1. Experimental Conditions

Glass centrifuge tubes (either 25 or 50 mL) were used as test systems. The tests were performed with triplicate soil samples.

In preliminary tests, the appropriate adsorption and desorption equilibration times and the stability of the test item were determined. Samples with a concentration of 5 mg/L were prepared to establish a material balance.

For the definitive phase the adsorption phase was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution at a soil-to-solution ratio of 1:5. The nominal test concentrations were 5.0, 1.0, 0.2 and 0.04 mg/L. The adsorption step was carried out for 21 to 24 hours (details not reported) under continuous agitation at 24-26 °C.

In the first desorption step, fresh aqueous 0.01 M $CaCl_2$ solution was added to pre-adsorbed soil specimens for all test concentrations. The resultant samples were re-equilibrated for 17 to 26 hours under continuous agitation. In the second desorption step, the procedure was repeated. The resultant samples were re-equilibrated for 21 to 23 hours under continuous agitation.

2. Analytical Procedures

After the adsorption step and each desorption step, the aqueous supernatant was separated by centrifugation and the radioactivity in the supernatant was determined by liquid scintillation counting (LSC).

Radioactivity in adsorption solutions of the highest test concentration was characterized by high performance liquid chromatography (HPLC).

For the material balance test radioactivity adsorbed to soil was determined by combustion of aliquots of samples and determination by LSC following the adsorption phase. The remaining soil was extracted using 0.5 M NH_4OH and radioactivity of the soil extracts was determined by LSC. Chromatographic analyses of soil extracts were not performed.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation, using the indirect method.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Recovered radioactivity determined in a material balance test accounted for 71 % of applied radioactivity (% AR) for soil Drummer, 67 % AR for soil Dupo and 67 % AR for soil Spinks.

B. STABILITY OF TEST ITEM

In the aqueous supernatants of the adsorption phase glyphosate was found at relative amounts of 50 % for soil Drummer, 48 % for soil Dupo and 59 % for soil Spinks. The metabolite aminomethylphosphonic acid (AMPA) was found at relative amounts of 14 % for soil Drummer, 47 % for soil Dupo and 32 % for soil Spinks.

B. FINDINGS

At the end of the adsorption phase 97.86-99.00 %, 87.84-93.05 %, and 96.92-98.78 % of the applied test material were adsorbed to soils Drummer, Dupo, and Spinks, respectively. The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 33 to 660 mL/g. The Freundlich exponents 1/n were in the range of 0.80 to 1.16. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3800 and 60000 mL/g.

After the desorption phase, between 0.70 and 7.88 % of the initially adsorbed radioactivity was desorbed from the respective soils.

Table 8.1.2.1-37: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values of triplicates)

	Test Concentration [mg/L]			
	5	1	0.2	0.04
Drummer	98.68	99.00	98.55	97.86
Dupo	87.84	91.84	93.05	91.98
Spinks	98.78	98.47	98.22	96.92

Table 8.1.2.1-38: [14C]Glyphosate: Percentage desorbed from soil (mean values)

Soil	Test Concentration [mg/L]			
	Desorption [1]			
	5	1	0.2	0.04
Drummer	1.56	0.93	1.36	2.30
Dupo	7.26	6.34	6.26	7.88
Spinks	0.90	0.70	1.27	2.17

[1] End of desorption phase, mean values expressed as percentage of applied radioactivity

Table 8.1.2.1-39: [14C]Glyphosate: Adsorption parameters in soil

Soil	Adsorption			
	$K_{F(ads)}$	1/n	R^2	$K_{F, OC(ads)}$
Drummer	324	0.92	0.9985	22300
Dupo	33	0.80	0.9999	3800
Spinks	660	1.16	0.9969	60000

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 33 to 660 mL/g. The respective $K_{F\ oc(ads)}$ values were in the range of from 3800 and 60000 mL/g. The Freundlich exponents 1/n were in the range of 0.80 to 1.16.

Assessment and conclusion by applicant:

The assessment of data in the test was performed using the indirect method to calculate adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, the data reported do not allow for the conclusion that the test substance was stable since no parental mass balance was established covering the soil phase following the adsorption step.

The data are therefore regarded as supportive information. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

An evaluation of information in study according to the EU OECD 106 Evaluators Checklist is presented for information only.

Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Spinks	Dupo	Drummer
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	- [1]	- [1]	- [1]
Adsorbed percentage	%	96.9-98.8	87.9-93.2	97.8-99.0
K_D x (soil:solution ratio)		31.5-81.4	7.3-13.8	44.6-99.0
$^{ads}K_F$ (95% confidence interval)	L/kg dw	828.5 (219.3-3128.7)	38.8 (16.4-91.6)	645.5 (71.4-5839.0)
$^{ads}1/n$ (95% confidence interval)	-	1.227 (0.9639-1.490)	0.903 (0.677-1.130)	1.123 (0.708-1.538)
$^{ads}R^2$	-	0.995	0.993	0.985
$^{ads}K_{F,OC}$	L/kg OC	75315	4459	44518
K_{FE} / K_F	-	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

[1] PMB was not established. Only aq. supernatants analysed by chromatographic methods (glyphosate recovery ≤59 %).

[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to missing parental mass balance providing the f-factor necessary for the calculations

Figure 8.1.2.1-9: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Spinks

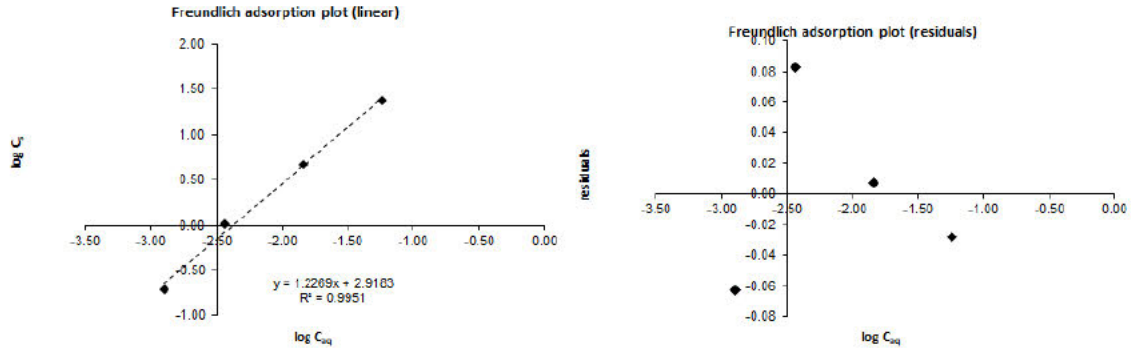


Figure 8.1.2.1-10: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Dupo

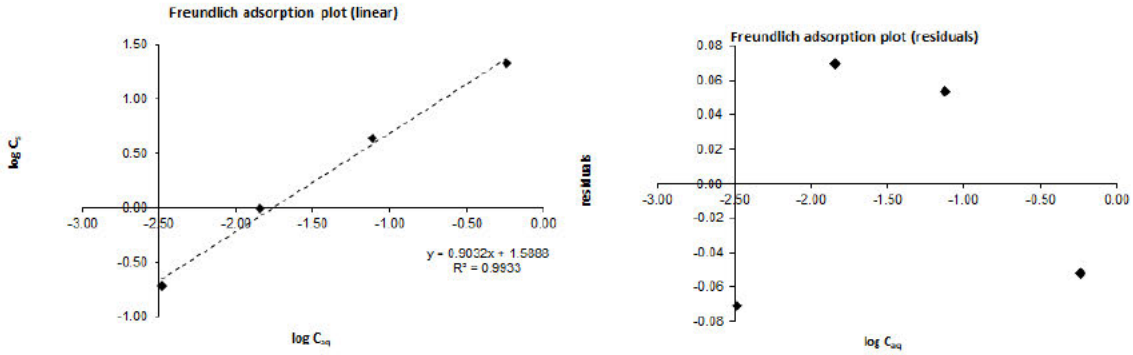
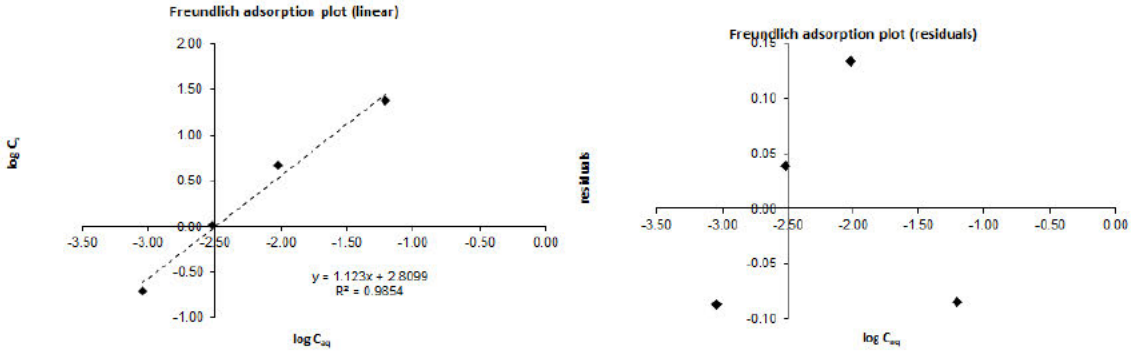


Figure 8.1.2.1-11: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Drummer



Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable. Please note that it only reports results for glyphosate as the two other substances studied do not provide any useful information for the glyphosate assessment.

Several deviations from OECD 106 are identified. There was no pre-equilibration of soils before the experiment, no preliminary test was performed to determine optimal soil to solution ratio, only 4 concentrations

were used, soil to solution ratio is not considered optimal (very high adsorption percentage measured), total mass balance is below 90% AR, and parental mass balance was not established.

As mentioned in the study report, glyphosate was not stable under study conditions, with AMPA formed up to 47% (Dupo soil) in the supernatant. No HPLC analysis in the soil extracts was performed. Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was glyphosate, results cannot be considered as reliable.The applicant indicates that the raw data of the study possibly could provide additional information to derive K_D for the concentration tested in the parental mass balance test by applying the direct method. This is not considered needed since no robust value could be obtained considering that inadequate soil to solution ratio was used.

The study is not considered acceptable.

[redacted], *1992*

Data point:	CA 7.1.3.1.1/008
Report author	[redacted]
Report year	1992
Report title	[14C-PMG] Glyphosate-Trimesium: Adsorption/desorption in four soils
Report No	RR92-016B
Guidelines followed in study	US EPA-FIFRA N-163-1 40 CFR, Sec. 158.130 and 158.50
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-equilibration of soils performed - Only 4 concentrations tested - Soil to solution ratio not optimal (very high adsorption percentage) - Recovery of radioactivity <90 % for at least two test concentrations for all soils - No detailed parental mass balance reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not presented nor mentioned in RAR (2015) nor in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate-trimesium (^{14}C-methyl-glycine)

Lot No.	PMS-363; 88J30
Specific activity	55.95 mCi/mmol
Radiochemical purity	95.0 ± 2.2 %

2. Test Soils

Table 8.1.2.1-40: Physico-chemical properties of test soils

Parameter	Results			
Soil Designation	Atterbery Silt Loam	Sorrento Loam	Visalia, CA Sandy Loam	Biggs, CA Clay
Country	UK	Italy	USA	USA
Textural Class (USDA)	Silt Loam	Loam	Sandy Loam	Clay
Sand (%)	16	38	57	21
Silt (%)	55	41	34	30
Clay (%)	28	21	9	49
pH (medium not reported)	5.6	6.8	7.4	6.1
Organic Carbon (%) [1]	1.5	2.0	0.4	1.2
Organic Matter (%)	3.0	3.9	0.8	2.3
Cation Exchange Capacity (meq (100/g))	19.2	17.3	7.6	31.5

Water Holding Capacity at 1/3 bar (%)	29.48	22.52	15.70	32.44

[1] Calculated as: OC [%] = OM [%] / 2.0 (calculated within report)
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes were used as test systems. The experiments were performed in duplicate.

In preliminary tests, the adsorption of glyphosate to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times and test item stability in soil extracts following the desorption phase (at highest test concentration) were determined. Additionally, a material balance test using sterile soils was performed at the highest test concentration including an adsorption and desorption step.

For the definitive phase the adsorption step was carried out using soils with a soil-to-solution ratio of 1:5. Glyphosate-trimesium was applied at nominal concentrations of 12.4, 1.24, 0.124, and 0.0124 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 4 hours in the dark at 25 ± 1 °C under continuous agitation.

For the desorption step, pre-adsorbed soil was supplied with fresh aqueous 0.01 M $CaCl_2$ solution was. The resultant samples were agitated for 8 hours at 25 ± 1 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and desorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity in the supernatants was determined by liquid scintillation counting (LSC). After desorption the radioactivity content in the soils was determined by combustion/LSC to establish full material balances of radioactivity.

For investigation of test item stability soils were extracted in an additional test after the adsorption and desorption phase using of 3 N aqueous hydrochloric acid (HCl) at 25 °C. Soil extracts were analysed by thin layer chromatography (TLC).

Adsorption isotherms were calculated by evaluation of the adsorption data via the indirect method according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances corrected for test vessel adsorption were 95.0 % AR for soil Atterbery Silt Loam (range from 83.0 to 111 % AR), 87.0 % AR for soil Sorrento Loam (range from 74.6 to 93.7 % AR), 92.3 % AR for soil Visalia Sandy Loam (range from 79.7 to 114 % AR), and 90.1 % AR for soil Biggs Clay (range from 71.9 to 103 % AR). Material balances for sterile soils were 99.0 % for soil Atterbery Silt Loam, 95.5 % for soil Sorrento Loam, 97.5 % for soil Visalia Sandy Loam and 77.0 % AR for soil Biggs Clay.

Table 8.1.2.1-41: Material balance at different test concentrations

		Test Concentration [mg/L]			
		12.4	**1.24**	**0.124**	**0.0124**
Atterbery Silt Loam	I	111	98.5	83.0	84.2
	II	107	101	88.3	87.3
Sorrento Loam	I	89.1	93.7	93.7	79.1
	II	88.8	88.7	74.6	87.9
Visalia Sandy Loam	I	92.0	92.0	82.8	79.7
	II	89.9	107	114	81.0
Biggs Clay	I	95.1	86.6	95.1	71.9
	II	103	91.8	90.8	86.3

B. STABILITY OF TEST ITEM

The relative amount of the test item in the soil extracts after the desorption phase accounted to 98.5 % for soil Atterbery Silt Loam, 98.4 % for soil Sorrento Loam, 97.1 % for soil Visalia Sandy Loam and 99.3 % for soil Biggs Clay.

The mean extraction efficiencies were 93.9 % for soil Atterbery Silt Loam, 78.1 % for soil Sorrento Loam, 96.9 % for soil Visalia Sandy Loam and 71.5 % for soil Biggs Clay.

Aqueous supernatants after adsorption and desorption were not analysed in the study.

C. FINDINGS

The adsorption coefficients $K_{F(ads)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the four test soils ranged from 31.5 to 2060 mL/g. The Freundlich exponents n were in the range of 0.909 to 1.14. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 2860 and 179000 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the three test soils ranged from 40.4 to 3230 mL/g. The Freundlich exponents n were in the range of 0.901 to 1.09. The corresponding, calculated $K_{F, OC(des)}$ values varied between 3030 and 281000 mL/g.

Table 8.1.2.1-42: [^{14}C]Glyphosate-trimesium: Percentage adsorbed / desorbed in soil

Soil	Replicate	Test Concentration [mg/L]							
		Adsorption [1]				Desorption [2]			
		12.4	1.24	0.124	0.0124	12.4	1.24	0.124	0.0124
Atterbery Silt Loam	I	98.7	98.6	99.0	98.7	0.64	0.83	0.62	0.79
	II	98.6	99.0	99.0	98.7	0.93	0.70	0.61	1.01
Sorrento Loam	I	90.7	94.5	94.7	94.4	7.24	5.60	5.97	4.91
	II	90.4	94.3	94.6	94.6	7.65	5.58	5.02	5.25
Visalia Sandy Loam	I	81.9	92.4	93.0	91.4	10.8	7.57	6.56	5.36
	II	81.9	91.8	94.0	91.8	11.1	6.01	8.46	6.80
Biggs Clay	I	99.6	99.7	99.6	99.2	0.19	0.14	0.29	0.66
	II	99.6	99.5	99.6	99.2	0.30	0.19	0.20	0.44

[1] End of adsorption phase, expressed as percentage of applied radioactivity
[2] End of desorption phase, expressed as percentage of applied radioactivity

Table 8.1.2.1-43: [^{14}C]Glyphosate-trimesium: Adsorption and desorption parameters in soil at 25 ± 1 °C

Soil	Adsorption				Desorption			
	K_F	n	R^2	$K_{F, OC}$	K_F	n	R^2	$K_{F, OC}$
Atterbery Silt Loam	376	1.02	0.9972	25100	550	1.00	0.9957	36700
Sorrento Loam	55.7	1.08	0.9968	2860	59.1	1.06	0.9986	3030
Visalia Sandy Loam	31.5	1.14	0.9879	7880	40.4	1.09	0.9962	10100
Biggs Clay	2060	0.909	0.9904	179000	3230	0.901	0.9782	281000

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the four test soils ranged from 31.5 to 2060 mL/g. The Freundlich exponents n were in the range of 0.909 to 1.14. The corresponding, calculated $K_{F, OC(ads)}$ values ranged from 2860 to 179000 mL/g. The desorption coefficients $K_{F(des)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the three test soils ranged from 40.4 to 3230 mL/g. The Freundlich exponents n were in the range of 0.901 to 1.09. The corresponding, calculated $K_{F, OC(des)}$ values ranged from 3030 to 281000 mL/g.

For soils Atterbery and Visalia relative test item recovery (regarding soil extracts only) was >90 % following the desorption phase. In combination with an extraction efficiency of >90 % for both soils total test item stability could be sufficient (i.e. >90 %).

For soils Sorrento Loam and Biggs Clay it is noted that the extraction efficiencies following the desorption phase were low (78.1 % AR for soil Sorrento Loam and 71.5% AR for soil Biggs Clay) resulting in formation of non-extractable residues (NER) of >20 % AR for both soils. Since NER are considered as degradation products of the parent test item the test item is considered unstable in the course of the study for soils Sorrento Loam and Biggs Clay.

Assessment and conclusion by applicant:

The test was performed using the indirect method for determination of adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. The results of the stability test (parental mass balance) are not reported in detail while the recovered amounts of the test item are reported for soil extracts following the desorption phase.

The data are therefore regarded as supportive information. It is noted that the raw data of the study possibly could provide additional information to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

An evaluation following EFSA Evaluators Checklist was not performed.

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. There was no pre-equilibration of soils before the experiment, only 4 concentrations were used, soil to solution ratio is not considered optimal (very high adsorption percentage measured except in 1 soil at the highest concentration – 82%), total mass balance is below 90% AR at least at 2 concentrations for all soils, and parental mass balance was not established.

No HPLC/TLC analysis of the radioactivity is presented in the study report. It is indicated that material bound to the soil was analysed by TLC in order to determine the radioactive areas but no result is then provided.

The stability of the test substance (glyphosate-trimesium) was performed separately from the definitive study. The study report indicates that 3g aliquot of each test soil was treated with 15mL of a 12.4µg/mL test solution, shaken in the dark for 4 hours at about 10 000 g to produce clear supernatant. The supernatants were decanted and the volumes replaced with 0.01 M $CaCl_2$. These samples were then equilibrated for 8h at 25°C and centrifuged as before. Clear supernatants were decanted. The soil pellet was then extracted with 2x 10mL aliquots of 3N HCl (platform shaker for 1h at 25°C for each extraction). These 3N HCl extracts have been analysed by TLC and the study reports that the test material was stable over the course of the test. No details are presented in the study. However it is understood that recovery in the supernatant was not analysed.

Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was glyphosate, results cannot be considered as reliable.

The applicant indicates that the raw data of the study possibly could provide additional information to derive K_D for the concentration tested in the parental mass balance test by applying the direct method. This is not considered needed since no robust value could be obtained considering that inadequate soil to solution ratio was used.

The study is not considered acceptable.

[redacted], *2001*

Data point:	CA 7.1.3.1.1/003
Report author	[redacted]
Report year	2001
Report title	Adsorption/desorption of glyphosate on soil
Report No	320164
Guidelines followed in study	OECD Guideline 106 US EPA OPPTS 835.1220 SETAC Procedures of Assessing the Environmental Fate and Ecotoxicity, Part 1, Section 4
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - preliminary test to determine optimum soil/solution ratio conducted at a single soil/solution ratio - No mass balance was carried out during the preliminary test - Soil to solution ratio not optimal in 3 soils at some concentrations (very high adsorption percentage measured) - Recovery of radioactivity <90 % for some soils in the definitive phase

	- Parental mass balance not available (no chromatographic analysis of soil), but test item not stable in supernatant in soil II after the adsorption step and not stable in supernatant in the 4 soils after desorption step
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C]glyphosate (PMG label)	
Lot No.	3415135
Specific activity	1.89 GBq/mmol (56.5 mCi/mmol by mass spectral analysis)
Radiochemical purity	99 %

2. Test Soils

The soils were collected before study fresh start from the upper soil horizon (0 to 22 cm), sieved to a particle size of ≤2 mm and air-dried prior to use. The soils history was known for the previous five years. A description of the soils used is summarised in the table below.

Table 8.1.2.1-44: Physico-chemical properties of test soils

Parameter	Results			
Soil Designation	Speyer 2.1	Cranfield 115	Cranfield 164	Cranfield 243
Horizon (cm)	20	0-10	15-22	5-15
Geographic Location				
City	Rheinzabern	Netherton, Evesham	Chelmorton, Buxton	Stoneleigh
State	Rheinland-Pfalz	Worcester	Derbyshire	Warwickshire
Country	Germany	United Kingdom	United Kingdom	United Kingdom
Textural Class (USDA)	Sand	Clay loam	Silt loam	Sandy loam
Sand (53 µm – 2 mm) (%)	90.2	43.74	15.95	71.93
Silt (2 µm – 53 µm) (%)	8.2	23.50	72.91	15.97
Clay (< 2 µm) (%)	1.7	32.76	11.14	12.10
pH				
- in $CaCl_2$	6.0	--	--	--
- in water	--	7.9	7.1	5.4
- in KCl	--	7.4	6.5	4.3
Organic Carbon	0.56	1.7	3.0	1.1
Organic Matter	0.97	2.9	5.2	1.9
Cation Exchange Capacity (meq/100 g)	4	19.6	18.1	3.3
Water Holding Capacity (%)	29	55.3	72.8	51.1
Moisture at 1/3 bar (%)	--	30.4	41.2	22.7

USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Polypropylene centrifuge tubes were used as test systems. The experiments were performed with duplicate soil samples.

In preliminary tests, the adsorption of glyphosate to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times, and the stability of glyphosate were determined.

For the definitive phase, the adsorption step was carried out using pre-equilibrated samples from air-dried soils in aqueous 0.01 M CaCl2 solution at a soil-to-solution ratio of 1:100. Glyphosate was applied at nominal concentrations of 5.0, 2.0, 1.0, 0.2, and 0.04 mg/L in aqueous 0.01 M CaCl2 solution. The adsorption step was carried out for 24 hours in the dark at 20 ± 2 °C under continuous agitation.

The desorption step was performed by supplying pre-absorbed soil samples with fresh aqueous 0.01 M CaCl2 solution. The resultant samples were re-equilibrated for 24 hours in the dark at 20 ± 2 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and desorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity in the supernatants was determined by liquid scintillation counting (LSC).

In the preliminary mass balance test, the aqueous supernatants were analysed by LSC and high performance liquid chromatography (HPLC) to determine the stability of glyphosate in aqueous supernatants. Soil samples were combusted followed by quantitation using LSC.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation, using the indirect method.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

For the definitive phase after the desorption stage, mean material balances after 48 h of equilibration ranged from 80.5 to 90.3 % for Speyer 2.1, from 78.3 to 84.6 % for Cranfield 115, from 88.8 to 92.1 % for Cranfield 164, and from 90.4 to 93.7 % for Cranfield 243.

B. STABILITY OF TEST ITEM

Following adsorption and desorption steps of the definitive phase, stability of test item was investigated in single aqueous supernatants by chromatographic analysis. The recovery of glyphosate in aqueous supernatants of samples after 24 h of adsorption was 97 % for Speyer 2.1, 80 % for Cranfield 115, 91 % for Cranfield 164, and 95 % for Cranfield 243. After desorption, recovery of glyphosate in aqueous supernatants was 84 % for Speyer 2.1, 16 % for Cranfield 115, 63 % for Cranfield 164, and 73 % for Cranfield 243. From these results, it appears that glyphosate was not stable during the test and degraded in the presence of soil, primarily during the desorption phase of the isotherms experiment.

C. FINDINGS

The adsorption coefficients KF(ads) of glyphosate calculated based on the Freundlich isotherms ranged from 57.4 to 56.9 mL/g for Speyer 2.1, 224 to 208 mL/g for Cranfield 115, 894 to 900 mL/g for Cranfield 164, and 222 to 223 mL/g for Cranfield 243. The Freundlich exponents 1/n were in the range of 0.59 to 0.73 across all soils. The corresponding, calculated $K_{F, OC}$(ads) values varied between 10 and 30 x 103 mL/g.

The desorption coefficients KF(des) of glyphosate calculated based on the Freundlich isotherms ranged from 139 to 148 mL/g for Speyer 2.1, 352 to 408 mL/g for Cranfield 115, 1460 to 1530 mL/g for Cranfield 164, and 362 to 366 mL/g for Cranfield 243. The Freundlich exponents 1/n were in the range of 0.62 to 0.72 across all soils. The corresponding, calculated $K_{F, OC}$(des) values varied between 21 and 51 x 103 mL/g.

Table 8.1.2.1-45: [^{14}C]Glyphosate: Adsorption parameters in soil at 20 °C

Soil	Replicate	Adsorption			
		K_F [10^2 mL/g]	1/n	R^2	$K_{F, OC}$ [10^3 mL/g]
Speyer 2.1	A	0.574	0.60	0.9879	10
	B	0.569	0.60	0.9840	10
Cranfield 115	A	2.24	0.67	0.9898	13

	B	2.08	0.64	0.9925	12
Cranfield 164	A	8.94	0.72	0.9925	30
	B	9.00	0.73	0.9952	30
Cranfield 243	A	2.22	0.59	0.9886	20
	B	2.23	0.59	0.9895	20

Table 8.1.2.1-46: [^{14}C]Glyphosate: Desorption parameters in soil at 20 °C

Soil	Replicate	Desorption			
		K_F [10^3 mL/g]	1/n	R^2	$K_{F, OC}$ [10^3 mL/g]
Speyer 2.1	A	0.139	0.71	0.9967	25
	B	0.148	0.72	0.9974	26
Cranfield 115	A	0.408	0.70	0.9897	24
	B	0.352	0.67	0.9893	21
Cranfield 164	A	1.53	0.72	0.9936	51
	B	1.46	0.71	0.9953	48
Cranfield 243	A	0.366	0.62	0.9934	33
	B	0.362	0.62	0.9937	33

III. CONCLUSIONS

The adsorption coefficients K_F(ads) of glyphosate calculated based on the Freundlich isotherms ranged from 0.574 to 9.00 × 102 mL/g across all soils. The corresponding, calculated $K_{F, OC}$(ads) values varied between 10 and 30 × 103 mL/g.

Assessment and conclusion by applicant:

The study had been assessed as invalid during AIR2 – following the citation in the RAR, 2015 (Outcome of the discussions in the Pesticides Peer Review Meeting 126, February 2015): "It was also noted that desorbed glyphosate was degrading in soil solution within the equilibrium time of batch experiments, though it was noted that the [redacted] experiment the equilibrium time was longer and more degradation of glyphosate was apparent. On balance the experts considered that the results of the [redacted] experiments should be excluded from the dataset as the longer batch equilibrium time (compared to other investigations or investigations where soils were sterilised) meant that degradation of glyphosate that occurred during the study resulted in lower confidence in these data." In light of the requirements of the EU Evaluators Checklist, the applicant agrees with this assessment.

Though the study does not fulfil the requirements as set out in the EU Evaluators Checklist, the results of the study were summarised formally below.

Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	**Speyer 2.1**	**Cranfield 115**	**Cranfield 164**	**Cranfield 243**
Adsorption method	-	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:100	1:100	1:100	1:100
Parental mass balance (at highest conc.)	%	- [1]	- [1]	- [1]	- [1]
Adsorbed percentage	%	21.9-76.3	57.5-93.5	89.4-98.3	57.1-96.3
K_D x (soil:solution ratio)		0.3-3.1	1.4-14.6	8.8-57.2	1.4-26.0
$^{ads}K_F$ (95% confidence interval)	L/kg dw	57.153 (49.374-66.158)	215.760 (181.947-255.857)	902.915 (726.288-1122.497)	222.843 (184.679-268.894)
^{ads}1/n (95% confidence interval)	-	0.603 (0.545-0.662)	0.656 (0.604-0.707)	0.729 (0.680-0.779)	0.593 (0.541-0.644)
$^{ads}R^2$	-	0.986	0.991	0.993	0.989
$^{ads}K_{F,OC}$	L/kg OC	10206	12692	30097	20259
K_{FE} / K_F	-	- [2]	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] A parental mass balance was not established in the course of the study.
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to a missing parental mass balance providing the f-factor necessary for the calculations.

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. No different soil to solution ratios were tested in preliminary test, selected soil to solution ratio is not optimal in 3 soils (very high adsorption percentage measured at some concentrations), total recovery after desorption step is below 90% for some soils, no parental mass balance was established.

The stability of the test substance in the supernatants was analysed by HPLC for each soil. Glyphosate was not stable in supernatant of soil II after the adsorption step, and it was not stable in supernatant of all 4 soils after the desorption step. No analysis of the stability of glyphosate in soil was performed. As previously highlighted in the RAR (2015), the equilibrium time in this study (24h) is longer than in other studies in which significant degradation was observed. Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was glyphosate, results cannot be considered as reliable.

The study is not considered acceptable.

[redacted], *1991*

Data point:	CA 7.1.3.1.1/010
Report author	[redacted]
Report year	1991
Report title	Behaviour of Glyphosate in water and soil, Part 2 Adsorption/desorption on soil.
Report No	PR90/002
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-tests for determination of soil-to-solution ratio and equilibration time performed - No pre-equilibration of soils performed - Only one concentration used - Method validation not reported (no LOD/LOQ available) - Soil to solution ratio not optimal (very high adsorption percentage) - Parental mass balance not available, but recovery of test item in soil extracts after desorption step >110 % or <50 % for two soils - No concentrations in aqueous supernatants reported - No adsorption coefficients reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentionned in RAR (2015) but not accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Glyphosate (non-labelled)	
Lot No.	00516
Chemical purity	99 %

2. Test Soils

Table 8.1.2.1-47: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	F3, 341	WO-41	2.1
Country	Not provided	Not provided	Not provided
Textural Class			
(630 µm – 2 mm) (%)	1.1	3.6	4.5
(200 µm – 630 µm) (%)	13.5	51.9	62.9
(63 µm – 200 µm) (%)	25.2	27.7	20.0
(20 µm – 63 µm) (%)	30.3	8.2	4.7
(6 µm – 20 µm) (%)	10.0	7.4	2.5
(2 µm – 6 µm) (%)	4.7	0.5	1.9
(< 2 µm) (%)	15.2	0.7	3.5
pH in water	7.3	3.8	6.1
Organic Carbon	1.20	2.76	0.70
Organic Matter (%) [1]	2.06	4.75	1.20
Cation Exchange Capacity (mval/100 g)	13	8	4.9
Water Holding Capacity			
maximum (g (100 g soil DW^{-1}))	45.7	--	31.9
Bulk density (g/1000 mL)			1365

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

The tests were performed with duplicate soil samples.

For the definitive phase the adsorption step was carried out in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:5. Nominal amount of glyphosate used 1 mg/L. The adsorption step was carried out for 16 hours under continuous agitation.

The desorption step was conducted using each soil with two desorption cycles. In each of the two desorption phases, pre-adsorbed soil specimens were supplied with fresh aqueous 0.01 M $CaCl_2$ solution. The resultant samples were re-equilibrated for 16 hours under continuous agitation followed by centrifugation.

2. Analytical Procedures

The aqueous supernatant after adsorption and after desorption was separated by centrifugation. Chromatographic analysis for glyphosate residues was reported to follow method iCD033E.

To determine the recovery of the test item, glyphosate was extracted from the soil with water and phosphoric acid following the desorption phase.

II. RESULTS AND DISCUSSION

A. STABILITY OF TEST ITEM

Total mean recoveries of glyphosate in soil extracts after the desorption phase were 117, 102, and 47 % for soil F3, 341, WO-41, and 2.1, respectively.

B. FINDINGS

More than 90 % of glyphosate was adsorbed at the soil phase and 2 % or less was desorbed following two desorption cycles.

Table 8.1.2.1-48: Glyphosate: Recovery in supernatant (mean values)

Soil	Percentage 1		
	Adsorption	Desorption Step 1	Desorption Step 2
F3, 341	6	2	1
WO-41	5.5	1	0.6
2.1	0	1.5	-

[1] Mean values expressed as percentage of applied glyphosate

III. CONCLUSIONS

More than 90 % of glyphosate was adsorbed at the soil phase and 2 % or less was desorbed following two desorption cycles. An evaluation according to EFSA Evaluators Checklist was not possible due to missing data (no concentrations reported).

Assessment and conclusion by applicant:

The study is considered as invalid due to various significant deviations from current OECD Guideline 106. No pre-tests for determination of soil-to-solution ratio and equilibration time were performed. For the adsorption step soils were not pre-equilibrated and the test item was applied together with the 0.01 M $CaCl_2$ solution. Furthermore, recovery of test item was >110 % or <50 % for two soils (F3, 341 and 2.1) and therefore outside of the acceptable range.

Finally, an evaluation according to EFSA Evaluators Checklist cannot be performed due to missing data of test item concentrations in the aqueous adsorption supernatants. Therefore, the results remain uncertain.

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable. In addition, very few information are presented in the study report.

Several deviations from OECD 106 are identified. No preliminary tests were conducted, no pre-equilibration of soils was performed, only one concentration was tested, information regarding method validation is not available, selected soil to solution ratio is not optimal (very high adsorption percentage measured), recovery of the test item in soil extracts after desorption is > 100% in 1 soil and < 50% in another soil, parental mass balance is not available, concentrations in supernatants are not reported.

The study is not considered acceptable.

[redacted], *1994*

Data point:	CA 7.1.3.1.1/006
Report author	[redacted]
Report year	1994
Report title	Adsorption and desorption of glyphosate on three types of soil
Report No	ALK-AA-15001
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-tests for soil:solution ratio, equilibration time, test item stability and adsorption of test item to test vessel surface - No pre-equilibration of samples - KCl instead of $CaCl_2$ solution used - Only 3 concentrations used, covering only one order of magnitude - Adsorption percentage not reported - No total mass balance nor parental mass balance established - No information on method validation
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, not mentionned in RAR (2015) but not accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Identification:	Glyphosate isopropylamine salt (non-labelled)
Batch No.:	451760292
Chemical purity:	99.3 %
Content referring to glyphosate acid	72.7%

2. Test Soils

The soils were sampled from the field, air dried, sieved to 2 mm and sterilized. The characterisation of test soils used is summarised in the table below.

Table 8.1.2.1-49: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	Sand	Loam	Clay
Geographic Location			
City	Nyíregyháza	Nyíregyháza	Tiszaadony
Country	Hungary	Hungary	Hungary
Textural Class	Sand	Sandy loam	Clayey loam
Sand (50 µm – 2 mm) (%)	-	-	-
Silt (2 µm – 50 µm) (%)	-	-	-
Clay (< 2 µm) [(%)	4.79	22.69	35.60
pH in KCl	5.27	7.64	4.42
Organic matter [%]	0.84	1.88	2.36
Cation Exchange S	14.63	30.93	28.20

B. STUDY DESIGN

1. Experimental Conditions

Centrifuge tubes were used as test vessels. The experiments were performed in triplicate.

Preliminary tests were not performed. Soils were sterilised prior to application.

For the definitive phase the adsorption step was carried out at a soil-to-solution ratio of approximately 1:10 (i.e. 1 g soil and 10 mL 0.01 M KCl solution). Glyphosate was applied each at nominal concentrations of 30, 100 and 300 mg/L in aqueous 0.01 M KCl solution. The adsorption step was carried out for 24 hours under continuous agitation.

For each of the three successive desorption steps in total, 4 mL fresh aqueous 0.01 M KCl solution was added to pre-adsorbed soil samples of the highest concentration (still containing approx. 6 mL of adsorption supernatant) and the resultant samples were re-equilibrated for 24 hours under continuous agitation. The procedure was repeated for two further desorption steps.

2. Analytical Procedures

After each adsorption and desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of glyphosate in the supernatants only was analysed by gas chromatography coupled with a thermionic ionization detector following derivatization of the samples. A method validation was not provided.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation using the indirect method.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

An overall recovery of test item in water and soil was not investigated.

B. STABILITY OF TEST ITEM

Stability of glyphosate was not demonstrated.

C. FINDINGS

The calculated concentrations in adsorption and desorption supernatants are shown in the tables below:

Table 8.1.2.1-50: Glyphosate: Concentration at start and after adsorption in supernatants (mean values of triplicates)

Soil	Initial concentration [µM/L]	Equilibrium concentration [µM/L]
Sand	177.42	85.12
	592.16	267.37
	1773.38	690.76
Loam	177.42	40.72
	592.16	195.69
	1773.38	477.34
Clay	177.42	12.34
	592.16	34.68
	1773.38	275.25

Table 8.1.2.1-51: Glyphosate: Concentration in desorption supernatants of samples of highest test concentration (mean values of triplicates)

Soil	Desorption time [h]	Initial concentration [µM/L]	Equilibrium concentration [µM/L]
Sand	24	1497.08	460.73
	48	1312.78	332.39
	72	1179.71	200.93
Loam	24	1588.35	475.79
	48	1398.03	639.49
	72	1142.23	337.44
Clay	24	1663.28	253.84
	48	1617.03	211.62
	72	1552.38	150.07

The adsorption coefficients KF(ads) of glyphosate calculated on the three test soils ranged from 0.0047 to 0.3595 mL/g and the desorption coefficient KF(des) values ranged from 0.1435 to 46.6874 mL/g.

Table 8.1.2.1-52: Glyphosate: Adsorption and desorption parameters in different soils

Soil	Adsorption			Desorption		
	$K_{F(ads)}$ [mL/g]	1/n	r^2	$K_{F(des)}$ [mL/g]	1/n	r^2
Sand	0.0047	1.1777	0.9992	0.1435	0.06264	0.8092
Loam	0.0466	0.88725	0.9823	46.6874	-0.2651	-0.4400
Clay	0.3595	0.68282	0.9675	8.2565	0.10188	0.9125

III. CONCLUSIONS

Values for the Freundlich adsorption coefficient $K_{F(ads)}$ of glyphosate ranged from 0.0047 to 0.3595 mL/g for the three soils tested. Values of the Freundlich exponent 1/n were in the range of 0.68282 to 1.1777. Values for the Freundlich desorption coefficient $K_{F(des)}$ of glyphosate ranged from 0.1435 to 46.6874 mL/g for the three soils tested. Values of the Freundlich exponent 1/n were in the range of -0.2651 to 0.10188 for desorption.

Assessment and conclusion by applicant:

The test was performed using the indirect method for determination of adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, no PMB was determined in this test to fulfil this criterion. Furthermore, potassium chloride solution was used instead of calcium chloride as aqueous phase and method validation is missing. The study is thus considered as invalid.

A further evaluation of the results in view of the EU OECD 106 Evaluators Checklist is therefore regarded as not necessary.

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from the OECD 106 are identified. No preliminary tests were conducted, no pre-equilibration of samples was performed, KCl solution was used instad of CaCl2, only three concentrations covering one order of magnitude only were used, adsorption percentages are not reported, no total mass balance nor parental mass balance were established (the available examples of chromatograms seem to indicate that glyphosate is not the only compound quantified), no information on method validation is available.

The study is not considered acceptable.

[redacted], *1978*

Data point:	CA 7.1.3.1.1/013
Report author	[redacted]
Report year	1978
Report title	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 0101
Report No	MSL-0207
Guidelines followed in study	None
Deviations from current test guideline	Deviations from OECD Guideline 106 (January 2000): - Soil was sieved to mesh size <500 µm - No pre-equilibration of samples - No preliminary tests for adequate equilibration time and soil to solution ratio - Only 4 concentrations used - $CaSO_4$ used instead of $CaCl_2$ - Stability of test items under study conditions not reported (neither in $CaSO_4$ solution nor in presence of soil) - No radioactivity material balance nor parental mass balance established
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not mentionned in RAR (2015) but not accepted in DAR (2001)
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: Adsorption/Desorption in soil Test item: [^{14}C] glyphosate, phosphonomethyl-label (94 % radiochemical purity) and [^{14}C] sodium sesqui salt of glyphosate (95 % chemical purity prior to labelling with [^{14}C]glyphosate) Test soils (soil type): Ray (silt loam), Spinks (sandy loam), Drummer (silty clay loam), Lintonia (sandy loam), Cattail (swamp sediment) pH: 8.1, 4.7, 6.2, 6.5, - (medium not stated) Organic matter: 1.2 %, 2.4 %, 3.4 %, 0.7 %, 1.5 % Soils were sieved to <500 µm or less. Experimental conditions: The adsorption phase was carried out at a soil to solution ratio of 1:4 for four hours at 25 °C. Test item was applied at concentrations of 0.1, 1.0, 10 and 20 mg/L in 0.01 N $CaSO_4$. For the desorption step fresh aqueous 0.01 N $CaSO_4$ solution was added to pre-adsorbed soil samples and the resultant samples were re-equilibrated for four hours under continuous agitation. Analytical procedures: Following each adsorption and desorption step soil and supernatant were separated by centrifugation. Radioactivity in supernatants was determined by LSC. Adsorption isotherms were calculated by evaluation of the adsorption data via the indirect method according to the Freundlich equation.

Short description of results:	Glyphosate: $K_{F, OC(ads)}$: 7500 (Ray), 2917 (Spinks), 1823 (Drummer), 3143 (Lintonia), - (Cattail sediment); 1/n: 0.902 (Ray), 0.944 (Spinks), 0.951 (Drummer), 0.782 (Lintonia), 1.010 (Cattail sediment) Sodium sesqui salt of glyphosate: $K_{F, OC(ads)}$: 9583 (Ray), 3333 (Spinks), 2000 (Drummer), 4286 (Lintonia), - (Cattail sediment); 1/n: 1.046 (Ray), 0.979 (Spinks), 0.971 (Drummer), 0.844 (Lintonia), 0.950 (Cattail sediment) Desorption was generally low for both test items and all soils (≤11.5 % of initially adsorbed)
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	Deviations from OECD Guideline 106 (January 2000): - $CaSO_4$ used instead of $CaCl_2$ - Soil was sieved to mesh size <500 µm - No preliminary tests for adequate equilibration time and soil to solution ratio - Stability of test items under study conditions not reported (neither in $CaSO_4$ solution nor in presence of soil) - No radioactivity material balance established - No pre-equilibration of samples

Assessment and conclusion by RMS:

As listed above, several deviations from OECD 106 are identified. The study summary was not completed by RMS since the study is not considered acceptable.

[redacted], *1986*

Data point:	CA 7.1.3.1.1/012
Report author	[redacted]
Report year	1986
Report title	HOE 017411, Adsorption/desorption in the soil/water system
Report No	A40783 (B)136/85
Guidelines followed in study	No information available (report not available)
GLP	No information available (report not available)
Previous evaluation	No, not mentioned in RAR (2015) nor in DAR (2001)
Acceptability/Reliability:	Study not relevant for glyphosate renewal

Short description of study design and observations:	No information available; From the report title, the compound number HOE 017411 is indicative for the active substance carbendazim. Presumably, the study was erroneously listed in the Monograph (2000).
Short description of results:	No information available
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS:

This study report is not available to the RMS but as indicated by the applicant, based on study title it does not concern glyphosate or AMPA. It is therefore considered as not relevant for glyphosate renewal and should not be listed in the available studies.

B.8.1.2.1.2. Relevant articles from literature search

Within the actual review of scientific literature for glyphosate (2010-2020), 20 articles were identified in total to potentially provide relevant information to the data point.

Table 8.1.2.1-53: Adsorption/desorption – relevant articles from literature search

Study	Study type	Substance(s)	Status
Albers *et al.*, 2018	Batch adsorption	Glyphosate	Reliable with restrictions
Dollinger *et al.*, 2018	Batch adsorption	Glyphosate	Reliable with restrictions
Skeff *et al.*, 2018	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions
Gómez *et al.*, 2017	Batch adsorption	Glyphosate	Reliable with restrictions
Munira & Farenhorst, 2017	Batch adsorption	Glyphosate	Reliable with restrictions
Munira *et al.*, 2017	Batch adsorption	Glyphosate	Reliable with restrictions
Zhelezova *et al.*, 2017	Batch adsorption	Glyphosate	Reliable with restrictions
Cassigneul *et al.*, 2016	Batch adsorption	Glyphosate	Reliable with restrictions
Munira *et al.*, 2016	Batch adsorption	Glyphosate	Reliable with restrictions
Sidoli *et al.*, 2016	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions
Dollinger *et al.*, 2015	Modelling study	Glyphosate	Reliable with restrictions
Kanissery *et al.*, 2015	Batch adsorption	Glyphosate	Reliable with restrictions
Tévez & dos Santos Afonso, 2015	Batch adsorption	Glyphosate	Reliable with restrictions
Jodeh *et al.*, 2014	Batch adsorption	Glyphosate	Reliable with restrictions
Rampoldi *et al.*, 2014	Batch adsorption	Glyphosate	Reliable with restrictions
Bergström *et al.*, 2011	Batch adsorption	Glyphosate	Reliable with restrictions
Maqueda *et al.*2017	Batch adsorption Dissipation in water	Glyphosate	Reliable with restrictions
Rampazzo N. *et al 2013*	Field adsorption		Reliable with restrictions
Tush D. *et al. 2018*	Adsorption Dissipation in field	Glyphosate	Reliable with restrictions
Paradelo M. *et al. 2015*	Adsorption	Glyphosate	Reliable with restrictions

Albers et al, 2018

Data point:	CA 7.1.3.1.1/014
Report author	Albers, C. et al.
Report year	2018
Report title	Soil Domain and Liquid Manure Affect Pesticide Sorption in Macroporous Clay Till
Report No	DOI 10.2134/jeq2018.06.0222 E-ISSN 1537-2537
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): 1mM $CaCl_2$ solution used instead of 0.010 M solution, 10 °C (standard: 20 – 25 °C); 4 test concentrations (standard: 5), no investigation of stability in soil by extraction, i.e. no parental mass balances reported no adequately validated analytical method including LOD and LOQ
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

In this study, it was observed that sorption of strongly sorbing pesticide, glyphosate, varied by more than an order of magnitude across soil domains in 5-m-deep clay till profiles with biopores and fractures.

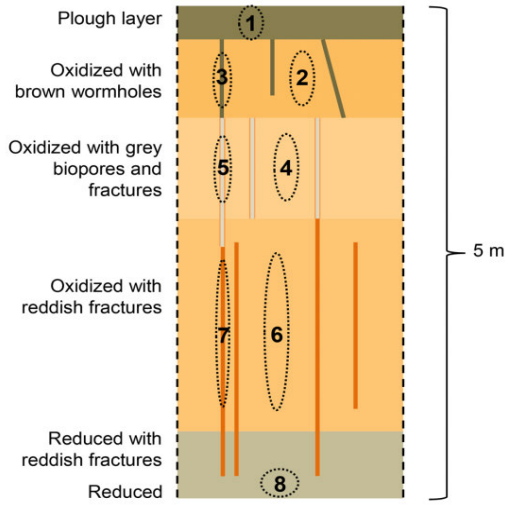
Eight soil domains were identified in each of the profiles: five matrix soils and three in the macropores. Glyphosate showed high variation in sorption between fractures and matrix soil from the same depths. The domain-specific sorption of both tebuconazole and glyphosate was, however, overruled by dilute liquid manure. Liquid manure unexpectedly had a greater effect on glyphosate sorption, which was strongly decreased by dissolved organic matter and phosphate in the manure. The variation in sorption across domains, as well as the effects of liquid manure, should be taken into account when assessing leaching risks.

Materials and methods

Soil sampling

Soil was sampled at two locations, Gjorslev (55°20.988´N, 12°23.672´E) and Lund (55°14.698´N, 12°17.418´E) in the Stevns area of southeastern Denmark. At both sites, soil profiles were excavated to a depth of ~5 m. We sampled composite soil samples from eight domains that were clearly separated on the basis of different soil horizons and the presence or absence of biopores and fractures (Figure 8.1.2.1-12). The surface of wormholes (Domain 3) was sampled by scraping off the outer 1 to 2 mm of the pore walls. Deeper soil pores surrounding decayed roots were dissected out, and the outer Fe oxides were scraped off with a knife to sample only the 5- to 10-mm wide, inner, greyish part (Domain 5). The surface of even deeper larger fractures with Mn and Fe oxide coatings was sampled by scraping off the outer 1 to 2 mm (Domain 7). At least 240 g soil was sampled from each fracture domain to have sufficient material for sorption experiments and analysis of sediment parameters. The matrix soil samples (bulk soil in the case of the plow layer, Domain 1) were also compositely sampled, comprising ~1 kg from 20 to 50 subsamples. All soil samples were sieved twice through a 2-mm sieve and stored at 2°C. Soil samples from the reduced zone were packed in airtight aluminum tape on location and sieved in a glove box under a reducing N_2/H_2 atmosphere. The fraction <2 mm was stored in anoxic jars at 2°C.

Figure 8.1.2.1-12: A schematic representation of the soil profiles in the Gjorslev and Lund sites and their associated soil domains (Domains 1-8). The approximate depth of the lower boundary of each matrix soil domain was (Gjorslev/Lund): Domain 1 (35/35 cm), Domain 2 (105/130 cm), Domain 4 (200/260 cm), and Domain 6 (390/420 cm)



Characterization of the Soil Domains

Soil texture was determined by sieving (0.063-2 mm) and by laser diffraction (<0.063 mm, Mastersizer 3000, Malvern). Water content was determined by drying at 105°C for 24 h. Total carbon and total organic carbon (TOC) were determined on an elemental analyzer (Leco CS-200) on dried (50°C) and crushed samples as they were (total C) or after acid treatment to remove carbonates (TOC). Total inorganic C was calculated as the difference between total C and TOC. The pH was determined in a 1:2.5 soil/liquid slurry with Milli-Q water or 10 mM $CaCl_2$. The $pH_{sorption}$ (i.e., the pH measured at conditions similar to those during the sorption experiments) was also determined with $CaCl_2$, pesticide,

and NaN_3 concentrations similar to those used in the sorption experiments. Soil-specific surface area was measured using a Coulter SA 3100 BET analyzer (Coulter Corporation) and calculated using the Brunauer-Emmett-Teller equation. Total Fe and ferrous Fe^{2+} were measured as described by Komadel and Stucki (1988). Iron and manganese oxides were extracted using the citrate-bicarbonate-dithionite (CBD) method and quantified by atomic absorption spectroscopy (PerkinElmer AANALYST 400). Amorphous Fe and Al oxides were extracted using ammonium oxalate solution. Cation exchange capacity was determined by standard method (Chapman, 1965). All analyses of soil parameters were single measurements.

Characterization of Liquid Pig Manure and Soil Extract

Liquid pig manure was sampled from a conventional farm that raised sows and offspring (weaner production) and was stored at 2°C for 4 wk. Topsoil extract was obtained by horizontal rolling of plow layer soil and Milli-Q water (1:1) for 24 h at 22°C. The liquid manure and the topsoil suspension were centrifuged (15 min, 3500 g), and the extracts were stored as frozen subsamples to be used in the subsequent sorption experiments. After thawing, the extracts were sonicated for 30 min before use in sorption experiments. Total organic C in soil and manure extracts and in the aqueous phase of selected sorption experiments was analysed on a TOC analyzer (TOC-Vcph, Shimadzu) after filtration (5 µm polyvinyl difluoride [PVDF], Millipore). Conductivity was determined using a conductivity probe (LE703, Mettler Toledo). The concentrations of major inorganic cations and anions were determined by ion chromatography (Metrohm 819 with a Metrosep A 150/4.0 column). Total Cu, Zn, Al, Ba, Fe, Mn, and S contents were measured on an inductively coupled plasma mass spectrometer (Elan 6100DRC, PerkinElmer) using a multielement scanning method (TotalQuant, PerkinElmer).

Chemicals

(P-methylene-^{14}C)-glyphosate (radiochemical purity = 99 %, specific activity = 122 MBq/mmol) were purchased from Izotop. Glyphosate (purity 97 %) was purchased from Dr. Ehrenstorfer, Germany.

Sorption of Glyphosate

Sorption experiments were performed using a batch-equilibrium method inspired by the Organization for Economic Cooperation and Development (OECD) guideline (OECD, 2000). Eleven-milliliter Pyrex glass vials with 15-mL polypropylene centrifuge vials were used for glyphosate. The final soil/liquid ratio was in all vials 1:10, which in general resulted in between 20 and 95 % sorption of the added pesticide. In each vial, 1 g of soil (wet weight) was mixed with 1 mM $CaCl_2$ solution (8.0 - 9.7 mL) and NaN_3 (20 µL of a 100 g/L solution) was added to repress biodegradation of the pesticides during incubation. One millimolar $CaCl_2$ was used, since it better represented the concentrations in the local soil water than the 10 mM $CaCl_2$ suggested in the OECD guideline. The soil-liquid slurries were then equilibrated at 10°C for 24 h by vertical rotation (7 revolutions/min) before addition of ^{14}C-labeled pesticide and, for the two highest pesticide concentrations, nonradioactive pesticide (both dissolved in 1 mM $CaCl_2$). Initial total concentrations of glyphosate were 30, 120, 1200 (thereof 120 µg/L radioactive glyphosate) and 12,000 µg/L (thereof 120 µg/L radioactive glyphosate). After addition of the pesticides, the vials were rotated at 10°C for another 24 h. The vials were then centrifuged at 1250 g (glass vials) or 3000 g (plastic vials) for 15 min. The pesticide concentration of the aqueous phase was determined by liquid scintillation counting (Tri-Carb 2810 TR, PerkinElmer) of the ^{14}C activity in duplicate 1-mL samples. The ^{14}C activity was counted for 30 min or until 1 % uncertainty (2S, 95 % confidence limit). Sorption to the vials was tested by including reagent blanks without soil, but no such sorption was found. The pesticide concentration in the solid phase (soil) was calculated based on pesticide missing in the aqueous phase.

The sorption experiments were all performed in duplicate. The difference in distribution coefficients between duplicates was <15%, and in most cases, it was <5 %. All sorption experiments with soil from reduced zones were prepared under a N_2 /H $_2$ atmosphere in a glove box with solutions that had been flushed with N_2.

Freundlich Sorption Models

Glyphosate sorption was described by an extended Freundlich equation, as suggested by de Jonge *et al.* (2001):

$$C_s = K_{Fcx} C_w^{n_{ex}} C_w^{-D}$$

where K_{Fex} is the extended Freundlich coefficient, n_{ex} is the extended Freundlich exponent, and D is a parameter that adds extra curvature to the line in a double-logarithmic plot (i.e., increases the concentration sensitivity compared with the simple Freundlich model). The extended Freundlich model was fitted to the experimental data by nonlinear optimization.

Results

Soil Domains

Both soil profiles had a characteristic depth zonation with eight visually different domains based on different layers and the presence or absence of macropores (Figure 8.1.2.1-12). At the Gjorslev site, the upper 35 cm was a relatively homogenous dark brown (10YR 3/2) plow layer (Ap horizon, Domain 1) rich in organic matter (Table 8.1.2.1-54). The plow layer was followed by an oxidized layer of variable color with a predominantly yellow-brown (10YR 4/4) matrix (Domain 2) perforated by brown (10YR 4/3), vertical wormholes where the soil was enriched in organic matter (Domain 3). Many of the wormholes were present within fractures (geological and desiccation) and extended to a depth of ~ 110 cm. The following layer (105–200 cm) was oxidized with a light brown (10YR 5/3) matrix (Domain 4) and numerous small biopores from decayed plant roots with a diameter of ~ 1 mm. The pores were surrounded by gray (10YR 8/1) pore soil with a diameter of 5 to 10 mm (Domain 5) and a thin, outer layer of Fe oxides. This layer also had gray fractures that extended into the next layer where they changed to reddish. The next layer (200–390 cm) was also oxidized with a light brown (10YR 5/3) matrix (Domain 6) and many parallel fractures (Domain 7). The surface of the larger fractures was coated with Fe and Mn oxides of variable reddish to almost black colors (10YR 4/6). This domain was devoid of visible biopores. The matrix was reduced at the bottom of the profile (Domain 8), as visible by its gray color (5Y 5/1). The oxidized reddish fracture surfaces extended ~ 50 cm into the reduced zone. Similar horizons and domains were present in the Lund profile, although at slightly different depths (Table 8.1.2.1-54). Soil parameters for both profiles are available in Table 8.1.2.1-54 and Table 8.1.2.1-55.

Table 8.1.2.1-54: Main soil parameters from the soil profiles

Domain	Sample depth		TOC†		$pH_{Sorption}$ ‡		Fe_{CBD} §		Mn_{CBD} ¶		Surface area	
	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund
	m		%				g kg^{-1}		mg kg^{-1}		m^2 g^{-1}	
1. Plough layer	0.1–0.3	0.2–0.3	0.89	0.67	7.33	7.38	5.6	4.2	286	204	4.1	4.2
2. Matrix	0.6–0.8	0.7–1.2	0.14	0.12	7.30	7.16	9.0	6.4	333	261	16.7	18.5
3. Wormholes	0.4–0.8	0.7–1.2	0.40	0.37	7.88	7.30	7.6	5.9	269	202	12.1	11.3
4. Matrix	1.5–1.6	2.0–2.6	0.05	0.05	8.20	8.26	4.7	3.9	170	153	12.4	13.0
5. Gray macropores	1.1–2.0	2.0–2.6	0.05	0.06	8.19	8.29	2.1	1.1	24	31	11.0	11.6
6. Matrix	2.5–3.5	3.0–3.5	0.05	0.05	8.16	8.31	4.5	2.9	97	97	11.8	12.2
7. Reddish fractures	2.5–3.5	3.0–3.6	0.05	0.06	8.09	8.28	18.8	12.6	798	910	16.0	21.2
8. Reduced zone	4.3–4.5	4.5–4.8	0.18	0.17	8.24	8.53	3.6	0.74	88	44	8.4	11.9

† TOC, total organic C.

‡ $pH_{Sorption}$, the pH measured at conditions similar to those during the sorption experiments.

§ Fe_{CBD}, total Fe oxides (extractable with citrate–bicarbonate–dithionite).

¶ Mn_{CBD}, total Mn oxides (extractable with citrate–bicarbonate–dithionite).

Table 8.1.2.1-55: Major soil parameters

Domain no.	Description	Sample depth (m) Gjorslev / Lund	$pH_{Sorption}$ Gjorslev / Lund	pH_{CaCl2} Gjorslev / Lund	pH_{H2O} Gjorslev / Lund
1	Plough layer	0.1-0.3 / 0.2-0.3	7.33 / 7.38	6.49 / 6.86	7.64 / 7.77
2	Matrix	0.6-0.8 / 0.7-1.2	7.30 / 7.16	6.81 / 6.86	8.09 / 7.92
3	Wormholes	0.4-0.8 / 0.7-1.2	7.88 / 7.30	7.21 / 6.80	8.35 / 7.80
4	Matrix	1.5-1.6 / 2.0-2.6	8.20 / 8.26	7.67 / 7.65	8.67 / 8.63
5	Grey macropores	1.1-2.0 / 2.0-2.6	8.19 / 8.29	7.64 / 7.79	8.77 / 8.77
6	Matrix	2.5-3.5 / 3.0-3.5	8.16 / 8.31	7.62 / 7.47	8.73 / 8.68
7	Reddish fractures	2.5-3.5 / 3.0-3.6	8.09 / 8.28	7.58 / 7.56	8.52 / 8.59
8	Reduced zone	4.3-4.5 / 4.5-4.8	8.24 / 8.53	7.54 / 7.59	N.D. / 8.27

Domain no.	Description	TOC (%) Gjorslev / Lund	TIC (%) Gjorslev / Lund	Surface (m^2/g) Gjorslev / Lund	CEC (cmol/kg) Gjorslev / Lund
1	Plough layer	0.89 / 0.67	0.32 / 0.20	4.1 / 4.2	11.0 / 9.25
2	Matrix	0.14 / 0.12	0.08 / 0.11	16.7 / 18.5	12.5 / 12.2
3	Wormholes	0.40 / 0.37	0.19 / 0.17	12.1 / 11.3	12.0 / 11.9
4	Matrix	0.05 / 0.05	1.95 / 2.41	12.4 / 13.0	8.77 / 7.52
5	Grey macropores	0.05 / 0.06	2.58 / 3.17	11.0 / 11.6	8.80 / 7.75
6	Matrix	0.05 / 0.05	1.88 / 2.98	11.8 / 12.2	8.53 / 7.15
7	Reddish fractures	0.05 / 0.06	1.83 / 2.67	16.0 / 21.2	9.46 / 8.26
8	Reduced zone	0.18 / 0.17	2.21 / 2.85	8.4 / 11.9	7.05 / 4.79

Domain no.	Description	Clay (%) Gjorslev / Lund	Silt (%) Gjorslev / Lund	Fine sand (%) Gjorslev / Lund	Med. sand (%) Gjorslev / Lund	Coarse sand (%) Gjorslev / Lund
1	Plough layer	6.5 / 6.3	41.1 / 39.4	35.9 / 35.7	10.6 / 12.3	6.0 / 6.3
2	Matrix	8.5 / 7.8	46.1 / 43.2	30.0 / 32.3	9.2 / 11.2	6.2 / 5.5
3	Wormholes	8.6 / nd	42.4 / nd	33.2 / nd	9.1 / nd	6.6 / nd
4	Matrix	13.1 / 13.4	42.7 / 43.2	28.8 / 28.5	8.7 / 8.9	6.7 / 6.0
5	Grey macropores	12.9 / 12.5	43.1 / 41.0	27.6/ 25.5	8.8 / 8.8	7.5 / 12.2
6	Matrix	12.9 / 13.6	45.0 / 43.5	27.1 / 26.4	9.0 / 7.4	6.0 / 9.1
7	Reddish fractures	10.6 / 14.1	40.8 / 45.5	24.8 / 26.0	8.8 / 9.9	15.1 / 12.0
8	Reduced zone	10.7 / 14.3	51.3 / 45.5	24.7 / 28.3	8.0 / 7.6	5.3 / 4.3

Sorption Is Domain Specific

Glyphosate sorption followed the Freundlich model with a high concentration dependence ($0.87 < n < 1.32$, Table 8.1.2.1-56).

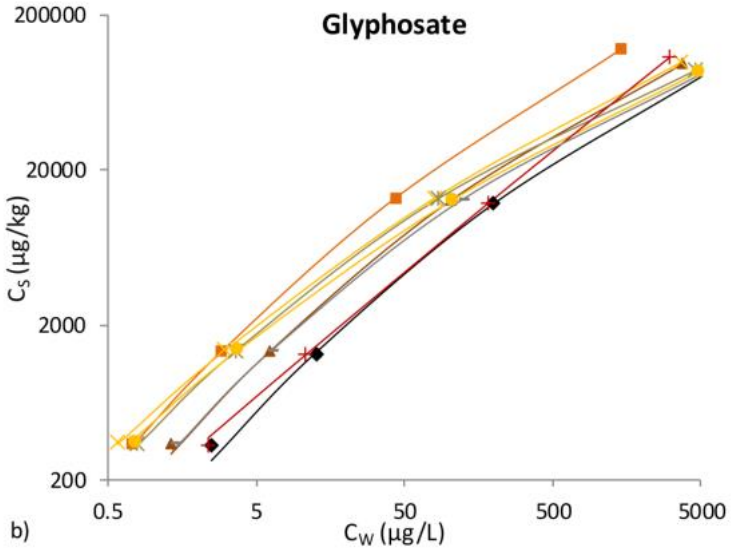
Table 8.1.2.1-56: Extended Freundlich parameters (glyphosate) for sorption to eight soil domains in the Gjorslev and Lund profiles

Domain	Glyphosate‡					
	K_{Fex}		*n*		*D*	
	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund
1	130	72.5	1.06	1.06	0.040	0.036
2	443	947	1.04	0.98	0.041	0.043
3	239	496	1.07	0.97	0.045	0.040
4	536	125	0.88	1.12	0.039	0.042
5	3849	353	1.01	1.01	0.053	0.053
6	424	359	0.87	0.91	0.037	0.037
7	124	58.1	0.92	0.93	0.011	0.000
8	217	251	1.08	1.32	0.049	0.071

‡ K_{Fex}, the extended Freundlich coefficient; *D*, a parameter that adds extra curvature to the line in a double-logarithmic plot. K_{Fex} equals the predicted distribution coefficient at 1 μg L^{-1} glyphosate.

The extended Freundlich model fitted the sorption data of glyphosate very well, though with a tendency to slightly underestimate sorption at the lowest concentration (Figure 8.1.2.1-13). Glyphosate sorption was very concentration dependent (Figure 8.1.2.1-13), which is why the extended Freundlich model fitted the sorption data better.

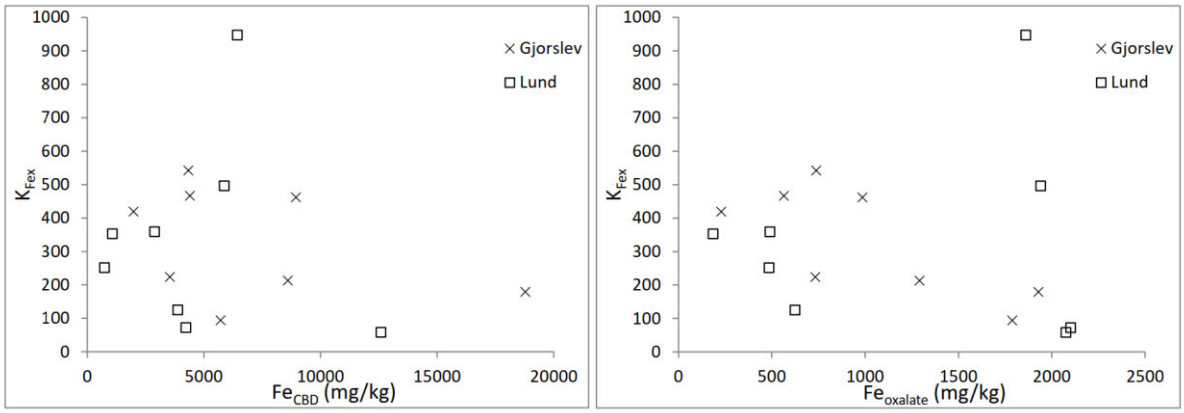
Figure 8.1.2.1-13: Sorption isotherms for glyphosate (extended Freundlich model) in the eight soil domains from the Gjorslev profile. Note: Cs is the pesticide concentration in the soil phase, and Cw is the pesticide concentration in the aqueous phase



The concentration dependence can be exemplified by Domain 6 (matrix soil), where the Kd at the lowest glyphosate equilibrium concentrations (0.8–0.9 mg/L) was 377 for Lund and 453 for Gjorslev, whereas at the highest equilibrium concentrations (4.2–4.8 mg/L), the Kd was only 18 for Gjorslev and 22 for Lund. Domain 7 (reddish macropores from same depth as Domain 6) was an exception with low sorption and little concentration dependence, with a Kd of 55 (Lund) to 137 (Gjorslev) at the lowest concentration and 33 to 34 at the highest. Hence, two very different sorption strengths and concentration dependencies were observed from the same soil depth. Also, at the 0.4- to 1.2-m depth, sorption of glyphosate varied in the two domains at both study sites, being twice as high in matrix soil (Domain 2) than in soil from the wormholes (Domain 3). This fits well with the much lower sorption of glyphosate to the plow layer, which shows some similarities with the wormholes.

There was no correlation between Fe oxide content (expressed either as total Fe oxides or amorphous Fe oxides) and glyphosate sorption (expressed as K_{Fex}) (Figure 8.1.2.1-14). There was also no correlation when K_{Fex} was plotted against any other measured soil parameter.

Figure 8.1.2.1-14: Relationship between total iron oxide concentration (Fe_{CBD}) or amorphous iron oxides ($Fe_{oxalate}$) and sorption of glyphosate (K_{Fex}) in the eight soil domains at the two study sites. K_{Fex} was determined at a µg/L basis and therefore denotes the calculated partitioning coefficient at 1 µg/L



It may be important to consider differences in pesticide sorption between soil domains from the same depth when modeling the risk of pesticide leaching. In clayey tills, most water transport takes place in the macropores; sorption studies, on the other hand, would normally be conducted on bulk soil samples, resembling the matrix samples in the present study. Most sorption studies are furthermore performed only with soil from the plow layer, and leaching in the actual fields may therefore be different from the leaching calculated from such sorption studies. For glyphosate, the leaching would most likely be higher than expected, since sorption to the soil of the upper biopores and especially to the surfaces of the metal oxide coated fractures is lower than in their corresponding matrix domains.

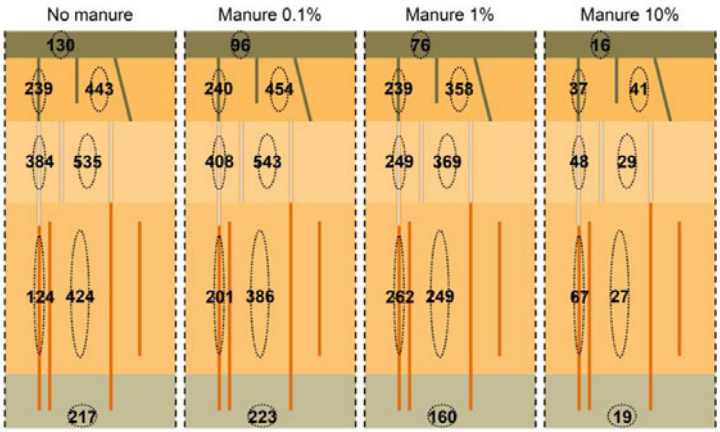
Topsoil Extract and Liquid Manure Extract Reduce Pesticide Sorption

The addition of topsoil extract had an effect on glyphosate sorption, decreasing sorption (K_{Fex}) by 3 to 37 % depending on the domain (Table 8.1.2.1-57), and the addition of liquid manure had an even larger effect. Ten percent liquid pig manure changed sorption (K_{Fex}) dramatically, with a decrease of 83 to 95 % in the Gjorslev Domains 1 to 6 and 8, and 76 to 83 % in the corresponding Lund domains (Figure 8.1.2.1-15). Manure additionally changed the other extended Freundlich parameters (n and D), as the sorption of glyphosate was less concentration dependent when manure was present.

Table 8.1.2.1-57: Sorption parameters for glyphosate with different liquid treatments. Control is without any additions

	Control			Topsoil extract (50%)			Manure (0.1%)			Manure (1%)			Manure (10%)		
Domain	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D
Gjor-1	130	1.06	0.040	86	1.16	0.044	96	1.17	0.046	76	1.09	0.037	16	1.14	0.028
Gjor -2	443	1.04	0.041	338	0.98	0.032	454	0.99	0.036	358	1.07	0.045	41	1.28	0.038
Gjor -3	239	1.07	0.045	193	1.07	0.042	240	1.09	0.048	239	1.08	0.045	37	1.13	0.028
Gjor -4	536	0.88	0.039	354	0.97	0.042	543	0.89	0.039	369	1.03	0.049	29	1.33	0.042
Gjor -5	384	1.01	0.053	295	1.02	0.049	408	0.98	0.049	249	1.14	0.057	28	1.28	0.039
Gjor -6	424	0.87	0.037	283	1.00	0.046	386	0.93	0.042	249	1.08	0.049	27	1.31	0.042
Gjor -7	124	0.92	0.011	142	0.99	0.023	201	0.86	0.012	262	0.98	0.030	67	1.14	0.032
Gjor -8	217	1.08	0.049	169	1.12	0.050	223	1.09	0.051	160	1.17	0.052	19	1.36	0.043
Lund-1	72	1.06	0.036	-	-	-	72	1.01	0.030	35	1.11	0.033	12	1.02	0.019
Lund-2	947	0.98	0.043	-	-	-	1026	0.96	0.044	657	1.00	0.043	47	1.20	0.035
Lund-3	496	0.97	0.040	-	-	-	472	0.96	0.038	262	1.07	0.045	23	1.25	0.036
Lund-4	125	1.12	0.042	-	-	-	244	0.98	0.039	253	1.09	0.050	30	1.27	0.040
Lund-5	353	1.01	0.053	-	-	-	350	1.04	0.055	258	1.09	0.053	18	1.39	0.044
Lund-6	359	0.91	0.037	-	-	-	354	0.97	0.045	241	1.07	0.047	30	1.27	0.040
Lund-7	58	0.93	0.000	-	-	-	116	0.85	0.000	259	0.88	0.016	88	1.12	0.031
Lund-8	251	1.32	0.071	-	-	-	264	1.33	0.073	265	1.33	0.073	14	1.29	0.035

Figure 8.1.2.1-15: Effect of liquid pig manure extract (%, v/v) on the sorption (K_{Fex}) of glyphosate in the Gjorslev soil domains. The K_{Fex} equals the predicted distribution coefficient at a glyphosate concentration of 1 µg/L



Why Do Topsoil Extract and Manure Reduce Glyphosate Sorption

Several soil water parameters have been suggested to influence glyphosate sorption. These include pH, phosphate, divalent metal ions like Cu^{2+} and Zn^{2+} and dissolved organic matter.

Change in pH cannot explain the general decrease in sorption when topsoil extract or pig manure was added.

The manure had a high conductivity (21,900 µS/cm, Table 8.1.2.1-58). In parallel experiments, it was observed an increase in sorption at increased ionic strengths (data not shown), which has also been reported previously in the literature. The high ionic strength in the manure therefore cannot explain the decreased sorption.

Table 8.1.2.1-58: Major analyzed parameters for the liquid manure and topsoil extracts. ND = not determined

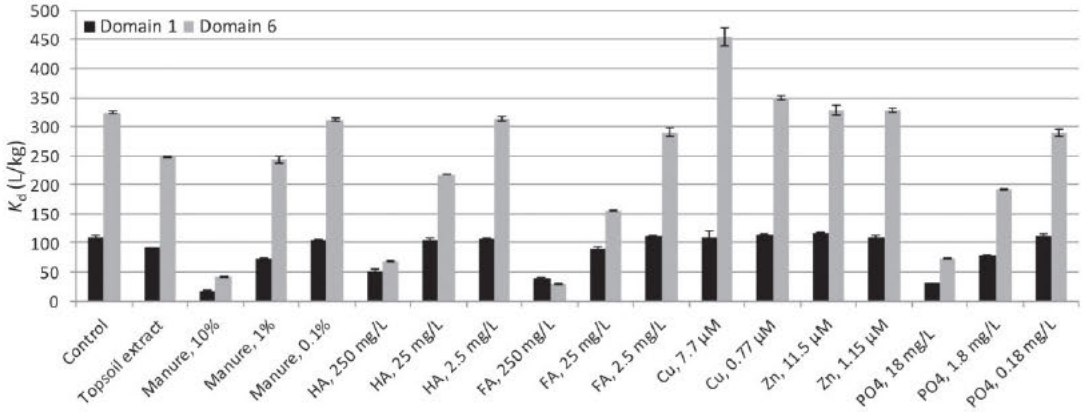
Parameter	Unit	Liquid pig manure	Topsoil extract
DOC	mg/L	2648	32
Dry matter	%	1.5	ND
Conductivity	µS/cm	21900	214
PO_4^{3-}	mg/L	182	<0.1
Cu	mg/L	4.9	0.03
Zn	mg/L	7.5	0.12
Fe	mg/L	10.2	22.7
Al	mg/L	0.28	32.0
Na^+	mg/L	623	0.35
K^+	mg/L	2500	0.57
Ca^{2+}	mg/L	<13	3.8
Mg^{2+}	mg/L	<8	0.15
F^-	mg/L	<0.5	1.2
Cl^-	mg/L	1481	4.5
Br^-	mg/L	30.7	0.86
NO_3^-	mg/L	0.64	33.1
SO_4^{2-}	mg/L	10.4	10.3
Ba	mg/L	0.2	0.16
Mn	mg/L	0.4	0.18
S	mg/L	668	11.2
Density	-	1.02	ND

Both the humic and fulvic acid fractions of soil organic matter decreased glyphosate sorption, when added to soil (Figure 8.1.2.1-16).

Divalent metal ions and phosphate would be relevant only with manure addition. Divalent metal ions (Cu^{2+} and Zn^{2+}) at concentrations corresponding to 1 and 10% pig manure had no effect on sorption in Domain 1 and increased sorption in Domain 6 (Figure 8.1.2.1-16). The Cu^{2+} and Zn^{2+} ions are therefore not likely to have caused the manure effect. Phosphate, on the other hand, reduced glyphosate sorption

at concentrations corresponding to those in the pig manure (Figure 8.1.2.1-16). Both phosphate and dissolved organic matter are therefore likely candidates to explain the manure effect on glyphosate sorption.

Figure 8.1.2.1-16: Effect of topsoil extract, liquid pig manure extract, organic matter (humic acids [HA] and fulvic acids [FA]), divalent metals (Cu and Zn), and phosphate on glyphosate sorption (expressed as the distribution coefficient, K_d) in Domains 1 and 6 from the Gjorslev site. Concentrations correspond to the tested manure concentrations. Error bars are minimums and maximums of duplicate samples. Results from experiments with topsoil extract and liquid manure are included for comparison. Controls are without any additions



Conclusion

The study has demonstrated that the sorption of glyphosate varies by an order of magnitude across eight identified soil domains in macroporous clayey till. It was expected that glyphosate would show the strongest sorption in domains with high Fe oxide content. This turned out to be wrong, since there was no correlation between glyphosate sorption and any measured soil parameter, including extractable Fe oxides. The domain-specific sorption of glyphosate was by far overruled by addition of liquid manure that strongly decreased glyphosate sorption due to its content of dissolved organic matter and phosphate. The variation across domains and the effects of solutes like the liquid fraction of manure should be taken into account when using sorption data in assessment of leaching risks. Our results suggest that hydrological modeling should focus more on sorption to fracture surfaces and pay less attention to traditional bulk sorption data when predicting pesticide transport through clay macropores. How much sorption influences leaching will, after all, also depend on general hydrological parameters such as pore size, connectivity, and climatic conditions.

Assessment and conclusion by applicant:

The article describes the sorption behaviour of glyphosate to different soil domains (top- and sub-soils) from two agricultural soils in Denmark. The set-up of the experiment was based on the OECD 106 guideline but with significant deviations: The study was conducted with 1 mM $CaCl_2$ solution (standard: 10 mM solution), at 10°C (standard: 20 – 25 °C); at 4 test concentrations (standard: 5), no validation of the analytical methods used, no concentrations in the solid phase were explicitly reported, i.e. no mass balances or parental mass balances were established.

The article is therefore classified as reliable with restrictions i.e. not used in risk assessment.

Assessment and conclusion by RMS:

RMS agrees with the applicant regarding the identified deviations from OECD 106.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Dollinger et al, 2018

Data point:	CA 7.1.3.1.1/015
Report author	Dollinger, J. et al.
Report year	2018
Report title	Contrasting soil property patterns between ditch bed and neighbouring field profiles evidence the need of specific approaches when assessing water and pesticide fate in farmed landscapes
Report No	DOI 10.1016/j.geoderma.2017.09.006 ISSN 0016-7061
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The authors' aim was to evaluate the specificity of ditch material properties to determine whether ditches require an approach that differs from that of field soils when studying water and pesticide fate in farmed landscapes. The authors thus analysed the variations in the pedological, herbicide sorption and flow properties of soil materials along a 2D cross-section of an intermittently flooded ditch in the Roujan catchment of southern France. They found that the upper part of the ditch bed soil profile is composed of 3 horizons that formed after the original creation of the ditch, most likely via the deposition of field-eroded particles and the accumulation of organic matter. These specific horizons have greater porosity, mostly due to their dense root systems, and contain up to 2 times more organic carbon than the neighbouring banks or field soils. Consequently, the hydraulic conductivity is greater, and the sorption of hydrophobic herbicides is up to 2 times greater in ditch bed materials than it is in soils located farther away from the ditch surface. Moreover, significant macroporal flow was evidenced in both profiles but with different contribution to the global flow. The contrasts in the hydrodynamic and sorption properties between both the ditch bed and banks materials likely results in significantly different water and pesticide infiltration patterns in ditches compared to crop fields. Given these differences, they recommend investigating the specific properties of ditch beds when studying and modelling water and pesticide fate in croplands.

Materials and methods

Study site

The studied ditch is located near the outlet of the Roujan catchment (Herault, France). This 91 ha catchment (Table 8.1.2.1-59) is cultivated mainly by vineyards and a dense network of ditches, 11 km total length, was implemented between the vine fields. Except on the plateau, the soils are directly developed over the Miocene loose sandstone and are organized along a toposequence. The soils depth increase and soil texture evolve consistently with the colluvial accumulations of clay and gravels in the glacis. Nearby the study site the soil is classified as a gleyic cambisol (IUSS Working Group WRB, 2014). A perennial groundwater has developed on the bottom part of the catchment and >5 km of ditches (47 % of the total length of ditches) drain this area.

The catchment is subjected to semi-arid Mediterranean climate characterized by scarce high-intensity rainfall events. This specific precipitation pattern results in the periodic flooding of ditches and the rapid fluctuation of the shallow water table in the bottom part of the catchment. The high reactivity of the water table leads to the alternation of downward and upward fluxes in ditch beds during storm events. The studied ditch is chosen near the catchment outlet in order to: i) represent the typical functions of

ditch in a perennial groundwater environment and ii) be representative of the soil type and ditch characteristics combination that prevail in the 33 ha of the bottom part of the catchment.

Figure 8.1.2.1-17: The ditch network over the Roujan catchment in relation with the soils and the perennial groundwater

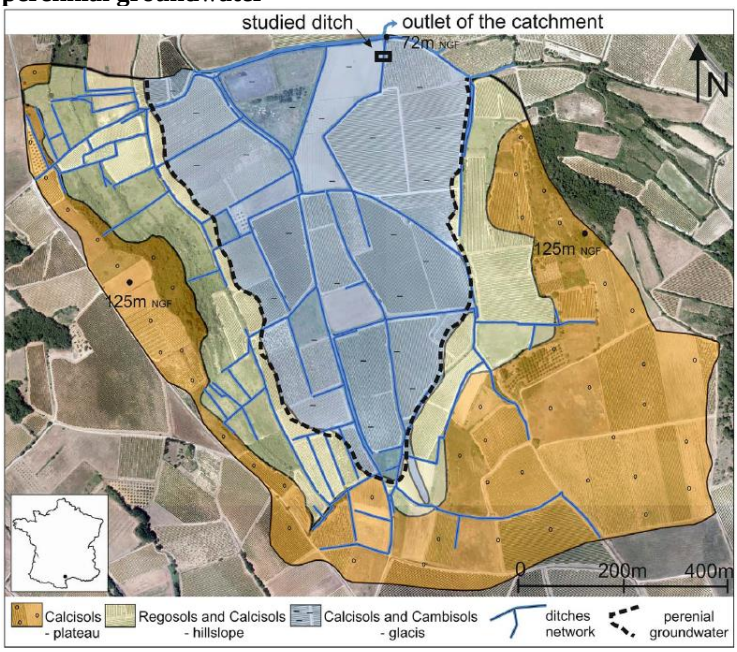


Table 8.1.2.1-59: The spatial variability of ditches network over the catchment

Topographic position	Dominant soil types (WRB 2014)	Soil depth	Surface area	Total length of ditch in the network
		m	ha	km
Plateau	Calcisols	0.4–2	36	1.49
Hillslope	Regosols and calcisols	0.2–1.5	22	4.78
Glacis	Calcisols and cambisols	1–4	33	5.48
Catchment			*91*	*11.75*

Experimental design

Characterization of soil properties and core sampling along the cross section

For characterizing and sampling soil heterogeneity of the ditch soil and its vicinity, a 1.50-m-wide, 1.50-m-deep trench was excavated across the ditch in February 2014. The studied ditch is densely vegetated, and roots are present along the entire soil profile to a depth of 1.5 m (Figure 8.1.2.1-18A).

A series of morphological parameters, including texture, structure, colour, stone and root abundances, were observed in the field. Soil horizons were determined based on these observations. Bulk densities (ρ_b) were measured by core sampling with 100-cm^3 cylinders, using 6 replicates per horizon. The ρ_b was determined as the ratio between the dry soil mass and the total core sampling volume. Samples of over 500 g were collected from each horizon for further laboratory characterization. Particle size distribution, pH, cation exchange capacity (CEC), organic carbon content (OC), and calcium carbonate ($CaCO_3$) content were measured at the INRA-ARRAS Laboratory (France) (see Table 8.1.2.1-60).

Figure 8.1.2.1-18: Morphology of the ditch cross-section soil profile. A) Description of the soil profile, B) core sampling scheme. The black lines represent core sampling locations within the soil profile. V and H represent the cores sampling axis direction being, respectively, vertical and horizontal

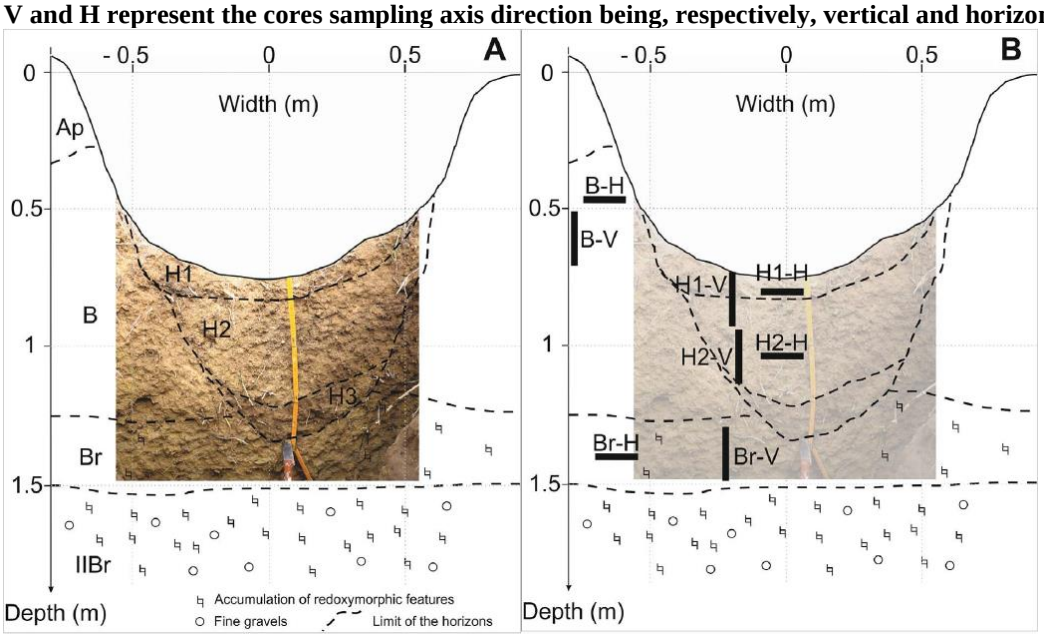


Table 8.1.2.1-60: Physico-chemical properties of ditch-bed and banks soils

Horizon	Depth from field topsoil	Structure	Sand	Silt	Clay	OC	CEC	pH	$CaCO_3$	ρ_b
	m		%	%	%	%	cmol kg^{-1}		g kg^{-1}	g cm^{-3}
B	0.4–1.30	Polyhedral subangular blocky – hydromorphic features	7.7	57.2	35.1	0.96	14.2	8.71	247.0	1.36 ± 0.01
H1	0.75–0.82	Stratified	35.9	39.1	25.0	1.58	12.5	8.44	150.0	1.25 ± 0.06
H2	0.82–1.15	Granular	40.3	33.1	26.6	1.56	12.1	8.54	160.0	1.26 ± 0.04
H3	1.15–1.35	Stratified and subangular blocky – hydromorphic features	10.4	56.7	32.9	1.17	14.0	8.59	248.0	1.21 ± 0.05
Br	1.30–1.50	Polyhedral blocky – hydromorphic and redoximorphic features	4.7	58.5	36.8	0.73	14.4	8.63	285.0	1.48 ± 0.03

Four undisturbed soil cores were sampled from each horizon except in the Ap horizon of the bank profile that has no counterpart in the ditch soil profile. These cores were collected by gently pushing stainless-steel cylinders with internal diameters of 15 cm and heights of 20 cm in the soil until the soil surface was approximately 5 cm from the top of the cylinder. The soil around the cylinders was then excavated to facilitate the undisturbed extraction of the monoliths. To characterize the anisotropy of downward vs. lateral water and solute flow, a series of monoliths was sampled vertically and a second series was sampled horizontally (Figure 8.1.2.1-18B). Due to the length of the sampled cores, the core sampled in the first horizon below the ditch also included the top of the second horizon; because the third horizon below the ditch was too narrow, it could not be sampled. After extraction, cores were stored at 4°C until undergoing tracer experiments.

Tracer displacement experiments

Tracer displacement experiments were performed on soil cores sampled vertically and horizontally in the ditch bed and bank profiles (Figure 8.1.2.1-18B) in order to characterize the water flow patterns of these materials. Because bromide is only present at trace concentrations in the environment and rarely sorbs to soil particles, it was selected as a conservative tracer of water flow for these displacement experiments.

Stainless-steel grids with 6-mm-diameter holes were sealed at the bottom of the columns to prevent soil loss occurring during the infiltration experiments without disturbing the water flux in the columns. Prior to tracer injections, the columns were gradually saturated via capillarity for 48 h to prevent the trapping of gas bubbles in soil pores.

The tracer solutions used for the displacement experiments contained 800 mg/L of bromide (Br^-). At the beginning of the displacement experiments, the saturated columns were manually ponded with a

30-mm water height of the tracer solution. This water height was chosen to mimic the infiltration conditions in the Roujan catchment during intermittent flooding and corresponds to the water level commonly monitored in ditches during flood events with a 1-month return period. The water height was kept constant during the infiltration by adjusting the supply of the tracer solution. A total of 85 mm of solution was supplied to the columns. Among the 16 columns, the pore volumes ranged from 63 to 82 mm. Therefore, the volume of tracer solution supplied during the displacement experiment was always higher than the pore volume of the columns. When the solution supply stopped, the decrease in water head was monitored during the remaining period of ponded water infiltration. Just after all the ponded solution had infiltrated, the columns were ponded again with a constant head of 30 mm, and the columns were flushed with 85 mm of tap water, following the same procedure. During the infiltration and flushing periods, 50 ml fractions of the percolates were collected in glass containers at the outlets of the columns. The sampling frequency varied from 15 s to about 6 min, depending on the columns drainage fluxes. The outlet flowrates were monitored using the timing of sample collection and their precise weights. The inlet flowrates were monitored by weighing the injection tank at 1-s intervals.

The concentrations of bromide in the percolate samples were measured using an ion-specific electrode (Hanna Instruments, HI4002, Lingolsheim). These concentration values were cross-validated with ion chromatography measurements of randomly selected samples. A good fit was found between the ion-specific electrode and the ion chromatograph results (data not shown). The electrical conductivity and pH were also measured in the percolate samples.

Dye tracing of the active macroporosity

Following the displacement experiments, dye tracing was performed on the columns to visualize and quantify the active macroporosity. The dye tracing experiments were also used to visualize the presence or absence of sidewall flow. The displacement experiments were validated when sidewall flow was absent or weak and discontinuous along the sides of the columns. In contrast, when continuous sidewall flow was detected along the sides of the columns, the corresponding displacement experiments were dismissed. A total of 8 columns, containing one sample per horizon and one sample per direction (vertical/lateral) were validated based on dye staining experiments.

The infiltration conditions of dye tracing were identical to those of the displacement experiments, as the fraction of active porosity mobilized for percolation in structured soils likely varies with initial moisture and water head conditions. The columns were thus saturated again via capillarity for 48 h; then, 57 mm of the fluorescent dye sulforhodamine B at a concentration 1 g/L was percolated through the columns with a constant water head of 30 mm. At a concentration of 1 g/L, the sorption sites of sulforhodamine B on soils in contact from all horizons were saturated, which guaranteed homogeneous staining among the columns. After percolation, the columns were sliced into cross-sections approximately 2 cm in height. A marker was placed on the sides of the slices to determine the orientation and superposition of the 7 slices within a given column. The slices were then imaged in a dark chamber with homogeneous LED lighting (3800 K) using a digital camera that was equipped with a 28-mm lens and was positioned 65 cm above the slice. The image resolution was 300 dpi, which corresponds to a pixel size of 71 µm. The illumination and hue saturation of the raw images were corrected using Nikon Capture NX2 software based on the grey and colour scales positioned next to the column slices during imaging. The RGB channels were split, and the colour thresholds were adjusted in each of the channels. The minimum/maximum thresholds applied to all images were 9/255, 118/253 and 4/255 for the R, G and B channels, respectively. The RGB channels were then merged, and the image was binarized. Both bright and dark isolated pixels were removed using the 'Noise' function of the ImageJ software, with a radius of 10 pixels, for white and black pixels, successively. The respective areas of both bright and dark pixels relative to the total area of the column cross-section were then calculated with ImageJ. The dark areas correspond to the stained areas on the cross-sections of the columns.

The volume of macroporosity mobilized during percolation relative to the total porosity (ω) was estimated from the dye coverage area. As dye diffusion in the matrix is limited, due to its short infiltration time, ω_i was calculated for each column cross-section (i) by multiplying the average dye coverage area per column slice (i.e., top and bottom coverage) by the slice height and dividing it by the total porosity (i.e., the total volume of soil in the slice multiplied by the soil porosity). The average ω per column was also calculated as a geometric mean of the respective ω_i values of the 7 column slices (i).

Inverse modelling of transport properties

Water flow and transport equations

Inverse modelling was performed with the HYDRUS-1D model that solves the Richards and convection-dispersion equations. Four modelling approaches were compared in the first place: single porosity, dual porosity, dual porosity + mobile-immobile (DP + MIM) and dual permeability (see Šimůnek *et al.*, 2003 for a detailed description of these approaches). Only the dual-permeability model provided satisfactory fits of the tracer displacement experiments for most of the columns and is thereby considered in this paper. The column H2-H was the only exception for which the model DP + MIM was better adapted than the dual permeability model. DP + MIM was used to simulate the bromide breakthrough curve of H2-H but is not described in this paper (for the description of the model please refer to Šimůnek *et al.*, 2003). Equations of the dual permeability model are briefly reviewed below.

The dual permeability model assumes that flow and solute transport occur within and between two distinct compartments, namely the macropore compartment, consisting in inter-aggregate or fracture porosities, and the micropore or matrix compartment, consisting in intra-aggregate porosity. The water flow equations in the macroporal and matrix compartments are assumed similar by HYDRUS 1D and given by:

$$\frac{\partial \theta_f(h_f)}{\partial t} = \frac{\partial}{\partial z}\left[K_f(h_f)\left(\frac{\partial h_f}{\partial z} + 1\right)\right] - S_f(h_f) - \frac{\Gamma_w}{\omega} \qquad (1a)$$

$$\frac{\partial \theta_s(h_s)}{\partial t} = \frac{\partial}{\partial z}\left[K_s(h_s)\left(\frac{\partial h_s}{\partial z} + 1\right)\right] - S_s(h_s) - \frac{\Gamma_w}{1-\omega} \qquad (1b)$$

where subscript *f* and *s* respectively refers to the fast macroporal compartment and the slow matrix compartment, θ is the water content [L^3/L^3], *h* is the pressure head [L], *K(h)* is the unsaturated hydraulic conductivity function, *S* is a sink or source term [T^{-1}], ω is the ratio of the macroporal volume of fast to the total poral volume of the soil (dimensionless) and Γ_w is the transfer rate between the two compartments [T^{-1}]. The water retention curve $\theta(h)$ and the unsaturated hydraulic function *K(h)* are defined for both compartments using the van Genuchten model. *K(h)* is described as the product of the relative hydraulic conductivity function *Kr* (dimensionless) and the saturated hydraulic conductivity *Ks* [L/T].

The transport equations associated with the dual-permeability formulation for water flow are based on the classical convection-dispersion equation for both the fast macroporal compartment and the slow matrix compartment with an exchange term between the two compartments:

$$\frac{\partial \theta_f c_f}{\partial t} + \rho\frac{\partial s_f}{\partial t} = \frac{\partial}{\partial z}\left(\theta_f D_f \frac{\partial c_f}{\partial z}\right) - \frac{\partial q_f c_f}{\partial z} - \phi_f - \frac{\Gamma_s}{\omega} \qquad (2a)$$

$$\frac{\partial \theta_s c_s}{\partial t} + \rho\frac{\partial s_s}{\partial t} = \frac{\partial}{\partial z}\left(\theta_s D_s \frac{\partial c_s}{\partial z}\right) - \frac{\partial q_s c_s}{\partial z} - \phi_s + \frac{\Gamma_s}{1-\omega} \qquad (2b)$$

$$\Gamma_s = \omega_{dp}(1-\omega)\theta_s(c_f - c_s) + \Gamma_w c^* \qquad (2c)$$

where *c* is the solute concentration [M/L^3], *s* is the sorbed solute concentration [M/M], ρ is the bulk density [M/L^3], *D* is the dispersion coefficient accounting for both molecular diffusion and hydrodynamic dispersion [L^2/T], *q* is the Darcian flux [L/T] ϕ is a sink-source term [$M/(L^3\ T)$], Γs is the mass transfer term for solute between the macroporal and the matrix compartments [$M/(L^3\ T)$] and *c** is equal to c_f. for $\Gamma_w > 0$ c_m for $\Gamma_w < 0$.

Inverse modelling design

The 0.15 m-soil profiles were densely discretized with 101 nodes to facilitate numerical convergence. An initial hydrostatic equilibrium with a zero-pressure at the top of the soil columns was considered. A variable head was imposed at the upper boundary condition. It was fixed at the ponding head value (3 cm) during the injection and rinsing phases and varied between both phases to correspond to the

ponding height decreases monitored during the experiments (see Section *Tracer displacement experiments*). The eight following parameters were fitted against the cumulative water outflows heights and bromide concentrations at the outlet of the soil column: θs_s, θs_f, Ks_s, Ks_f, ω, $Disp_s$, $Disp_f$, ω_{dp} with θs and θr respectively the saturated and residual soil water content and *Disp*, the dispersion coefficient [L]. To avoid local minimum, the stability of the fitted parameter set estimated was evaluated using different sets of initial parameters, including the estimated sets themselves. The other hydrodynamic parameters were set according to the textural composition and bulk density of the soils using Rosetta, except θr_f that was set to zero. Note however that since the soil column remained saturated during the whole experiment, the van Genuchten parameters alpha, n and l were not sensitive. The Bromide diffusion coefficient was fixed to $1.67\ 10^{-9}\ m^2\ s^{-1}$. K_{sat} was not adjusted but calculated from the experimental outflow data using Darcy's law. Θs was also not adjusted but calculated from the bulk density data using a pedotransfer function.

Sorption properties of selected herbicides

Two herbicides, diuron and glyphosate, were selected to assess the heterogeneity of sorption properties along the profile of the ditch cross-section. Diuron was extensively used on the Roujan catchment for weed control in vineyards. After it was banned from the list of allowed active molecules in France in 2008, it was replaced by the broad-spectrum herbicide glyphosate. Both herbicides were still measured in the water column of the ditch at the outlet of the catchment in 2016. Glyphosate and diuron exhibit very different physicochemical properties (Table 8.1.2.1-61), which may lead to contrasting sorptive patterns along the soil profiles.

The adsorption parameters were assessed according to the procedure described in Dollinger *et al.* (2016), which was adapted from the OECD Guideline 106. Briefly, the soils were air-dried to a target humidity of 10 % then sieved to a size of 2 mm. 10 mL of the ^{14}C-labelled pesticide solution, with concentrations ranging from 5 to 1000 µg/L, were equilibrated with 1 and 2 g of dry soil in glass centrifuge tubes for glyphosate and diuron adsorption experiments, respectively. The tubes were shaken for 24 h, and the radioactivity in the supernatant was measured after centrifugation. Pesticide concentrations in soils were assessed by mass balance between initial and equilibrium concentrations. Both linear (Eq. 3) and Freundlich models (Eq. 4) were fitted to the experimental data.

$$C_s = Kd\ C_w \quad (3)$$

$$C_s = Kf\ C_w^{\,n} \quad (4)$$

$$H = \frac{n_{des}}{n_{ads}} \quad (5)$$

where C_s is the amount of sorbed pesticides in the soil at equilibrium (µg kg^{-1}), C_w is the equilibrium concentration in the supernatant (µg/L), *Kd* is the linear sorption coefficient (L/kg), *Kf* ($\mu g^{(1-n)}\ L^n/kg$) and n are the Freundlich coefficients and H is the apparent hysteresis index with n the non-linearity parameter of the Freundlich model and subscripts *ads* and *des* standing for adsorption and desorption isotherms, respectively.

The detailed procedure for the determination of herbicide desorption parameters can be found in Dollinger *et al.* (2016). Briefly, after 24 h of equilibration with a 100 µg/L pesticide solution, the activity in the supernatant was measured and the residual supernatant was removed. An equivalent volume of fresh electrolyte was added, and the tubes were shaken again for 24 h. Five successive desorption steps of 24 h each were then performed. The amount of pesticides sorbed to soils at each step was calculated by mass balance based on radioactivity counting, and experimental data were fitted to Freundlich isotherms (Eq. 5). The hysteresis between adsorption and the corresponding desorption isotherms was represented by the H parameter (Eq. 3), which was calculated as proposed by Barriuso *et al.* (1994). Sorption is considered to be hysteretic when H <0.7; the lower the value of H is, the more irreversible the sorption is.

Table 8.1.2.1-61: Physico-chemical properties of the studied pesticides

Properties		Glyphosate	Diuron
Formula		$C_3H_8NO_5P$	$C_9H_{10}Cl_2N_2O$
Molecular mass	g mol^{-1}	169.1	233.1
Aqueous solubility at 20 °C	g l^{-1}	10.5 to 12.0	0.42
Log Kow at pH 7		− 4.1 to − 3.2	2.7
pKa_1 – pKa_2 – pKa_3		2.2–5.5–10.2	13.2

From ANSES, 2017, FOOTPRINT, 2015, ChemID, 2017 and chemicalize.org 2017.

Results

Morphology of the ditch bed and bank profiles

Based on field morphological descriptions, two different soil profiles were distinguished along the cross-section: (i) the bank profile, which is composed of 4 horizons, and (ii) the ditch bed profile, which is composed of 5 horizons (Table 8.1.2.1-60 and Figure 8.1.2.1-18).

The bank soil profile corresponds to the soil pit observed in the vicinity of the ditch by Andrieux *et al.* (1993). According to the World Reference Base, this soil is a tilled gleyic Cambisol (colluvic, clayic). The structure of the first horizon (Ap, which extends from the surface to a depth of 0.4 m) is affected by tillage and deep ploughing operations. The upper cambic horizons B and Br (described in Table 8.1.2.1-59) are developed above another deep cambic horizon IIBr (Figure 8.1.2.1-18A) that feature both a high clay content and high bulk density values. However, more hydromorphic features and denser root systems are observed closer to the ditch bank surface than they are in the bank soil profile, which is located further away. The ditch bed soil profile corresponds to a succession of 3 ditch-specific horizons (H1, H2, and H3) and the Br and IIBr horizons, which are shared with the bank profile. The H1, H2, and H3 horizons are significantly different from the other horizons. H1 and H2, which are enriched in sand and have platy structures, are different from the bank horizon B, which is siltier and is dominated by a subangular blocky structure. The third horizon, H3, is similar to the bank horizon B in terms of texture but features a stratified structure that differs from that of the bank horizons. These differences indicate that the H1, H2, and H3 horizons were formed by the deposition of field-eroded particles during successive flood events subsequent to the creation of the ditch. The contours of the 3 horizons specific to the ditch bed (H1, H2 and H3; Figure 8.1.2.1-18A) thus likely delimit the section of the original ditch. The shape of the horizons is probably due to the regular management of the ditch, including dredging operations. At the location where the profiles were observed, the original ditch only slightly incises the Br horizon that prevailed prior to the creation of the ditch.

The B and Br horizons have very similar physicochemical properties (Table 8.1.2.1-60), although horizon B has a slightly greater organic carbon content and a lower bulk density. However, the porosities of horizons B and Br are larger in the vicinity of the ditch surface due to the higher density of the ditch vegetation root system. The upper two ditch bed soil horizons (H1 and H2) contain 1.5 to 2 times more organic carbon than horizon B, which is consistent with the presence of vegetation and higher water contents during the year. Moreover, the bulk densities of the specific ditch bed horizons are significantly lower than those of the other horizons, which is in accordance with their textures, organic matter contents, and dense root channels network. Therefore, the overall porosity of the ditch bed soil profile is higher than that of the bank profile.

In summary, the ditch bed profile and the bank profile have contrasting textural, chemical and structural properties. Moreover, the vertical gradient of the analysed soil properties across the ditch bed horizons is sharper than that across the bank horizons. Both lateral and vertical gradients of soil properties, such as organic matter content or bulk density, are present within the limited spatial area of one square metre between the ditch and the bank. It is therefore expected that flow and sorption properties differ between the ditch and bank soils.

Heterogeneity and anisotropy of water pathways and associated soil pore structure

The results of the displacement experiments and dye staining of the active macroporosity (ω_{dye}) allowed us to compare the hydraulic conductivity and preferential flow patterns of the different horizons and sampling axes in the two soil profiles (

Figure 8.1.2.1-19, Table 8.1.2.1-62) and to relate these flow patterns to the macroporosity patterns (Figure 8.1.2.1-20 and Figure 8.1.2.1-21). The inverse modelling procedure provides a complementary estimation of the flow mechanisms and soil hydrodynamic properties (Table 8.1.2.1-62). The main interest of the modelling results is the opportunity to estimate the contribution of the fast flow to the global outflow.

The horizontal and vertical saturated hydraulic conductivity values (K_{sat}) at the column scale calculated from the percolation flux data range from very large ($1.7 \cdot 10^{-4}$ m s^{-1} for H2-V) to rather small ($6.9 \cdot 10^{-6}$ m s^{-1} for Br-H) values (Table 8.1.2.1-62). Regardless of the horizon, no systematic differences were observed in the measured K_{sat} values between the two sampling axes. With the exception of the second horizon in the ditch bed (H2), the anisotropy of the hydraulic conductivity was small in all samples. Therefore, a mean saturated hydraulic conductivity value was calculated for each horizon, and these values are reported in Table 8.1.2.1-62. The mean saturated hydraulic conductivity of the B horizon is slightly smaller than those of the H1 and the H2 horizons, despite important differences in their textures, organic matter contents and structures, as observed in Section *Morphology of the ditch bed and bank profiles*. Horizon H2 is the most conductive horizon due to the large value of its observed vertical K_{sat}, which may be caused by specific local macropore features, such as the snail shells observed in this horizon. The Br horizon is 4 to 15 times less conductive than the other horizons. Accordingly, as generally observed in structured soils, both ditch bed and bank soil profiles exhibit decreasing soil hydraulic conductivity with depth (e.g., Sammartino *et al.*, 2015; Udawatta and Anderson, 2008), but the upper horizons of the ditch bed profile have higher permeability values than those of the bank profile.

The dye tracing experiments reveal information about the active macroporosity patterns (Figure 8.1.2.1-20) and, thus, about the heterogeneity of the soil structures between the horizons. In all columns, the dye percolated across the column demonstrating the presence of connected macroporosity along the height of the column. However, the magnitude of this connected macroporosity varied greatly between the horizons. Roots were found to be the main source of flow paths in most horizons, as most stained areas surrounded living or decayed root channels. However, not all living or decayed root channels were stained. Roots were present throughout the entirety of both profiles, but denser networks were located near the ditch surface. Consistently, on average, the active porosity was largest in the cores sampled in the upper horizons of the ditch bed profile (Figure 8.1.2.1-18 and Figure 8.1.2.1-20, Table 8.1.2.1-62). The B horizon exhibits a large anisotropy in ω_{dye}, yielding a very large value of approximately 20 % in the horizontal direction. This anisotropy may be partly explained by the fact that the sampling location of the horizontal column is almost in the ditch sidewalls and is slightly further away for the vertical column (Figure 8.1.2.1-18). The H1 and H2 horizons both exhibit a large active porosity, as H2 has the largest ω_{dye} values of all of the horizons. The numerous snail shells, combined with the granular structure present in H2, are likely responsible for its greater active porosity than H1. The active porosity of the Br horizon is significantly smaller than those of the other horizons. Finally, in accordance with the observed variations in saturated hydraulic conductivity, the ditch bed profile exhibits, on average, a larger active porosity than the bank profile. Indeed, although the linear correlation is not statistically significant, K_{sat} generally increases when ω_{dye} increases (Figure 8.1.2.1-21).

Figure 8.1.2.1-19: Bromide breakthrough curves. The black dashed lines represent the shift between contaminated and clear water injection

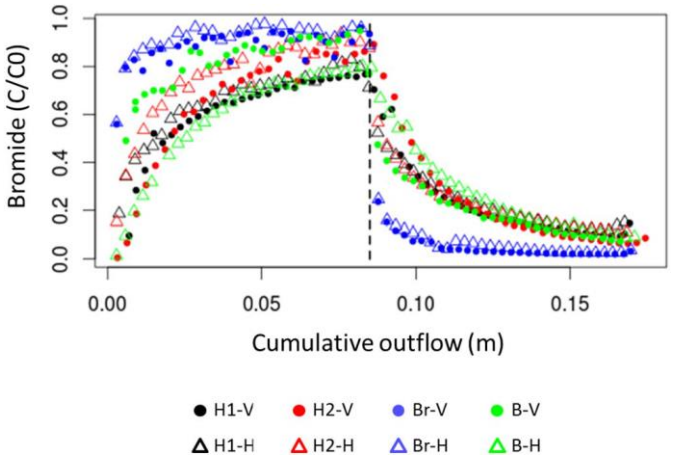


Figure 8.1.2.1-20: Imaging of preferential flow patterns within the soil columns. The black areas represent the stained areas at different depths along the soil cores

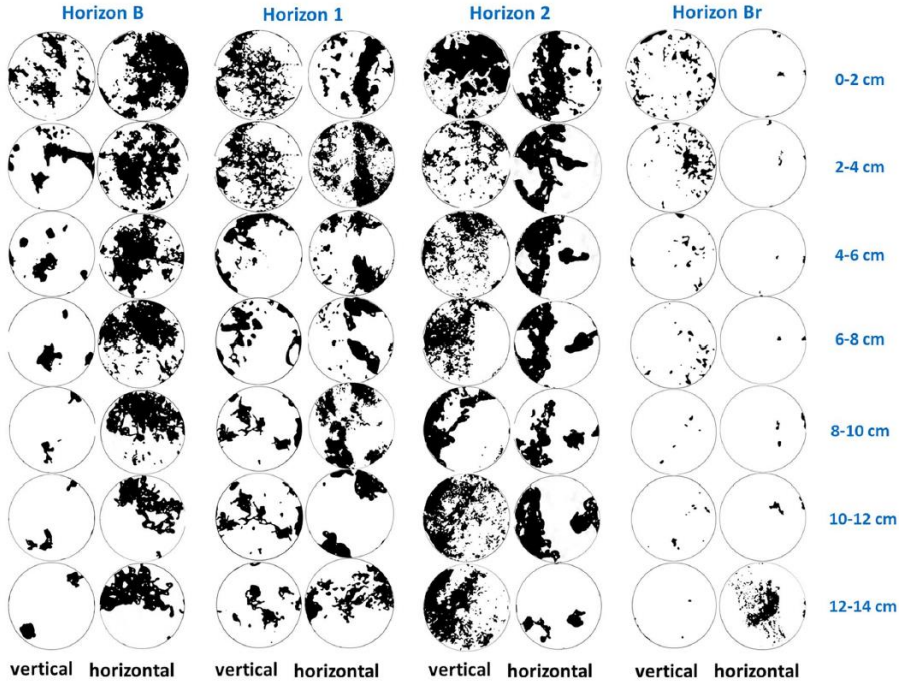


Figure 8.1.2.1-21: Evolution of the hydraulic conductivity with the active macroporosity fraction in the set of soil columns

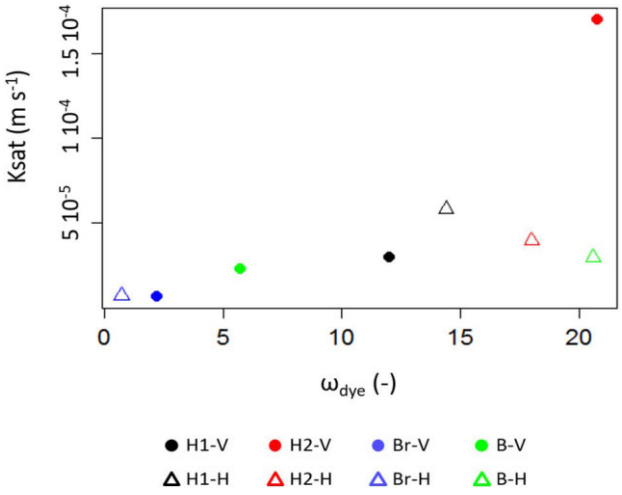


Table 8.1.2.1-62: Hydrodynamic properties of the ditch-bed and banks soil horizons

Columns	[a]K_{sat}	[a]*Mean* K_{sat}*	[a]ω_{dye}	[b]θr_s	[b]θs_s	[b]Ks_s	[b]θs_f	[b]Ks_f	[b] ω	[b]$Disp_s$	[b]$Disp_f$	[b]θs_{Tot}	[a]θs	[b]Relative contribution of preferential flow
	$m\,s^{-1}$	$m\,s^{-1}$	%	$m^3\,m^{-3}$	$m^3\,m^{-3}$	$m\,s^{-1}$	$m^3.m^{-3}$	$m\,s^{-1}$	%	m	m	$m^3\,m^{-3}$	$m^3\,m^{-3}$	%
B-V	$2.34\cdot10^{-5}$	$2.68\ 10^{-5}$	5.7 ± 3.5	0.093	0.55	$3.01\cdot10^{-5}$	0.150	$3.07\cdot10^{-4}$	10.0	0.13	0.5	0.51	0.48	53.1
B-H	$3.02\cdot10^{-5}$		20.6 ± 3.5	0.093	0.45	$8.95\cdot10^{-6}$	0.492	$7.76\cdot10^{-5}$	21.0	0.27	24.25	0.46	0.48	69.7
H1-V	$2.99\cdot10^{-5}$	$4.41\ 10^{-5}$	12.0 ± 3.6	0.075	0.41	$1.35\cdot10^{-5}$	0.367	$1.13\cdot10^{-4}$	17.0	0.1	0.07	0.40	0.52	63.2
H1-H	$5.82\cdot10^{-5}$		14.4 ± 2.8	0.075	0.45	$2.26\cdot10^{-5}$	0.450	$3.79\cdot10^{-4}$	10.0	0.23	30	0.45	0.52	65.1
H2-V	$1.70\cdot10^{-4}$	$1.05\ 10^{-4}$	20.8 ± 5.2	0.077	0.55	$4.91\cdot10^{-5}$	0.440	$5.98\cdot10^{-4}$	22.0	0.1	0.1	0.52	0.52	77.5
H2-H**	$3.99\cdot10^{-5}$		18.0 ± 3.5	0.077	0.49	$1.18\cdot10^{-5}$	0.430	$3.0\cdot10^{-4}$	10.0	0.03	25.84	0.48	0.52	73.3
Br-V	$6.90\cdot10^{-6}$	$7.38\ 10^{-6}$	2.2 ± 2.0	0.092	0.40	$1.08\cdot10^{-6}$	0.300	$9.80\cdot10^{-5}$	6.0	0.1	0.47	0.39	0.44	85.3
Br-H	$7.86\cdot10^{-6}$		0.7 ± 1.1	0.092	0.40	$1.08\cdot10^{-6}$	0.300	$9.80\cdot10^{-5}$	7.0	0.1	0.47	0.39	0.44	87.2

[a] Values obtained from the experimental data.
[b] Values obtained from the inverse modelling of the bromide breakthrough curves.
* Mean K_{sat} is the mean saturated hydraulic conductivity for a given horizon.
** The values of the hydrodynamic parameters obtained from inverse modelling result from adjustments of a dual permeability model except for the column H2-H for which a dual porosity + mobile-immobile model was used. θr_F always equals 0. For H2-H the saturated water content of immobile compartment: $\theta s_{IM} = 0.35$, the Ks_s and Ks_f are the K_{sat} values in the slow and fast porous compartments and the K_{sat} of the immobile compartment is null.

Figure 8.1.2.1-19 depicts the succession of bromide concentrations measured at the column outlets during displacement experiments. The results are expressed as the ratio of outlet to inlet concentrations. These concentration evolutions could all be satisfactorily simulated using a dual-permeability model except the H2-H for which a dual-porosity model with a mobile-immobile conceptualization of the matrix compartment was needed. For each set of parameters allowing a good reconstitution of the water flow and the bromide leaching pattern (Table 8.1.2.1-62), the estimated ω were statistically equivalent to the one obtained with dye tracing (linear correlation: slope = 0.97, intercept = 0, $R^2 = 0.92$, p-value = $5\cdot10^{-5}$). This highlights the reliability of the simulated parameters, despite the equifinality issue inherent to the large number of fitted parameters.

For all displacement experiments, quantifiable bromide concentrations were measured in the first 50 ml of leachates, which were collected between 15 s and 7 min after injection began. This suggests that preferential flow occurred in all of the columns (e.g., Paradelo *et al.*, 2016), which is consistent with the observation of connected macroporosity in the columns. Additionally, two major shapes of breakthrough curves can be distinguished.

In the columns of horizons B and H, the curve features a gentle increase and decrease in concentration during the injection and rinsing phases, respectively, as well as a maximum concentration that is less than the injected concentration. If it is assumed that macropore flow occurred almost instantaneously at a concentration close to the injected concentration, it follows that, throughout the displacement experiment in these columns, matrix flow was a significant contributor to outflow, as bromide concentration remained below the injected concentration. Although the volume of the injected bromide solution was chosen to be larger than the overall pore volume of the columns, this volume was likely not sufficient to ensure a renewal of matrix pore water. This hypothesis is confirmed by the modelling results indicating that even if preferential flow contributed up to 77 % to the global outflow for this group of columns, the hydraulic conductivity of the fracture never exceeded 25 time that of the matrix (Table 8.1.2.1-62).

The other breakthrough curve shape is observed in the columns of the Br horizon and exhibits a sharp increase and early plateau in bromide concentrations during the injection phase, in which the plateau concentration is close to the injected concentration value. Additionally, a sharp decrease in concentration is observed during the rinsing phase. This pattern is not consistent with the small observed macroporosity of the Br horizon but can be explained by the very poor permeability of the soil matrix. In this case, most of the flow bypasses the soil matrix and flows through a few connected macropores. This hypothesis is confirmed by the modelling results indicating that preferential flow contributed to >85 % to the global outflow for this group of columns and that the hydraulic conductivity of the fracture was >90 time higher than that of the matrix (Table 8.1.2.1-62).

In accordance with recent studies relating soil macroporosity and hydraulic conductivity in structured soils, K_{sat} generally rises along with an ω increase (Figure 8.1.2.1-21). As ω is related to the root channels

density, which decreases with the distance from the ditch surface, the saturated hydraulic conductivity is overall greater in the upper horizons of the ditch bed than in the banks. The contribution of preferential flow to the global outflow, is however greater in the deep bed and bank horizon. This can be explained by the contrasted hydraulic conductivity of the macroporal compartment relative to that of the matrix compartment (Ks_f/Ks_s) and by ω (R^2= 0.95, p-value = $6 \cdot 10^{-4}$).

In sum, it is mainly in their upper horizons that the bank and ditch bed profiles differ in their patterns of water and solute transport. The top horizons of the ditch bed exhibit larger transport properties due to their larger active macroporosities, which are related to their denser rooting patterns. Thus, in contrast with the soil profile from which it originates, the ditch bed profile exhibits larger infiltration and percolation capacities. However, the deeper percolation of water and solutes is limited in both profiles by their common bottom Br horizon, which exhibits low permeability. The differences in the flow patterns may induce significant contrasts in the transfer and retention of herbicides. Indeed, water pathways determine the material surface area that is in contact with the soil solution and its effective contact time during downward seepage. This conditions the herbicide sorption equilibria.

Pesticide sorption heterogeneities among the ditch bed and bank soil profiles

The heterogeneities in herbicide sorption properties among the horizons are presented in

Table 8.1.2.1-63. The H1 and H2 horizons exhibit the greatest diuron adsorption capacities and lowest desorption capacities, whereas adsorption on B and Br is low and very easily reversible. Therefore, the sorption capacities of the ditch bed profile are larger than those of the bank profile. For glyphosate, the adsorption coefficient of the B horizon is higher than that of the H1 horizon but is lower than that of the H2 horizon, and the desorption hysteresis of the B horizon is smaller and larger than those of the H1 and H2 horizons, respectively. The Br horizon exhibits a lower adsorption coefficient than the B horizon, but they both exhibit a similar desorption hysteresis. Based on the properties of these horizons, it remains unclear whether the ditch bed profile or the bank profile has the greater retention capacity.

Table 8.1.2.1-63: Sorption coefficients of the herbicides on ditch soils

Molecule	Horizon	Kf_{ads}	n_{ads}	Kd_{ads}	Kf_{des}	n_{des}	H
		$\mu g^{(1-n)} l^n kg^{-1}$	–	$l\,kg^{-1}$	$\mu g^{(1-n)} l^n kg^{-1}$	–	–
Diuron	B	4.49	0.84	1.86	110.58	0.12	0.98
	H1	14.79	0.83	5.17	114.66	0.32	0.39
	H2	10.57	0.82	3.52	118.74	0.25	0.31
	Br	4.12	0.81	1.49	114.31	0.09	0.97
Glyphosate	B	157.66	0.94	109.90	666.99	0.28	0.29
	H1	77.60	0.93	51.69	675.64	0.14	0.15
	H2	165.24	0.96	129.86	533.29	0.36	0.37
	Br	124.36	0.91	75.38	505.00	0.29	0.32

In summary, the heterogeneities in the sorption coefficients of the two studied pesticide within a given profile are more substantial under the ditch bed than in the banks. Generally, due to the enrichment in organic matter of the ditch bed horizons, the sorption capacities of hydrophobic molecules in the ditch bed profile should be greater than those in the bank profile. Concerning ionisable compounds with low hydrophobicity, a higher sorption capacity of ditch bed profiles is not straightforward. The desorption hysteresis are generally significant in the ditch bed and are weaker or null in the bank soils. This should lower the release of pesticides previously adsorbed in the ditch bed soils as compared to the bank soils.

Conclusion

This study provides the first description of the range of soil properties influencing the magnitude of the water and pesticide exchanges occurring between surface water and groundwater along a ditch cross section profile. These ditch bed soil properties were also for the first time compared with those of the surrounding field soils. The in-situ and laboratory characterization of the physico-chemical properties evidenced distinct soil profiles between both the ditch bed and banks profiles. The ditch bank profile was equivalent to the surrounding field profile. In particular, the ditch bed upper horizons contain up to

2 times more organic carbon than the bank soils. These upper ditch bed horizons being also located closer to the ditch surface than the bank soils, they contain a denser network of plant roots which increases their active macroporosity and in turn their hydraulic conductivity. The deeper horizons share however, great similarities in both profiles, in particular their poor macroporosity, hydraulic conductivity and organic carbon content.

In conclusion, the physicochemical and sorptive properties of specific ditch bed horizons contrast with those of the ditch banks and neighbouring field soils. These differences may thus have different effects on the risk of groundwater contamination by pesticides. On one hand, ditch beds exhibit higher organic matter contents than field soils, possibly limiting the percolation of hydrophobic pesticides due to increased retention in the soil matrix. On the other hand, the upper horizons of ditch beds present larger active macroporosity and transport property values, which favour percolation. The final balance between the two effects, in terms of overall groundwater contamination risk, depends on the local hydrological conditions.

Assessment and conclusion by applicant:

The article reports the properties of a soil from a ditch in an agricultural area in the south of France. Mainly, the hydraulic parameters of the different soil layers of the ditch and the surrounding banks are considered and modelled and tracer experiments with bromide are presented.

Sorption experiments with glyphosate were conducted and Freundlich sorption coefficients for the different soil horizons including topsoil and subsoil are reported. However, there was no detailed reporting of data to assess the validity (i.e. mass balances, chemical properties of test substance, solvents used, information about analytical methods and their validation including, LOD, LOQ, temperature, test concentrations, demonstration of stability of the test item).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

Agrees with the applicant that the needed information to assess the validity of the study against OECD 106 criteria are not reported. However it is noted that the article indicates that "the detailed procedure for the determination of herbicide desorption parameters can be found in Dollinger et al. (2016)." Dollinger et al. (2016) seem to be the sorption study, the aim of Dollinger *et al.* (2018) reported here being mainly to assess the aspect of ditch properties and the impact of these characteristics on the behaviour of pesticides. Reported $Kfoc_{,ads}$ range 4911-17035 with 1/n of 0.91-0.96.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment. However, a data gap is set for the applicant to provide the Dollinger *et al.* (2016) study.

Skeff et al, 2018

Data point:	CA 7.1.3.1.1/016
Report author	Skeff, W. et al.
Report year	2018
Report title	Adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid, and 2-aminoethylphosphonic acid on three typical Baltic Sea sediments
Report No	DOI 10.1016/j marchem.2017.11.008 E-ISSN-1872-7581
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

A batch experiment was conducted to study the adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid (AMPA), and 2-aminoethylphosphonic acid (2-AEP) in marine sediments (mud, silt, and sand) from the Baltic Sea. The experiment took into account the influence of pH, salinity, and temperature on the adsorption behaviors of the studied compounds. In contrast to glufosinate, glyphosate exhibited an adsorption affinity for the three types of sediments. AMPA and 2-AEP showed similar adsorption behaviors on mud and silt, while their adsorption on sand was negligible. The equilibrium adsorption data for glyphosate, AMPA, and 2-AEP on mud and silt fit well with the linear partitioning and Freundlich isotherms, whereas the data for glyphosate on sand could only be fitted with the Freundlich isotherm. The Freundlich distribution coefficients (kf) were in the range of 6.1-259.5 L/kg for glyphosate, 9.2-39.5 L/kg for AMPA, and 7.7–38.5 L/kg for 2-AEP under the experimental conditions of pH 8.1, temperature = 21°C, and a salt concentration of 8 g/L. The adsorption kinetic was better described by the pseudo-second-order than the pseudo-first-order model, suggesting chemisorption as the adsorption mechanism. The order of adsorption of the compounds on the sediments was: glyphosate >AMPA ≥2-AEP >glufosinate. The adsorption capacity of sediments followed the sequence: mud >silt >sand. Increasing the pH, salinity, or temperature of the solution significantly reduced the adsorption capacity of the compounds. The data obtained in this study provide valuable information on the fate and distribution of the investigated phosphonates in the Baltic Sea.

Materials and methods

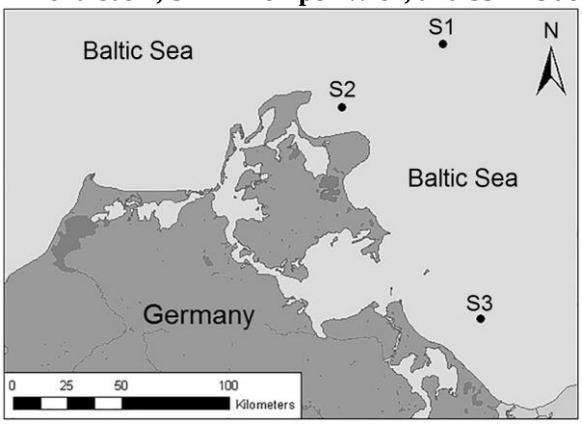
Chemicals and reagents

Standards of glyphosate, a glyphosate internal standard (1-2-13C215N glyphosate), AMPA, an AMPA internal standard (13C 15N AMPA), and glufosinate were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 2-AEP was supplied by Sigma-Aldrich (Taufkirchen, Germany). Stock solutions of these compounds, except the internal standards, were prepared in polypropylene volumetric flasks at a concentration of 100 mg/L by dissolving 5 mg of each compound in 50 mL of LC-MS grade water (VWR International GmbH, Darmstadt, Germany). The stock solutions were stored at 5°C in the dark. A stock solution (66.6 mM) of 9-fluorenylmethyl chloroformate (FMOC-Cl) (purity 99.0 %, Sigma-Aldrich) was prepared by dissolving 1 g in 58 mL of acetonitrile (Walter-CMP GmbH, Kiel, Germany). Borate buffer at pH 9 was prepared by dissolving 1 g of sodium tetraborate decahydrate (Sigma-Aldrich) in 50 mL of Milli-Q water (Merck KGaA, Darmstadt, Germany). Artificial sea salt, contains all 70 trace elements found in natural seawater in the exact proportions found in nature, was purchased from Tropic Marin®, Germany. Chloroform was supplied by VWR AnalaR Normapure (Germany).

Sediment collection and characterization

Three types of sediment typical of the Baltic Sea were collected from the German Baltic Sea (Figure 8.1.2.1-22) which are: S1 from Arkona basin (54° 50′ N, 13° 30′ E), S2 from Tromper Wiek (54° 39′ N, 13° 35′ E), and S3 from Oder Bank (54° 04′ N, 14° 03′ E). The sediments were collected using a multiple corer during research cruise EMB76, in June 2014. Samples of the uppermost sediment were sealed in glass jars, stored at -20°C until dry. No sieving was done but large items such as stones, leaves, grass and animals were removed and the samples were manually homogenized. The bulk sediments were freeze-dried using a Chaist ALPHA 1-4 LD freezer dryer and used as sorbents in this study. The grain sizes of the sediments were determined using a CILAS 1180 particle size analyzer. The TOC content of the sediments was analyzed with an elemental analyzer according to (Leipe et al., 2011). The major and trace elements in the sediments were measured using inductively coupled plasma optical emission spectrometry after acid total digested of the samples. The sediment grain sizes were distributed among the different classes: clay (<2 µm), silt (2-63 µm), and sand (>63 µm). The sediment S1 contained 6.6 % clay, 92.3 % silt, and 1.1 % sand, with a median grain size 20.1 µm. The sediment S2 contained 3.6 % clay, 69.9 % silt, and 26.5 % sand, with a median grain size 41.2 µm. The sediment S3 contained 1.7 % clay, 10.7 % silt, and 87.6 % sand, with a median grain size 156.8 µm. The sediment S1, with organic-rich silt-size sediments, was classified as mud, while the sediment S2 as silt and the sediment S3 as fine sand. The sediment TOC, total phosphorus, and major and trace elements followed the order: mud >silt >fine sand.

Figure 8.1.2.1-22: Location of the sampling stations in the German Baltic Sea. The station S1 is in Arkona basin, S2 in Tromper Wiek, and S3 in Oder Bank



Batch sorption experiment

To investigate the possible adsorption of the analytes onto the walls of the centrifuge tubes, the hydrolysis and degradation of the test compounds during the experiment, a control set of tubes was established in which sediment-free artificial seawater samples were spiked with 250 µg of the analytes/L for 48 h. An additional set of tubes containing sediments with unspiked artificial seawater controlled for possible desorption and the contamination of the sediments and media with the target compounds.

To initiate the experiment, artificial seawater was prepared at a salt concentration of 8 g/L. The pH of the solution was 8.1, measured using a conductivity meter (WTW Inolab cond® 720, Germany). Chloroform (0.1 %) was added to the media to inhibit microbial activity. 1 g dry weight of each sediment type was distributed in 15-mL polypropylene centrifuge tubes and mixed with 10 mL of artificial seawater. The tubes were mechanically shaken and incubated for at least 2 days, after which the samples were centrifuged (Megafuge 1.0, Heraeus Instruments) for 3 min at 2500 rpm. Then, 8 mL of each supernatant was transferred to a sediment-free polypropylene centrifuge tube. The samples were then spiked with the target compounds, well shaken at 300 rpm using a mechanical shaker, and 200 µL were then drawn and analyzed for their initial concentrations of the compounds. Thereafter, the spiked medium was returned to the respective sediment tube, which was then vigorously shaken. This process was conducted in order (i) to avoid any possible adsorption of the compounds onto the sediments at the start of the experiment (T = 0 h) and (ii) to allow the analysis of the phosphonates in same sample matrices during the experimental time, whereas a variety of sample matrices might lead to analytical errors. The experiment was conducted for 48 h at room temperature (21°C), with samples from the aqueous phase taken for analysis at 0, 1, 3, 5, 7, 24, and 48 h. The phosphonates were tested at the following concentrations: 120, 300, 600, 900, and 1200 µg/L. All experiments were performed in duplicate and each sample was measured in triplicate. The target compounds were measured in the aqueous phase. The amounts adsorbed onto the sediments (qt, µg/g) at time t were calculated according to Eq. (1):

$$q_t = (c_0 - c_t)v/m \qquad (1)$$

where c0 is the initial concentration (µg/L), ct is the concentration at time t (µg/L), v is the volume of the solution (L), and m is the dry mass of the sediment (g).

Analysis of organophosphonates

A volume of 200 µL of each supernatant was transferred to 2-mL reaction tubes (Eppendorf, Germany) and diluted to 700 µL using LC-MS grade water. The samples were then treated with 100 µL of the glyphosate and AMPA internal standard solutions, prepared in the same matrix, to obtain a final concentration of 15 µg/L. To derivatize the samples, the pH was adjusted to 9 using 100 µL of 0.07 M borate buffer, after which 100 µL of 33.3 mM FMOC-Cl in acetonitrile was added. The samples were shake-incubated at room temperature for 4 h to allow complete derivatization, filtered through a 45-µm Phenex-RC 15-mm syringe filter (Phenomenex, Germany), and analyzed by LC-MS/MS according to a

previously described method (Skeff et al., 2015, 2016). Glyphosate was quantified using the glyphosate internal standard, and AMPA, glufosinate, and 2-AEP using the AMPA internal standard.

Statistical analysis

All adsorption experiments were conducted in duplicate, and the measurements in triplicate. The adsorption study measured the initial and equilibrium concentrations of the target compounds. A p-value <0.05 was considered to indicate statistical significance. A one-way ANOVA followed by a Holm-Sidak post-hoc test was carried out using SigmaPlot software (version 13.0, Systat Software Inc.).

Results

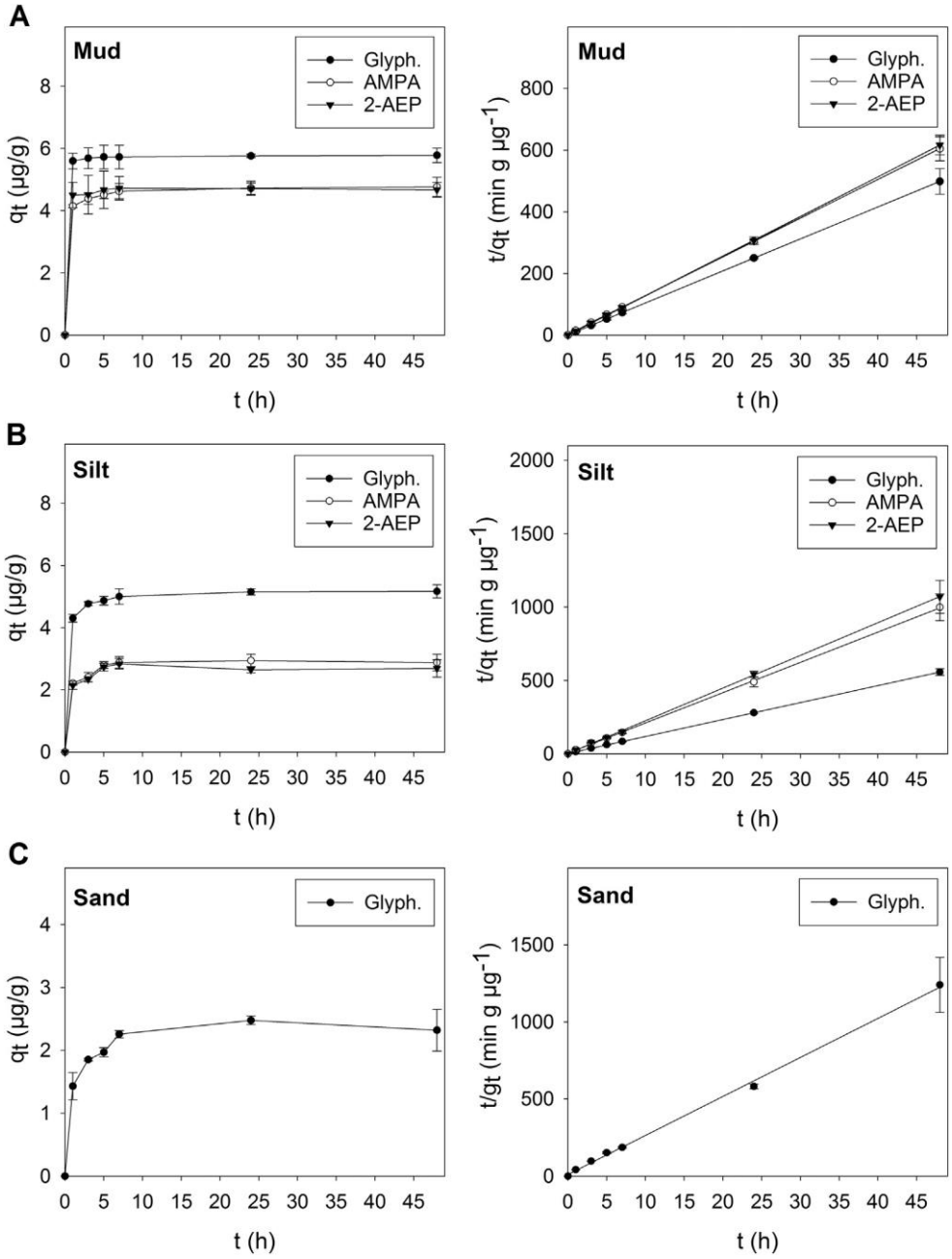
Control experiments

A successful adsorption investigation requires the proper controls to rule out both contamination of the aqueous phase or adsorbents with the sorbates and the loss of the sorbates due either to their degradation during the experiment or their adsorption onto the tubes. Controls for both possibilities were therefore established. Data from the first control experiment, in which the compounds were incubated in artificial seawater without sediments, showed a high degree of measurement stability and thus high biological stability of the sorbates during the 48 h and negligible adsorption onto the tubes as well. Furthermore, the stable measurements indicate that the C-P bonds in the organophosphonates are relatively stable and no hydrolysis occurs. Data from the second control experiment, in which the sediments were incubated without sorbates, failed to show the compounds in the aqueous phase and thus confirmed the lack of contamination or desorption. The results of both control experiments demonstrated the validity of the adsorption study.

Kinetic studies and models

The mechanism of glyphosate, glufosinate, AMPA, and 2-AEP absorption onto marine sediments was examined in kinetic studies. The qt (µg/g) values of glyphosate, AMPA, and 2-AEP between 0 and 48 h are shown in Figure 8.1.2.1-23. Whereas glyphosate had an affinity for all three types of sediments, AMPA and 2-AEP adsorbed to mud and silt but not sand. Glufosinate concentrations measured in the aqueous phase remained comparable during the 48 h of the experiment, indicating the lack of significant adsorption (p >0.05) onto the sediments. The presence of a methyl group on the phosphonate of glufosinate might obstruct the formation of surface complexes, thus limiting its adsorption compared to glyphosate.

Figure 8.1.2.1-23: Adsorption equilibrium time (left) of glyphosate, AMPA, and 2-AEP on A. mud, B. silt, and C. sand, and the respective pseudo-second order kinetics (right). The figures in the left column are based on a concentration of 600 µg of each compound/L



As shown in Figure 8.1.2.1-23, glyphosate, AMPA, and 2-AEP followed similar adsorption kinetics in the mud and silt sediments, and on sand for glyphosate. The adsorption kinetics consisted of two distinct stages: a fast adsorption process in the first hour followed by slow adsorption. The adsorption equilibrium of glyphosate, AMPA, and 2-AEP was reached in 24 h. The amount adsorbed of glyphosate was higher than those of AMPA and 2-AEP for the three types of sediment. The adsorption rate of glyphosate was the highest on mud, followed by silt and sand (96.3 %, 86.2 %, and 38.6 %, respectively). The adsorption rates of AMPA and 2-AEP on mud and silt were similar (~80 % and ~50 %, respectively). The latter observation can be explained that AMPA and 2-AEP own the same functional groups (i.e. each contains phosphonate and amino group), resulting in their similar interactions with sediments.

Lagergren pseudo-first-order and pseudo-second-order models were employed as kinetic models to investigate the rate-controlling steps involved in the adsorption of glyphosate, AMPA, and 2-AEP onto sediments. The linearized Lagergren pseudo-first-order [Eq. (2)] and pseudo-second-order [Eq. (3)] equations are as follows:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (2)$$

$$t/q_t = 1/k_2\, q_e^2 + t/q_e \quad (3)$$

where q_e and q_t are the amount of phosphonates (µg/g) adsorbed onto the marine sediments at equilibrium and time *t* (min), respectively, and k_1 (min^{-1}) and k_2 (g/µg min) are the equilibrium rate constants of the pseudo-first-order and pseudo-second-order models, respectively. The best-fit model was selected based on the values of the linear regression correlation coefficient (R^2). The pseudo-second-order kinetic model efficiently predicted the kinetic behavior of the three compounds on sediments, based on the high R^2 values (0.9982-0.9999), whereas a poor fit of the data was obtained with the pseudo-first-order kinetic model (R^2 <0.85). The rate constant k_2, the q_e values, and the corresponding linear regression correlation coefficient (R^2) were calculated from the linear plots of t/q_t vs. *t* (Figure 8.1.2.1-23) and are shown in Table 8.1.2.1-64. The good fit obtained with the pseudo-second-order model suggested chemisorption as the rate-limiting step, presumably between the functional groups of the compounds (i.e., the phosphonate, carboxylate, and amino groups) and the sediment surfaces through the sharing or exchange of electrons. As can be seen from Table 8.1.2.1-64, the calculated adsorption capacity values ($q_{e\ cal}$) from the second order model are well comparable to the experimental adsorption capacity values ($q_{e\ exp}$). Thus, the adsorption kinetics of the three phosphonates on the sediments is more precisely described by the mechanism of surface site-sorbates reaction of pseudo-second-order model. The adsorption capacity of the three compounds followed the sequence glyphosate >AMPA ≥2-AEP.

Table 8.1.2.1-64: Pseudo-second order kinetic parameters for the adsorption of glyphosate, aminomethylphosphonic acid (AMPA), and 2-aminoethylphosphonic acid (2-AEP) onto Baltic Sea mud, silt, and fine-sand sediments under the experimental condition of 600 µg/L initial concentrations, 8 g salt/L, pH = 8.1, temperature = 21 °C

Sediment	Pseudo-second-order				
	Equation	R^2	$q_{e.\ exp}$ (µg/g)	$q_{e.\ cal}$ (µg/g)	K_2 (g/ µg min)
Mud					
Glyphosate	Y = 0.1730X + 0.4567	0.9999	5.7804	5.7789	0.0655
AMPA	Y = 0.2093X + 2.4457	0.9999	4.7778	4.7633	0.0179
2-AEP	Y = 0.2146X + 1.4743	0.9998	4.6772	4.6705	0.0312
Silt					
Glyphosate	Y = 0.1879X + 2.3688	0.9999	4.6998	5.1685	0.0157
AMPA	Y = 0.3371X + 3.6885	0.9997	2.9070	2.9318	0.0308
2-AEP	Y = 0.5931X + 6.7319	0,9997	2.6925	1.6432	0.0522
Sand					
Glyphosate	Y = 0.4218X + 10.449	0.9982	2.3708	2.8043	0.0170
AMPA	NA	NA	NA	NA	NA
2-AEP	NA	NA	NA	NA	NA

NA: not applicable.

Adsorption isotherms

Linear partitioning and Freundlich models are common adsorption isotherms that were applied in this study to describe the adsorption equilibrium of glyphosate, AMPA, and 2-AEP on marine sediments. Linear partitioning is described by Eq. (4) and the linear formula of the Freundlich isotherm is shown in Eq. (5):

$$q_e = k_d\, c_e \quad (4)$$

$$\log q_e = \log k_f + 1/n \log c_e \quad (5)$$

where c_e is the concentration (µg/L) in the aqueous phase at equilibrium, and k_d (L/g) the distribution coefficient for the sediment/solution ratio (1/10). The k_d values (Table 8.1.2.1-65) were obtained from the slope of the linear plots of q_e (µg/g) vs. c_e (µg/L) (Figure 8.1.2.1-24). k_f (µg/g) is the Freundlich constant (i.e. sorption capacity), and 1/n an empirical parameter related to the intensity of adsorption. The values for k_f and 1/n (Table 8.1.2.1-65) were determined from the intercept and slope of the plots log q_e vs. log c_e (Figure 8.1.2.1-24). As shown in Figure 8.1.2.1-24, both isotherms described the equilibrium adsorption of the three phosphonates on mud and silt. The Freundlich model had a slightly better fit than the linear partitioning model based on the higher R^2 values (0.96 and 0.99), which suggests that the adsorption takes place on heterogeneous surfaces. It is important to point out that the concentrations of the compounds tested in this study of marine sediments were lower than those typically used in soil adsorption studies, as they were considered representative of conditions in the marine ecosystem. Thus, fitting of the data to both models might be a result of the narrow concentration range (120-1200 µg/L) tested in this study.

The k_d values obtained from linear partitioning were in the range of 55.2 to -259.5 L/kg for glyphosate, 10.0-39.5 L/kg for AMPA, and 7.7-38.5 L/kg for 2-AEP. Data on glyphosate adsorption in the sandy sediment could only be fitted with the Freundlich model (R^2 =0.99), not with linear partitioning (R^2 = 0.607), which suggested adsorption saturation by the sand as the glyphosate concentration increased. The AMPA and 2-AEP concentrations measured in the aqueous phase were relatively stable, indicative of their difficult adsorption onto sand.

In the Freundlich isotherm, higher k_f values represent a larger adsorption capacity. The calculated k_f values for glyphosate, AMPA, and 2-AEP were in the range 129.8-397.7 L/kg, 25.3-73.5 L/kg, and 19.9-70.1 L/kg, respectively. The k_d and k_f values obtained in this study clearly demonstrated the higher adsorption capacity of glyphosate than of the other studied compounds. The parameter 1/n represents the linearity of the relationship between C_{aq} and $C_{sediment}$, with a lower 1/n value indicating a less homogeneous distribution of the adsorption site energy on the sediments. For all of the tested compounds, the 1/n values were <1: 0.527-0.917 for glyphosate, 0.784-0.899 for AMPA, and 0.779-0.882 for 2-AEP. The higher 1/n value of glyphosate implied that the variability of the sediment adsorption sites had a smaller effect on its adsorption than was the case for AMPA or 2-AEP. The 1/n values for the three compounds decreased according to the sequence mud >silt >sand, reflecting the increasingly difficult (i.e., concentration-dependent) adsorption process.

The influence of sediment organic carbon content on the adsorption of glyphosate, AMPA, and 2-AEP was determined by examining their correlations. The sediment organic carbon normalized distribution coefficient (k_{oc}) was calculated from the Freundlich isotherm using Eq. (6). The results are provided in Table 8.1.2.1-65:

$$K_{oc} = (k_f \times 100)/TOC\% \quad (6)$$

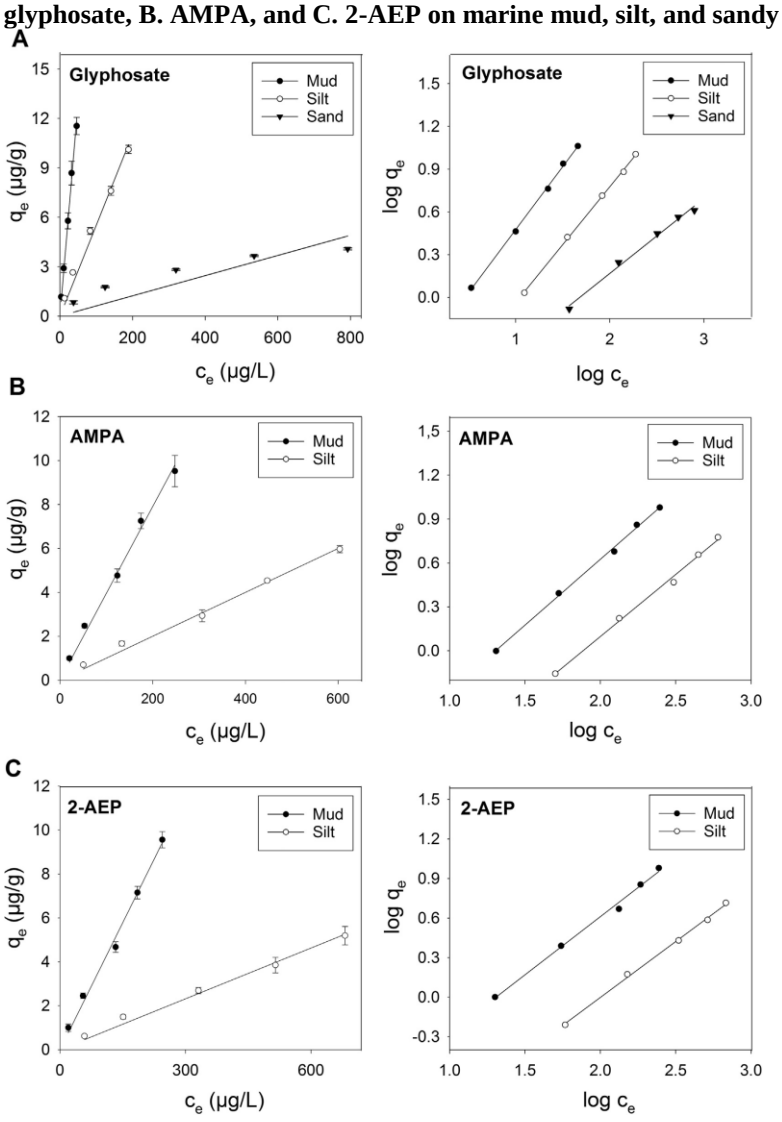
The koc values of glyphosate were in the range of 5706-86,540 L/kg, but were higher in the sandy sediment, which had the lowest TOC content (0.15 %). This result demonstrated that sediment organic carbon content is not a determining factor in glyphosate adsorption. For AMPA and 2-AEP, the koc values decreased with the decreasing TOC content, which suggested that the adsorption of both compounds was more sensitive to the organic carbon content of the sediments than glyphosate. The soil mineral composition, which includes aluminium and iron oxides, is a major factor governing glyphosate and AMPA adsorption. In this study, a positive correlation was also determined between the aluminium and iron contents of the sediments and the adsorption of glyphosate, AMPA, and 2-AEP.

Table 8.1.2.1-65: Parameters obtained from the linear partitioning and Freundlich adsorption isotherms of glyphosate, AMPA, and 2-AEP on mud, silt, and sandy sand sediments under the experimental condition of 8 g salt/L, pH =8.1, temperature = 21 °C

Sediment	Linear partitioning model		Freundlich model			
	K_d (L/kg)	R^2	K_f (L/kg)	K_{oc} (L/kg)	1/n	R^2
Mud						
Glyphosate	259.5	0.994	397.7	7152.9	0.917	0.999
AMPA	39.5	0.992	73.5	1321.9	0.889	0.998
2-AEP	38.5	0.992	70.1	1259.0	0.882	0.994
Silt						
Glyphosate	55.2	0.990	141.5	5706.1	0.849	0.999
AMPA	9.2	0.988	25.3	1019.4	0.784	0.996
2-AEP	7.7	0.987	19.9	802.4	0.779	0.998
Sand						
Glyphosate	6.1	0.607	129.8	86,540.0	0.527	0.992
AMPA	NA	NA	NA	NA	NA	NA
2-AEP	NA	NA	NA	NA	NA	NA

NA: not applicable.

Figure 8.1.2.1-24: Linear partitioning isotherm (left) and Freundlich isotherm (right) of A. glyphosate, B. AMPA, and C. 2-AEP on marine mud, silt, and sandy sediments



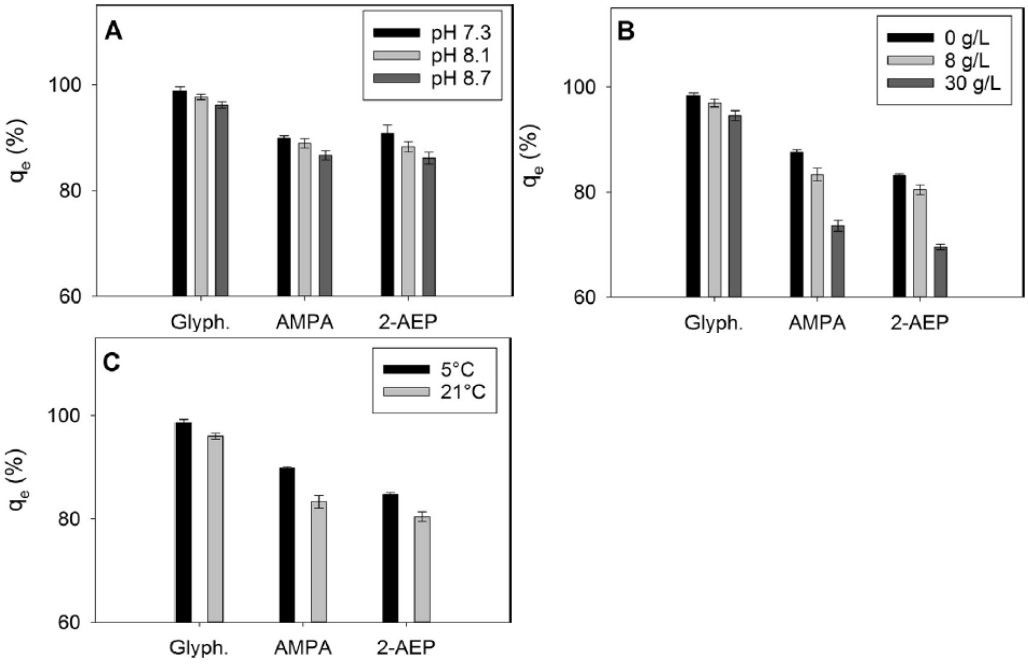
Effect of environmental factors

The impact of environmental factors, including the pH, salinity, and temperature of the medium, on the adsorption behaviors of glyphosate, AMPA, and 2-AEP was investigated in the mud sediment. The choice of this sediment is due to its greater adsorption capacity for the compounds than silt and sandy sediments. The initial concentrations of the three compounds in the salinity and temperature tests was 300 µg/L, and in the pH test 120 µg/L.

Effect of the medium pH

To elucidate the effect of the pH of the medium on phosphonate adsorption onto the mud sediment, artificial seawater (a salt concentration of 8 g/L, 0.1 % CHCl3) was adjusted to three different pH values (7.3, 8.1, 8.7) reflecting the variability of the pH of Baltic Sea water. The pH was adjusted using concentrated HCl and NaOH. The mud sediment samples were incubated for 48 h in the corresponding medium and then spiked with the test compounds. The experiment was then conducted as described for the standard experiment at 21°C. As seen in Figure 8.1.2.1-25A, adsorption of the three compounds increased significantly ($p < 0.05$) as the pH decreased from 8.7 to 7.3. The results suggested the similar effects of a change in seawater pH on 2-AEP and AMPA. As the pH of the medium increases, the positive surface charge of the sediment decreases and may become negative. Thus, in this study, the decreased adsorption may have been due to the reduced coordination between the phosphonate group of the compounds and the surface of the sediments. In addition, a higher pH may enhance the release of native organic matter from the sediment into solution, thereby reducing the sediment adsorption capacity of the target compounds.

Figure 8.1.2.1-25: The influence of A. pH, B. salinity, and C. temperature on the adsorption of glyphosate, AMPA, and 2-AEP onto mud sediment. The data are based on duplicate experiments, each consisting of triplicate measurements. The initial concentrations of the compounds were 120 µg/L in the pH experiment and 300 µg/L in the salinity and temperature experiments



Effect of solution salinity

Salinity (ionic strength) may have an important influence on the adsorption behavior of amphoteric compounds, including glyphosate, AMPA, and 2-AEP, in seawater-sediment systems. To investigate its effect, media containing three different salt concentrations (0, 8, 30 g/L) were prepared. The salt-free medium (0 g/L) consisted of LC-MS grade water, presumably free of salt. The experiment was run at pH 8.1 and 21°C. The results revealed the negative correlation between the adsorption of the compounds and the salinity of the medium (Figure 8.1.2.1-25B). The adsorption capacity increased significantly (p

<0.05) as the salt concentration decreased from 30 g/L to 0 g/L, an effect attributable to ion exchange. At pH 8.1, glyphosate and AMPA carry negative charges related to the phosphonate (both molecules) and carboxylate (glyphosate) groups and positive charges related to the amino group (both compounds). Most sediment surfaces carry a net negative charge but at pH 8.1 positive charges in sediment organic matter might be exposed. Therefore, changing the ionic composition of the medium may influence the adsorption process, by promoting competition for ion-exchangeable sites. Alternatively, complexes between the phosphonates and cations such as Ca2+ and Mg2+, present in the medium, may form that have a lower adsorption affinity for the sediments than do free compounds, such that their adsorption decreases with increasing salinity. According to the results, the various salt concentrations had similar effects on the adsorption behaviors of 2-AEP and AMPA, perhaps because they have the same functional groups.

Effect of temperature

To examine the influence of temperature on the adsorption behaviors of glyphosate, AMPA, and 2-AEP, two different temperatures (5°C and 21°C) were tested. As shown in Figure 8.1.2.1-25C, the amount of adsorbed compounds increased significantly ($p < 0.05$) as the temperature decreased, indicating that adsorption was an exothermic process. The amount of adsorbed glyphosate, AMPA, and 2-AEP increased differentially as the temperature decreased; at rates of 1.5 %, 6.5 %, and 4.3 %, respectively. This may have been due to the different effects of temperature on the water solubility of the compounds. In general, the solubility of chemical substances improves as the temperature rises, such that the amounts entering the solid phase will be lower when equilibrium is reached. Moreover, an increase in the temperature of the medium could increase the solubility of organic matter in sediments, thus increasing the competition with phosphonates for sediment adsorption.

Environmental implications

Mud sediments can act as a sink for glyphosate as well as for AMPA and 2-AEP, based on the high adsorption affinities of these compounds (>96 % and >78 %, respectively). In silt, the three compounds were distributed between the water and adsorption to the sediment, with a higher tendency of the latter. This result clearly supports the need for bioavailability and toxicity studies of benthic as well as pelagic organisms. Sandy sediments had a weak adsorption capacity for glyphosate, and a negligible adsorption capacity for glufosinate, AMPA, and 2-AEP. Therefore, these compounds can be easily moved from Baltic Sea regions characterized by sandy sediments to those with mud or silt sediments. The pH, salinity, and temperature data demonstrated that the variability of these parameters significantly influences the adsorption behaviors of glyphosate, AMPA, and 2-AEP. A decrease in either the seawater pH or the temperature enhanced the adsorption of these compounds onto marine sediments. Thus, their mobility is a more important factor in warmer than in colder marine systems. Furthermore, the warming effect induced by climate change may influence the fate of phosphonates in the marine environment. The negative correlation between salinity and adsorption suggested the greater mobility of these compounds in marine than in freshwater systems. In the Baltic Sea, salinity varies greatly from south to north, and from east to west, increasing from 2 to 4 in the northern area up to 20-30 in the southwestern area of the sea. Thus, the distribution of glyphosate, AMPA, and 2-AEP in Baltic Sea water and sediments is most likely spatially dependent. These results provide basic information about the fate of these phosphonates in the Baltic Sea and highlight the importance of monitoring these compounds in marine water and sediments, especially in semi-closed seas such as the Baltic Sea, where contaminants may cause acute effects.

Conclusion

In this work, the adsorption of glyphosate, glufosinate, AMPA, and 2-AEP onto mud, silt, and sandy sediments of the Baltic Sea was investigated. Glufosinate had no adsorption affinity for any of the sediments tested. Data on the adsorption kinetics of the other compounds could be well fitted with a second-order rate model. The adsorption rate followed the order glyphosate >AMPA ≥2-AEP >glufosinate. Linear partitioning and Freundlich isotherms described the adsorption of glyphosate,

AMPA, and 2-AEP on mud and silt. However, only glyphosate showed important adsorption onto the sandy sediment and its behavior could be well modeled with the Freundlich isotherm. The adsorption capacity of the sediments decreased in the order mud >silt >sand. Inverse correlations between the pH, salinity, and temperature of the medium and the adsorption of glyphosate, AMPA, and 2-AEP were determined. This study showed that a small difference in the chemical structure of amphoteric substances such as glyphosate and glufosinate can lead to large differences in their adsorption behaviors.

Assessment and conclusion by applicant:

The article describes the sorption of glyphosate and AMPA to sediments of the Baltic Sea. Sediments are out of scope of EU data requirements for adsorption data. There was no detailed reporting of data to assess the validity (i.e. mass balances, test items not sufficiently described, information about LOD, LOQ).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

This article assessed the influence of types of sediment, pH and salinity over the adsorption of glyphosate and AMPA. The marine sediment used for this batch study cannot be considered as representative of agricultural soils in EU. Additionally, information needed to ensure the validity of the study against OECD criteria are lacking from the report. The concentrations tested do not cover two order of magnitude but only one. The salt concentration of the sediment and water used was 8 g/L and the study indicates that the concentration of salt has a significant influence over the adsorption of glyphosate and AMPA.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Gomez Ortiz et al, 2017

Data point:	CA 7.1.3.1.1/017
Report author	Gómez Ortiz, A.M. et al.
Report year	2017
Report title	Sorption and desorption of glyphosate in mollisols and ultisols soils of Argentina
Report No	DOI 10.1002/etc.3851 E-ISSN 1552-8618
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

In Argentina, glyphosate use has increased exponentially in recent years as a result of the widespread adorption of no-till management combined with genetically modified glyphosate-resistant crops. This massive use of glyphosate has created concern about its potential environmental impact. Sorption–desorption of glyphosate was studied in 3 Argentinean soils with contrasting characteristics. Glyphosate sorption isotherms were modeled using the Freundlich equation to estimate the sorption coefficient (Kf). Glyphosate sorption was high, and the Kf varied from 115.6 to 1612 mg1−1/nL1/n/kg. Cerro Azul soil had the highest glyphosate sorption capacity as a result of a combination of factors such as higher clay content, cation exchange capacity, total iron, and aluminum oxides, and lower available phosphorus and pH. Desorption isotherms were also modeled using the Freundlich equation. In general, desorption was

very low (<12%). The low values of hysteresis coefficient confirm that glyphosate strongly sorbs to the soils and that it is almost an irreversible process. Anguil soil had a significantly higher desorption coefficient (Kfd) than the other soils, associated with its lower clay content and higher pH and phosphorus. Glyphosate high sorption and low desorption to the studied soils may prevent groundwater contamination. However, it may also affect its bioavailability, increasing its persistence and favoring its accumulation in the environment. The results of the present study contribute to the knowledge and characterization of glyphosate retention in different soils.

Materials and Methods

Soils

Soil samples were taken from agricultural fields of Cerro, Tandil, and Anguil, The studied soils are located in areas of high agronomic land use and have different edaphoclimatic conditions. Four composite soil samples from the top 15 cm of topsoil were collected from each field. Samples were homogenized, air-dried, and sieved to a particle size of 2 mm. A subsample of each replicate was used for physicochemical analysis of the soils (see Table 8.1.2.1-66). Particle size distribution was measured using the pipette method; organic carbon content was measured according to the Walkley-Black method; CEC was determined by displacement with 1M ammonium acetate at pH 7; soil pH was measured by electrode in a soil:water ratio of 1:2.5; available phosphorus (P-Bray) was determined according to Bray and Kurtz; total iron (Fe) was determined by atomic absorption spectrophotometry; and exchangeable aluminum (Al) was measured according to the Al method.

Table 8.1.2.1-66: Main characteristics of the sampled locations and soil physicochemical properties

	Soil		
	Anguil	Cerro Azul	Tandil
Altitude (masl)	157	280	256
Annual average temperature (°C)	15.3	20.5	13.7
Mean annual precipitation (mm)	760	1844	993
Latitude	36°35′54″S	27°39′42″S	37°36′0.1″S
Longitude	63°58′31″W	55°26′25″W	59°04′29″W
Soil type	Mollisol	Ultisol	Mollisol
Main textural class	Loam	Clay	Loam
pH	6.3 A	4.9 C	5.4 B
Clay (%)	14.7 C	78.5 A	23.0 B
Silt (%)	45.6 A	15.4 C	40.9 B
Sand (%)	39.6 A	6.1 C	36.0 B
Organic carbon (%)	1.3 C	2.4 B	3.4 A
P-Bray (mg/kg)	29.6 A	7.6 C	17.1 B
CEC (meq/100 g)	17.4 C	20.6 B	25.2 A
Ca^{2+} (meq/100 g)	8.1 B	5.6 B	14.7 A
Mg^{2+} (meq/100 g)	2.9 B	3.2 B	5.1 A
K^{+} (meq/100 g)	3.2 A	1.2 A	2.8 A
Na^{+} (meq/100 g)	0.3 A	0.2 A	0.5 A
Al^{3+} (meq/100 g)[b]	0.15 B	0.69 A	0.11 B
Total Fe (%)[b]	1.08 B	8.40 A	0.81 B

[a]Different letters indicate differences among soils ($p < 0.05$).
[b]From Gianelli et al. [8].
CEC = cation exchange capacity; P-Bray = available phosphorus.

Chemicals

Stock solutions for the standard curves and the isotherm study solutions were prepared using analytical pure glyphosate (99.9%). For analytical procedures HPLC - grade methanol and HPLC - grade acetonitrile were purchased commercially. Nanopure water was obtained by purifying demineralized water.

Sorption isotherms

The sorption isotherms were performed according to the batch equilibrium method. First, 2 g of soil was shaken with 40 mL of a 0.01M CaCl2 solution. After 24 h, glyphosate was spiked at different initial concentrations (C0): 0, 0.5, 1, 5, 10, and 20 mg/L. The suspensions were shaken for another 24 h at

constant temperature (20°C). Afterward, tubes were centrifuged, and an aliquot (3 mL) of the aqueous solution was analyzed for glyphosate concentration. Each initial concentration was tested by duplicate for each soil sample. These laboratory duplicates were averaged, finally obtaining data of 4 replicate isotherms per soil.

Desorption isotherms

The desorption isotherms were performed using the spiked soil with the C0: 5 mg/L solution from the sorption isotherm studies. This concentration is equivalent to the commonly used dose in the field per year (6 L/ha/yr) considering 5 cm depth of soil. After the sorption study, the aqueous phase was carefully discarded to avoid any soil loss during manipulation. The volume of the solution that was removed was replaced with 0.01M CaCl2, and the soil was re-suspended and shaken at a constant temperature for another 24 h. Then, samples were centrifuged and glyphosate was measured in the aqueous solution to quantify the glyphosate that desorbed from the soil matrix. This procedure was repeated at 48 and 72 h by removing the aqueous solution and adding again CaCl2. The amount of adsorbed glyphosate at each desorption step was calculated as the difference between the initially adsorbed concentration and the desorbed amount.

Glyphosate analysis

To quantify the remaining glyphosate in the aqueous solution, an aliquot of 3 mL was transferred to a 15-mL polyethylene flask, and 0.5 mL of borate buffer solution (0.04 mM Na2B4O7 . 10 H2O, pH 9) and 0.5 mL of acetonitrile were added. Samples were shaken vigorously, then derivatized with 0.5 mL of 9-fluorenylmethylchloroformate dissolved in acetonitrile (6 g/L) and incubated overnight at room temperature. As a cleanup step, CH2Cl2 was added to the samples to remove any organic impurities and minimize matrix effects. The aqueous fraction was separated from the organic solvent by centrifuging. The supernatant was collected and filtered and then analyzed by liquid chromatography coupled to a tandem mass spectrometer (MS/MS).

Chromatographic analysis was carried out using a Waters ACQUITY1 ultra-performance liquid chromatography system. Target molecules were detected by a triple quadrupole MS/MS Quattro Premier XE (Waters). The equipment was operated with an electrospray ionization source in positive mode. To take into account the matrix effect of each soil, standard curves were prepared using a background solution of each soil obtained after shaking with CaCl2 0.01 M. After separating the solid phase from the aqueous phase, the solution was used to prepare each point of the standard curves by adding the corresponding glyphosate concentration. A sample without any glyphosate was also analyzed to check the concentration of presorbed glyphosate. In all cases, the background solution had non-detectable levels of glyphosate. The limit of detection was 0.1 µg/L, and the limit of quantification was 0.5 µg/L.

Sorption modeling

Following the experimental design proposed by the Organisation for Economic Co-operation and Development Guidelines for the Testing of Chemicals, Test No. 106, the measured glyphosate in the aqueous solution was used to estimate the remaining glyphosate sorbed to the soil (Cs).

$$Cs = Ms/Msoil = (C0 – Cw)V0/Msoil \quad (1)$$

where Cs is the concentration of glyphosate adsorbed to the soil at equilibrium (mg/kg), Ms is the mass of glyphosate sorbed to the soil at sorption equilibrium (mg), Msoil is the dry mass of the soil sample (kg), C0 is the initial tested concentration of glyphosate in contact with the soil sample (mg/L), Cw is the analytically measured mass concentration of glyphosate in the aqueous phase at sorption equilibrium (mg/L), and V0 is the initial volume of the aqueous phase in contact with the soil sample (mL).

The Freundlich equation was used to describe sorption and desorption isotherms

$$Cs = KfCw1/n \quad (2)$$

where Kf (mg1–1/n L1/n/kg) is the Freundlich sorption coefficient and 1/n is the Freundlich exponent (Kf and 1/n will hereafter refer to sorption and Kfd and 1/nd to desorption). The Kf coefficient indicates the affinity of the substance to the soil matrix, and 1/n indicates the degree of linearity between the amounts adsorbed and the concentration in the solution.

The hysteresis coefficient (H) for the sorption/desorption isotherms was calculated according to the equation

$$H = (1/nd)/(1/n) \quad (3)$$

where 1/n and 1/nd are the Freundlich slopes obtained for the sorption and desorption isotherms, respectively.

Statistical analysis

For the isotherm sorption and desorption studies, each soil sample was analyzed in duplicate. Laboratory duplicate samples were averaged, and the isotherm curves were then modeled using the NLIN procedure of SAS software. Statistical analyses of the soil properties and of the estimated sorption and desorption parameters were performed using a completely randomized design with 4 replicates per soil. Analysis of variance was performed using the PROC GLM procedure to evaluate differences in the Freundlich parameters at a significance level of 5%.

Results and Discussion

Soil characteristics

Tandil and Anguil soils correspond to a loam texture, while Cerro Azul is classified as clay. Cerro Azul soil had a significantly higher clay content, followed by Tandil and then Anguil ($p<0.05$). On the other hand, the organic carbon content and CEC were significantly higher in Tandil, followed by Cerro Azul and Anguil soil ($p<0.05$). Anguil soil had significantly higher pH and P-Bray values than Tandil and Cerro Azul ($p<0.05$). Regarding the exchangeable cations, significant differences were observed only for Ca2+ and Mg2+, following the order Tandil>Cerro Azul>Anguil ($p<0.05$). The highest Al3+ and Fe contents were found in Cerro Azul soil, denoting its Ultisol origin.

Sorption isotherms

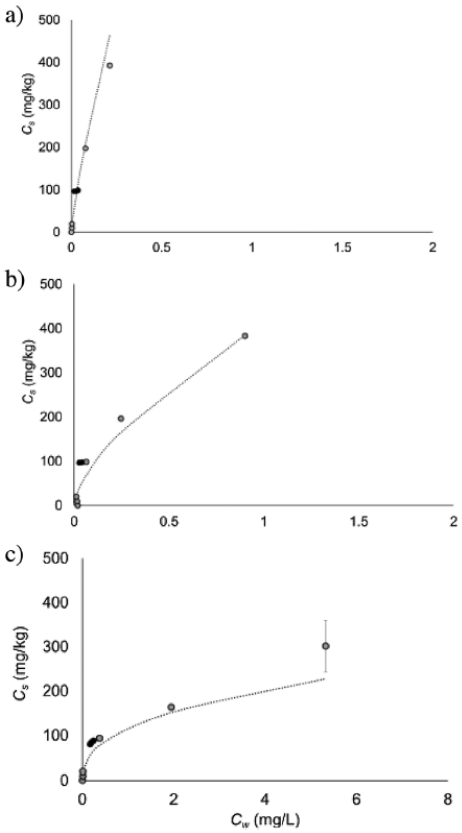
Glyphosate sorption and desorption isotherms are shown in Figure 8.1.2.1-26. The K_f values for glyphosate were very high and ranged from 115.6 to 1612 (Table 8.1.2.1-67), being generally higher than those usually reported in the literature. Glyphosate K_f was significantly higher in Cerro Azul compared with Tandil and Anguil soil ($p<0.05$) (Table 8.1.2.1-67). The values of 1/n ranged from 0.4 to 0.8 (Table 8.1.2.1-67). Isotherms exhibited an L-type ($1/n<1$) curve according to the classification of Giles *et al.* This indicates that sorption is not constant as the concentration of the herbicide increases and that the sorption sites become saturated with increasing glyphosate concentration. In the case of Tandil and Anguil soils, glyphosate was almost completely sorbed to the soil at low initial concentrations; and as the concentration increased, sorption became less efficient (Figure 8.1.2.1-26). Isotherms of this type occur when the adsorbent has a high initial affinity for the herbicide until the sorption sites become saturated. In contrast, the Cerro Azul isotherm exhibits an almost linear relationship between the amount of sorbed glyphosate and its concentration at equilibrium in the solution (Figure 8.1.2.1-26), with 1/n values closer to 1 (Table 8.1.2.1-67). Therefore, it can be assumed that the number of sorption sites remains almost constant even at high concentrations. The reason glyphosate sorption was significantly higher in Cerro Azul soil can be explained by the soil's textural composition. At the soil's pH, the negatively charged glyphosate molecule can be complexed with cations released from the clays via a cation exchange reaction with solution protons. On the other hand, Fe and Al oxides also play an important role in glyphosate sorption because the phosphonate group of glyphosate establishes coordination links with the interchangeable surfaces of Fe^{3+} and Al^{3+} cations. In this sense, the lower soil pH of Cerro Azul could also be favoring sorption via Fe and Al oxides because as the pH decreases, these oxides become more protonated, increasing the affinity toward the negatively charged glyphosate molecule. Therefore, aside from cation exchange reactions, glyphosate may strongly bond through ligand exchange with the metal ions (Fe or Al) at the surface of the clay minerals. This mechanism has been proposed for other organic weak acids, and hence it can be applied to glyphosate.

Table 8.1.2.1-67: Glyphosate Freundlich sorption and desorption parameters for Anguil, Cerro Azul, and Tandil soils[a]

	Sorption			Desorption[b]							
							Percentage[c]				
Soil	K_f ($mg^{1-1/n}L^{1/n}/kg$)	$1/n$	r^2	K_{fd} ($mg^{1-1/n}L^{1/n}/kg$)	$1/n_d$	r^2	1°	2°	3°	Total[d]	H[e]
Cerro Azul	1612.0 (859.8) A	0.8 (0.5) A	0.97–0.99	101.2 (2.9) C	0.01 (0.0) C	0.99–0.99	0.7 (0.1)	0.6 (0.0)	0.5 (0.1)	1.6 (0.8)	0.01 (0.0) B
Tandil	412.6 (50.9) B	0.5 (0.07) AB	0.98–0.99	105.4 (1.7) B	0.02 (0.0) B	0.99–0.99	0.8 (0.1)	0.6 (0.0)	0.3 (0.0)	1.9 (0.5)	0.04 (0.0) B
Anguil	115.6 (12.9) B	0.4 (0.2) B	0.90–0.99	117.5 (0.6) A	0.20 (0.0) A	0.99–0.99	4.5 (0.3)	3.6 (0.1)	3.3 (0.3)	12.3 (4.1)	0.4 (0.2) A

[a]Mean values of 4 replicates; standard deviation in parentheses. Different letters indicate significant differences among soils ($p < 0.05$).
[b]Desorption from initial glyphosate aqueous concentration $C_0 = 5$ mg/L.
[c]Percentage of desorbed glyphosate in the 1°, 2°, and 3° desorption cycle.
[d]Total desorbed glyphosate after 3 successive desorption cycles.
[e]Hysteresis coefficient ($H = 1/n_d/1/n$).

Figure 8.1.2.1-26: Adsorption (gray dots) and desorption (black dots) isotherms for (a) Cerro Azul, (b) Tandil, and (c) Anguil soils. Error bars represent standard deviation. Black dotted line represents the Freundlich model fit. Note different x axis scale for Anguil soil. Cs = concentration of glyphosate adsorbed to the soil at equilibrium; Cw = analytically measured mass concentration of glyphosate in the aqueous phase at sorption equilibrium



Desorption isotherms

The K_{fd} values of the studied soils ranged from 101.2 to 117.5 $mg^{1-1/n}kg^{-1}L^{1/n}$ (Table 8.1.2.1-67). Anguil soil had the highest K_{fd}, while Cerro Azul had a significantly lower desorption coefficient than the rest ($p<0.05$). The total desorbed glyphosate at the end of the desorption study was 1.6 and 1.9% for Cerro Azul and Tandil, respectively, whereas in Anguil soil desorption reached 12% (Table 8.1.2.1-67). The values of $1/n_d$ ranged from 0.01 to 0.2 (Table 8.1.2.1-67). The irreversibility of glyphosate sorption was confirmed by the lower values of $1/n_d$ with respect to 1/n. The more pronounced curvature of the desorption isotherms suggests that more energy is required to desorb the molecules than that needed for the sorption process. In consequence, hysteresis coefficients were low, ranging from 0.01 to 0.4 (Table 8.1.2.1-67). When comparing the 3 soils, desorption and hysteresis coefficients were significantly higher in Anguil. This can be explained by the lower clay content and lower CEC, as well as the significantly higher pH and available phosphorus, which affect glyphosate sorption mechanisms in an inverse way, as explained before. Nevertheless, desorption hysteresis can be considered significant in all the studied

soils because the hysteresis coefficient was <0.7, indicating that glyphosate sorption is nearly an irreversible process.

The fact that glyphosate binds strongly to the studied soils and that desorption was very low has a major implication for glyphosate bioavailability. Glyphosate's biological degradation is strongly limited in soils that have high glyphosate affinity and low desorption.

The results obtained in the present study indicate that sorption of glyphosate increases in soils with high contents of Al3+, Fe, and clays as well as low pH and phosphorus content. This situation favors greater glyphosate retention and, therefore, lower desorption, which would reduce the likelihood of leaching and therefore the potential risk of groundwater contamination. However, glyphosate bioavailability can also be reduced, increasing its persistence and therefore contributing to its accumulation in the environment. These results contribute to the knowledge about glyphosate retention in soils and allow the identification of behavior patterns of this extensively applied herbicide in different edaphic scenarios. This is of major importance for the development of decision-making tools and criteria to reduce the potential negative impacts on soil and groundwater resources.

Assessment and conclusion by applicant:

The article describes an adsorption/desorption experiment with glyphosate on three different agricultural soils from Argentina which have not been tested for their applicability to EU conditions due to the supportive character of the overall information in the article (insufficient information to assess validity, i.e. no mass balance, previous exposure to other chemicals not documented).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

This article focuses on the adsorption and desorption of glyphosate on three Argentinian soils. The concentrations used in the experiment do not cover two order of magnitude and results are only presented as mean of calculated Kf. Detailed results are lacking to assess the validity of the study against OECD 106 criteria (*e.g.* additional information on the stability of the substance, recovery, fittings).

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Munira, Farenhorst, 2017

Data point:	CA 7.1.3.1.1/018
Report author	Munira, S., Farenhorst, A.
Report year	2017
Report title	Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate
Report No	DOI 10.1080/03601234.2017.1361773 E-ISSN 1532-4109
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) - Temperature: 5 °C, - 0.01 M KCl - Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Phosphate fertilizers and herbicides such as glyphosate and MCPA are commonly applied to agricultural land, and antibiotics such as tetracycline have been detected in soils following the application of livestock manures and biosolids to agricultural land. Utilizing a range of batch equilibrium experiments, this research examined the competitive sorption interactions of these chemicals in soil. Soil samples (0–15 cm) collected from long–term experimental plots contained Olsen P concentrations in the typical (13 to 20 mg/kg) and elevated (81 to 99 mg/kg) range of build–up phosphate in agricultural soils. The elevated Olsen P concentrations in field soils significantly reduced glyphosate sorption up to 50 %, but had no significant impact on MCPA and tetracycline sorption. Fresh phosphate additions in the laboratory, introduced to soil prior to, or at the same time with the other chemical applications, had a greater impact on reducing glyphosate sorption (up to 45 %) than on reducing tetracycline (up to 13 %) and MCPA (up to 8 %) sorption. The impact of fresh phosphate additions on the desorption of these three chemicals was also statistically significant, but numerically very small namely <1 % for glyphosate and tetracycline and 3 % for MCPA. The presence of MCPA significantly reduced sorption and increased desorption of glyphosate, but only when MCPA was present at concentrations much greater than environmentally relevant and there was no phosphate added to the MCPA solution. Tetracycline addition had no significant effect on glyphosate sorption and desorption in soil. For the four chemicals studied, we conclude that when mixtures of phosphate, herbicides and antibiotics are present in soil, the greatest influence of their competitive interactions is phosphate decreasing glyphosate sorption and the presence of phosphate in solution lessens the potential impact of MCPA on glyphosate sorption. The presence of chemical mixtures in soil solution has an overall greater impact on the sorption than desorption of individual organic chemicals in soil.

Materials and Methods

Chemicals

Analytical grade glyphosate (99.9 %), MCPA (99 %), tetracycline (98 %), potassium dihydrogen phosphate (KH2PO4), (99 %) and potassium chloride (100 %) were obtained commercially. Radioactive [phosphonomethyl–14C] glyphosate (99 %; specific activity 50 mCi/mmol), [2–methyl–4–chlorophenoxyacetic acid 14C] MCPA (98 %; specific activity 55 mCi/mmol) and [7–3H (N)] tetracycline (98 % radiochemical purity; specific activity 20 Ci/mmol) were obtained commercially.

Table 8.1.2.1-68: Selected soil physical and chemical properties as mean with standard error

Organic Carbon [a] (%)	pH[b]	Fe_2O_3[c] (mg kg^{-1})	Al_2O_3[c] (mg kg^{-1})	Ca[d] (mg kg^{-1})	Clay[e] %	Silt[e] %	Sand[e] %
2.81 ± 0.04	4.7± 0.02	237±7.93	6.41±0.64	2,252±35	20	20	60

[a]Soil organic carbon content was determined using combustion technique with a high temperature induction furnace.[39]
[b]Soil pH was determined using a 10 ml 0.01M $CaCl_2$ solution and 2 g soil solution ratio.[40]
[c]Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*DTPA*) [41] and 0.01M $CaCl_2$, [42] respectively, and extracts were analyzed by ICP.
[d]Extractable Ca was also measured by ICP using ammonium acetate as an extractant [43]
[e]data adapted from Grant et al.[36]

Soil characteristics and experimental design

Soil samples (0–15 cm) were collected in spring 2013 from experimental plots that were arranged in a randomized complete block design with four replications and were located at the University of Manitoba Carman Field Research Station, Manitoba, Canada. All plots were under a flax and durum wheat rotation and received urea fertilizers at an annual rate of 50 and 90 kg N/ha, respectively. For this study, samples were collected from the replicated plots that had also received eight years (2002–2009) of annual mono ammonium phosphate (MAP) applications at rates of 80 kg P/ha, as well as from control plots that did not receive MAP application during these years. The rotation was continued from 2010 to 2013 but after 2010 no phosphate was applied. In each plot, composite soil samples were collected using a Dutch auger with ten samples per plot and the auger was cleaned in between plots. The soil is classified as an Orthic Black Chernozem based on the Canadian System of Soil Classification, which is approximately equivalent to the Udic Boroll subgroup in the U.S. Soil Taxonomy. Key soil properties are listed Table 8.1.2.1-68.

Impact of phosphate in solution on herbicides and antibiotic sorption and desorption

Batch equilibrium procedures using 50–mL centrifuge Teflon tubes (duplicates) followed the OECD guideline 106 with air–dried soil (2 g) and a soil/solution ratio of 1:5 with 0.01 M KCl as the background electrolyte Soil slurries were rotated in the dark at 5°C from 0 to 24 h (pre–incubation), from 24 to 48 h (sorption) and from 48 to 72 h (desorption) with phosphate added at 0 h, 24 h and/or 48 h, or never

added, depending on the treatment (Table 8.1.2.1-69). Radiolabelled chemical solutions contained 1 mg/L analytical–grade glyphosate, MCPA or tetracycline, with 6.67 x 10^5 Bq/L ^{14}C–labelled glyphosate, 3.83 x 10^5 Bq/L ^{14}C–labelled MCPA or 4.17 x 10^5 Bq/L ^{3}H–labelled tetracycline, respectively. The concentration 1 mg/L represented environmentally–relevant concentrations of herbicides and antibiotics detected in agricultural soils or animal manure. At 48 h, tubes were centrifuged at 10,000 rev/min for 10 min and subsamples (1 mL) of the supernatant (duplicates) were added to scintillation vials (7 mL) containing 5 mL 30 % Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method). The sorption distribution constant, Kd (L/kg), of glyphosate, MCPA or tetracycline was quantified by Cs/Ce, where Cs is the concentration of the organic chemical in soil at equilibrium (mg/kg) and Ce is the concentration of the organic chemical in the equilibrium solution (mg/L). The concentration of the organic chemical in soil was calculated by the difference between the radioactivity in the initial solution and the equilibrium solution. The soil organic carbon coefficient, Koc (L/kg) of glyphosate, MCPA or tetracycline was calculated by dividing the Kd value by 0.0281 which was the fraction of soil organic carbon in soil.

Table 8.1.2.1-69: Addition of phosphate during pre–incubation, sorption and desorption steps

Code	Pre-incubation from 0 h to 24 h	Sorption from 24 h to 48 h	Desorption from 48 to 72 h
n,n,n	No P added	No P added	No P added
n,n,P	No P added	No P added	P added at 48 h
P,n,n	P added at 0 h	No P added	No P added
P,n,P	P added at 0 h	No P added	P added at 48 h
n,P,P	No P added	P added at 24 h	P added at 48 h

n = no phosphate added during pre-incubation, sorption and/or desorption step; P = phosphate added at time 0 h at the start of the pre-incubation step or at time 24 h at the start of the sorption step; or at time 48 h at the start of the desorption step.

Impacts of MCPA and tetracycline in solution on glyphosate sorption and desorption in the presence and absence of fresh phosphate

Experiments followed similar protocols as described for n,n,n; n,n,P; and P,n,P in Table 8.1.2.1-69 above and also added to soil (at 0 h) were MCPA, tetracycline (Tetra), or their mixtures (M/T). MCPA, Tetra, and M/T were added at concentrations of 1 or 11 mg/L. The glyphosate solution was always added at 24 h and contained 1 mg/L analytical–grade glyphosate with 6.67 x 105 Bq/L 14C–labelled glyphosate.

Table 8.1.2.1-70: Effect of phosphate fertilizer on MCPA and tetracycline sorption and desorption in soil. See Table 8.1.2.1-69 for an explanation of the treatment labels

	Kd (L kg^{-1})		Desorption (%)	
Treatment	MCPA	Tetracycline	MCPA	Tetracycline
n,n,n	5.37 A	134.49 A	27.45 B	0.51 B
n,n,P	5.28 A	129.02 A	29.63 A	0.73 A
P,n,n	5.00 B	117.50 B	29.04 A	0.69 A
P,n,P	5.00 B	122.55 B	30.18 A	0.71 A
n,P,P	4.99 B	117.55 B	29.91 A	0.74 A

Effect of the pre–sorbed phosphate on the sorption of glyphosate, MCPA and tetracycline

This batch equilibrium experiment only used the soil samples obtained from the plots that had not received phosphate fertilizer applications.

Effect of the pre–sorbed MCPA on glyphosate sorption

Experiments followed similar protocols as described for the pre–sorbed phosphate above. The glyphosate solution contained 1 mg/L analytical–grade glyphosate with 6.67 x 105 Bq/L 14C–labelled glyphosate.

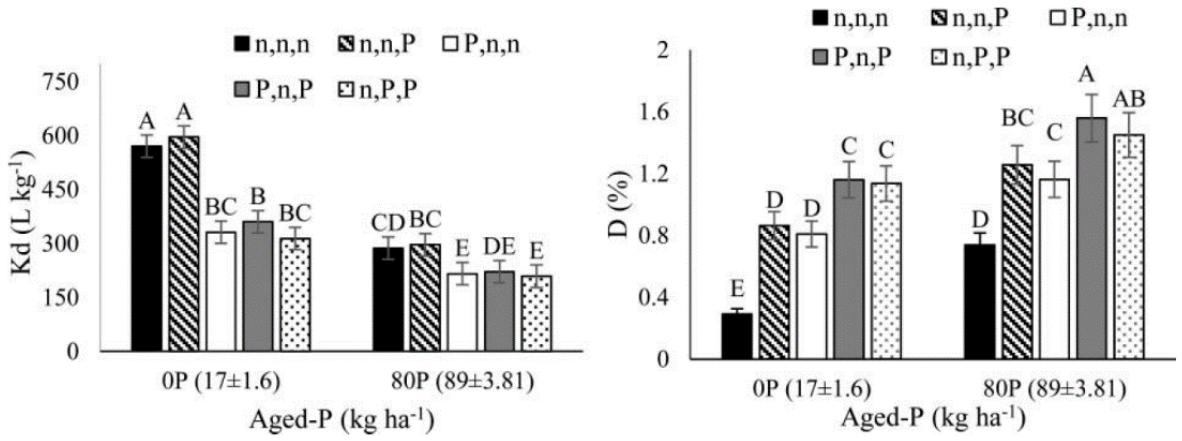
Statistical analysis

Statistical analyses were carried out using SAS software version 9.4 for Windows. Prior to each analysis, data sets were checked for outliers, normality of residuals and homogeneity of variances. Residuals were normally distributed and variances were homogeneous. For the Kd values, data were analyzed by using normal distribution and for the % desorption by beta distribution. Two–way ANOVA in PROC GLIMMIX was used to quantify the effect of field aged–P (0P, 80P) and fresh–P addition (0, 11 mg/L) on Kd values and % desorption of MCPA, tetracycline, and glyphosate in soil. One–way ANOVA in PROC GLIMMIX was utilized to determine the effect of retained phosphate in soil on glyphosate, MCPA and tetracycline sorption, and of retained MCPA in soil on glyphosate sorption. Both in the presence and absence of fresh phosphate, two–way ANOVAs in PROC GLIMMIX were carried out to quantify the effect of field aged–P (0P, 80P) and of the concentrations (0, 1, 11 mg/L) of MCPA, tetracycline, or MCPA–tetracycline mixtures on glyphosate Kd values. For fresh phosphate added at 48 h only, or at both 0 h and 48 h, and in the absence of fresh phosphate, two way ANOVAs in PROC GLIMMIX were carried out to quantify the effect of field aged–P (0P, 80P) and of the concentration (0, 1, 11 mg/L) of MCPA, tetracycline, or MCPA–tetracycline mixtures on the percent of glyphosate desorbed. For all ANOVAs, the separation of treatment means was performed using the Tukey's test ($p < 0.05$).

Results

Kd values on average ranged from 209 to 596 L/kg for glyphosate (Figure 8.1.2.1-27), from 118 to 135 L/kg for tetracycline, and from 4.99 to 5.37 L/kg for MCPA (Table 8.1.2.1-70). Koc values ranged from 6105 to 25,496 L/kg for glyphosate, from 3,928 to 4,901 L/kg for tetracycline, and from 156 to 209 L/kg for MCPA. These results are within the ranges observed in previous studies of the sorption of glyphosate, tetracycline and MCPA in soils. Glyphosate (<2 %) (Figure 8.1.2.1-27) and tetracycline (<1 %) desorption was always small but MCPA desorption ranged from 26 to 31 % (Table 8.1.2.1-70). Phosphate significantly reduced glyphosate sorption in soil (Figure 8.1.2.1-27). Without laboratory-added phosphate, glyphosate Kd values were 50 % smaller in soil containing 81 to 99 mg/kg Olsen P than in soil containing 13 to 20 mg/kg Olsen P. Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged–P always significantly reduced glyphosate Kd values. When phosphate was added to soil solution at either 0 h or 24 h, it had the same significant effect on reducing glyphosate sorption with glyphosate Kd values being reduced by 37–45 % in field soils containing 13 to 20 mg P/kg, and by 23–27 % in field soils containing 81 to 99 mg P/kg (Figure 8.1.2.1-27).

Figure 8.1.2.1-27: Effect of phosphate fertilizer on glyphosate sorption and desorption in soil. Potassium dihydrogen phosphate was added prior or during glyphosate addition for the sorption study and prior, during and/or post stage of glyphosate addition for the desorption study (see Table 8.1.2.1-69 for labels and details)



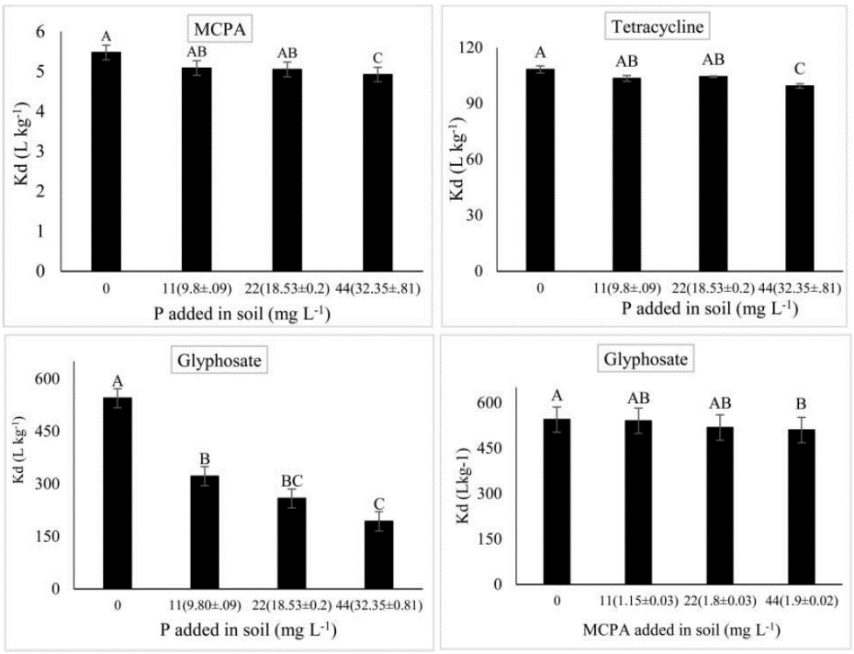
In the presorbed phosphate experiment, the soil retained 9.8, 18.5 and 32.4 mg P/kg for the additions of 11, 22, 44 mg P/L respectively, and glyphosate sorption was significantly reduced by 41 % (11 mg P/L), 52 % (22 mg P/L) and 65 % (44 mg P/L) (Figure 8.1.2.1-28).The amount of field aged–P in soil had no significant impact on MCPA and tetracycline sorption in soil. However, fresh phosphate added to soil solution significantly reduced tetracycline Kd values by 8–13 % and MCPA Kd values by 7–8 % (Table 8.1.2.1-70). The competitive effect of phosphate on MCPA and tetracycline sorption was not dependent on when the phosphate was added in the laboratory (either 0 h or 24 h) (Table 8.1.2.1-70). In the presorbed phosphate experiment, phosphate significantly reduced MCPA sorption by 10 % and tetracycline sorption by 8 % for the addition of 44 mg P/L (Table 8.1.2.1-72, or Figure 8.1.2.1-28). However, there was no impact on MCPA or tetracycline sorption when phosphate additions were 11 or 22 mg P/L. Glyphosate desorption was significantly greater in field soils containing 81 to 99 mg/kg Olsen P (0.74 %) than in soils containing 13 to 20 mg/kg Olsen P (0.29 %) (Figure 8.1.2.1-27). Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged–P always significantly increased glyphosate desorption. Fresh phosphate additions at 0 h, 24 h or/and 48 h to soil solutions in the laboratory also significantly increased glyphosate desorption by 0.52–0.84 % in soils containing 13 to 20 mg/kg Olsen P and by 0.52–0.82 % in field soils containing 81 to 99 mg/kg Olsen P (Figure 8.1.2.1-27). The amount of field aged–P in soil had no significant impact on MCPA and tetracycline desorption in soil, but the addition of fresh phosphate to soil solutions in the laboratory significantly increased desorption of MCPA by 2–3 % and tetracycline by 0.18–0.23 % (Table 8.1.2.1-70).

Table 8.1.2.1-71: Effect of MCPA (0, 1, 11 mg/L), tetracycline (0, 1, 11 mg/L) and MCPA/tetracycline mixtures (0, 1, 11 mg/L) on sorption and desorption of glyphosate in soil in the presence and absence of phosphate

		No P		P at 48 h		P at 0 h and 48 h	
Chemicals	Concentration (mg L^{-1})	Kd (Lkg^{-1})	D (%)	Kd (Lkg^{-1})	D (%)	Kd (Lkg^{-1})	D (%)
MCPA	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	409.73 A	0.53 A	424.99 A	1.11 A	271.09 A	1.42 A
	11	370.88 B	0.60 B	382.32 B	1.16 A	278.44 A	1.43 A
Tetracycline	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	415.64 A	0.54 A	426.02 A	1.04 A	283.50 A	1.36 A
	11	415.94 A	0.55 A	426.02 A	1.08 A	271.72 A	1.45 A
MCPA-tetracycline mixtures	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	426.02 A	0.48 A	444.58 A	1.12 A	283.50 A	1.39 A
	11	318.05 B	0.66 B	386.72 B	1.15 A	290.51 A	1.44 A

The competitive effect of phosphate on MCPA, tetracycline and glyphosate desorption was not dependent when phosphate was added to soil solution (either at 0 h, 24 h or 48 h). The number of times that phosphate was added had no significant effect on MCPA and tetracycline desorption (Table 8.1.2.1-70). However, glyphosate desorption was greater when phosphate was added twice (*P,n,P*, or *n,P,P*) rather than once (*P,n,n* or *n,n,P*) but glyphosate desorption remained <2 % in all cases (Figure 8.1.2.1-27). MCPA and MCPA/tetracycline mixtures added at 11 mg/L significantly reduced glyphosate Kd values and increased glyphosate desorption, but only when no phosphate was added to the soil solution (Figure 8.1.2.1-29, Table 8.1.2.1-71). MCPA and MCPA/tetracycline mixtures added at 1 mg/L had no significant effect on glyphosate sorption and desorption (Table 8.1.2.1-71). Tetracycline had no significant effect on glyphosate Kd values and desorption, regardless of whether it was added to soil at 1 or 11 mg/L, and whether or not phosphate was added to soil solution (Table 8.1.2.1-71). Thus, the effect of MCPA/tetracycline mixtures on glyphosate sorption and desorption was due to MCPA.MCPA addition significantly reduced glyphosate Kd values by 14 % (Figure 8.1.2.1-29) and glyphosate desorption by 0.1 % (Figure 8.1.2.1-29). In the pre–sorbed MCPA experiment, the addition of 11, 22, 44 mg MCPA/L the soil retained 1.2, 1.8 and 1.9 mg MCPA/kg, respectively. The pre–sorbed MCPA significantly reduced glyphosate sorption by 6 % for the addition of MCPA at 44 mg/L, but there was no impact on glyphosate sorption when additions were at 11 or 22 mg/L (Table 8.1.2.1-72, Table 8.1.2.1-70 S, or Figure 8.1.2.1-28).

Figure 8.1.2.1-28: Effect of pre–sorbed phosphate concentrations on MCPA, tetracycline and glyphosate sorption, and of pre–sorbed MCPA concentrations on glyphosate sorption in soil. Numbers on x–axis in parenthesis refer to mean (± standard error) of measured pre–sorbed phosphate and MCPA



Discussion

The addition of phosphate at either 0 h or 24 h yielded the same impact on glyphosate sorption (Figure 8.1.2.1-27), in agreement with the findings of Gimsing et al. (2004) who also reported that the timing of phosphate additions had no significant effect. Glyphosate and phosphate have shown to compete for the same sorption sites in soil. Application of phosphate with glyphosate in solution reduced glyphosate sorption because phosphate is preferentially sorbed over glyphosate by available sorption sites. Glyphosate Kd values were significantly smaller in soils containing elevated Olsen P concentrations than in soils containing typical Olsen P concentrations. This elevated Olsen P concentrations resulted from eight years of annual phosphate application from 2002 to 2009, with soils being sampled for this study in 2013. These results indicate that phosphate persists in agricultural soils and occupies sorption sites that otherwise would be available sorption sites for glyphosate. In–addition, in the pre–sorbed phosphate experiment, glyphosate sorption was also reduced with increasing phosphate application to soil thus indicating that phosphate from recently fertilizer applications will also occupy sorption sites otherwise available for glyphosate sorption. Given the moderately acidic conditions (soil pH 5), the sorption sites that phosphate occupies are positively charged Fe/Al–oxides. When phosphate (H2PO4-1) is retained by Fe/Al–oxides, the Fe/Al–oxides will yield a net negative charge, leading to an electrostatic repulsion between the Fe/Al–oxides and glyphosate (H2G-) in soil. However, a portion of glyphosate molecules that were sorbed by available positively charged Fe/Al–oxides. The addition of phosphate after this sorption increased glyphosate desorption (Figure 8.1.2.1-27) possibly because phosphate is able to displace glyphosate bound to Fe/Al–oxides as the bonding forces between phosphate and Fe/Al–oxides are stronger than the bonding forces between glyphosate and Fe/Al–oxides. Under the experimental conditions with the soil slurries being at a pH 5, the molecules of MCPA (pKa D 3.73) are predominantly negatively–charged.

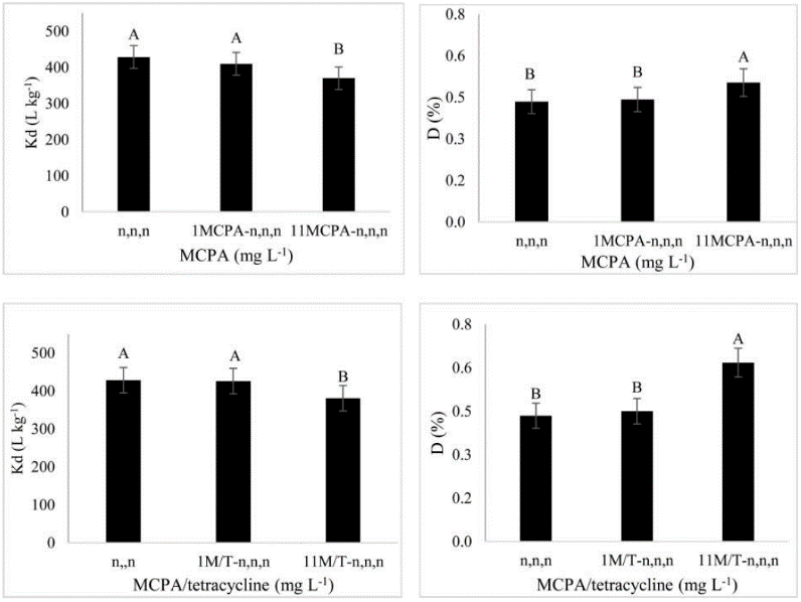
Table 8.1.2.1-72: Effect of pre–sorbed phosphate (0, 11, 22, 44 mg/L) on glyphosate, MCPA and tetracycline sorption and pre–sorbed MCPA on glyphosate sorption (L/kg) in soil

Concentration (mg L^{-1})	Glyphosate	MCPA	Tetracycline	Glyphosate
0	544.60 A	5.48 A	108.22 A	544.6 A
11	321.78 B	5.09 AB	103.39 AB	540.8 AB
22	258.49 BC	5.05 AB	104.35 AB	518.25 AB
44	192.96 C	4.93 C	99.32 C	510.25 B

MCPA and tetracycline sorption was only significantly reduced at the highest rate because more Fe/Al–oxides were net negatively charged and repelling MCPA and tetracycline molecules. The effect of phosphate on reducing sorption was less for MCPA and tetracycline than for glyphosate. Under moderately acidic conditions, Fe/Al–oxides are the dominant sorption sites for glyphosate and phosphate because both have a phosphonic acid group. However, MCPA (i.e., carboxyl and phenyl groups) and tetracycline (i.e., tricarbonylamide carbonyl, amine and hydroxyl groups) have other functional groups and sorption sites for MCPA and tetracycline can include under moderately acidic conditions humic substances and clay minerals in addition to Fe/Al–oxides in soils. MCPA had no longer a significant effect on glyphosate sorption when phosphate was added to the soil solution. The molecular size of phosphate (0.25 nm) is smaller than glyphosate (0.43 nm) and MCPA (0.77 nm). Therefore, it is possible that phosphate is preferentially sorbed over glyphosate and MCPA. Thus, when both phosphate and MCPA were added to the soil solution, phosphate occupied the sorption sites that may otherwise be available to MCPA and suppressed the effect of MCPA on glyphosate sorption. In the pre–sorbed experiment, in the absence of phosphate additions, MCPA reduced glyphosate sorption because pre–sorbed MCPA occupied some sorption sites which may otherwise be accessible to glyphosate.

MCPA was weakly retained with Koc values ranging from 156 to 209 L/kg while glyphosate and tetracycline were strongly retained with Koc values ranging from 6,105 to 25,496 and 3,928 to 4,901 L/kg, respectively. It has been reported that organic molecules are considered relatively mobile when Koc value ranges from 150 to 500 L/kg. Thus, given these Koc values, MCPA is relatively mobile in soil because it is only weakly retained, unlike glyphosate and tetracycline. Glyphosate is very strongly retained in soil and is less likely to be mobile in matrix flow than MCPA, regardless of the amounts of phosphate or MCPA that can compete with glyphosate for sorption sites in soil. In contrast, the presence of recent phosphate applications to agricultural soils may increase the mobility of MCPA to deeper depths but only when applied at relatively large phosphate fertilizer rates.

Figure 8.1.2.1-29: Effect of MCPA and MCPA/tetracycline mixtures on glyphosate sorption and desorption in soil. Potassium dihydrogen phosphate with MCPA or MCPA/tetracycline were added prior glyphosate for the sorption study and prior, or post stage of glyphosate addition for the desorption study: (see Table 8.1.2.1-69 for labels and details)



Conclusion

Field–aged phosphate had no significant effect on MCPA and tetracycline sorption and desorption but significantly reduced glyphosate sorption up to 50 % and increased glyphosate desorption by 0.45 %. Pre–sorbed phosphate had a greater impact on reducing glyphosate sorption than on reducing MCPA and tetracycline sorption. The addition of fresh phosphate in the laboratory also significantly decreased glyphosate sorption (up to 45 %) and increased glyphosate desorption (up to 0.87 %) and the impact on reducing MCPA and tetracycline sorption (<13 %) and increasing MCPA and tetracycline desorption

(<3 %) was significant but smaller than the impact on glyphosate. Glyphosate and tetracycline were strongly retained in soil with Kd values >100 L/kg and desorption less than 2 %. In contrast, MCPA was weakly retained in soil with Kd values <6 L/kg and desorption was above 25 %. Hence, even in soils with a large phosphate build–up, glyphosate will be less mobile in matrix flow than MCPA. MCPA but not tetracycline additions significantly decreased glyphosate sorption, but only when MCPA was present at concentrations ten times greater than typically detected in agricultural soils and there was no phosphate added to the herbicide solutions.

Assessment and conclusion by applicant:

The article describes an OECD 106 experiment with glyphosate on a Canadian soil considering the influence of phosphate additions. The article shows some deviations from the validity criteria for EU guidelines (temperature, usage of 0.01 M KCl instead of 0.01 M CaCl2, no mass balance and no demonstration of test item stability).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

This article presents the analysis of adsorption of glyphosate under different conditions, such as the presence of MCPA, of pre-sorbed phosphate or tetracycline before adding glyphosate. Pre-incubation, the soil slurries were rotated at 5°C. A KCl solution was used instead of $CaCl_2$. The article lacks many details to assess the validity of the study against OECD 106 criteria (e.g. lack of mass balance, analytic efficacy or stability of the test item).

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Munira, 2017

Data point:	CA 7.1.3.1.1/019
Report author	Munira, S. et al.
Report year	2017
Report title	Phosphate and glyphosate sorption in soils following long-term phosphate applications
Report No	DOI 10.1016/j.geoderma.2017.10.030 ISSN 0016-7061
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Phosphate and glyphosate molecules compete for sorption sites in soil. The objective of this study was to quantify the impact of Olsen P concentrations in two contrasting soils on phosphate and glyphosate sorption. Soils were a sandy clay loam soil rich in iron oxides (SCL-Fe2O3) and a clay loam soil rich in calcium carbonates (CL-CaCO3). The phosphate Freundlich sorption coefficient (Kf) ranged from 3 to 68 L1/n mg1-1/n kg−1 in the SCL-Fe2O3 and from 21 to 76 L1/n mg1−1/n kg−1 in the CL-CaCO3. Glyphosate sorption coefficient (Kd) ranged from 293 to 1173 L/kg in the SCL-Fe2O3 but only 99 to 141 L/kg in the CL-CaCO3. Glyphosate Kd and phosphate Kf values decreased significantly with increasing Olsen P concentrations in both soils. Glyphosate Kd values were further significantly reduced when phosphate was added to the slurry solutions, but phosphate Kf values were not impacted by the presence of glyphosate in solutions. We conclude that annual phosphate fertilizer applications leave

phosphate concentrations in Prairie soils to the extent that soils have a lesser capacity to retain glyphosate and phosphate that are subsequently applied, but glyphosate residues will not influence phosphate sorption.

Methods

Chemicals

Chemicals used were analytical grade glyphosate (99.9% purity) from Sigma-Aldrich Co., St. Louis, MO; [phosphonomethyl-14C]glyphosate (99% radiochemical purity; specific activity 50 mCi/mmol) from American Radiolabeled Chemicals Inc., St. Louis, MO; Roundup Ultra2® (49% active ingredient and 51% other ingredients, CAS No. 70901-12-1) from Monsanto Chemical Company; and analytical grade potassium dihydrogen phosphate (KH2PO4) (99% chemical purity), potassium chloride (100% chemical purity) and calcium chloride, dehydrate (> 95% chemical purity) from Fisher Scientific, Fair Lawn, NJ.

Soil characteristics and experimental design

This study utilized soil samples (0–15 cm) obtained from long-term experimental plots under a durum wheat and flax rotation near Carman (49° 29.7′ N, 98° 2.4′ W) and near Forrest (50° 1.2′ N, 99° 53.3′ W) Manitoba, Canada. Soil profiles at both sites were classified based on the Canadian System of Soil Classification as Orthic Black Chernozems, which is equivalent to the Udic Boroll subgroup in the U.S. Soil Taxonomy. The experimental design at each site was a randomized complete block design with four mono ammonium phosphate fertilizer treatments and four replicates plots. Treatments were a control (no phosphate applications), and plots receiving annual applications of mono ammonium phosphate fertilizers at 20, 40, and 80 kg P/ha or 20P, 40P, and 80P, respectively, from 2002 to 2009. For all plots that received mono ammonium phosphate, 20 kg P/ha was placed near the seed to enhance fertilizer use efficiency, a common practice in Canadian Prairie agriculture. For the 40 and 80 kg P/ha treatments, to avoid seedling toxicity, the additional mono ammonium phosphate was broadcast and then incorporated. From 2010 to 2013, the rotation was continued but no phosphate was applied. Application of urea fertilizer differed by year. Generally, durum wheat received 90 kg N/ha and flax 50 kg N/ha· From each plot, composite samples were collected in spring, 2013 using a Dutch auger with ten (Carman) to eight (Forrest) samples per plot and cleaning the auger between plots. Soil samples were air-dried and sieved (< 2 mm) prior to soil property analyses and sorption experiments. The Carman soil has a sandy clay loam texture and is relatively high in iron oxides (SCL-Fe_2O_3), whereas the Forrest soil has a clay loam texture and is relatively high in calcium carbonates (CL- $CaCO_3$) (Table 8.1.2.1-73). Available phosphate was extracted using the Olsen (0.5 M $NaHCO_3$, pH 8.5) phosphorus test. 2 g of air-dried soil and 40 mL of 0.5 N $NaHCO_3$ solution was mixed in a 50 mL Erlenmeyer flask. Flasks (duplicates) were shaken horizontally (200 excursions/min). Equilibrium solutions were filtered through Whatman No. 2 filter paper and phosphate concentrations were determined colorimetrically.

Table 8.1.2.1-73: Selected soil physical and chemical properties as mean with standard error

Soil	Organic Carbon[a] (%)	pH[b]	Fe_2O_3[c] (mg kg^{-1})	Al_2O_3[c] (mg kg^{-1})	Ca[d] (mg kg^{-1})	Clay[e] %	Silt[e] %	Sand[e] %
SCL-Fe_2O_3	2.81 ± 0.04	4.7 ± 0.02	237 ± 7.93	6.41 ± 0.64	2252 ± 35	20	20	60
CL-$CaCO_3$	3.2 ± 0.07	7.3 ± 0.02	12.52 ± 0.22	1.07 ± 0.47	4791 ± 158	30	39	31

[a] Soil organic carbon content was determined using combustion technique with a high temperature induction furnace (Nelson and Somemers, 1996).
[b] Soil pH was determined using a 10 mL 0.01 M $CaCl_2$ solution and 2 g soil solution ratio (Jones, 2001).
[c] Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*DTPA*) (Whitney, 2011) and 0.01 M $CaCl_2$, (Barnhisel and Bertsch, 1982) respectively, and extracts were analyzed by *ICP*.
[d] Extractable Ca was also measured by *ICP* using ammonium acetate as an extractant (Warncke and Brown, 2011)
[e] Data adapted Grant et al., (2013)

Phosphate sorption

Phosphate sorption was determined by batch equilibrium using either 0.01 M CaCl2 or 0.01 M KCl as the background electrolyte. Batch equilibrium procedures followed standard protocols using a soil/solution ratio of 1:10 and an equilibrium time of 24 h. Two experiments were conducted utilizing soil samples: (1) from all plots at each site to quantify the effect of Olsen P concentrations on phosphate

sorption in soil and (2) from control and 80P plots at each site to quantify the effect of Roundup Ultra2 additions to soil slurries on phosphate sorption in soil.

Effect of field-aged phosphate concentrations on sorption of phosphate

In the first experiment, potassium dihydrogen phosphate solutions (20 mL) at concentrations of 5, 10, 25, 50, 100, 150, 250 or 500 mg P/L were added to air-dried soil (2 g) in 50-mL centrifuge tubes (duplicates) and shaken horizontally (120 excursions/min) at room temperature (23 ± 2 °C) for 24 h. Equilibrium solution was centrifuged (6100 G for 10 min) and filtered (0.45 μm). Phosphate concentration was determined colorimetrically by the molybdate blue method. Linearized Freundlich isotherm has been specified as: The phosphate sorption coefficient, Kf (L1/n mg1−1/n kg−1), was calculated using the linearized form of Freundlich equation: log q= log Kf + 1/n log C. Where q represents phosphate sorption in soil at equilibrium (mg/kg), C represents phosphate concentration of equilibrium solution (mg/L), and 1/n represents the Freundlich slope. In addition, the Freundlich P sorption isotherm was used to determine the equilibrium P concentration (EPCo) at log q = 0, which is the concentration at which neither sorption nor desorption occurs and hence can be used to define whether a soil is likely to act as a sink (sorption) or source (desorption) of P. EPCo levels above 0.025 mg/L suggest an increased risk of eutrophication because of P transport in soluble form.

Effect of glyphosate formulation on sorption of phosphate

In the second experiment, stock solutions of 150 mg P/L were prepared with and without 100 mg/L Roundup Ultra2 in the solution. The 100 mg/L Roundup Ultra 2 was equivalent to 378 mg glyphosate/kg soil. The 150 mg P/L solution was used because previous studies have proposed that this parameter (P150) is the most optimum single point in the isotherm reflective of the phosphate sorption capacity in soils. Batch equilibrium procedures were carried out as described above. The phosphate sorption coefficient, Kd (L/kg), was calculated by q/C, where q represents phosphate sorption by soil at equilibrium (mg/kg) and C represents phosphate concentration of equilibrium solution (mg/L).

Glyphosate sorption

Glyphosate sorption was determined by batch equilibrium with the initial glyphosate solution containing 1 mg/L analytical-grade glyphosate and 6.67 × 104 Bq/L 14C–labelled glyphosate. Two experiments were conducted utilizing soil samples: (1) from all plots to quantify at each site the effect of Olsen P concentrations on glyphosate sorption, and (2) from control and 80P plots to quantify at each site the effect of fresh phosphate additions to soil slurries on glyphosate sorption in soil.

Impact of field-aged phosphate concentrations on sorption of glyphosate

Batch equilibrium procedures followed the OECD guideline 106 using a soil/solution ratio of 1:5, an equilibrium time of 24 h and 0.01 M CaCl2 or 0.01 M KCl as background electrolyte. Glyphosate solutions (10 mL) were added to air-dried soil (2 g) in 50-mL centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark at 5 °C for 24 h. Equilibrium solution was centrifuged (6100 G for 10 min) and subsamples (1 mL) of supernatant were added in duplicated 7-mL scintillation vials containing 5 mL of 30% Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method) (LS 6500 Beckman Instruments, Fullerton, CA). The glyphosate sorption distribution constant, Kd (L/kg), was calculated by Cs/Ce, where Cs represents glyphosate sorption by soil at equilibrium (mg/kg) and Ce represents glyphosate con- centration of equilibrium solution (mg/L). The difference between the added radioactivity and radioactivity in the supernatant was assumed to be the proportion of glyphosate having been sorbed.

Impact of fresh phosphate addition on sorption of glyphosate

Experiments followed similar batch equilibrium sorption protocols as described above. In this experiment, potassium dihydrogen phosphate was added to the initial glyphosate solution at rates equivalent to 11, 22 and 44 mg P/kg soil, or an estimated 20, 40 and 80 P kg/ha, respectively, when assuming the fertilizer being present in the top 15-cm layer of a soil with a bulk density of 1200 kg/m3.

Statistical analysis

Statistical analyses were carried out using SAS software version 9.3 for Windows (SAS Institute Inc. 2002–2010). Prior to each analysis, data sets were checked for outliers, normality of residuals and homogeneity of variances. Residuals were normally distributed and variances were homogeneous. The paired t-test ($P < 0.05$) was used to test for the effect of background electrolyte solution (0.01 M CaCl2 versus 0.01 M KCl) on glyphosate Kd or phosphate Kf and EPCo. For both background electrolyte solutions and at each site, simple linear regression analyses ($P < 0.05$) were carried out to estimate glyphosate Kd and phosphate Kf values using Olsen P concentration as the independent variable. In each of the glyphosate Kd and phosphate Kf figures, the slopes of regression lines developed for SCL-Fe2O3 and CL- CaCO3 were compared by including dummy variables in PROC REG to test whether the responses of sorption to increasing Olsen P concentrations was influenced by soil type. Simple linear regression analysis was also carried out to estimate glyphosate Kd values by using the added fresh phosphate concentration as an independent variable. The slopes of the regression lines developed for the 0P (control) and 80P plots in both soils were compared by including dummy variables in PROC REG to test whether the responses of sorption to increasing potassium dihydrogen phosphate concentration was influenced by Olsen P concentrations (0P, 80P). Simple linear regression analyses were carried out to determine the relationship between glyphosate Kd and phosphate Kf values by using Kf as an independent variable. Simple linear regression analyses ($P < 0.05$) were also carried out to estimate EPCo values by using Olsen P as an independent variable for CL-CaCO3 soil. Graphical plot fitting of EPCo as a function of Olsen P showed that data did not fit well with simple linear regression for the SCL-Fe2O3 soil.

Results

Effect of background electrolyte solutions on sorption of phosphate and glyphosate

The types of ions in solution had a significant effect on phosphate and glyphosate sorption, except for glyphosate sorption in the CL-$CaCO_3$ soil (Table 8.1.2.1-74). Phosphate K_f values in both soils were significantly greater in experiments with 0.01 M $CaCl_2$ than experiments with 0.01 M KCl (Table 8.1.2.1-74).

Table 8.1.2.1-74: Statistical parameters (Paired t-tests) on the effect of background electrolyte solution (0.01 M $CaCl_2$ versus 0.01 M KCl) on glyphosate (L/kg) and phosphate sorption coefficient ($L^{1/n}$ mg $^{1-1/n}$ kg^{-1}) in soils

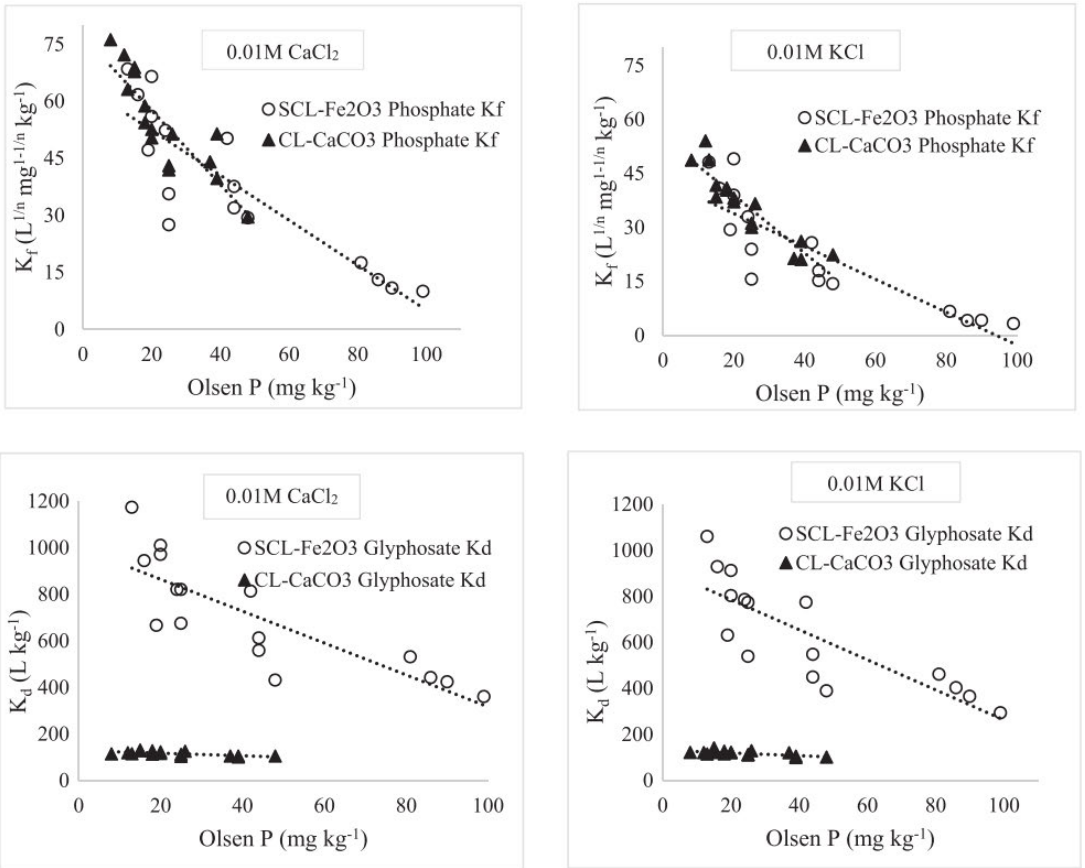
Sorption Parameter	Soil	Mean		DF	t Value	Pr > |t|
		0.01 M $CaCl_2$	0.01 M KCl			
Phosphate sorption coefficient, K_f	SCL-Fe_2O_3	38.47	23.11	15	11.14	< 0.0001
	CL-$CaCO_3$	54.08	36.04	15	10.94	< 0.0001
Glyphosate sorption coefficient, K_d	SCL-Fe_2O_3	703	632	15	5.89	< 0.0001
	CL-$CaCO_3$	116	117	15	− 1.55	0.1430
Phosphate equilibrium concentration, EPCo	SCL-Fe_2O_3	0.007	0.04	15	− 2.29	< 0.0366
	CL-$CaCO_3$	0.006	0.015	15	− 4.72	< 0.0003

Phosphate K_f values were on average 54 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 38 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} SCL-Fe_2O_3 with $CaCl_2$ but on average 36 L1/n mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 23 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} SCL-Fe_2O_3 with KCl. Thus, when 0.01 M $CaCl_2$ was used with the SCL- Fe_2O_3 and CL-$CaCO_3$ soils but also when KCl was used with the CL-$CaCO_3$ soil, phosphate likely formed stable complexes with a portion of Ca^{2+} in soil solution and precipitated. In batch equilibrium experiments with 0.01 M $CaCl_2$, precipitation with Ca^{2+} occurs more readily for phosphate than glyphosate. For glyphosate sorption, K_d values were on average 116 L/kg in CL-$CaCO_3$ and 703 L/kg SCL- Fe_2O_3 with $CaCl_2$, and on average 117 L/kg in CL-$CaCO_3$ and 632 L/kg SCL-Fe_2O_3 with KCl. In calcareous soils, Ca^{2+} in forms a bridge between negatively charged soil colloids and glyphosate molecules in soil and, because of the already high free calcium content in the CL-$CaCO_3$ soil, the addition of Ca with 0.01 M $CaCl_2$ solution had no impact on glyphosate sorption. For the SCL-Fe_2O_3 soil, glyphosate sorption was greater with 0.01 M $CaCl_2$ than 0.01 M KCl, suggesting that glyphosate was able to form complexes with Ca^{2+} in solution for enhanced sorption.

Effect of field-aged phosphate concentrations on sorption of phosphate

Despite being exposed to similar long-term phosphate fertilizer treatments, Olsen P ranged from 13 to 99 mg/kg in the acidic SCL-Fe_2O_3 soil but only from 8 to 48 mg/kg in the calcareous CL-$CaCO_3$ soil. Olsen P concentrations by treatment were on average 17 (control), 24 (20P), 44 (40P) and 89 (80P) mg/kg in the SCL- Fe_2O_3 soil and 13 (control), 18 (20P), 24 (40P) and 41 (80P) mg/kg in the CL-$CaCO_3$ soil. The Olsen P test was originally developed for calcareous soils and can overestimate plant available P in acidic soils, such as the SCL-Fe_2O_3. Olsen P measures the $NaHCO_3$ extractable phosphate in soil, but calcareous soil may also contain slow release inorganic phosphate (apatite minerals) extracted by 1 M HCl. Olsen P concentrations ranged from 8 to 99 mg/kg in this research which is within the typical range of 8 to 114 mg/kg that has been reported for soils in North America. Hence, the findings from this research on the sorption pattern of phosphate and glyphosate in soil would be applicable to a wider range of soils in North America. Phosphate K_f values significantly decreased with the increasing concentrations of Olsen P in soil (Figure 8.1.2.1-30).

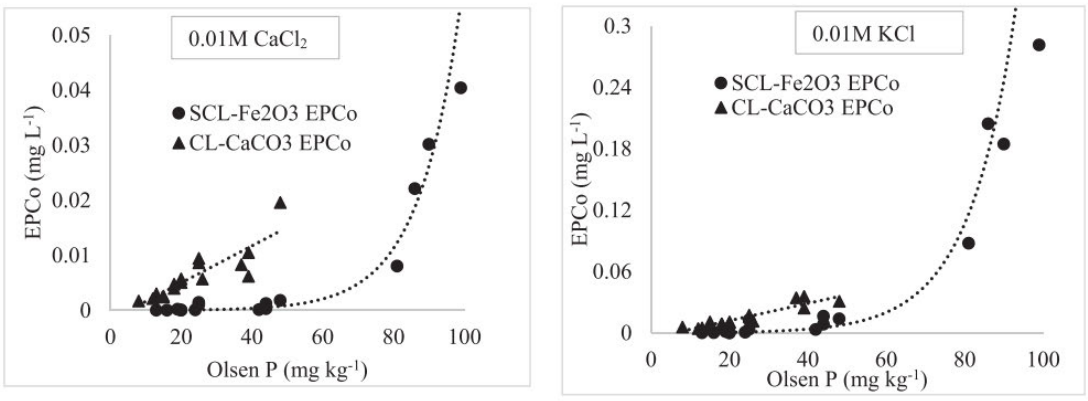
Figure 8.1.2.1-30: Effect of Olsen P concentrations in soil on glyphosate and phosphate sorption in SCL-Fe2O3 and CL-CaCO3 soils, as determined by batch equilibrium experiments using 0.01 M CaCl2 or 0.01 M KCl as background electrolyte solutions. All regression equations are significant at P < 0.05



The SCL- Fe2O3 and CL-CaCO3 soils showed relatively similar phosphate sorption (Figure 8.1.2.1-30). Phosphate Kf values ranged from 3.2 to 68 L1/n mg 1–1/n kg−1 in the SCL- Fe2O3 soil with 1/n values between 0.37 and 0.92, and from 21 to 76 L1/n mg 1–1/ n kg−1 in the CL-CaCO3 soil with 1/n values between 0.68 and 0.92. These values are within the range of other studies (Bertrand et al., 2003; Jalali, 2007; Shafqat and Pierzynski, 2014). A maximum reduction of phosphate Kf value was observed in SCL- Fe2O3 soil. The phosphate Kf value in SCL- Fe2O3 was reduced by 95% in soil containing 99 mg/kg Olsen P relative to soil containing 13 mg/kg Olsen P. Thus, P accumulation in soil reduced the capacity of soil to hold Wang et al. (2015) also reported that sorption of P decreased with the in- creasing concentrations of Olsen P because long-term application of P fertilizer leads to the accumulation of P in soil. In their study, they showed that long-term (5 to 15 years) application of phosphate significantly reduced phosphate sorption by 56% in soil containing 53 mg/kg Olsen P relative to soil containing 15 mg/kg Olsen P. Olsen P concentrations significantly predicted phosphate Kf (Figure 8.1.2.1-30) in both SCL- Fe2O3 and CL-CaCO3. The effect of Olsen P concentrations on reducing phosphate sorption was more pronounced for SCL- Fe2O3 than CL-CaCO3. For the phosphate Kf, the regression slopes were significantly different between the soils in case of 0.01 M KCl but not with 0.01 M CaCl2 because the presence of Ca in solution led to the possibility of precipitation of phosphate-Ca2+ complexes in both soils. Generally, in calcareous soil, Ca forms precipitation with the added phosphate in soil solution. For 0.01 M KCl, the CL- CaCO3 showed a significantly steeper slope than SCL- Fe2O3 (Figure 8.1.2.1-30) because, with increasing Olsen P concentrations, more sorption sites remained available in SCL- Fe2O3. CL-CaCO3 soil has less sorption sites available for the added phosphate than SCL- Fe2O3 soil because calcareous soils contain slow-release phosphate (e.g., octacalcium phosphate and apatite) which occupy sorption sites that otherwise would be available for the added phosphate.

EPCo significantly increased with increasing concentrations of Olsen P in both SCL- Fe_2O_3 and CL- $CaCO_3$ (Figure 8.1.2.1-31). EPCo values ranged from 0 to 0.281 mg/L, depending on the background electrolyte solution and soil (Figure 8.1.2.1-31). EPCo values in both soils were significantly greater in the experiments with 0.01 M KCl than experiments with 0.01 M $CaCl_2$ (Table 8.1.2.1-74) because of the formation of Ca^{2+}-phosphate complexes in both soils with 0.01 M $CaCl_2$. All EPCo levels were below the threshold value of 0.025 mg/L except in the 80P plots. The average calculated EPCo values for the four replicated 80P plots was 0.031 mg/L for CL-$CaCO_3$ and 0.190 mg/L for SCL- Fe_2O_3 with 0.01 M KCl, and 0.025 mg/L for SCL- Fe_2O_3 with 0.01 M $CaCl_2$. Although this suggest that prairie soils have a low risk for soluble P transport, a recent review reported that a significant portion of phosphate in Prairie soils can be transported as dissolved P during snow melt runoff. Phosphate can be transported from the agricultural soil when phosphate fertilizer is applied in excess of crop requirements and also from plant residues during snow melt.

Figure 8.1.2.1-31: Effect of Olsen P concentrations in soil on the phosphate equilibrium concentration, (EPCo) in SCL-Fe2O3 and CL-CaCO3 soils determined by batch equilibrium experiments using 0.01 M CaCl2 or 0.01 M KCl as background electrolyte solutions. Olsen P All regression equations are significant at P < 0.05



Effect of glyphosate formulation on sorption of phosphate

Commercially available glyphosate formulation had no impact on phosphate sorption in soil because there were no significant differences in phosphate sorption between treatments with and without Roundup Ultra2 additions to soil slurries. Gimsing and Borggaard (2001) also found that, when glyphosate was added following phosphate additions to goethite, glyphosate did not displace the sorbed phosphate. In a recent article that was published in the magazine "No-Till Farmer", a statement was made that "20-25 percent of the dissolved reactive phosphorus in runoff is caused by glyphosate [use]" because of the assumption that glyphosate residues in soil decreases phosphate retention in soil. However, in our batch-equilibrium study that utilized very high rates of Roundup Ultra2, there was no significant difference in phosphate sorption between treatments with and without Roundup Ultra2 additions to soil slurries. Thus, given our findings, the recent concerns stated in Barrera (2016) are unlikely to be applicable to the Prairie soils that were included in our studies.

Effect of field-aged phosphate on sorption of glyphosate

Glyphosate Kd values significantly decreased with the increasing concentrations of Olsen P in both SCL- Fe2O3 and CL-CaCO3 (Figure 8.1.2.1-30). Glyphosate Kd values ranged from 293 to 1173 L/kg in the acidic SCL- Fe2O3 soil and from only 99 to 141 L/kg in the calcareous CL- CaCO3 soil (Figure 8.1.2.1-30), and these values are within the range of other studies (Farenhorst et al., 2008; Kumari et al., 2016; Sørensen et al., 2006). Long-term application of phosphate fertilizer in soil reduced glyphosate sorption because pre-sorbed phosphate occupied the sorption sites that would otherwise be available to glyphosate. A maximum reduction in glyphosate Kd value was observed in SCL- Fe2O3 soil. The Kd value was reduced by 75% in soil containing 99 mg/kg Olsen P relative to soil containing 13 mg/kg Olsen P in SCL- Fe2O3. Thus, results indicate that glyphosate and phosphate compete for the same sorption sites in soil. Similar observations have been made by de Jonge et al. (2001) who reported that long-term (60 to 100 years) application of phosphate significantly reduced glyphosate sorption by 50% in soil containing 59 mg/kg Olsen P relative to soil containing 6 mg/kg Olsen P.

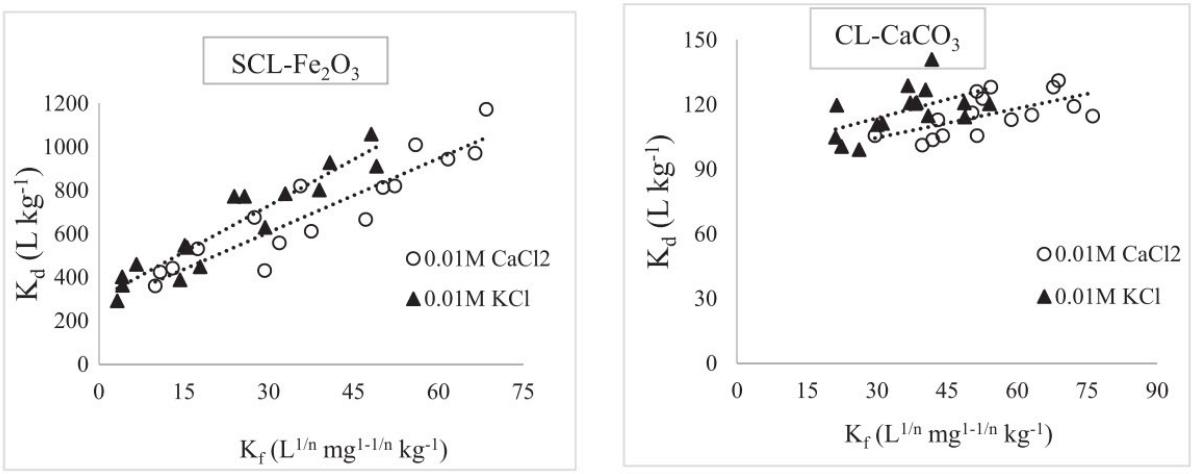
Olsen P concentrations significantly predicted glyphosate K_d (Figure 8.1.2.1-30) in both SCL- Fe_2O_3 and CL-$CaCO_3$. With both 0.01 M $CaCl_2$ and 0.01 M KCl, the slopes of the regressions predicting glyphosate K_d were significantly different between soils with the SCL- Fe_2O_3 showing steeper slopes than CL-$CaCO_3$ (Figure 8.1.2.1-30). Regardless of the solution used, the sorption of glyphosate was greater in SCL- Fe_2O_3 than CL-$CaCO_3$ be- cause of the importance of Fe_2O_3 in providing sorption sites for the negatively charged glyphosate in acidic soils. Research findings indicate that the presence of iron-oxide and soil pH had a stronger influence on glyphosate than phosphate sorption. The SCL- Fe_2O_3 soil contained 94% more Fe-oxides and 83% more Al-oxides than the CL-$CaCO_3$ soil (Table 8.1.2.1-73), and glyphosate sorption was greater in SCL-Fe_2O_3 soil because glyphosate sorption has been shown to be positively correlated with Fe/Al-oxides. In addition, glyphosate sorption was greater in SCL- Fe_2O_3 (pH 4.7 to 5) than CL-$CaCO_3$ (pH 7.3 to 7.5) soil because glyphosate sorption is negatively correlated with soil pH. This is because with increasing soil pH, an increasing portion of the glyphosate molecules become negatively charged with glyphosate molecules existing as HG^{2-} (~ 100%) (net negative charge of glyphosate is 2^-) at pH 7.3–7.5, and soil colloid deprotonation increases with soil colloids having a net negative charge in Prairie soils when soil pH > 6. Hence, regardless of the background electrolyte solutions, the sorption of glyphosate was always relatively low in the CL-$CaCO_3$ soil (Figure 8.1.2.1-30). Thus, the effect of Olsen P concentrations on reducing glyphosate sorption was more pronounced for SCL-Fe_2O_3 than CL-$CaCO_3$. For example, with 0.01 M KCl, glyphosate K_d was reduced by 39% when the phosphate concentration increased from 17 mg/kg (control) to 44 mg/kg (40P plots) in SCL-Fe_2O_3 but by only 11% when the phosphate concentration increased from 13 mg/kg (control) to 41 mg/kg (80P plots) in CL-$CaCO_3$.

Association between glyphosate Kd and phosphate Kf in relation to field-aged phosphate

Phosphate Kf and glyphosate Kd values were positively correlated (Figure 8.1.2.1-32). Thus, agreeing with previous studies suggesting phosphate and glyphosate have similar sorption pattern in soil. However, regardless of the background electrolyte solution, phosphate Kf and glyphosate Kd were more strongly correlated in SCL-Fe2O3 than CL-CaCO3. Hence, glyphosate and phosphate may compete more strongly for sorption sites in acidic soils with high Fe/Al-oxides content than in calcareous soils. In both soils and under both electrolyte background solutions, phosphate sorption was more strongly

reduced by Olsen P concentrations than glyphosate sorption was reduced by Olsen P concentrations. Thus, long-term application of phosphate fertilizer has an overall greater impact on reducing phosphate sorption than glyphosate sorption.

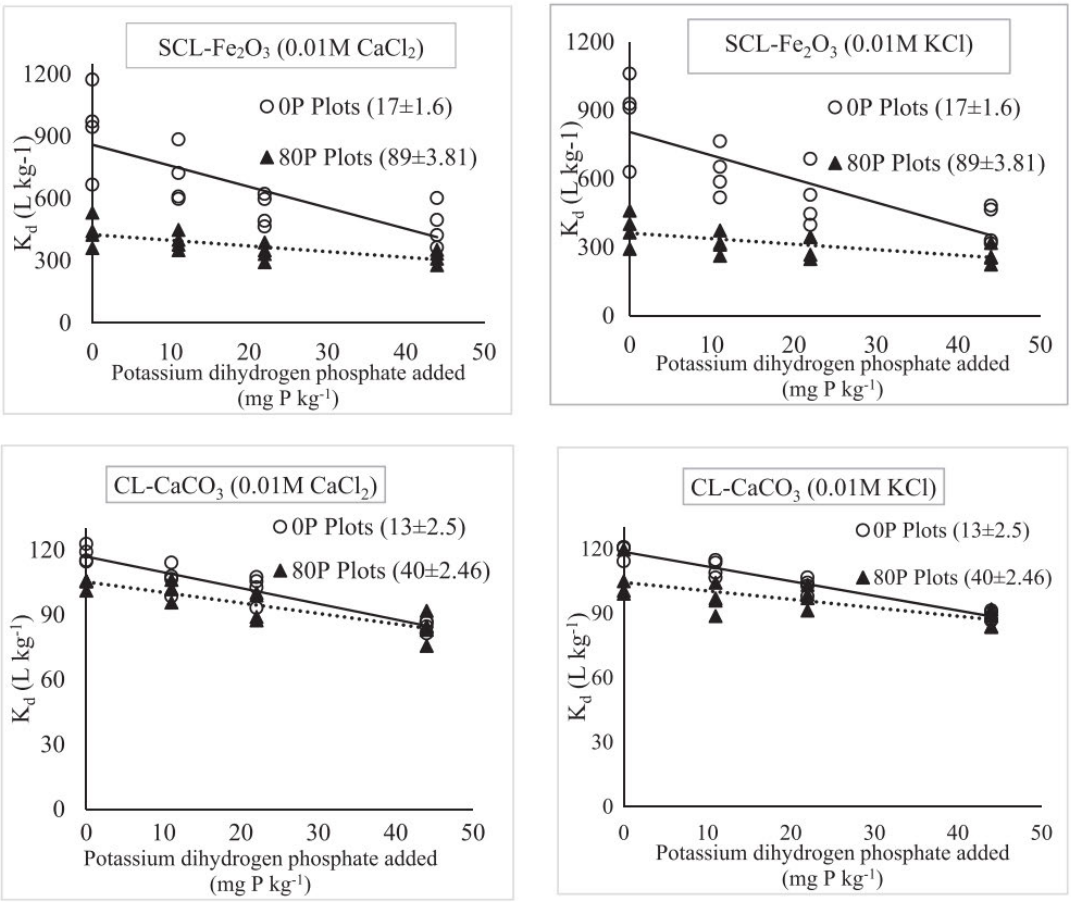
Figure 8.1.2.1-32: Association between glyphosate Kd and Phosphate Kf in SCL-Fe2O3 and CL-CaCO3 soils with sorption being determined by batch equilibrium experiments using 0.01 M CaCl2 or 0.01 M KCl as background electrolyte solutions. All regression equations are significant at P < 0.05



Effect of fresh phosphate addition on the sorption of glyphosate

Regardless of the background electrolyte solution and soil, the potassium dihydrogen phosphate additions to soil slurries significantly decreased glyphosate Kd values (Figure 8.1.2.1-33). Addition of fresh phosphate significantly reduced glyphosate sorption because the chemicals competed for the same sorption sites as they have similar phosphonate functional groups. Gimsing and Borggaard (2002) studied the competitive sorption effect of fresh phosphate on glyphosate in soil and concluded that phosphate is preferentially sorbed over glyphosate. In addition to this, sorption of phosphate lowers the zero point charge of sorption sites such as Fe/Al-oxides, potentially increases the net negative charge on the oxide surfaces and thereby increasing the electrostatic repulsion between glyphosate and soil oxides.

Figure 8.1.2.1-33: Effect of potassium dihydrogen phosphate concentrations on glyphosate sorption in SCL-Fe2O3 and CL-CaCO3 soils with low (0P) or high (80P) Olsen P concentrations. Potassium dihydrogen phosphate was added to glyphosate in soil slurries during batch equilibrium experiments using 0.01 M CaCl2 and 0.01 M KCl. All regression equations are significant at P < 0.05. The values in parentheses in each legend represent mean values of Olsen P and standard error



Fresh phosphate significantly predicted glyphosate Kd (Figure 8.1.2.1-33) in both SCL- Fe2O3 and CL-CaCO3. The regression slope was significantly steeper for 0P plots (control) than 80P plots in both soils and regardless of the background electrolyte solution (Figure 8.1.2.1-33). Thus, the effect of potassium dihydrogen phosphate addition in reducing glyphosate Kd values was less in soils that had greater Olsen P concentrations because less sorption sites were available for the added phosphate to compete with glyphosate molecules. This impact of phosphate already in soil was larger in SCL- Fe2O3 than CL-CaCO3 because in CL-CaCO3 soil at pH 7.3–7.5, glyphosate molecule existed as HG2− (~ 100%) leading to less sorption, both in the presence and absence of fresh phosphate. Thus, the competitive effect of phosphate on glyphosate is stronger in soils that are acidic and contain substantial amount of Fe-oxides than in calcareous soils.

Conclusion

The sorption of phosphate and glyphosate was reduced due to the long-term addition of phosphate fertilizer in two Prairie soils. The impact of Olsen P on reducing glyphosate sorption was more pronounced in the acidic (iron-oxide rich) sandy clay loam than the calcareous (calcium carbonate rich) clay loam soil, both with or without the addition of potassium dihydrogen phosphate. Regardless of the background electrolyte and soil type, phosphate sorption was more strongly reduced by the Olsen P concentrations than glyphosate sorption. The reduction of glyphosate sorption due to the application of potassium dihydrogen phosphate was greater in soils containing low Olsen P concentrations. The equilibrium phosphate concentration was above the threshold level for eutrophication only in soils that had exceptionally high phosphate concentrations i.e., the soils had received annual applications of mono ammonium phosphate at rates of 80 kg/ha for eight years. Commercially formulated glyphosate had no influence on phosphate sorption suggesting that glyphosate residues in soils have no impact on phosphate sorption or mobility.

Assessment and conclusion by applicant:

The article describes a sorption experiment with phosphate and glyphosate to Canadian agricultural soils. Some validation criteria of the underlying OECD 106 study protocol were not met, or insufficient information is reported (i.e. no material balance, stability of test item not demonstrated, no pre-equilibration of samples).

The article is therefore classified as reliable with restrictions and was. not used in risk assessment.

Assessment and conclusion by RMS:

Two soils from Canada were used for the tests performed in this study. The two soils come from long-term experimental plots. Among other significant deviations, the stability of test item was not tested and all of the radioactivity was presumed to be glyphosate (on the tests with glyphosate). The mass balances were not provided either, nor the efficacy of the analytical method.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Zhelezova, 2017

Data point:	CA 7.1.3.1.1/020
Report author	Zhelezova, A. et al.
Report year	2017
Report title	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides
Report No	DOI 10.1007/s11270-017-3392-7 ISSN 0049-6979
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:

The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/010). The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Cassigneul, 2016

Data point:	CA 7.1.3.1.1/021
Report author	Cassigneul, A. et al.
Report year	2016
Report title	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study
Report No	DOI 10.1016/j.scitotenv.2015.12.052 E-ISSN: 1879-1026
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:

The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/011). The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Munira, 2016

Data point:	CA 7.1.3.1.1/022
Report author	Munira, S. et al.
Report year	2016
Report title	Phosphate fertilizer impacts on glyphosate sorption by soil, Chemosphere 153 (2016) 471-477
Report No	DOI 10.1016/j.chemosphere.2016.03.028
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

This research examined the impact of field-aged phosphate and cadmium (Cd) concentrations, and fresh phosphate co-applications, on glyphosate sorption by soil. Soil samples were collected in 2013 from research plots that had received, from 2002 to 2009, annual applications of mono ammonium phosphate (MAP) at 20, 40 and 80 kg P/ha and from products containing 0.4, 70 or 210 mg Cd/kg as an impurity. A series of batch equilibrium experiments were carried out to quantify the glyphosate sorption distribution constant, Kd. Extractable Cd concentrations in soil had no significant effect on glyphosate sorption. Glyphosate Kd values significantly decreased with increasing Olsen-P concentrations in soil, regardless of the pH conditions studied. Experiments repeated with a commercially available glyphosate formulation showed statistically similar results as the experiments performed with analytical-grade glyphosate. Co-applications of MAP with glyphosate also reduced the available sorption sites to retain glyphosate, but less so when soils already contain large amounts of phosphate. Glyphosate Kd values in soils ranged from 173 to 939 L/kg under very strong to strongly acidic condition but the Kd was always <100 L/kg under moderately acidic to slightly alkaline conditions. The highest Olsen-P concentrations in soil reduced Kd values by 25 - 44% relative to control soils suggesting that, under moderately acidic to slightly alkaline conditions, glyphosate may become mobile by water in soils with high phosphate levels. Otherwise, glyphosate residues in agricultural soils are more likely to be transported off-site by wind and water-eroded sediments than by leaching or runoff.

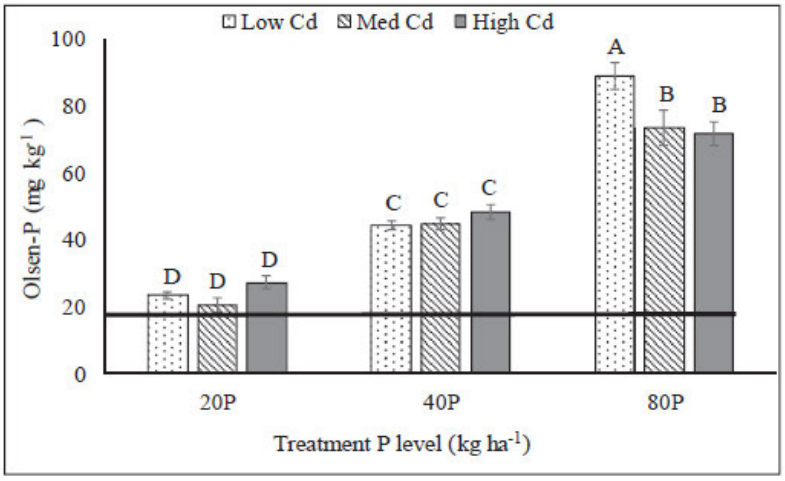
Materials and Methods

Experimental design and soil characteristics

Soil samples (0 -15 cm) with a sandy clay loam texture were collected in the spring 2013 from research plots situated under a durum wheat and flax rotation near Carman, Manitoba, Canada. The soil is classified as an Orthic Black Chernozem. The experimental plot was a randomized complete block design with 10 treatments and 4 replicates per treatment. In each of the forty plots, the composite soil sample consisted of ten samples collected in the plot using a Dutch augur. Treatments were a control (neither phosphate nor Cd applications), and plots receiving from 2002 to 2009 annual applications of mono ammonium phosphate (MAP) fertilizers that originated from three different phosphate rock sources containing 0.4, 70 or 210 mg Cd/kg, or low, medium and high Cd, respectively (Grant et al., 2013). MAP from these three sources was applied to plots at 20, 40 and 80 kg P/ha, or 20P, 40P and 80P, respectively. For all plots that received MAP, 20 kg P/ha was placed near the seed to enhance fertilizer use efficiency, a common practice in Canadian Prairie agriculture. For the 40 and 80 kg P/ha treatments, to avoid seedling toxicity, the additional MAP was broadcasted and then incorporated in

soil. From 2010 to 2013, the rotation was continued but no phosphate or Cd was applied. Nitrogen fertilizer varied by year to optimize yields. The typical rate of N applied was 90 kg N/ha in durum wheat and 50 kg N/ha in flax. Soil samples were air-dried and sieved (<2 mm) prior to soil properties analysis and sorption studies. Soil was digested with nitric acid and total Cd was determined by inductively coupled plasma (ICP). Extractable Cd was extracted with diethylene triamine pentaacetic acid (DTPA) ICP. Various factors have been shown to influence the efficiency of micronutrient extraction by DTPA, including extraction temperature and shaking time. Available phosphate was extracted using Olsen (NaHCO3) phosphorus test. Soil physical and chemical properties that are known to influence glyphosate and phosphate sorption by soil, but did not significant vary across the plots by treatment, were also determined. Soil organic carbon content was determined using combustion technique with a high temperature induction furnace. Extractable Fe2O3 and Al2O3 were extracted with DTPA and 0.01 M CaCl2, respectively, and extracts were analyzed by ICP. Extractable Ca was also measured by ICP using ammonium acetate as an extractant. Results were soil organic carbon content: 2.80% (mean) ±0.04 (standard error) (n = 16, number of plots analyzed); extractable Fe2O3: 246 ± 5 mg/kg (n = 40), extractable Al2O3: 6.4 ± 0.65 mg/kg (n = 16); and extractable Ca: 2252 ± 40.57 mg/kg (n = 16). Given that the study focused on Cd and P applications as treatments, the concentrations of extractable and total Cd, as well as Olsen-P in all plots were determined. We did not expect to see treatment differences for the other parameters that were measured (i.e., extractable Fe2O3, Al2O3, and Ca). Fe2O3 was also measured in all plots as previous studies have demonstrated that there is a strong positive association between Fe2O3 concentrations and phosphate or glyphosate sorption in soils. Since our results indicated no treatment differences induced by Cd and P applications on Fe2O3 concentrations extractable Al2O3, and Ca were quantified for 16 plots only (i.e., Control, 20P, 40P and 80P plots).

Figure 8.1.2.1-34: Effect of phosphate fertilizers with different Cd levels on Olsen P concentrations in soil. The solid line indicates the concentration of Olsen P in control plots

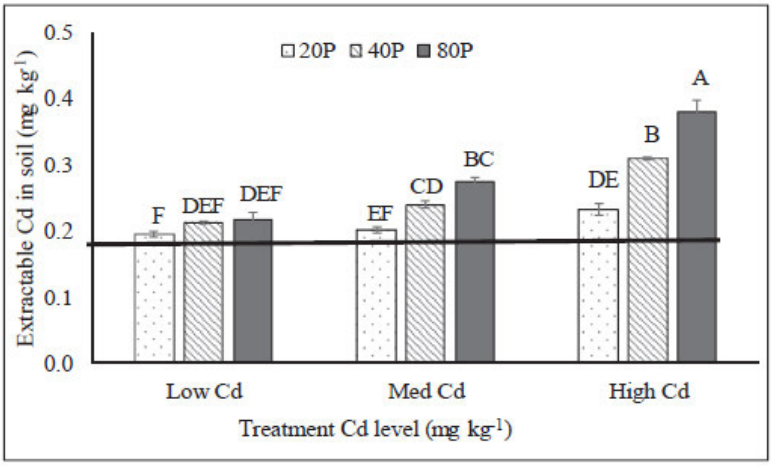


Sorption studies

Chemicals used in the sorption studies were: analytical grade ammonium phosphate monobasic (98% chemical purity) and glyphosate (99.9% purity), 14C-labelled glyphosate [phosphonomethyl-14C] (99% radiochemical purity; specific activity 50 µCi), and Roundup Ultra 2 (49% active). Active ingredient was potassium salt of N-(phosphonomethyl) glycine. Glyphosate sorption was determined by batch equilibrium with the initial solution containing 1 mg/L glyphosate and 6.67 x 104 Bq/L 14C-labelled glyphosate. Batch equilibrium procedures followed the OECD guideline 106 using a soil/solution ratio of 1:5 and an equilibrium time of 24 h (OECD, 2000). Initial solution was added to soil in centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark for 24 h. A constant 5°C temperature was utilized to minimize risks for biodegradation. Equilibrium solution was centrifuged and subsamples of supernatant were added in duplicated scintillation vials containing Scintisafe scintillation cocktail. Vials were lightly shaken and stored in the dark for 24 h to disperse the chemiluminescence before the radioactivity was measured. Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method). The glyphosate sorption distribution constant, Kd (L/kg)

was calculated by Cs/Ce, whereby Cs = glyphosate sorption by soil at equilibrium (mg/kg), and Ce = glyphosate concentration of equilibrium solution (mg/L). The effects of field-aged phosphate and Cd concentrations on glyphosate sorption were examined at pH conditions ranging from 3.6 to 7.3. This first experiment utilized soils from all forty plots and the range in pH was induced using different types of ions in the initial solution (0.01M HCl, 0.01M CaCl2, 0.01M KCl, 0.01M KOH or dH2O). For the control and high Cd 80P plots, the experiments were repeated but then using the Tier 2 parallel method with tubes being sampled at 1, 2, 4, 6, 8 and 24 h. The two subsequent experiments utilized soils from the plots labelled as low Cd and with 20P, 40P or 80P levels. In one experiment, for slurry pH conditions ranging from 3.6 to 7.3, batch equilibriums procedures were repeated but using Roundup Ultra 2 in 0.01 M HCl, 0.01 M CaCl2, 0.01 M KCl, 0.01 M KOH or dH2O to verify experimental results for a formulated product. In the other experiment, for slurry pH conditions range from 4.7 to 5.4, the effect of fresh phosphate additions on glyphosate sorption by soil was examined by adding analytical grade MAP to analytical glyphosate in 0.01 M CaCl2, 0.01 M KCl and dH2O solutions. The amounts of MAP added was equivalent to 11, 22 and 44 mg P/kg, or an estimated 20, 40 and 80 P kg/ha, respectively, assuming the fertilizer being present in the top 15 cm layer of a soil with a bulk density of 1200 kg/m3.

Figure 8.1.2.1-35: Effect of phosphate fertilizers with different Cd levels on DTPA-extractable Cd in soil. The solid line indicates the concentration of extractable Cd in control plots



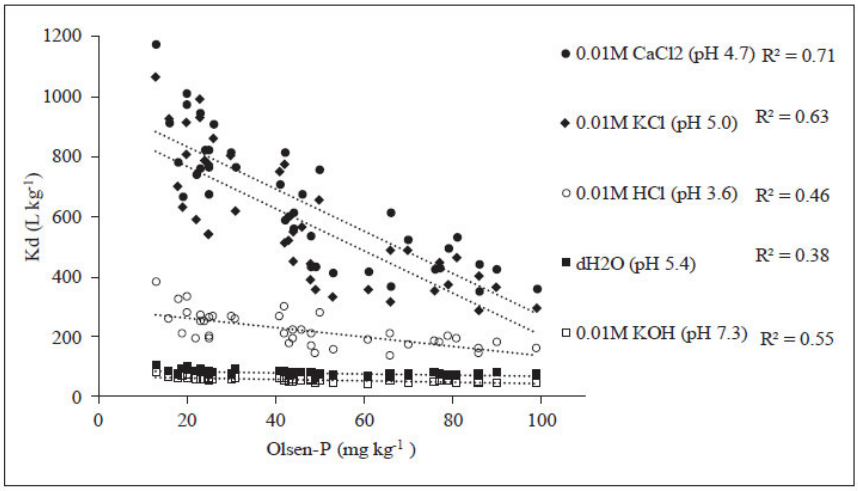
Statistical analyses were completed using SAS software version 9.3 for Windows. Two-way analysis of variance (ANOVA) and multiple means comparison (Tukey's) tests were conducted to determine the effect of phosphate fertilizer (20P, 40P, 80P) and Cd (low, medium, high) treatment on Olsen-P concentrations, extractable Cd concentrations and total Cd concentrations in soil. For each pH (ionic solution), multiple linear regression analyses were carried out to predict glyphosate Kd values by using Olsen-P and extractable Cd concentrations as independent variables. Repeated measure analysis was used to determine the effect of shaking time (0.5, 1, 2, 4, 6, 8 and 24 h) by using phosphate levels and time as independent variables. Two way ANOVA and multiple means comparison (Tukey's) tests were utilized to quantify the effects of field-aged (20P, 40P, 80P) and fresh phosphate additions (11, 22 and 44 mg P/kg) on glyphosate Kd values. One-way ANOVA and multiple means comparison (Tukey's) tests were applied to quantify the impact of using Roundup Ultra 2 versus analytical-grade glyphosate on Kd values in soils.

Results and Discussion

Glyphosate Kd values ranged from 43 to 1173 L/kg which is in agreement with glyphosate Kd values reported in agricultural soils. There were no significant differences in glyphosate sorption by soil when using either Roundup Ultra 2 or analytical-grade glyphosate, suggesting that other ingredients in the commercial formulation had no impact on the sorption behaviour of the active ingredient glyphosate in soil. The additions of MAP fertilizers from 2002 to 2009 had a significant effect on phosphate concentrations in 2013 (Figure 8.1.2.1-34). Olsen-P concentrations ranged from 13 to 99 mg/kg across plots and significantly decreased from 80P > 40 P > 20P plots. Total Cd concentrations in soil ranged from 0.42 to 0.98 mg/kg across plots but there were no significant treatment effects. Thus, the amount of Cd in the MAP fertilizers applied had no significant effect on the total Cd concentrations in 2013.

DTPA-extractable Cd concentration ranged from 0.19 to 0.41 mg/kg, within the typical range of 0.1 - 0.5 mg/kg reported for soils (International Cadmium Association, 2015). There was a significant interaction, between the rate of phosphate fertilizer applied and the amount of Cd that the phosphate fertilizer contained, on extractable Cd concentrations in soil (Figure 8.1.2.1-35). For the 80P plots, extractable Cd concentrations significantly decreased in the order of high Cd > med Cd > low Cd. For the 40P plots, extractable Cd concentrations significantly decreased in the order of high Cd > (med Cd = low Cd). In 20P plots, only the high and low Cd treatments had significantly different extractable Cd concentrations. Despite these significant differences, extractable Cd concentrations in soil had no significant influence on glyphosate Kd values. The Cd concentrations in our field plots are those typically encountered in agricultural soils, but we recognize that in a batch equilibrium experiment, Zhou et al. (2004) demonstrated that the co-application of exceptionally large quantities of Cd to glyphosate solutions (i.e., 562 mg Cd/kg soil) can increase glyphosate sorption by approximately 1.6 times fold, relative to control soil. Increased Olsen-P concentrations in soil was a significant factor ($P < 0.0001$) in the regression analysis to explain reduced glyphosate Kd values in soil.

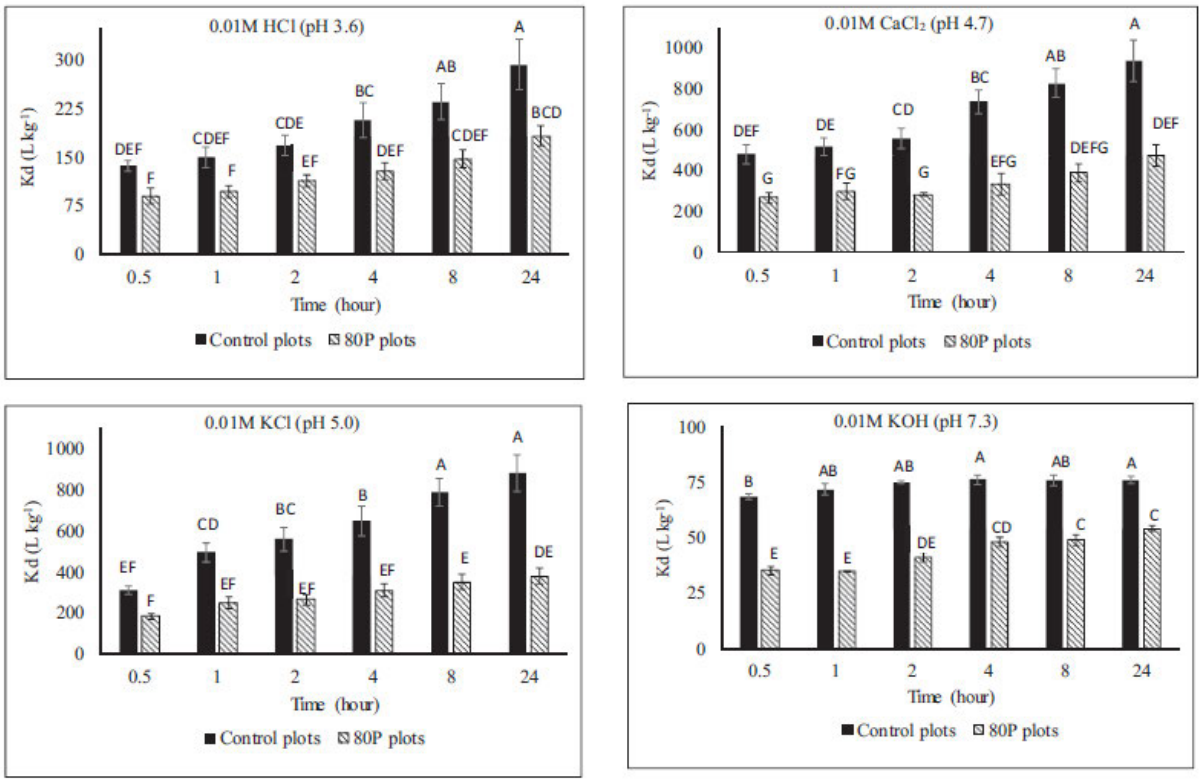
Figure 8.1.2.1-36: Relation between Olsen-P concentrations in soil and the glyphosate sorption distribution constant, Kd, with soil slurries being under different pH conditions. All regression equations are significant at P < 0.0001



Regardless of the ionic solution used in the batch equilibrium experiments, increased Olsen P concentrations significantly decreased glyphosate sorption by soil (Figure 8.1.2.1-36). A maximum reduction in glyphosate sorption occurred at a pH of 5 (0.01 M KCl solution) when the Olsen-P concentrations was on average 89 mg/kg Olsen P and the glyphosate Kd value was reduced by 57%, relative to the control plots that contained on average 18.75 mg/kg Olsen-P (Figure 8.1.2.1-36). Our results are in agreement with the findings of de Jonge et al. (2001) who also reported that field-aged phosphate in soil reduces glyphosate sorption by soil. The iron oxides content of the Orthic Black Chernozem used is within the range of that observed in other Prairie soils in Canada suggesting the competitive effect of phosphate on glyphosate sorption could be applicable to a wider range of soils in the Prairie region of Canada particularly with low pH and high Fe content. At pH 5.4, in both 80P and control, time had no significant effect on glyphosate Kd values and sorption was always significantly smaller in 80P than control plots. For all other pH conditions, glyphosate sorption approached equilibrium at approximately 8 h because there were no significant differences in glyphosate Kd values between 8 and 24 h (Figure 8.1.2.1-37). For these pH conditions, glyphosate sorption was almost always significantly smaller in 80P than control plots, regardless of the time, except for 0.5, 1 and 2 h under pH 3.6 and 0.5 h under pH 5.0 (Figure 8.1.2.1-37). In general, longer shaking hours resulted in greater numerically differences in glyphosate Kd values between control and 80P plots. Regardless of the ionic solution used (Figure 8.1.2.1-38), there was a significant interaction ($P < 0.01$) between field-aged and fresh phosphate on glyphosate sorption. In general, regardless of the amount of aged phosphate in soil, the addition of fresh MAP to the ionic solutions numerically reduced glyphosate Kd values, suggesting that phosphate and glyphosate compete for the same sorption sites in soil and that phosphate is preferentially sorbed when added with glyphosate to soil. Additions of 11 mg P/kg to the 0.01 M CaCl2

solutions had no significant effect on glyphosate Kd values, except in the 20 P plots containing relatively small Olsen-P concentrations (Figure 8.1.2.1-38). The addition of 22 or 44 mg P/kg to the 0.01 M CaCl2 solutions always significantly reduced glyphosate Kd values, except the addition of 22 mg P/kg to 80 P plots (Figure 8.1.2.1-38). For the largest co-application (44 mg P/kg), glyphosate Kd values were reduced on average by 52% in 20P plots, but by only 37% in the 80P plots. Additions of 11, 22 or 44 mg P/kg to 0.01 M KCl solutions always significantly reduced glyphosate Kd values except for 80 P plots for which only the addition of 44 mg P/kg resulted in a significant reduction in glyphosate Kd values (Figure 8.1.2.1-38).

Figure 8.1.2.1-37: Time dependent sorption study of glyphosate Kd values in control and 80P plots



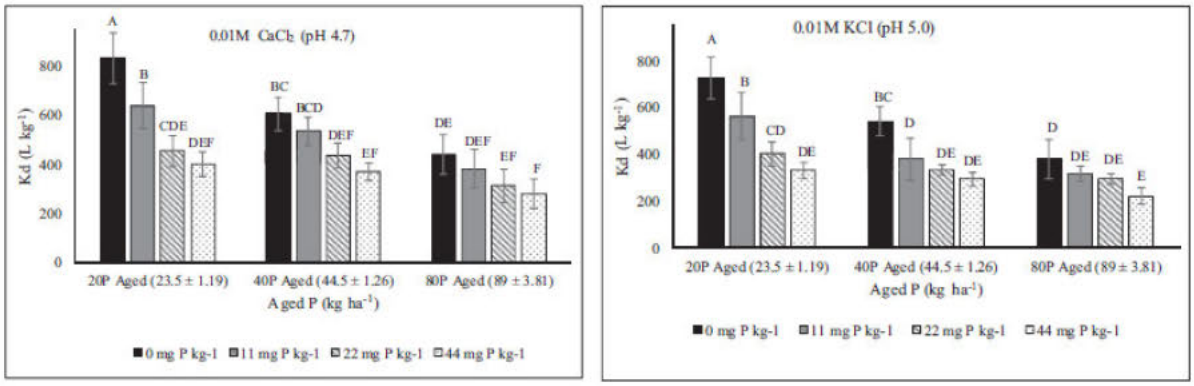
For the 44 mg P/kg co-application, glyphosate Kd values were reduced on average by 54% in 20P plots, but by 42% in the 80P plots. Thus, the largest impact of fresh MAP applications on reducing sorption sites for glyphosate occurred in soils with smaller field-aged phosphate concentrations because more sorption sites were available for competition in the plots that had low field-aged phosphate concentrations. In general, glyphosate Kd values were largest at pH 4.7 (0.01 M CaCl2) when glyphosate molecules mainly exist as H2G- (~85%) and HG2- (~15%), and at pH 5.0 (0.01M KCl) when glyphosate molecules mainly exist as H2G- (~75%) and HG2- (~25%). The soil used in this study had already a relatively large Ca2+ content (2252 ± 40.57 mg kg-1), and using 0.01 M KCl, would allow K+ to replace Ca2+ on the exchange site of organic-clay complexes which may interact with glyphosate forming stable complexes. Glyphosate Kd values were greater at pH 3.6 (0.01 M HCl), than pH 5.4 (dH2O) (Figure 8.1.2.1-36). At pH 3.6, a greater amount of soil colloids is net positively-charged, promoting the sorption of glyphosate molecules that mainly exist as H2G- (~95%) and H3G (~5%). Sorption was less at pH 5.4 than at pH 3.6 because the amount of negatively- charged soil colloids increases with soil pH, and glyphosate molecules mainly exist as H2G- (~60%) and HG2- (~40%) at pH 5.4. The lowest sorption was observed at pH 7.3 (0.01 M KOH), as the negatively charged soil colloids increased and glyphosate molecules existed as HG2- (~100%).

Conclusion

Analytical-grade glyphosate showed similar results as a commercially-available glyphosate formulation. Long-term additions of phosphate fertilizers to soils will reduce the capacity of the soil to bind

glyphosate under a wide range of pH conditions, but the impurities of Cd in these fertilizers have no impact on glyphosate sorption.

Figure 8.1.2.1-38: Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch equilibrium experiments using 0.01 M CaCl2 (pH 4.7) and 0.01 M KCl (pH 5.0)



Fresh applications of phosphate fertilizers to most soils will significantly reduce the availability of sorption sites for glyphosate. However, this reduction in sorption site availability will be small in soils that have exceptionally high phosphate levels and do not have many sorption sites available for phosphate or glyphosate. Cd concentrations typically found in agricultural fields are not high enough to influence the binding capacity of glyphosate in soil.

Assessment and conclusion by applicant:

The article describes a sorption experiment with glyphosate on a Canadian agricultural soil considering different treatments with phosphate fertilizer. Some information on soil and study design are not reported (i.e. soil characteristics, mass balances, amount of soil, no information on chromatographic methods used, stability of test item not demonstrated), so no final validity check is possible.

The article is therefore classified as reliable with restrictions. Therfore, data were not used in risk assessment.

Assessment and conclusion by RMS:

This article focuses on the impact of phosphate fertilizer on the sorption of glyphosate. Some information are missing to assess the validity of the study against OECD 106 criteria: information on soils tested, on stability of the compound, on efficacy of the analysis and on mass balance. For information, the adsorption of glyphosate was found to be pH dependant in this article, with lower adsorption at lower pH values.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Sidoli, 2016

Data point:	CA 7.1.3.1.1/023
Report author	Sidoli, P. et al.
Report year	2016
Report title	Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules
Report No	DOI 10.1007/s11356-015-5796-5 E-ISSN 1614-7499
Guidelines followed in study	OECD Guideline 106 (2000)

Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Adsorption of the herbicide glyphosate and its main metabolite AMPA (aminomethylphosphonic acid) was investigated on 17 different agricultural soils. Batch equilibration adsorption data are shown by Freundlich adsorption isotherms. Glyphosate adsorption is clearly affected by equilibration concentrations, but the nonlinear AMPA adsorption isotherms indicate saturation of the adsorption sites with increasing equilibrium concentrations. pHCaCl2 (i.e. experimental pH) is the major parameter governing glyphosate and AMPA adsorption in soils. However, considering pHCaCl2 values, available phosphate amount, and amorphous iron and aluminium oxide contents by using a nonlinear multiple regression equation, obtains the most accurate and powerful pedotransfer rule for predicting the adsorption constants for these two molecules. As amorphous iron and aluminium oxide contents in soil are not systematically determined, we also propose a pedotransfer rule with two variables—pHCaCl2 values and available phosphate amount—that remains acceptable for both molecules. Moreover, the use of the commonly measured pHwater or pHKCl values gives less accurate results compared to pHCaCl2 measurements. To our knowledge, this study is the first AMPA adsorption characterization for a significant number of temperate climate soils.

Materials and Methods

Soil properties

Seventeen surface top soils were sampled in different agricultural plots with variable land uses and fertilization practices under intensive agriculture. The sample site is located in a quaternary fluvio-glacial corridor near Lyon in southeastern France. They are loamy to sandy-loamy soils, characterized by a decarbonation state on surface and large amounts of amorphous iron and aluminium oxides issued from the weathering of primary minerals (Table 8.1.2.1-75). Fresh soil samples were air-dried, sieved to 2 mm, and stored in the dark at 4 °C, before measuring their physicochemical properties. Crystallized oxy-hydroxides (Fe_{DCB} and Al_{DCB}) were extracted by the Mehra-Jackson method (1960), and amorphous oxy-hydroxides (Fe_{ox} and Al_{ox}) by the Tamm method (1992) (Table 8.1.2.1-75). The experimental 1:5 soil pH_{CaCl2}, hereafter referred to as 'pH_{CaCl2}', was measured in batch supernatants with a pH microelectrode (Inlab Flex-Micro). These soils showed wide ranges of pH_{CaCl2} (5.1 to 7) and clay content (8.9 to 15.3 %) and, except soil 11, contained less than 2 % organic carbon (Table 8.1.2.1-75).

Chemical reagents and analysis

Glyphosate adsorption was studied with its 14C-radiolabeled form (phosphonomethyl-14C)-glyphosate (4.36 MBq/mg, radiochemical purity 96.32 %) purchased from Izotop (Hungary). Unlabeled solid glyphosate and AMPA products (purity ≥98 %) were purchased from Dr Ehrenstorfer (CIL Cluzeau, Sainte-Foy la Grande, France). Stock solutions were prepared in MilliQ water (storage at 4 °C for 1 month).

Table 8.1.2.1-75: Physicochemical properties of studied soils. Crystallized oxy-hydroxides (Fe_{DCB} and Al_{DCB}) and amorphous oxy-hydroxides (Fe_{OX} and Al_{OX}) were extracted by the Mehra-Jackson method (1960) and the Tamm method, respectively

Soil	pH_{CaCl2}	pH_{water}	pH_{KCl}	Organic C (g kg^{-1})	Olsen P (g kg^{-1})	Al_{DCB} (g kg^{-1})	Fe_{DCB} (g kg^{-1})	Al_{ox} (g kg^{-1})	Fe_{ox} (g kg^{-1})	Clay %	Silt %	Sand %	CEC (meq 100 g^{-1})
1	6.8	7.7	6.8	14.1	0.10	2.1	9.9	1.7	3.1	12.3	36.4	49.1	7.6
2	5.1	6.1	4.9	14.0	0.05	11.1	2.7	2.2	2.7	15	37.3	45.5	8.3
3	5.8	6.5	5.7	13.0	0.10	2.4	10.9	2.0	2.9	15.1	39.1	43.6	8.2
4	5.9	6.9	6.0	13.6	0.07	2.6	11.2	1.9	2.8	14.9	38.1	45	8.1
5	5.9	7.5	6.6	16.0	0.09	2.5	12.8	2.0	2.7	15.3	41.5	40.8	8.2
6	5.8	7.0	6.1	14.7	0.07	2.4	11.5	1.9	2.7	15	40.3	42.5	8.3
7	5.9	6.9	6.1	13.4	0.08	2.2	11.5	1.8	2.6	15	39	44.2	8.4
8	6.1	7.2	6.3	8.3	0.11	1.8	8.8	1.5	2.6	11.4	34.3	52.8	6.0
9	6.2	7.3	6.5	14.8	0.06	2.5	13.5	2.1	3.2	15.4	36.3	45.7	8.2
10	5.5	6.7	5.8	17.3	0.06	2.5	11.5	2.0	2.9	14.8	35.6	47.3	7.7
11	7.0	8.0	7.2	23.1	0.20	1.5	8.6	1.2	3.0	11.8	42.3	42.8	9.6
12	6.1	7.0	6.1	16.1	0.08	2.1	11.8	1.9	3.0	13.9	34.1	49.5	8.2
13	5.1	6.3	5.4	12.9	0.04	10.6	2.1	1.8	2.8	14.6	40	43.8	6.6
14	6.2	7.3	6.5	9.2	0.08	1.6	6.8	1.3	2.2	8.9	31.5	58.1	5.0
15	6.3	7.4	6.5	9.1	0.12	1.6	6.9	1.4	2.1	8.9	29.5	60.4	5.6
16	5.4	6.5	5.6	7.2	0.11	1.8	7.7	1.5	2.5	9.7	30.6	58.3	4.4
17	5.9	6.7	5.9	15.0	0.14	2.0	10.4	1.7	2.7	12.7	32.4	52.7	7.6
Mean	5.9	7.0	6.1	13.6	0.09	3.1	9.3	1.8	2.7	13.2	36.4	48.4	7.4
Standard deviation	0.5	0.5	0.5	3.7	0.04	2.8	3.2	0.3	0.3	2.2	3.7	5.9	1.4
Min	5.1	6.1	4.9	7.2	0.04	1.5	2.1	1.2	2.1	8.9	29.5	40.8	4.4
Max	7.0	8.0	7.2	23.1	0.20	11.1	13.5	2.2	3.2	15.4	42.3	60.4	9.6

The glyphosate concentration was obtained by measuring 14C-glyphosate activity, which was counted with a liquid scintillation analyzer (Packard Tricarb® 2300TR). After adding a scintillator (Aquasafe 300 Plus, Zinsser Analytic), the radioactivity was measured in 2 mL of supernatant. The minimal measured 14C-glyphosate radioactivity is 30 dpm/mL which corresponds to 0.09 µg/L.

AMPA analysis was done on an Acquity ultra-performance liquid chromatography system (UPLCTM, Waters) interfaced to a triple quadrupole mass spectrometer (Quattro Premier XE, Waters). Due to its low molecular weight, a derivatization step with FMOC-chloride in the presence of a borate buffer is required prior to analysis. Extraction is done online with an SPE cartouche (Oasis HLB 25 µm 2.1×20 mm) before separation in an Acquity UPLC HSS column (T3 1.8 µm×2.1 mm×100 mm). The quantification limit is 0.05 µg/L.

Isotherm adsorption coefficients (Kf)

Sorption experiments were run according to a normalized method (OECD guideline 106, 2000) with a 1/5 soil-weight/solution-volume ratio in 15-mL centrifuge plastic tube. Equilibrium - tested with a 1 mg/L solution - was obtained after 24 h. After 12 h of pre-equilibration with a CaCl2 solution (0.01 M), the equilibrated soil suspensions were spiked with a pesticide solution and agitated during 24 h (darkness, 20 °C). After centrifugation (3000 rpm, 30 min, 20 °C), the supernatants were filtrated with 0.2 µm cellulose acetate and analyzed for pesticide concentrations. Blanks (each soil without spiking) did not reveal any presence of either molecule in the soils before the experiments. No adsorption was measured on tubes and filters used for batch experiments. The adsorption isotherm was obtained by the relationship between adsorbed concentration per weight (Cs, mg/kg) compared to the equilibrium concentration per volume of solution (Ce, mg/L) according to the Freundlich equation. Six solute concentrations were tested, 0.05, 0.2, 0.5, 1.0, 3.0 and 5.0 mg/L, for both glyphosate and AMPA. For glyphosate, which was studied with its 14C radiolabeled form, the initial radioactivity was 6000 dpm/mL in tubes. The experiments were run as triplicates. The Freundlich parameters Kf and 1/nf were estimated by using a nonlinear fitting programme (XLStat, Excel 5.0).

Parametric linear and nonlinear regression for pedotransfer rule determination

The relationship between the Kf parameter and soil properties was studied for each pesticide (XLStat, Excel 5.0) by multiple linear and nonlinear regression analyses.

Results and discussion

Freundlich adsorption isotherms

The Freundlich isotherm equation adjusts accurately the experimental data (R^2>0.99). High experimental glyphosate K_f values, $K_{f\text{-}exp}$, were obtained, ranging between 32 and 540 mg/kg(L/mg)$^{-nf}$ in agreement with previous studies. In the case of AMPA, $K_{f\text{-}exp}$ values between 33 and 392 mg/kg (L/mg)$^{-nf}$ are in the same high adsorption range as glyphosate.

Table 8.1.2.1-76: Experimental Freundlich isotherm coefficients $K_{f\text{-}exp}$ (mg kg^{-1} (L mg $^{-1}$)$^{-nf}$) and $1/n_{f\text{-}exp}$ (-) for glyphosate and AMPA, and K_f recalculated for averaged $1/n_{f\text{-}exp}$ glyphosate ($1/n_{f\text{-}avg}$ = 0.93) and AMPA ($1/n_{f\text{-}avg}$ = 0.78)

Soils	Glyphosate					AMPA				
	$K_{f\text{-}exp}$	$1/n_{f\text{-}exp}$	R^2	K_f (for $1/n_{f\text{-}avg}$)	R^2	$K_{f\text{-}exp}$	$1/n_{f\text{-}exp}$	R^2	K_f (for $1/n_{f\text{-}avg}$)	R^2
1	34	0.86	1.00	36	1.00	63	0.74	1.00	67	1.00
2	540	0.97	0.99	475	1.00	392	0.80	1.00	365	1.00
3	143	0.93	1.00	143	1.00	140	0.72	1.00	162	1.00
4	92	1.09	1.00	73	0.99	212	0.79	1.00	206	1.00
5	135	0.92	0.99	139	1.00	226	0.81	1.00	208	1.00
6	132	0.90	0.99	140	0.99	191	0.80	1.00	183	1.00
7	101	0.90	1.00	106	0.99	143	0.75	1.00	154	1.00
8	99	0.94	1.00	97	1.00	119	0.81	1.00	111	1.00
9	107	0.91	1.00	110	1.00	158	0.81	0.99	147	0.99
10	233	0.94	1.00	230	1.00	242	0.77	1.00	253	1.00
11	32	0.83	1.00	34	1.00	33	0.78	1.00	33	1.00
12	91	0.94	1.00	89	1.00	134	0.82	1.00	122	1.00
13	285	0.99	0.99	243	0.99	284	0.79	1.00	210	1.00
14	42	0.98	1.00	40	1.00	91	0.80	1.00	87	1.00
15	41	0.88	1.00	43	1.00	56	0.73	1.00	61	1.00
16	233	0.94	1.00	229	1.00	198	0.78	1.00	198	1.00
17	111	0.97	1.00	103	1.00	107	0.82	1.00	99	1.00
Min	32	0.83		34		33	0.72		33	
Max	540	1.09		475		392	0.82		365	
Mean	144	0.93		137		164	0.78		157	
Standard deviation	121	0.06		106		88	0.03		79	

Table 8.1.2.1-77: Glyphosate and AMPA K_f coefficients calculated by multiple nonlinear regression from Eq. (2): $Kf = C\, e \sum_{i=1}^{n} a_i X_i$

Soil variables X_n	Constant	pH measurements			Olsen P (g kg^{-1})	Al_{ox} (g kg^{-1})	Fe_{ox} (g kg^{-1})	R^2
		1:5 soil pH_{CaCl2}	pH_{KCl}	pH_{water}				
Regression coefficient values	C	a_1	a'_1	a''_1	a_2	a_3	a_4	
4 variables (pH, Olsen P, Al_{ox}, Fe_{ox})								
Glyphosate	**5.1×10^5**	**−1.7**			**−1.1**	**−0.4**	**1.0**	**0.94**
	3.6×10^5		−1.25		−5.6	−0.1	0.1	0.65
	1.3×10^6			−1.30	−7.3	−0.4	0.3	0.61
AMPA	**1.8×10^4**	**−0.9**			**−4.4**	**0.6**	**−0.1**	**0.92**
	3.6×10^3		−0.50		−5.4	1.3	−0.7	0.81
	3.8×10^3			−0.40	−5.4	1.3	−0.8	0.78
2 variables (pH, Olsen P)								
Glyphosate	1.9×10^6	−1.6			−2.6			0.88
	3.5×10^5		−1.24		−5.3			0.65
	6.7×10^5			−1.17	−6.0			0.60
AMPA	6.1×10^4	−0.9			−7.7			0.88
	2.1×10^4		−0.7		−9.6			0.73
	3.2×10^4			−0.7	−9.8			0.70
1 variable (pH)								
Glyphosate	1.33×10^6	−1.59						0.82
	2.23×10^5		−1.24					0.61
	2.74×10^5			−1.12				0.51
AMPA	4.67×10^4	−0.97						0.69
	1.18×10^4		−0.63					0.37
	1.18×10^4			−0.63				0.37

For both molecules, the pedotransfer rule including four variables and the simplified rule including two or one variable(s) are calculated for pH_{CaCl2}, pH_{KCl} or pH_{water}. Value parameters corresponding to the pedotransfer rule including the four pH_{CaCl2}, Olsen P, Al_{ox} and Fe_{ox} variables are indicated in bold

These values are consistent with those obtained by Baez *et al.* (2015) for molisols and alfisols. Experimental values of the $1/n_{f\text{-}exp}$ coefficients vary between 0.83 and 1.09 for glyphosate, and between 0.72 and 0.82 for AMPA. As the $1/n_{f\text{-}exp}$ values are different, the glyphosate and AMPA $K_{f\text{-}exp}$ datasets cannot be compared directly. Indeed, even if the $K_{f\text{-}exp}$ value is similar, the isotherm can be very different because of the $1/n_{f\text{-}exp}$ value. For each molecule, the $1/n_{f\text{-}exp}$ values had a low standard deviation, allowing to calculate an average $1/n_{f\text{-}exp}$ value, i.e. $1/n_{f\text{-}avg}$, of 0.93 (±0.06) and 0.78 (±0.03) for glyphosate and AMPA, respectively. New $K_{f\text{-}}$ Freundlich coefficients were recalculated for each soil by using this averaged $1/n_{f\text{-}avg}$ value. For both molecules, this second fit to the Freundlich equation is very precise ($R^2\geq0.99$) and allows the comparison between soils. For AMPA, the $1/n_{f\text{-}avg}$ value of less than one (i.e. 0.78) indicates that adsorption is strongly limited by the availability of sorption sites. However, the high glyphosate $1/n_{favg}$ value of 0.93 means that adsorption is less governed by the availability of adsorption sites than AMPA. Therefore, despite the similar atomic composition of glyphosate and AMPA, they probably do not sorb in the same way onto the studied soils.

Pedotransfer rule for glyphosate and AMPA adsorption prediction

Regression analysis was restricted to soils with a higher experimental pH_{CaCl2} than both glyphosate pKa_3 and AMPA pKa_2, i.e. $pH_{CaCl2}>5.4$. This limitation allowed defining adsorption rule when the same ionic form of either glyphosate or AMPA dominates in solution. Thus, soils 2 and 13 (pH_{CaCl2} values=5.1) were excluded from the data analysis. First, a linear multiple regression was tested to relate K_f to every combination of measured soil properties, but the adjustment accuracy was very weak ($R^2<0.75$). In our study, nonlinear consideration sharply improves the fit of both glyphosate and AMPA K_f ($R^2>0.92$). Of the ten variables studied, nonlinear regression analysis appears optimized when considering the four variables: pH_{CaCl2} value, available phosphate, and amorphous aluminium and iron oxide amount, for both glyphosate and AMPA K_f adsorption coefficients. As in earlier studies, the highest correlation was found between glyphosate K_f and pH - in our study pH_{CaCl2} - (Table 8.1.2.1-78). For the pH_{CaCl2} here studied (between 5.4 and 7.0), deprotonation of the phosphonic group results in the dominant glyphosate net-2^- (2^-) and dominant AMPA net-1^- charged (1^-) forms (Figure 8.1.2.1-40). For soils with high pH

values, high repulsion forces with negative charges act on the amorphous oxide surfaces and sorption is reduced. In the regression analysis, both glyphosate and AMPA K_f values positively correlate with amorphous iron and aluminium oxides (Table 8.1.2.1-78), with higher correlations calculated for amorphous aluminium oxides that probably are more reactive in the studied soils. A negative correlation between available phosphate and glyphosate K_f values is observed (Table 8.1.2.1-78) where both molecules compete for the same adsorption sites on oxide surfaces, thus reducing glyphosate adsorption in the presence of phosphate (Gimsing *et al.* 2004b).

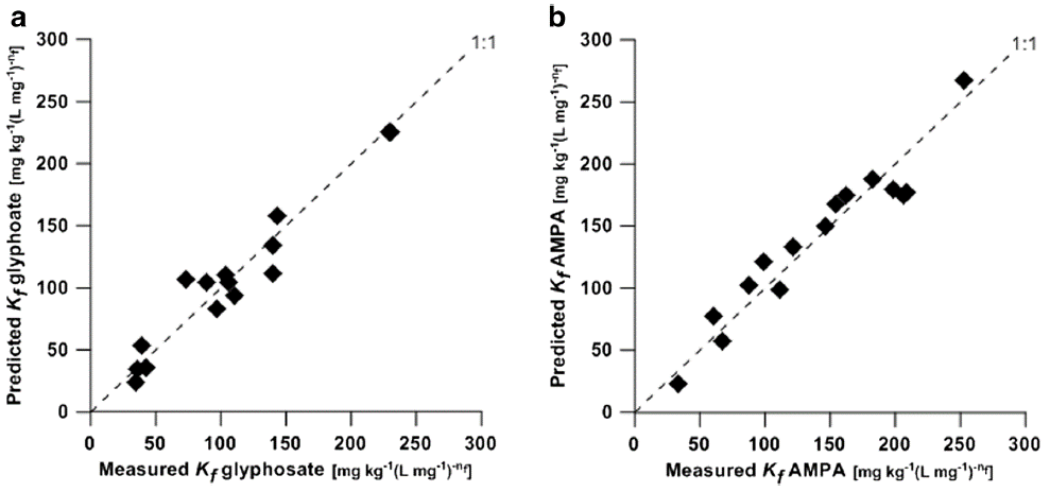
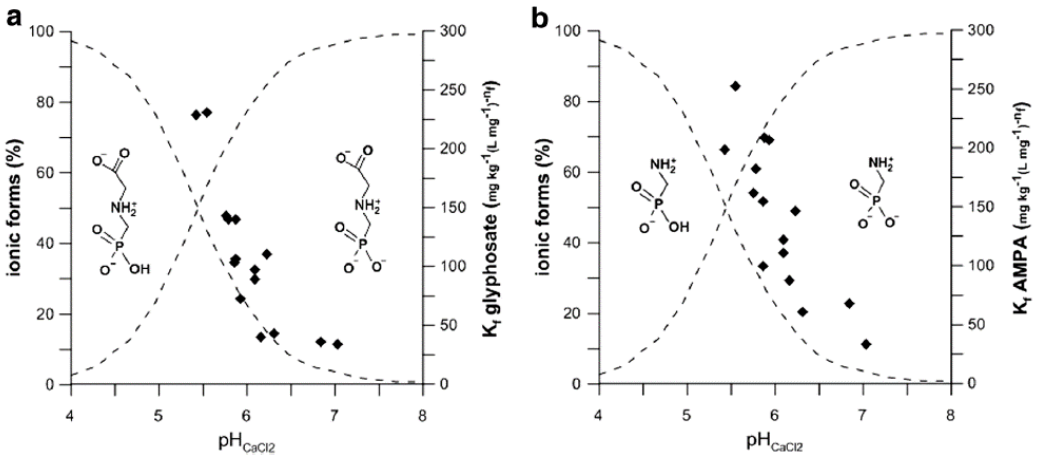


Figure 8.1.2.1-39: Comparison between measured and predicted Kf coefficients with a nonlinear pedotransfer rule for a glyphosate (with l/nf-avg = 0.93) and b AMPA (with l/nf-avg = 0.78). The dotted lines represent a 1 to 1 straight line. Glyphosate and AMPA pedotransfer rules include pH CaCl2, Olsen P, AlX and FeOX variables

A negative correlation between AMPA K_f values and available phosphate suggests a similar competition for adsorption on oxide surface sites by reducing AMPA sorption when phosphate is present. The more strongly negative correlation between phosphate and K_f values for AMPA than for glyphosate (Table 8.1.2.1-78) indicates a higher competitive adsorption between phosphate and AMPA than glyphosate.

To evaluate the sensitivity of the variables affecting the pedotransfer rule, the number of variables was initially reduced to two dominant parameters, i.e. pH_{CaCl2} and available phosphate amount. The resulting equation explains 88 % of the variations in the K_f of glyphosate and AMPA (see R^2). Considering only variable pH_{CaCl2} - the most significant of all four variables – decreases the accuracy adjustment for AMPA (R^2 0.69), whereas that for glyphosate is only slightly modified (R^2 0.87). Thus, it seems possible to arrive at an acceptable estimate of glyphosate adsorption with an equation with just one variable, i.e. pH_{CaCl2}, whereas for AMPA, the two variables pH_{CaCl2} and available phosphate amount are needed.

Figure 8.1.2.1-40: Distribution of Freundlich coefficients Kf as a function of pHCaCl2 and dominance of dissociated glyphosate forms (a) and dissociated AMPA forms (b) in solution. Bjerrum diagram taken from (Sheals et al. 2002) for glyphosate (a) and same diagram suggested as hypothesis for AMPA (b). Glyphosate pKa_3 = 5.46 (Tomlin 1997), AMPA pKa_3 = 5.4 (Chen et al. 2009)



Constraints in applying pedotransfer adsorption equations

To verify the accuracy of the proposed sorption multiple regression, as Paradelo *et al.* 2015 showed that this might be dependent upon the study site, we collected published data concerning pedotransfer rules for testing them in our model. To our knowledge, AMPA adsorption instead of glyphosate sorption is rarely described in the literature. Nevertheless, none of the published work describes all four variables - pH_{CaCl2}, available phosphate, and amorphous iron and aluminium contents - for several soils. We thus carried out an in-depth study on the effect of the pH-measuring method on predicting the glyphosate K_f The parameters for the adsorption equations with four and two variables, or even one variable, were recalculated for pH_{water} or pH_{KCl} values, as these are more commonly measured parameters than experimental pH_{CaCl2}. We then did the same work for AMPA equations as a comparison. The choice of pH clearly affected the accuracy of an equation with four variables, as R^2 varied from 0.94 with pH_{CaCl2} to ≤0.65 with pH_{water} and pH_{KCl} for glyphosate, and from 0.92 to ≤0.81 for AMPA. This decrease in the adjustment accuracy was obviously also noted for regressions with two variables - R^2 going from 0.88 to ≤0.65 for glyphosate and from 0.88 to ≤0.73 for AMPA—and one variable (R^2 going from 0.88 to ≤0.61 for glyphosate and from 0.69 to ≤0.37 for AMPA). Glyphosate K_f coefficients are much more affected by the pH measurement method than those of AMPA, but the pH variable in exponential glyphosate equations is systematically associated with higher correlation coefficients than in the AMPA ones (Table 8.1.2.1-78). These results clearly show that the type of pH measurement plays a crucial role for the prediction of glyphosate and AMPA adsorption coefficients. Since no simple relationship can be established between experimental pH_{CaCl2} and pH_{water} (R^2 0.76) or pH_{KCl} (R^2 0.80), a model validation for glyphosate cannot be based on available published data.

Table 8.1.2.1-78: Correlation matrix of Kf glyphosate ($1/n_{f\text{-}avg}$ = 0.93) and K_f AMPA ($1/n_{f\text{-}avg}$ = 0.78) with soil parameters by multiple nonlinear regression

Correlation matrix of K_f glyphosate ($1/n_{f\text{-}avg}$=0.93) and K_f AMPA ($1/n_{f\text{-}avg}$ =0.78) with soil parameters by multiple nonlinear regression

Variables	pH $_{CaCl2}$	Olsen P (%)	Al_{ox} (%)	Fe_{ox} (%)
K_f glyphosate	−0.83	−0.36	0.43	0.05
K_f AMPA	−0.82	−0.67	0.68	0.10

Conclusions

High adsorption coefficients calculated for glyphosate and AMPA molecules depend upon the experimental $pHCaCl_2$ value (1:5 soil/solution), the available phosphate content, and the amorphous aluminium- and iron oxide contents. These four key soil parameters combined in an exponential regression equation provide a precise description of a pedotransfer rule for Kf prediction. To our

knowledge, our AMPA dataset contains the first published data for adsorption on a significant number of natural soils. Because of low 1/nf values, prediction of the AMPA Kf is strongly related to the soil-solution concentration, contrary to glyphosate.

Changes in pH strongly affect adsorption by modifying the ionic state of glyphosate and AMPA and the available amorphous oxide surface sorption sites. Phosphate competes with both molecules for adsorption, but more strongly with AMPA. Considering only the two variables, pH_{CaCl2} and available phosphate content, leads to a satisfactory prediction of the adsorption constants. For both molecules, pH_{CaCl2} is the most reliable explanatory variable. However, pH_{CaCl2} is only rarely measured during batch experiments, even though most of such experiments use $CaCl_2$ as the solute. Replacing pHCaCl2 by pH_{KCl} or by pHwater - that are more frequently measured in soils - as the variable in the pedotransfer rule does not allow adjusting Kf with sufficient precision. Since simple relation does not exist between pH_{KCl} and pH_{CaCl2} - or between pHwater and pH_{CaCl2} - a complementary soil characterization with pH_{CaCl2} value therefore appears necessary for the application of the pedotransfer rule. However, such a measurement is easier and faster than the implementation of sorption experiments. The acquisition of supplementary data on various soils will lead to a better validation of the pedotransfer rules for glyphosate and AMPA. Finally, the strong adsorption observed in the studied soils, which are rather depleted in organic carbon, takes place on mineral fractions. Hence, the—little studied—geological materials present in the unsaturated zone might also strongly adsorb glyphosate and AMPA. The adsorption of these molecules on such materials should thus be studied as well.

Assessment and conclusion by applicant:

The article describes batch adsorption experiments with glyphosate and AMPA on 17 soils from France. The OECD 106 guideline was considered. However, not all parameters were reported to check the validity of the study (i.e. no material and mass balances established, stability of test item not reported, chromatographic method for analysis of glyphosate not reported).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

The lack of valuable information to determine the validity of the study (as stated by the applicant, mass balance, stability of the test items, efficacy of the method) prevents its use for deriving endpoints for the risk assessment. The article however focuses on the pH dependency of the Kf of glyphosate and AMPA with a significant correlation observed with pH_{CaCl2} for glyphosate (based on data from 17 soils). No correlation was observed between Kf and pH_{KCl} or pH_{H2O}.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Dollinger, 2015

Data point:	CA 7.1.3.1.1/024
Report author	Dollinger, J. et al.
Report year	2015
Report title	Glyphosate sorption to soils and sediments predicted by pedotransfer functions
Report No	DOI 10.1007/s10311-015-0515-5 ISSN 1610-3653
Guidelines followed in study	None
Deviations from current test guideline	Not relevant, modelling study with no experimental data determined
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate is the most applied herbicide for weed control in agriculture worldwide. Excessive application of glyphosate induces water pollution. The transfer of glyphosate to freshwater and groundwater is largely controlled by glyphosate sorption to soils and sediments. Sorption coefficients are therefore the most sensitive parameters in models used for risk assessment. However, the variations in glyphosate sorption among soils and sediments are poorly understood. Here we review glyphosate sorption parameters and their variation with selected soils and sediment. We use this knowledge to build pedotransfer functions that allow predicting sorption parameters, Kd, Kf and n, for a wide range of soils and sediments. We gathered glyphosate sorption parameters, 101 Kf, n and equivalent Kd, and associated soil properties. These data were then used to perform stepwise multiple regression analyses to build the pedotransfer functions. The linear (Kd) and Freundlich (Kf, n) pedotransfer functions were bench marked against experimental data. We found the following major points: (1). Under current environmental conditions, sorption is best predicted by the Kd pedotransfer function. (2) The pedotransfer function is Kd = 7.20*CEC – 1.31 *Clay + 24.82 (Kd in L/kg, CEC in cmol/kg and clay in %). (3) Cation exchange capacity (CEC) and clay content are the main drivers of Kd variability across soils and sediments. Freundlich parameters are additionally influenced by pH and organic carbon. This suggests that the formation of complexes between glyphosate phosphonate groups and soil–exchanged polyvalent cations dominates sorption across the range of analyzed soils.

Materials and Methods

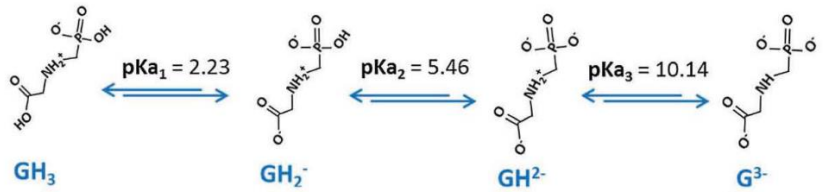
Physical and chemical properties of glyphosate

Glyphosate [N–(Phosphonomethyl)glycine] is a weak acid with strong hydrophilicity and very high water solubility (Table 8.1.2.1-79). Speciation of this zwitterionic molecule varies with the pH of the surrounding environment (Figure 8.1.2.1-41). The main species within the soil pH range are GH_2^- and GH^{2-}, corresponding to net negative charges of one and two, respectively (Figure 8.1.2.1-41).

Table 8.1.2.1-79: Physicochemical properties of glyphosate

Properties		References
Formula	$C_3H_8NO_5P$	ANSES (2015), FOOTPRINT (2015)
Molecular mass (g mol^{-1})	169.1	ANSES (2015), FOOTPRINT (2015)
Aqueous solubility at 20 °C (g L^{-1})	10.5 to 12.0	ANSES (2015), FOOTPRINT (2015)
Log Kow at pH 7	−4.1 to −3.2	ANSES (2015), FOOTPRINT (2015)
pKa_1–pKa_2–pKa_3	2.2–5.5–10.2	ANSES (2015), FOOTPRINT (2015)
Vapor pressure at 25 °C (mPa)	1.31×10^{-2}	ANSES (2015), FOOTPRINT (2015)
Henry's law constant at 25 °C (Pa m^3 mol^{-1})	2.10×10^{-7}	ANSES (2015), FOOTPRINT (2015)

Figure 8.1.2.1-41: Speciation of glyphosate through the entire soil pH range from Albers et al. (2009), Borggaard (2011) and Maqueda et al. (1998)



Data mining

We extensively reviewed the literature to assemble a database of observed glyphosate sorption coefficients to both soils and sediments and the associated substrate properties (Table 8.1.2.1-80). We found 23 studies reporting sorption parameters for one or more soils or sediments. The soils or sediments for which glyphosate sorption measurements were carried out originated from four continents (Europe, Asia and North and South America) and exhibited highly varied texture and properties. The

experimental conditions varied greatly. For example, the initial concentrations in the liquid phase ranged from 0.01 to more than 1000 mg/L. Only coefficients of sorption to unmodified soils or sediments were included in the database. Measured coefficients of sorption to organic soils were included in the database, but only those measured for sorption to mineral soils, i.e., with an organic matter content lower than 20 % (IUSS 2014) were used for the statistical analyses. Several studies have reported that sorption coefficients depend strongly on the background electrolyte. Therefore, only sorption coefficients obtained with classical background electrolyte, either Milli–Q water or $CaCl_2$, were included in the database. Among the 101 sorption parameters registered in the database (Table 8.1.2.1-80), 69 were measured with $CaCl_2$, as the background electrolyte. Statistical analyses were only performed for sorption parameters measured with $CaCl_2$ (designated as "sample A").

Sorption isotherms

For sample A, approximately two–thirds of the sorption models were nonlinear Freundlich (Table 8.1.2.1-81). To establish a pedotransfer function for Kd, we approximated equivalent Kd values by linearizing the Freundlich models over the actual range of the initial aqueous concentrations of the batch experiment used for model fitting (Table 8.1.2.1-81). The relative difference between Kf and its equivalent, Kd (Kd_{eq}), was approximately 30 % on average.

Statistical analyses

Pedotransfer functions aim to predict the sorption parameters Kd, Kf and n from selected substrate properties. Some of the properties, especially CEC, iron– and aluminum oxides or phosphorus content, were not available for all soils or sediments (Table 8.1.2.1-80). This lack of data induced a subsampling of sample A for the establishment of pedotransfer functions for the Kd and Kf parameters. This sample is designated as "sample B". The sample used for the establishment of the pedotransfer function for the n parameter excluded sorption studies that investigated only one concentration and, thereby, did not consider the possibility that n differs from 1. This sample is designated as “sample C”.

The statistical analyses were performed using the R statistical computing software. Correlation analyses were performed using the default "1 m" function of the R software. The three pedotransfer functions for the estimation of linear and nonlinear sorption models were established by forward and backward stepwise multiple regression analyses of the substrate properties and the Kdeq, Kf and n parameters. The stepwise multiple regression analyses were performed using the default "step" function of the R software.

The validity of the Kd pedotransfer function is strongly supported by the fact that sorption processes do not depend on the pesticide concentration. However, in sample A, the n values ranged from 0.48 to 1.05, with a mean value of 0.83, indicating saturation of the sorption sites at high glyphosate concentrations. A complementary multiple regression between n, the substrate properties and the experimental conditions (Cmax and R) was performed. The resulting equation (see Eq. 4) indicates the linearity range under various conditions.

Finally, we evaluated the accuracy of the predicted equilibrium partitioning of glyphosate between the soil and water by using the sorption parameters provided by the Kd or Kf/n pedotransfer functions. The evaluation was performed for 11 initial concentrations (0.01, 0.04, 0. 10, 0.40, 1, 4, 10, 40, 100, 400 and 1000 mg/L) in the liquid phase by comparing the predicted soil–to–water glyphosate concentration ratios, as obtained by the pedotransfer–estimated sorption parameters, to those obtained by the batch–fitted sorption parameters. The aqueous and soil concentrations were calculated for all sample B soils and sediments using the numerical solver as described previously.

Table 8.1.2.1-80: Glyphosate sorption parameters and associated soil or sediment properties' database

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/ liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Italy)	63.4:22.6:14.0	8.11[a]	na	0.70	14.00	na	na	43.01	0.79	0.20–120.00	1/5	$CaCl_2$	Accinelli et al. (2005)
Soil (USA)	93.5:2.7:3.8	7.20 [a]	na	0.94	3.80	na	na	62.16	0.88	0.20–120.00	1/5	$CaCl_2$	Accinelli et al. (2005)
Soil (France)	Sandy loam	5.10 [a]	na	0.82	10.50	1240.00	298.73	34.50	0.97	0.73–60.13	1/5	$CaCl_2$	Al-Rajab et al. (2008)
Soil (France)	Silt clay loam	6.30 [a]	na	1.45	30.60	3240.00	48.57	33.60	1.00	0.73–60.13	1/5	$CaCl_2$	Al-Rajab et al. (2008)
Soil (France)	Clay loam	7.90 [a]	na	1.91	34.90	2740.00	35.67	16.60	1.00	0.73–60.13	1/5	$CaCl_2$	Al-Rajab et al. (2008)
Soil (Finland)	Sandy loam	6.10[b]	na	0.47	17.00	1.60	na	166.00	0.97	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	5.80[b]	na	2.88	46.00	3.00	na	55.00	0.92	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	5.60[b]	na	0.54	58.00	1.10	na	249.00	0.91	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	5.80[b]	na	2.57	13.00	27.40	na	44.00	0.90	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	5.70[b]	na	0.72	4.00	17.10	na	55.00	1.00	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	6.00[b]	na	7.06	41.00	4.10	9.66	97.00	1.03	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	6.00[b]	na	2.96	47.00	1.50	11.22	41.00	1.02	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	6.40[b]	na	5.93	4.00	3.30	9.26	97.00	0.85	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	5.90[b]	na	1.77	4.00	0.90	6.70	51.00	0.86	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	8.10[b]	na	2.67	41.00	38.70	na	58.00	0.93	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	7.90[b]	na	2.50	30.00	22.40	na	113.00	0.87	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	7.10[b]	na	2.35	21.00	8.80	na	93.00	0.90	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	6.80[b]	na	0.75	8.00	2.80	na	90.00	0.86	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	6.00[b]	na	7.05	41.00	5.80	na	179.00	1.26	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	6.30[b]	na	5.93	4.00	6.40	na	121.00	0.98	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Silty loam	5.40[a]	na	7.90	5.00	10.00	4.48	159.00	0.93	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Silty loam	5.60[a]	na	4.50	4.00	3.60	4.29	102.00	1.05	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Silty loam	5.40[a]	na	1.30	8.00	3.50	4.57	37.00	0.76	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Muddy clay	6.90[b]	na	12.60	57.00	52.00	na	84.00	0.91	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Organic soil	5.20[b]	na	26.00	79.00	5.10	17.09	303.00	1.11	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Sediment (France)	35.9:39.1:25.0	8.44[a]	12.50	1.58	25.00	19.00	2.78	51.69	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	40.3:33.1:26.6	8.54[a]	12.10	1.56	26.60	6.00	2.62	129.86	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	4.7:58.5:36.8	8.63[a]	14.40	0.73	36.80	5.00	3.27	75.38	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	7.7:57.2:35.1	8.71[a]	14.20	0.96	35.10	5.00	3.48	109.90	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	9.7:54.6:35.7	7.30[a]	23.20	0.54	35.70	11.00		302.63	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	25.5:42.0:32.5	7.93[a]	26.10	3.88	32.50	73.00	na	262.41	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	77.9:13.9:8.2	6.03[a]	6.34	0.47	8.20	142.00	na	89.07	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	7.8:62.6:29.6	6.19[a]	17.60	3.67	29.60	144.00	na	318.82	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Soil (Sweden)	7.3:46.2:46.5	7.20[a]	28.40	4.00	46.50	na	na	118.00	0.95	0.01–0.10	1/10	$CaCl_2$	Bergström et al. (2011)
Soil (Sweden)	3.3:40.6:56.1	7.40[a]	33.60	0.00	56.10	na	na	165.00	1.03	0.01–0.10	1/10	$CaCl_2$	Bergström et al. (2011)

continued

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/ liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Sweden)	87.8:4.5:7.7	7.40[a]	4.70	2.00	7.70	na	na	40.00	0.92	0.01–0.10	1/10	$CaCl_2$	Bergström et al. (2011)
Soil (Sweden)	95.4:4.6:0.0	6.40[a]	1.80	1.00	0.00	na	na	28.70	0.82	0.01–0.10	1/10	$CaCl_2$	Bergström et al. (2011)
Soil (Spain)	82.0:11.0:7.0	7.90[a]	5.20[c]	1.00	7.00	na	36.70	93.00	0.78	0.10–5.00	2/5	H_2O	Candela et al. (2007)
Soil (Spain)	59.0:13.0:10.0	7.30[a]	4.60[c]	0.00	10.00	na	126.50	154.00	0.74	0.10–5.00	2/5	H_2O	Candela et al. (2007)
Soil (Malaysia)	Sandy loam	6.70	7.10[c]	1.30	10.00	na	na	83.80	0.85	0.10–5.00	1/5	$CaCl_2$	Cheah et al. (1997)
Soil (Malaysia)	Muck	4.70	54.10[c]	30.50	32.50	na	na	417.00	0.78	0.10–5.00	1/5	$CaCl_2$	Cheah et al. (1997)
Soil (France)	10.0:44.0:46.0	7.60[a]		3.90	46.00	na	4.40	33.90	1.00	0.10–5.00	1/5	H_2O	Dousset et al. (2007)
Soil (France)	53.0:25.0:22.0	6.60[a]		1.50	22.00	na	5.10	51.80	1.00	0.10–5.00	1/5	H_2O	Dousset et al. (2007)
Soil (Denmark)	Sandy	7.67	12.60	1.70	15.00	na	na	30.90	0.78	0.03–67.00	1/20	H_2O	de Jonge and Wollesen de Jonge (1999)
Soil (Denmark)	Sandy	6.13	12.60	1.70	15.50	na	na	78.50	0.75	0.03–67.00	1/20	$CaCl_2$	de Jonge and Wollesen de Jonge (1999)
Soil (Denmark)	Sandy	6.30	12.60	1.70	15.50	na	na	78.40	0.75	0.03–67.00	1/20	$CaCl_2$	de Jonge and Wollesen de Jonge (1999)
Soil (Denmark)	Sandy	6.53	12.60	1.70	15.50	na	na	48.40	0.77	0.03–67.00	1/20	$CaCl_2$	de Jonge and Wollesen de Jonge (1999)
Soil (Chili)	Clay loam	4.79[a]		6.60	18.00	na	na	12.13	1.00	1000.00–2500.00	1/10	H_2O	Kogan et al. (2003)
Soil (Finland)	Clay	6.02[a]		7.10	41.00	4.20	9.66	98.69	1.02	2.00–10.00	1/5	H_2O	Laitinen et al. (2008)
Sediment (Germany)	Sandy	7.7[a]		0.34	0.00	0.00	0.61	1.89	0.48	0.10–100.00	1/2	$CaCl_2$	Litz et al. (2011)
Soil (France)	Rendzina over limestone	8.4[a]	6.40	1.86	8.80	3590.00	2.51	17.60	0.76	0.20–10.00	1/5	H_2O	Mamy and Barriuso (2005, 2007)
Soil (France)	Rendzina over limestone	8.2[a]	7.10	1.86	9.30	2720.00	2.58	34.80	0.80	0.20–10.00	1/5	$CaCl_2$	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	8.36[a]	17.80	2.00	37.60	2490.00	15.20	32.90	0.86	0.20–10.00	1/5	H_2O	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	8.2[a]	20.60	1.69	37.70	2760.00	14.96	41.90	0.80	0.20–10.00	1/5	$CaCl_2$	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	6.3[a]	16.40	1.01	27.40	1460.00	8.58	60.50	0.88	0.20–10.00	1/5	H_2O	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	7.6[a]	15.90	0.96	23.50	1310.00	7.00	276.00	0.77	0.20–10.00	1/5	$CaCl_2$	Mamy and Barriuso (2005, 2007)
Soil (Greece)	13.4:64.1:22.6	8.0[a]	28.30[c]	0.00	22.60	na	3.40	13.79	0.77	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (UK)	46.3:36.8:17.0	5.8[a]	18.30[c]	3.73	17.00	na	63.10	40.64	0.77	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (Germany)	81.5:12.6:6.0	4.6[a]	30.70[c]	9.23	6.00	na	14.80	51.14	0.58	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (France)	1.7:82.4:16.0	8.3[a]	11.40[c]	0.45	16.00	na	22.80	152.85	0.44	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (Brazil)	46.0:7.0:47.0	5.2[a]	14.18	3.20	47.00	89.00	222.80	162.90	0.98	0.42–6.72	1/5	$CaCl_2$	Prata et al. (2003)
Soil (Brazil)	38.0:60.0:56.0	5.0[a]	12.32	2.50	56.00	59.00	277.10	215.70	0.99	0.42–6.72	1/5	$CaCl_2$	Prata et al. (2003)

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Denmark)	Sandy	5.3[a]	na	na	1.64	na	2.21	403.50	1.00	0.30	1/5	$CaCl_2$	Strange-Hansen et al. (2004)
Soil (China)	–	7.20[a]	23.60	4.57	34.30	na	209.50	31.19	0.82	21.00–169.00	1/25	H_2O	Wang et al. (2006)
wetland Sediment (Canada)	Sandy	6.70[a]	13.30	3.20	9.10	na	na	172.90	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	6.40[a]	16.80	3.80	11.30	na	na	152.60	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	6.40[a]	24.80	6.80	4.90	na	na	251.90	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	7.20[a]	33.10	8.70	5.60	na	na	193.10	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	7.10[a]	31.90	9.60	6.70	na	na	124.90	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Soil (Denmark)	Sandy	3.70[b]	na	1.32	4.20	50.55	3.21	107.40	0.62	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	3.60[b]	na	1.06	4.20	63.49	2.47	80.60	0.62	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	3.60[b]	na	1.28	4.20	87.39	2.98	83.50	0.66	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	3.80[b]	na	1.06	4.20	72.15	2.53	79.40	0.68	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.20[b]	na	1.20	4.20	18.32	2.73	121.20	0.60	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.30[b]	na	1.33	4.20	27.70	3.36	141.20	0.63	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.20[b]	na	1.30	4.20	44.58	3.43	118.10	0.67	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.30[b]	na	1.40	4.20	44.50	3.24	111.50	0.62	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.50[b]	na	1.26	4.20	12.07	3.23	126.80	0.56	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.70[b]	na	1.26	4.20	18.56	2.95	120.00	0.58	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.60[b]	na	1.21	4.20	36.31	2.76	92.00	0.59	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.90[b]	na	1.36	4.20	32.08	3.27	116.20	0.61	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.20[b]	na	1.33	4.20	9.08	3.46	154.00	0.62	0.04—65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.50[b]	na	1.14	4.20	13.07	3.44	138.80	0.65	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.40[b]	na	1.29	4.20	24.28	3.65	136.60	0.63	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.50[b]	na	1.20	4.20	25.57	3.32	119.80	0.63	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.20[b]	na	1.28	10.80	6.19	6.45	214.70	0.56	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.20[b]	na	1.26	10.80	13.26	5.26	165.10	0.60	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.30[b]	na	1.25	10.80	24.52	5.35	137.60	0.67	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.40[b]	na	1.23	10.80	58.74	5.71	106.40	0.71	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.30[b]	na	1.21	10.80	8.75	5.28	171.70	0.58	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.30[b]	na	1.40	10.80	15.56	5.04	144.00	0.59	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.30[b]	na	1.44	10.80	24.79	4.94	151.30	0.65	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Brazil)	81.7:2.0:16.3	4.00[a]	2.00	0.83	16.30	28.77	212.00	31.32	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	81.7:2.0:16.3	5.00[a]	2.00	0.83	16.30	28.77	212.00	17.89	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	81.7:2.0:16.3	6.00[a]	2.00	0.83	16.30	28.77	212.00	19.59	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	81.7:2.0:16.3	7.00[a]	2.00	0.83	16.30	28.77	212.00	15.52	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	4.00[a]	4.00	0.38	30.30	3.12	289.40	0.87	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	5.00[a]	4.00	0.38	30.30	3.12	289.40	0.83	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	6.00[a]	4.00	0.38	30.30	3.12	289.40	1.37	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	7.00[a]	4.00	0.38	30.30	3.12	289.40	0.90	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	4.00[a]	11.00	2.56	54.30	16.93	236.70	4.45	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	5.00[a]	11.00	2.56	54.30	16.93	236.70	3.44	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	6.00[a]	11.00	2.56	54.30	16.93	236.70	2.51	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	7.00[a]	11.00	2.56	54.30	16.93	236.70	2.01	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)

Note that sorption parameters included in the database were measured on unmodified soil or sediment and with background electrolyte being either water (H_2O) or calcium chloride ($CaCl_2$). Some parameters were not included in the database because of unit incoherencies (e.g., Jacobsen et al. 2008)

na not available

[a] pH_{H2O}

[b] pH_{CaCl2}

[c] CEC meq/100 g

Table 8.1.2.1-81: Statistical characteristics of the database subsamples

	Sample A					Sample B					Sample C				
	Nobs.	Mean	Median	Min	Max	Nobs.	Mean	Median	Min	Max	Nobs.	Mean	Median	Min	Max
Kf (L kg^{-1} n^{-1})	69	108.16	111.50	0.83	403.50	36	96.32	63.00	0.83	297.02	51	118.89	118.10	1.89	297.02
n	69	0.83	0.88	0.48	1.05	36	0.94	1.00	0.75	1.05	51	0.77	0.75	0.48	1.05
Kd_{eq} (L kg^{-1})	69	73.96	38.89	0.06	403.50	36	87.02	39.05	0.83	318.82	51	72.61	44.78	0.06	318.82
pH	69	6.10	6.30	3.60	8.71	36	6.68	6.85	4.00	8.71	51	6.20	6.30	3.60	8.71
OC (%)	69	1.79	1.29	0.00	9.60	36	2.62	1.69	0.00	9.60	51	1.50	1.29	0.00	6.44
CEC (cmol kg^{-1})	36	13.25	12.55	1.80	33.60	36	13.25	12.55	1.80	33.60	19	15.21	12.60	1.80	33.60
Clay (%)	69	18.32	10.80	0.00	56.10	36	25.36	24.25	0.00	56.10	51	16.11	10.80	0.00	56.10
P_{OLSEN} (mg kg^{-1})	52	297.88	25.18	0.00	3240.00	26	321.32	16.93	3.12	2760.00	40	382.36	29.89	0.00	3240.00
Fe_{ox}–Al_{ox} (g kg−1)	49	80.87	5.04	0.61	298.73	19	157.32	212.00	2.58	289.40	36	28.00	3.44	0.61	298.73

Sample A all data with $CaCl_2$ as the background electrolyte. *Sample B* data used for the calibration of Kd and Kf pedotransfer functions. *Sample C* data used for the calibration of the **n** pedotransfer function

OC organic carbon, *CEC* cation exchange capacity, Fe_{ox}–Al_{ox} iron- and aluminum oxides, *Nobs.* number of observations

Results and Discussion

Database and sample characteristics

The soils and sediments used in the glyphosate sorption measurements displayed great variability in their origins and properties. This variability was preserved in the subsampling of the database for pedotransfer function calibration, as seen in Table 8.1.2.1-81. Indeed, the three subsamples of the database displayed similar distributions of properties and parameters values. The 0.01–1000 mg/L concentration range was also preserved by the subsampling of the database. This range covers all possible environmental glyphosate concentrations from concentrations found during spraying to those found in runoff and groundwater. It is interesting to note that the data presented in Table 8.1.2.1-83 exhibited highly significant correlations between some basic soil properties: The CEC was correlated with organic carbon or iron– and aluminum oxides, and the clay content was correlated with iron– and aluminum oxides. In contrast, there was no correlation between clay and CEC, suggesting a large influence of the within–sample variation in clay mineralogy (Table 8.1.2.1-83).

Table 8.1.2.1-82: Initial aqueous concentration used for the linear approximation if Freundlich isotherms

	Initial aqueous concentrations (mg L^{-1})	Frequency of use in experimental design (%)
Class 1	0.01–0.02–0.04–0.06–0.08	56
Class 2	0.10–0.20–0.40–0.60–0.80	59
Class 3	1.00–2.00–4.00–6.00–8.00	55
Class 4	10.00–20.00–40.00–60.00–80.00	32
Class 5	100.00–200.00–400.00–600.00–800.00–1000.00	12

Table 8.1.2.1-83: The Pearson correlation coefficients matrix among soil properties

Parameters	CEC (cmol kg^{-1})	OC (%)	Clay (%)	Phosphorus (mg kg^{-1})	Fe_{ox} and Al_{ox} (g kg^{-1})
pH	0.407*	NS	0.250*	0.339*	NS
	(36)	(69)	(69)	(52)	(49)
CEC	–	0.666***	NS	NS	−0.691**
		(36)	(36)	(26)	(19)
OC	–	–	NS	NS	NS
			(69)	(52)	(49)
Clay	–	–	–	NS	0.631***
				(52)	(49)
Phosphorus	–	–	–	–	NS
					(49)

The number in brackets corresponds to the number of observations for the given correlation. "***", "**" and "*" represent correlation significance levels of 0.001, 0.01 and 0.05, respectively

NS correlation is not significant, *OC* organic carbon, *CEC* cation exchange capacity, Fe_{ox}–Al_{ox} iron- and aluminum oxides

Table 8.1.2.1-84: The Pearson correlation coefficients matrix between sorption parameters and soil properties or experimental conditions

Parameters	pH	CEC (cmol kg^{-1})	OC (%)	Clay (%)	Phosphorus (mg kg^{-1})	Fe_{ox} and Al_{ox} (g kg^{-1})	C_{max} (mg L^{-1})	log(R)
Kd_{eq}	NS	0.659***	0.380**	NS	NS	NS	−0.364**	NS
	(69)	(36)	(69)	(69)	(52)	(49)	(69)	(69)
Kf	NS	0.659***	0.255*	NS	NS	−0.527***	−0.551***	−0.432***
	(69)	(36)	(69)	(69)	(52)	(49)	(69)	(69)
n	0.531***	NS	0.351*	0.760***	0.361*	0.560***	−0.666***	0.489***
	(51)	(19)	(51)	(51)	(40)	(36)	(51)	(51)

The number in brackets corresponds to the number of observations for the given correlation. "***", "**", and "*", represent correlation significance levels of 0.001, 0.01 and 0.05, respectively

NS correlation is not significant, *OC* organic carbon, *CEC* cation exchange capacity, Fe_{ox}–Al_{ox} iron- and aluminum oxides, C_{max} maximal initial concentration (mg L^{-1}), *log(R)* log-transformed solid–liquid ratio (g mL^{-1})

Table 8.1.2.1-85: Pedotransfer function for the estimation of linear (Kd) and Freundlich (Kf–n) sorption isotherms

Pedotransfer function	Sample	Soil parameters	Equation	R^2	RMSEP
Kd	B	CEC, Clay	Kd = 24.821 + 7.199 * CEC − 1.307 * Clay	0.48***	7.59 (8.7 %)[a]
Kf	B	CEC, Clay, OC	Kf = 50.904 + 9.246 * CEC − 1.985*Clay − 11.811 * OC	0.52***	16.33 (16.9 %)[b]
n	C	Clay, pH	*n* = 0.505 + 0.007 * Clay + 0.024 * pH	0.62***	0.006 (0.8 %)[c]

OC organic carbon (%), *CEC* cation exchange capacity (cmol kg^{-1}), clay (%)

*** Correlations are significant at the level 0.001

[a] RMSEP expressed as a percentage of the mean Kd value (87.02 L kg^{-1})

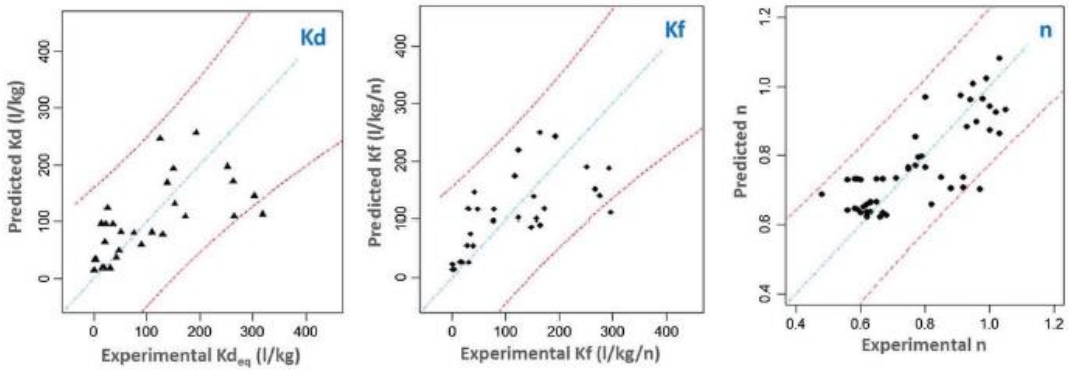
[b] RMSEP expressed as a percentage of the mean Kf value (96.32 L kg^{-1} n^{-1})

[c] RMSEP expressed as a percentage of the mean n value (0.77)

Glyphosate sorption: mechanisms and prediction

The Pearson correlation coefficients (Table 8.1.2.1-84) showed that the Kd, q and Kf values are primarily correlated with CEC and, secondarily, with organic carbon content and Fe_{ox}–Al_{ox} content. They also show that n exhibits significant correlation with all of the selected soil properties, with the exception of CEC. The multiple regression analysis (Table 8.1.2.1-85) provided pedotransfer functions that accurately fit the observed Kd_{eq}, Kf and n values. The functions account for 48–62 % of the variation in the sorption parameters. Visual inspection of the disparity between the measured and predicted Kd_{eq}, Kf and n sorption parameters did not reveal systematic departures from the regression, except for one outlier corresponding to high Kd_{eq} and Kf values measured on a sediment containing a particularly high organic carbon content (Figure 8.1.2.1-42). The multiple regression analyses high–lighted the points that CEC is the main predictor of Kd_{eq} and Kf variation and that clay is a useful predictor. Furthermore, we found that organic carbon was a predictor for Kf only. The analyses also revealed that clay and pH are significant predictors of *n*. These results suggest that the formation of complexes between the glyphosate phosphonate groups and the soil exchanged polyvalent cations is the dominating sorption mechanism across the entire range of analyzed soils. This is indicated by the primary role of CEC in controlling Kd_{eq} and Kf variability. Given the high correlation between CEC and Fe_{ox}–Al_{ox} in our sample, it is likely that the influence of the latter property was masked by that of the former. Additionally, we found that clay content explained only approximately 5 % of the Kd_{eq} and Kf variability (Table 8.1.2.1-84, Table 8.1.2.1-85). Significant correlations were found between organic carbon and Kd_{eq} or Kf (Table 8.1.2.1-84), although organic carbon only slightly increased the R^2 value obtained in the multiple regression analyses of Kf. Organic carbon appeared to be strongly correlated with CEC, indicating the significant contribution of organic matter to CEC; this correlation may explain the correlation of organic carbon with the sorption parameters. There is a general consensus that a rise in pH negatively affects the sorption of glyphosate. However, the multiple regression analyses did not detect any influence of pH on Kd_{eq}, and Kf variability.

Figure 8.1.2.1-42: Multiple regression analysis of the sorption coefficients (Kd, Kf, n) and soil properties. The sorption coefficients predicted from the pedotransfer functions were plotted against the sorption coefficients (Kf, n) fitted from the experimental data and for Kdeq, against the linearized sorption coefficients



Here, pH and clay explained most of the n parameter variability (Table 8.1.2.1-84 and Table 8.1.2.1-85). The positive correlation of n with pH may be related to the increased negative charges for both glyphosate (Figure 8.1.2.1-41) and the soil, favoring the formation of complexes with soil–exchanged polyvalent cations. Despite the increasing electrostatic repulsion, a rise in pH appears to reduce the potential saturation of sorption sites for high initial concentrations by favoring cation bridging between glyphosate and the soil. The variability of the sorption parameters that is not predicted by the multiple regressions may be largely attributed to the varying experimental conditions among the studies measuring glyphosate sorption to soils and sediments (Table 8.1.2.1-80). If different parameters are considered to be possible predictors in the multiple regressions, they enable a fit to a regression function (Eq. 4) with a better performance (R^2 = 0.69) than that of the regression using only basic soil properties as predictors for *n*.

n = 0.920 - 0.028 x log(Cmax (mg/L)) + 0.064 x log (R(g m/L)) + 0.005 x clay (%) (4)

A small R implies a limited amount of sorption sites.

Correlations between Kf, Kd_{eq} and the solid–to–liquid ratio or the maximal initial concentration (Table 8.1.2.1-84) are further evidence of the influence of the experimental conditions on the sorption. However, unlike the case of the *n* parameter, inclusion of the experimental conditions (C_{max}, R) in the multiple regression analyses did not increase the predictive performance of the regression for Kd_{eq} and Kf. It must be noted that the pedotransfer functions could be improved with additional experimental sorption studies designed to closely mimic the environmental conditions and with pH and CEC analyzed with the standardized methods [pH_{H2O}, Metson CEC (cmol/kg)].

Use of pedotransfer functions for risk assessment

The linear sorption coefficient Kd can be predicted by a pedotransfer function requiring the knowledge of only two properties, the clay content and CEC. The prediction performance is good with an RMSEP of less than 10 % of the mean glyphosate Kd_{eq} (Table 8.1.2.1-85). However, Figure 8.1.2.1-43a shows that the errors in the predicted soil–to–water concentration ratios vary largely according to the initial concentration of water. The errors are moderate for initial liquid–phase concentrations below 10 mg/L, indicating that the Kd pedotransfer function predicts sorption relatively accurately for concentrations below this threshold. The 10 mg/L may correspond to the threshold above which the concentration independence of the sorption process can no longer be assumed. This assumption can be checked by examining the variation in n given by Eq. 4. Figure 8.1.2.1-44 presents the departure from linearity assumed to occur when *n* is below 0.9 across a range of clay content values and initial glyphosate concentrations.

Figure 8.1.2.1-43: Distributions of the prediction errors for linear sorption isotherms and nonlinear sorption isotherms. Δ represents the difference (%) at a given initial concentration between the predicted and the measured ratio of concentrations between soil and water. a Ratios predicted by the Kd pedotransfer function (linear isotherm estimation) and b ratios predicted by the Kf and n pedotransfer function (nonlinear isotherm estimation)

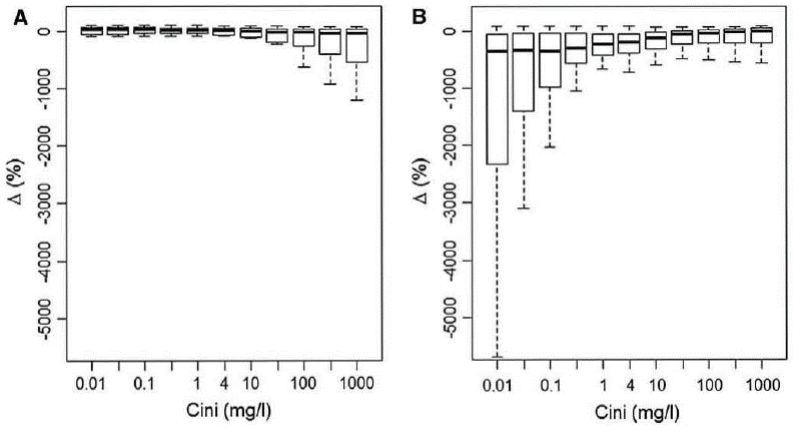
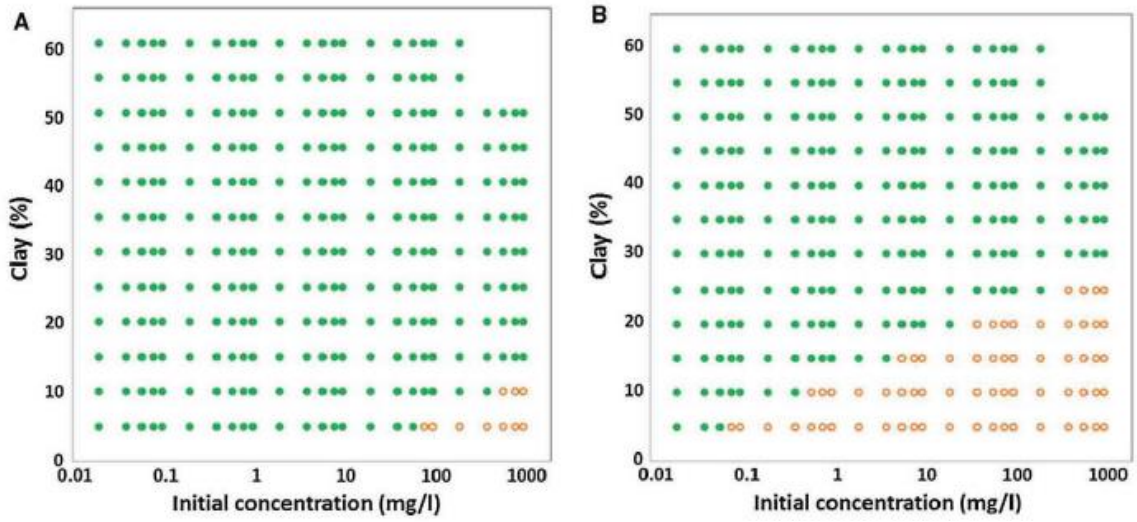


Figure 8.1.2.1-44: Linearity range of sorption isotherms in relation to the clay content and initial glyphosate concentrations in the liquid phase. Plain green dots represent 0.9 <n <1.05, and empty orange dots represent n values lower than 0.9 (bottom right), A n values were calculated from Eq. 4 with a solid–to–liquid ratio of 1:1 (g/ml). B n values were calculated from Eq. 4 with a solid–to–liquid ratio of 1:20 (g/ml). Note that for a solid–to–liquid ratio of 1:1, the saturation of sorption sites occurs at initial concentration higher than 100 mg/L for clay content varying between 0 and 10 %, whereas for the 1:20 ratio, the saturation for the same clay content starts at initial concentrations of approximately 0.1 mg/L



The Freundlich isotherms can be satisfactorily predicted by two pedotransfer functions requiring the knowledge of four properties, namely the organic carbon and clay contents, CEC and pH (Table 8.1.2.1-85). As seen in Figure 8.1.2.1-44, the prediction errors of the soil–to–water concentration ratio exceed 1000 % for concentrations between 0.01 and 0.40 mg/L and 500 % for concentrations up to 10 mg/L (Figure 8.1.2.1-43b). Thus, the sorption estimated by the combination of *n* and Kf pedotransfer functions is significantly underestimated for initial concentrations below 10 mg/L. This may be due to the multiplication of properties used to estimate the sorption parameters and the accumulation of inherent bias of the two pedotransfer functions. However, it must also be noted that for concentrations higher than 10 mg/L, the predictions using the estimated Freundlich model parameters show slightly smaller errors than those using the estimated linear isotherms. The application of the Kf pedotransfer function is therefore only advisable for estimating sorption for very high liquid–phase concentrations, a condition that is relatively rarely found in the current environmental conditions.

Conclusion

Sorption to soils and sediments controls the fate of glyphosate in the environment and thus the potential risk of freshwater and groundwater contamination. Glyphosate sorption appeared to be controlled mainly by cation exchange capacity, clay and organic carbon content and pH. This suggests that the mechanism driving glyphosate sorption over the range of soil and sediment investigated is the complex formation between the phosphonate group of glyphosate and the soil–exchanged polyvalent cations. Robust pedotransfer function for the estimation of glyphosate Kd was built from multiple regression analysis of the literature data. This Kd pedotransfer function enables prediction of glyphosate sorption for a wide range of soils and sediments with a limited number of properties and with reasonable accuracy for most environmental conditions.

Assessment and conclusion by applicant:

The article estimates pedotransfer functions for the adsorption of glyphosate to soil based on review of existing published data. However, no new experimental data is presented neither existing data is evaluated regarding their quality in conduct according to OECD 106 or the EU Evaluators Checklist.

The article is therefore classified as reliable with restrictions, i.e. not used in risk assessment.

Assessment and conclusion by RMS:

This article provides a review of existing published data and observes correlation of adsorption parameters of glyphosate and characteristics of the soil. In this article, the correlation of Kd from glyphosate and pH H_2O based on data from 101 soils did not indicate a significant dependency. However the quality of the data used cannot be assessed.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Kanissery, 2015

Data point:	CA 7.1.3.1.1/025
Report author	Kanissery, R.G. et al.
Report year	2015
Report title	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate
Report No	DOI 10.2134/jeq2014.08.0331 E-ISSN 1537-2537
Guidelines followed in study	USEPA guidelines for adsorption studies (USEPA, 2008)
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:

The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/013). The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Tévez, 2015

Data point:	CA 7.1.3.1.1/026
Report author	Tévez, H., dos Santos, A.M.
Report year	2015
Report title	pH dependence of Glyphosate adsorption on soil horizons
Report No	DOI 10.18268/BSGM2015v67n3a13 ISSN 1405-3322
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Pesticides bring many problems to the environment and to human health. The first rationale for their use is increased food production. Glyphosate N-(phosphonomethyl)glycine (PMG) is a non-selective, post emergent, and broad spectrum herbicide, very well known for its extensive application in agriculture worldwide. PMG adsorption experiments were carried out in three horizons of a Typic Haplustoll soil from the Province of Santiago del Estero, Argentina. Adsorption isotherms were fitted using Freundlich and Langmuir models. The affinity constants (KF and KL), the adsorption intensity (l/n) and the

maximum surface coverage (Γmax) were obtained. The results show the dependence of the parameters KL and Γmax with pH and also with the different horizons and particle size.

Materials and Methods

Chemicals

All chemicals utilized were of analytical reagent grade and were used without further purification. All solutions and soil dispersions were prepared using Milli-Q water. All PMG solution concentrations ranged from 0.05 to 10 mM prepared daily.

Study area

Climate is semiarid mesothermal, with an average annual temperature of 19.6 °C and rainfall of between 600 and 750 mm per year concentrated in the spring-summer period. Samples were taken up to 130 cm of depth from three very well differenced horizons classified as Ap (0 - 18 cm), AB (18 - 50 cm) and BC (105 - 130 cm).

Characterizations

The fresh soil samples were air-dried and ground. pH was measured in 0.01 M CaCl2 solution. Organic matter (OM) content and soils chemical analysis were determined by the dichromate oxidation method. The available phosphorus (P) is the inorganic P, that is extractable at pH 8.5 and was determined following the experimental procedure described in Olsen et al., 1954 and Page et al., 1982. The total surface area (Sw) was measured by H20 adsorption (Torres-Sanchez and Falasca, 1997). The total iron oxides (Fetot) and amorphous iron oxides (Feamorph) were established by dithionite (Holmgren, 1967) and oxalate method (McKeague, 1967), respectively. Soils samples were mixed with Lithium Metaborate/Lithium Tetraborate (LiBO2, /Li2B4O7) and fused in a furnace. The molten melt was completely dissolved in acidic media of 5 % nitric acid. This solution was analyzed for major and selected trace elements by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). The sample composition is reported as oxide percentage. The mineralogical composition and quantitative analysis of the soils were determined by X-ray Diffraction (XRD) and using the Rietveld method (Rietveld, 1969). Point of zero net proton charge (PZNPC) or point of zero salt effect (PZSE) is the pH where the net adsorption of protons and hydroxyl ions on the surfaces is independent of electrolyte concentration. Titration curves, when surface charge is plotted against pH, frequently showed a common intersect ion point that match with PZNPC.

Table 8.1.2.1-86: Characteristics of agriculture soils profile from Santiago del Estero/Argentina

Horizon	pH ($CaCl_2$ 1:2.5)	OM (g C.Kg^{-1})	P ($\mu g.g^{-1}$)	Sw ($m^2.g^{-1}$)	Fe_{amorph} ($mg.g^{-1}$)	Fe_{tot} ($mg.g^{-1}$)	PZNPC (pH)
Ap	5.90	23.30	43.34	188	0.239	1.66	7.1
AB	5.75	17.10	6.67	259	0.158	1.91	7.4
BC	6.02	12.10	1.19	242	0.095	0.99	8.1

Adsorption experiment

The adsorption of herbicide by the soils was studied using batch experiments. Solutions of different concentration of glyphosate was added to soil samples dispersions. Dispersions were kept in constant agitation overnight at constant pH, ionic strength and room temperature to reach equilibrium. The sample was filtered and adsorbed glyphosate was calculated from the difference between the total added ligand and the supernatant concentration (Ce). PMG was evaluated by ion chromatography. Two plastic anion columns were coupled in series to serve both as pre-column and analytical chromatographic column. The typical experimental error is lower than 5 % for all results.

Table 8.1.2.1-87: Chemical Analysis of agriculture soils profile from Santiago del Estero, Argentina

Horizon	Chemical Analysis (%)						
	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O
Ap	64.6	12.25	3.51	1.47	1.14	1.70	2.43
AB	63.0	13.65	4.10	1.34	1.40	1.50	2.53
BC	63.0	14.05	4.42	1.44	1.62	1.56	2.66

pH effect

The pH dependence of the glyphosate uptake by soil horizons was investigated using batch isotherm experiments in a pH range from 2 to 8 with a soil concentration of 9.1 g/L and different initial concentrations of PMG at a constant ionic strength of 0.1 M of KNO3. The pH was measured using a Metrohm 644 pH-meter with a combined glass microelectrode. Adsorption experiments were conducted in triplicate following the procedure described above. There were no significant differences within each replicate ($p < 0.01$). The expressed values represent the average of the obtained results.

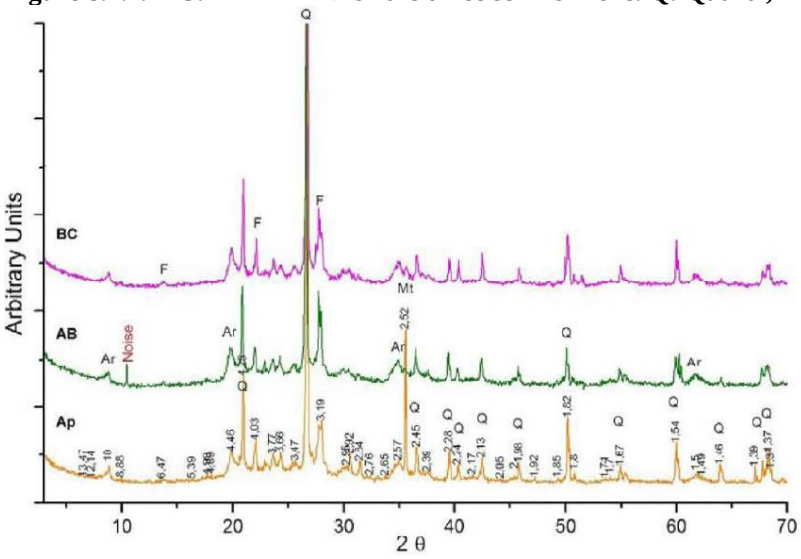
Isotherms Modeling

The relationship between the ligand uptake and the sorbate equilibrium concentration as constant temperature is known as the adsorption isotherm. The adsorbent capacity of a certain material is related to the material balance adsorption: the sorbate that disappears from solution must be in the adsorbent. Freundlich and Langmuir models were chosen and applied for describing the equilibrium data.

Table 8.1.2.1-88: Mineralogical Composition of agriculture soils profile from Santiago del Estero, Argentina. Values in parenthesis represent estimated standard deviations

Horizon	Mineralogical Composition (%)				
	Quartz	Sanidine Feldspar	Andesine Feldspar	Illite	Magnetite
Ap	45.2 (0.4)	9.6 (0.9)	24.7 (0.8)	18.6 (1.4)	1.3 (0.2)
AB	39.8 (0.5)	9.6 (0.8)	23.5 (0.7)	25.9 (1.5)	1.2 (0.3)
BC	46.2 (0.4)	9.3 (0.9)	19.9 (0.9)	24.7 (1.3)	1.2 (0.3)

Figure 8.1.2.1-45: XRD or the three soil horizons. Q: Quartz, Ar: Clay, F: Feldspar, Mt: Magnetite



Results and Discussion

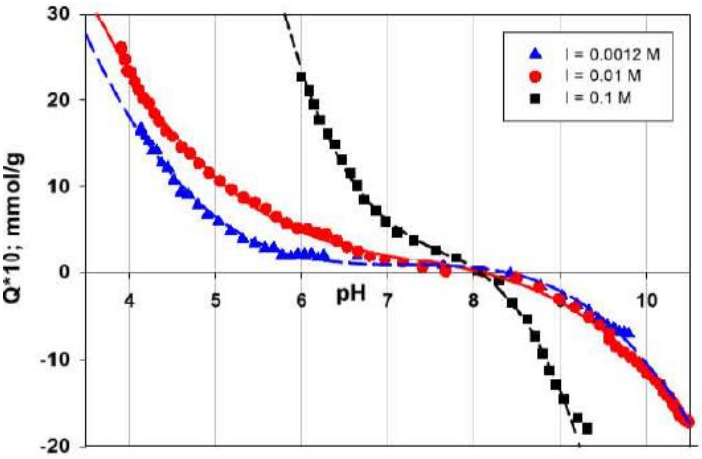
Soil characteristics, chemical analysis, mineralogical composition and quantitative analysis are presented in Table 8.1.2.1-86, Table 8.1.2.1-87 and Table 8.1.2.1-88 respectively. XRD of the three soil

horizons are shown in Figure 8.1.2.1-45. The experimental curves of PZNPC recorded for the BC horizon are illustrated in The Langmuir model was also applied to make an interpretation of PMG adsorption isotherms on soil dispersions equilibrated at different pH values. This is shown in Figure 8.1.2.1-47, where solid lines are calculated using this model and Γmax and *KL* are given.

Figure 8.1.2.1-46. Similar behavior was found for all the horizons that showed PZNPC values in the range of 7.1 - 8.1 (Table 8.1.2.1-86) following the sequence: Ap<AB<BC. PZNPC value can be explained by the absence of clay minerals with a negative permanent charge, while the presence of 2: 1 clays shift the PZNPC to lower pH values (Table 8.1.2.1-88). The higher PZNPC value for the horizons corresponds to horizon BC that contains similar amount of quartz, lower amount of feldspars (andesine) and high amount of illite. PZNPC increase with andesine feldspar content and OM decrease. The determination coefficients of a linear fit were $R^2_{andesine}$ = 0.9971 and R^2_{OM} = 0.9189. The analysis of the three parameters variations in a 3D plot presented a determination coefficient of R^2 = 1.0000 and a constant variance test of p < 0.0001. The PMG adsorption isotherms of soils dispersions equilibrated at different pH values are shown in Figure 8.1.2.1-47. The Freundlich model parameters values (K_Γ , and l/n) were calculated and are given in Table 8.1.2.1-89. The 1/n values vary between 0.1 and 1, which indicates that this model could be used for interpreting the data. The correlation between experimental and calculated curves had a p-level between 0.137 and 0.0035; the determination coefficients were between 0.7578 and 0.9953 for different pHs and horizons.

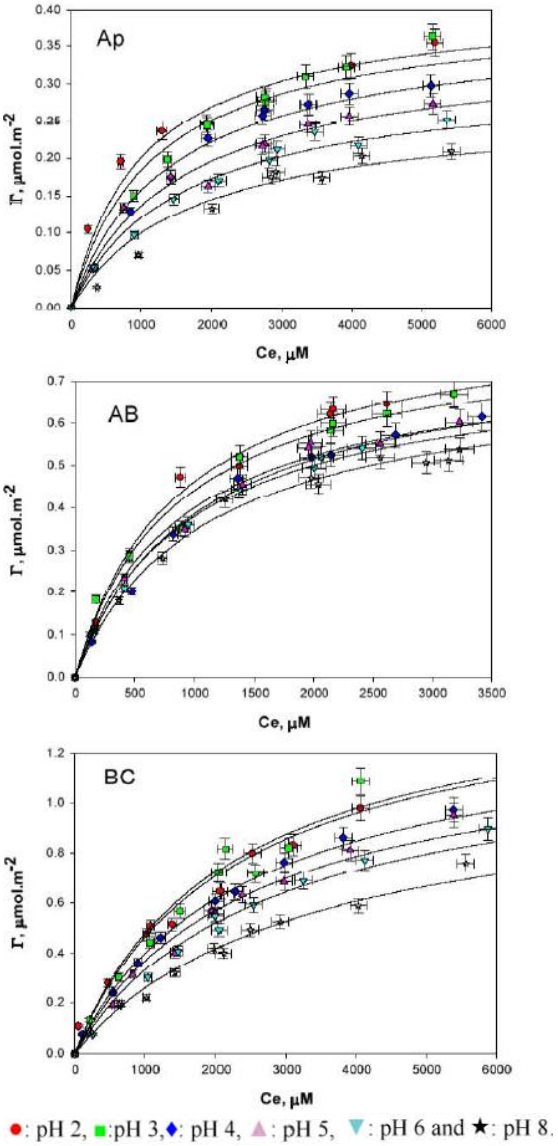
The Langmuir model was also applied to make an interpretation of PMG adsorption isotherms on soil dispersions equilibrated at different pH values. This is shown in Figure 8.1.2.1-47, where solid lines are calculated using this model and Γ_{max} and K_L are given.

Figure 8.1.2.1-46: Potentiometric titration curves or the dispersions or the BC horizon at three ionic strengths (I = ½ΣiCiZi)



The isotherm model parameters were obtained by a non-linear optimization using the Solver-Excel tool. The parameters values were obtained from the plot of the inverse of the surface coverage as a function of the inverse of the equilibrium concentration. Results of the adsorption and surface coverage calculations were normalized with Sw data and the various horizons were contrasted. The correlation between experimental and calculated curves had a p-level between 0.050 and 0.001; the determination coefficients (R^2) obtained were between 0.9300 and 0.9999; and were higher than those obtained using the Freundlich model. Thus, the Langmuir model would better represent the adsorption process of PMG on the Santiago del Estero Province soil.

Figure 8.1.2.1-47: Adsorption isotherm or PMG on horizon Ap, AB and BC. Solid lines are calculated using Langmuir model with constants and maximum surface coverage



The dependence of the surface coverage with PMG concentration in the various horizons at constant pH = 5 is shown in Figure 8.1.2.1-48. Horizon Γ_{max} sequence is Ap<AB<BC. This behavior is similar to those found for PZNPC. The dependence of the surface coverage with pH in the various horizons is also shown in Figure 8.1.2.1-47. The adsorption capacity increases from pH 8 to 2. This pH effect was normally observed during the adsorption of anionic species. Consequently, PMG interaction with the surface occurs throughout the anionic chemical groups (carboxylate or phosphonate) and not through the amine gr up (pKa = 10.14) that is positively charged at the studied pH range (Figure 8.1.2.1-49). The surface coverage decrease, $\Delta\Gamma_{max}$ for horizon Ap is around 41 % for this pHs range (Table 8.1.2.1-90). This difference is lower for horizons BC, 27 %, and AB, 12 %. The highest adsorption capacity is obtained by horizon BC followed by horizon AB, and the lowest for horizon Ap. A similar sequence was obtained for PZNPC (Table 8.1.2.1-86), indicating that the horizon with higher positive surface charge presents higher PMG surface coverage. The ratio of the Γ_{max} of the horizons ($R_{H1/H2}$) was calculated where H1 and H2 denote two different horizons, Γ_{maxH1} and Γ_{maxH2} indicate the maximum coverage of H1 and H2 horizons, respectively. This ratio between the horizons BC and AB was $R_{BC/AB}$ = 46 %, between horizons BC and Ap was $R_{BC/AP}$ = 72 % and between horizon AB and Ap was $R_{AB/Ap}$ = 50 %. These percentages are opposed to the phosphate content that follows the order Ap > AB > BC. The highest adsorption constants correspond to horizon AB (Table 8.1.2.1-90). The changes in the adsorption affinity between horizon BC and AB reach ΔK_L = 46 % while horizon BC decreases 73 % in respect to horizon Ap.

Figure 8.1.2.1-48: Adsorption isotherm of PMG on horizon AP, AB and BC at pH 5

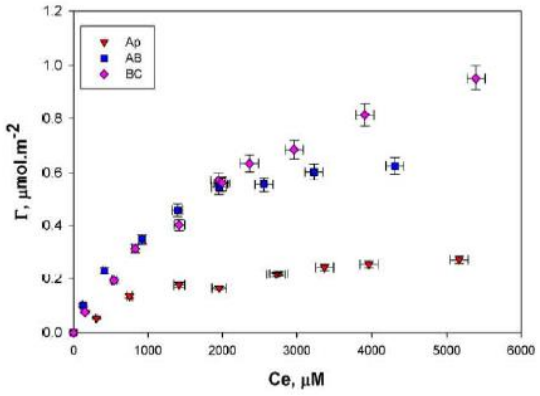
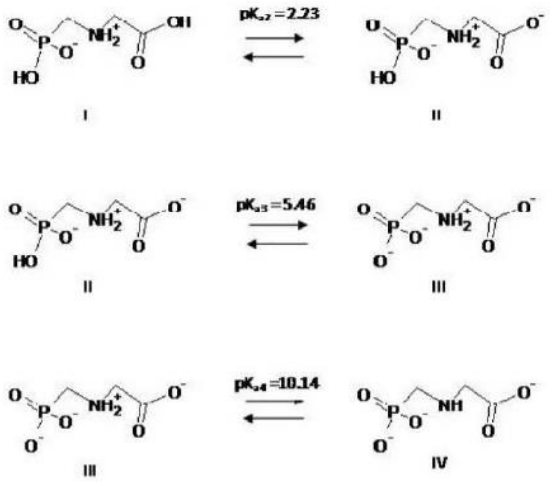


Figure 8.1.2.1-49: PMG acid-base equilibrium



The greater slope of the adsorption curves in the AB horizon indicate that PMG binds more strongly to the active sites of this horizon. Thus, the active site of PMG adsorption on the AB horizon could be the surface iron atoms and the higher adsorption in this horizon is directly related to higher iron content.

The adsorption on horizon BC does not reach maximum coverage in experimental conditions. The adsorption isotherms with a low initial slope describe an adsorption process with characteristic adsorption constants of low energy interaction (Figure 8.1.2.1-47). The constant and the equilibrium reactions of acid-base dissociation of glyphosate (Barja and dos-Santos-Afonso, 1998) are shown in Figure 8.1.2.1-49, where I, II and III are the main species presents in the studied pH range.

Table 8.1.2.1-89: Freundlich parameters (in $\mu mol^{1-1/n}.m^{-2}$) for glyphosate adsorption on Santiago del Estero Province soils

Horizon	Ap			AB			BC		
pH	K_F*10^3	1/n	R^2	K_F*10^3	1/n	R^2	K_F*10^3	1/n	R^2
2	7.3	0.46	0.9449	19.7	0.44	0.9743	3.3	0.64	0.9842
3	6.7	0.47	0.9953	18.4	0.45	0.9894	4.9	0.65	0.9759
4	5.9	0.47	0.9646	16.2	0.46	0.9654	4.7	0.63	0.9852
5	5.6	0.46	0.9729	16.2	0.45	0.9652	4.6	0.63	0.9892
6	5.0	0.48	0.7578	15.1	0.46	0.9862	4.3	0.62	0.9812
8	3.8	0.47	0.9453	14.3	0.45	0.9749	3.0	0.64	0.9893

Conclusions

The major factor in PMG adsorption on soil samples is given by the pH, which could be due to the influence of this parameter on the PMG molecule and on the surface charge of the soil particles. PMG adsorption increase with acidity, and this increase correspond to the adsorption of a ligand with a

negative net charge. Sorption of glyphosate in soils is similar to the adsorption of the organic molecule on the soil components such as clay minerals, iron oxides and OM. For these soils with a low organic matter contents and/or similar amounts of clay in the various horizons, the adsorption would be determined by the content of phosphorous, iron oxide and the specific surface. Regarding the relative adsorption capacity of the soil, the adsorption process has a different behavior profile, where the deeper horizon (BC) has a higher capacity retention for this herbicide.

Table 8.1.2.1-90: Langmuir parameters for PMG adsorption on soils or Santiago del Estero Province, Argentina

Horizon	Ap			AB			BC		
pH	Γ_{max}, µmol.m^{-2}	K_L, L.mmol^{-1}	R^2	Γ_{max}, µmol.m^{-2}	K_L, L.mmol^{-1}	R^2	Γ_{max}, µmol.m^{-2}	K_L, L.mmol^{-1}	R^2
2	0.41	0.91	0.9572	0.81	0.93	0.9936	1.50	0.40	0.9331
3	0.40	0.87	0.9723	0.82	0.93	0.9833	1.49	0.42	0.9664
4	0.38	0.73	0.9874	0.78	0.92	0.9925	1.36	0.41	0.9955
5	0.34	0.67	0.9769	0.76	0.92	0.9932	1.27	0.40	0.9854
6	0.31	0.63	0.9633	0.74	0.91	0.9974	1.23	0.39	0.9858
8	0.24	0.61	0.9424	0.72	0.90	0.9926	1.10	0.28	0.9830

The lower adsorption in the AB and Ap horizons could be influenced by the higher content of phosphorus. However, the strength of the interaction, as given by the Langmuir Model Constant KL is larger on horizon A B and would be linked to the illite and iron oxide content that have a better distribution in AB. It should be noted that the Langmuir adsorption model is the best fit to the adsorption experimental results in these soils, although the Freundlich model has a good fit for some pHs. Given the adsorption extent found in this study, it is expected that pesticides will be retained in these soils. This strong interaction could prevent the pesticides movement into the ground water. On the other hand, this retention rate could result in the release of the herbicide on the environment due to displacement by runoff.

Assessment and conclusion by applicant:

The article describes the adsorption of non-labelled glyphosate to topsoil and subsoil of an agricultural soil from Argentina. The pH-dependency was investigated in addition. However, there was no detailed reporting of data to assess the validity (i.e. mass balances, detailed chemical properties of test substance, solvents used, information about analytical methods and their validation including, LOD, LOQ, temperature, test concentrations).

The article is therefore classified as reliable with restrictions, i.e. no use of data in risk assessment.

Assessment and conclusion by RMS:

The lack of valuable information to determine the validity of the study against OECD 106 criteria (as stated by the applicant, mass balance, stability of the test items, efficacy of the method) prevents its use for deriving endpoints for the risk assessment. The article however focuses on the behaviour of Kf of glyphosate when applied solutions of different pH. This approach is not in line with the OECD guidance.

The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Jodeh, 2014

Data point:	CA 7.1.3.1.1/027
Report author	Jodeh S. et al.
Report year	2014
Report title	Fate and Mobility of Glyphosate Leachate in Palestinian Soil Using Soil Column
Report No	ISSN 2028-2508

Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

In recent years, pesticides were used heavily in Palestine, which led to the contamination of soil and water and causing many diseases. Many studies focused on the impact of pollutants such as pesticides and oil on soil, humans, animals, plants and the environment in general. Using column study the amount of glyphosate in soil decreases with increasing depth of soil, where it is for 0–30 cm (11 ppm) >30–60 cm (6 ppm) >60–100 cm (2 ppm) due to organic content and metal oxides founded in soil that can form stable complexes with glyphosate. When we increased the concentration of glyphosate, the amount of glyphosate (contaminant) in leachate where found to be 25 x (15.96 ppm) >15 x (3.91) >5 x (3 ppm) column. The behavior of glyphosate leachate fits the first order reaction and the isotherm is in according with the Freundlich adsorption equation with R2 value 0.98, k value 6.4 and n value 1.07 which indicates good adsorption to soil.

Materials and Methods

Chemicals

Glyphosate (purity 98.5 %) was purchased commercially. Other chemicals like carbon disulfide, copper nitrate and chloroform were available at the university department of chemistry. All chemicals and solvents used in the experiment were of high performance liquid chromatography and high purity.

Acid digestion of soil

To find the metals in soil and $HClO_4$ (70 %) and HF (40 %) were added then heated to incipient (near dryness). HF were added again and heated to dryness then $HClO_4$ and distilled water were added and heated to incipient. The remaining residue was dissolved in HCl and water. Volume was made up to the 100 mL volume and stored in polyethylene bottle. Fe and Cu in the supernatant were determined by AAS. The physicochemical soil properties (Table 8.1.2.1-91) were determined using standard methods.

Sampling site and Collection

The soil was sampled in three layers; 0–30 cm, 30–60 cm and 60–100 cm from agricultural locations in Nablus, Mount Gerizim before herbicide treatment of the fields. The soil samples were mixed well separately. The soil used for chemical analysis was air dried, sieved to 2 mm stored in the dark at room temperature and protected from humidity. Basic physicochemical properties of soil were conducted on soil before any treatment with glyphosate.

Table 8.1.2.1-91: Physico–chemical characteristics of the soil column

Soil texture • Sand [%] • Silt [%] • Clay [%] •	35% 57.5% 7.5%
Moisture **%**	3.3%
Moisture correction factor (mcf)	1.033
pH	7.62
Organic Carbon %	2.11%
Organic Matter %	3.63%
Conductivity(µs)	530
N%	0.1934%
Ca CO_3 (mg)	0.795
Cu (mg/kg)	44
Fe (mg/kg)	1982.27
Available Phosphorous (P) (mg/kg)	62.41

Leachate extraction columns

Leachate extraction columns consist of four columns of polyvinyl chloride (PVC) pipe. A metal mesh screen was placed at the bottom end of each column and a plastic bottle was placed under each column to collect water. Soil column was washed with distilled water to remove air bubbles from soil and to ensure that the pH of leachate water from each column is neutral.

Glyphosate application to soil–column experiment

Glyphosate contains the monoisopropylamine salt of glyphosate (N–(phosphonomethyl)–glycine) (360 g/L) was applied to each column with concentrations; 5 X, 15 X and 25 X, where X equals amount of glyphosate applied to soil yearly (nearly 2 L/dunom), numbers (5, 15, 25) are the years of applying glyphosate to soil. Blank soil samples were used as controls without glyphosate addition. The concentrations of glyphosate added to soil columns are listed in Table 8.1.2.1-92.

Leachate

Leachate was collected from each column in plastic bottle at the end of every period. Leachate volumes were determined gravimetrically. Leachate water was centrifuged to remove solid particles and then the supernatant was filtered before analysis. Glyphosate extracted by the method described below and derivatized using the method shown below then measured by Spectrometer at 435 nm.

Procedure for Solid–Phase Extraction (SPE) of glyphosate from water samples

A cation exchange resin was used for the pre concentration and cleanup of glyphosate. A slurry of the Amberlite IR–120, Na–ion exchange resin (cationic) was made in 10 mL distilled water and packed into a narrow glass column, plugged with glass wool at the bottom. The resin was rinsed with distilled water and then with 1 M HCl at a flow rate of 2 mL/min several times before sample application. The pH of water sample spiked with glyphosate was adjusted to 2 and amine group of glyphosate was converted into its protonated form. The protonated sample (25 mL) was passed through the column at a flow rate of 0.5 mL/min in order to have maximum exchange of protonated sample. After the loading step, the sorbent was washed with 25 mL of 2 M NaCl solution (used as eluent) at the same flow rate. The eluted solution was evaporated to about 10 mL at 70ºC then evaluated by the proposed method.

Table 8.1.2.1-92: Main characteristics of soil after application of glyphosate at different depths

Column	Depth (cm)	PH	C %	O.M %	N %	Available P mg/kg	$CaCO_3$ mg/kg	Fe mg/kg	Cu mg/kg
Blank	0-30	7.45	1.56	2.69	0.145	7.91	0.211	1941	43
	3-60	7.78	1.53	2.64	0.082	5.3	0.245	1997	38
	60-100	7.7	1.36	2.33	0.024	5.27	0.292	2008	52
5x	0-30	7.55	2.08	3.58	0.321	66.62	0.147	1853	30
	30-60	7.86	2.05	3.53	0.270	48.49	0.161	1953	35
	60-100	7.72	2.03	3.49	0.250	45.71	0.199	2000	64
15x	0-30	7.68	2.08	3.58	0.373	72.57	0.194	1909	35
	30-60	7.75	2.02	3.48	0.356	66.1	0.197	2053	44
	60-100	7.88	1.99	3.42	0.305	53.26	0.208	2103	52
25x	0-30	7.49	2.21	3.80	0.425	95.04	0.178	1909	24
	30-60	7.56	2.05	3.53	0.375	88.31	0.206	1985	29
	60-100	7.66	2.01	3.46	0.319	74.13	0.200	2032	34

Derivatization procedure of glyphosate

Glyphosate was derivatized using carbon disulfide to convert the amine group into dithiocarbamic acid. The dithiocarbamate group was used as chelating group for reaction with transition metal ion Cu (II). The resultant yellow colored complex was measured at 435 nm using UV–Spectrophotometer. Carbon disulfide (1 % CS2) solution was prepared and an aliquot of glyphosate were added to a series of 100 mL separating funnels followed by the addition of CS2 solution. Then the mixture was shaken for 3 minutes for the formation of dithiocarbamic acid. An ammonical solution of Cu(II) (1000 mg/L) was added to the mixture, shaken again vigorously to form complex with dithiocarbamic acid and then kept for separation of two phases. The yellow colored chloroform layer containing the complex was separated in a 10 mL flask and diluted with ethanol. The absorbance of the complex was measured at 435 nm.

Soil columns after glyphosate application

At the end of the experiment, soil columns were cut into three parts. Three samples were taken from each part, air dried and stored in an air tight polythene bottle to analyze their parameters in soil lab at An Najah National University. Glyphosate were extracted from the three parts of soil columns, derivatized and measured spectrophotometrically.

Batch sorption experiment

Sorption kinetics was analyzed by altering the contact time at a constant concentration of 20 and 30 ppm per vessel for determination of an appropriate equilibrium time at room temperature for the sorption isotherm experiments. They were shaken for 1, 2, 4, 6, 8, 24, 48 and 72 hours, respectively. Samples were equilibrated and processed.

Adsorption isotherm experiment

Soil samples were air–dried, sieved, stored in the dark at room temperature (23°C), and protected from humidity. Sorption experiments were carried out using the standard batch equilibration method. A series of five selected glyphosate concentrations were carried out to determine the adsorption isotherms of glyphosate on soil. The adsorption measuring steps were as follows:

200 mL of a PTFE vessels containing 25 g air dried weight soil.

100 mL aqueous solutions containing 0–50 mg/L glyphosate were equilibrated for 24 h at room temperature on a reciprocating shaker at low speed 120 excursions per minute.

The supernatant equilibrium concentration is obtained after centrifuging at 3000 rpm (round per minute) for 20 minutes.

Blank without glyphosate was also equilibrated. The equilibrium concentrations of each soil were measured spectrophotometrically after derivatization.

Consequently, the differences between the initial and equilibrium concentrations were assumed to be due to sorption onto soil. Sorption isotherms were obtained by plotting the amount of glyphosate sorbed per weight of soil at equilibrium (Qe, µg/g) versus the amount of glyphosate per volume of solution at equilibrium (Ce, µg/mL). The sorption data were described using the Freundlich equation:

Qe = Kf .Cenf eq. 1

where Qe is the concentration of glyphosate sorbed onto the solid phase (µg/g), Ce is the concentration of glyphosate in solution at equilibrium (µg m/L), and Kf (in µg1−nf mLnf g−1) and nf are empirical constants which are related to the adsorption phenomenon and calculated by regression analysis. Kf can be considered as a characterisation of the intensity of sorption, modulated by the deviation from the unity of the nf exponent.

Glyphosate extraction from soil samples

Homogenized soil sample (10 g) was extracted for 60 min with 25 mL of 2 M NH4OH solution. The extraction was repeated three times. The pH of eluted sample was re–adjusted to pH 5.4 and was evaluated by the proposed method. Each recovery was performed in triplicates.

Results and Discussion

Batch sorption experiments

The sorption kinetics of the soil were studied to determine an appropriate shaking time for the sorption isotherm experiments. Readings were recorded until 72 hours, no changes in concentrations were observed after 24 hours for all samples, and therefore 24 hours were chosen as equilibrium time for the sorption isotherm experiment due to the quick degradation of glyphosate. The equilibrium adsorption data over the range of concentrations studied here were used to fit Freundlich adsorption equation (eq. 1). The values of n within the range of 2–10 represent good adsorption. Higher values of *k* indicate high adsorption capacity. The isotherm equilibrium results for the examined soil are shown in Figure 8.1.2.1-50. Freundlich isotherm constants (*k* & n) for glyphosate, the correlation coefficient "R" were obtained from Figure 8.1.2.1-50 and listed in Table 8.1.2.1-93. Glyphosate sorption at 25°C in the studied soils was evidenced to be a kinetics process, with a reasonable equilibration time of 24 hours. Literature usually reports Freundlich adsorption constants for glyphosate adsorption by soils which are consistent with that founded in our study. It is indicated from Table 8.1.2.1-93 and Figure 8.1.2.1-50 that "n" of glyphosate adsorption is higher than 1. The adsorption isotherms for the soil is of S–type, which indicates the easiness of the adsorption, mainly at higher concentrations.

Figure 8.1.2.1-50: Adsorption isotherm of glyphosate for Palestinian soil

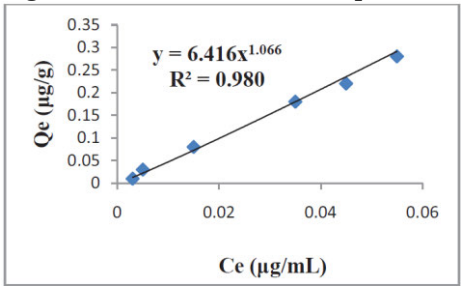


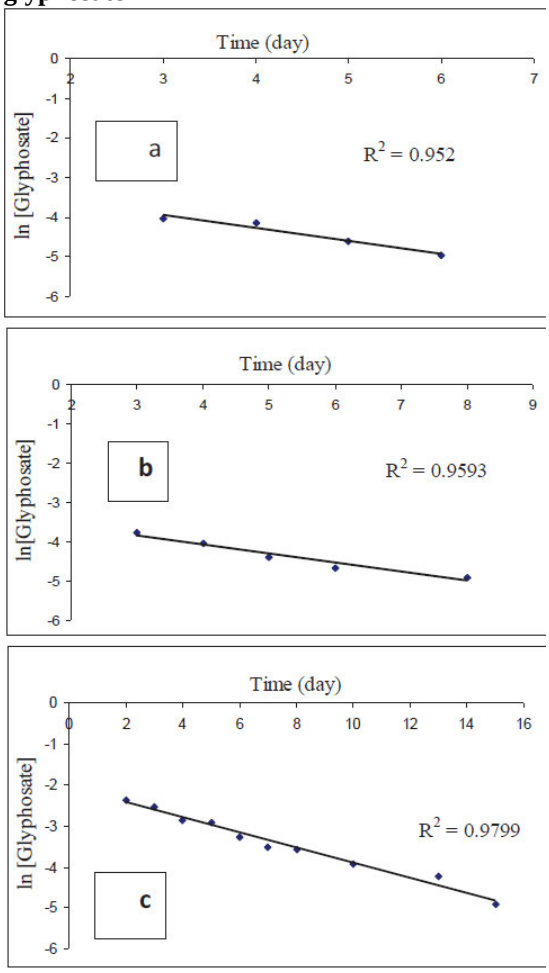
Table 8.1.2.1-93: Freundlich isotherm constants for glyphosate

Coefficient	K	1/n	n	R^2
Glyphosate	6.41	0.93	1.07	0.98

Glyphosate in leachate

It is indicated that the amount of glyphosate detected in leachate decreases with increasing time. It takes time for 25 x >15 x >5 x until the inability to detect glyphosate in leachate for concentrations less than 1 ppm. Doubling the concentration of glyphosate increases the amount glyphosate (contaminant) in leachate. The above resulting curves shows that the best fit of the glyphosate degradation data was obtained using a first–order reaction as shown in Figure 8.1.2.1-51. DT_{50} values of glyphosate was 2, 3 and 3.75 days for 5 x, 15 x & 25 x column respectively. This indicate relatively rapid degradation.

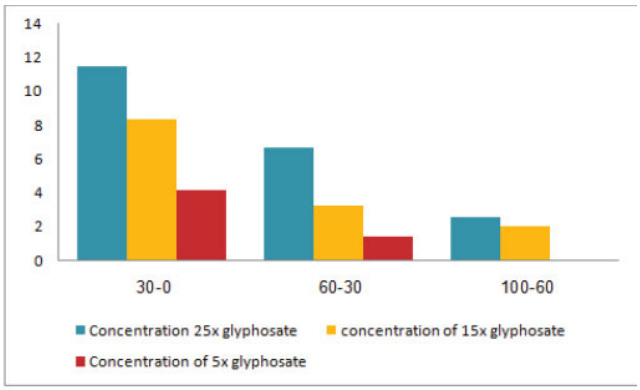
Figure 8.1.2.1-51 Plot of time vs. Ln concentration for 5 X (a), for 10 X and (c) for 25 X times glyphosate



Glyphosate in column soil

The results indicated that the glyphosate mobility in the soil columns increased with application rate. With more glyphosate applied, more glyphosate in the soil columns was capable of moving out of the columns. Amount of glyphosate detected in soil columns was increased in the order: 25 x >15 x >5 x. The amount of glyphosate was decreased with depth increasing due to decreasing organic content. It means that the adsorption tendency decreases as the depth increases. No glyphosate detected in 60–100 cm depth as shown in Figure 8.1.2.1-52. This due to low concentration of glyphosate less than 1 ppm that couldn't be measured by the method used here. Lowest concentration was used also most of glyphosate adsorbed on the upper layer of soil (0–30 cm). This study indicates that glyphosate can be extensively mobile in soil environment if it is applied on soils unable to retain the molecule long enough for its microbial degradation. This may also lead to herbicide leaching to lower soil layers where a limited biological activity occurs.

Figure 8.1.2.1-52: Concentration (mg/L) of glyphosate in soil column at different depths



The effect of organic matter

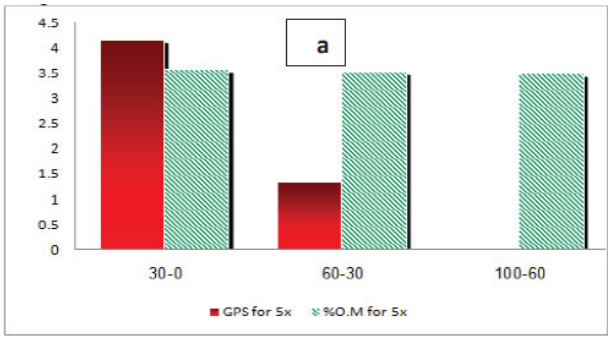
Soil organic matter consists of a variety of components. These include, in varying proportions and many intermediate stages:

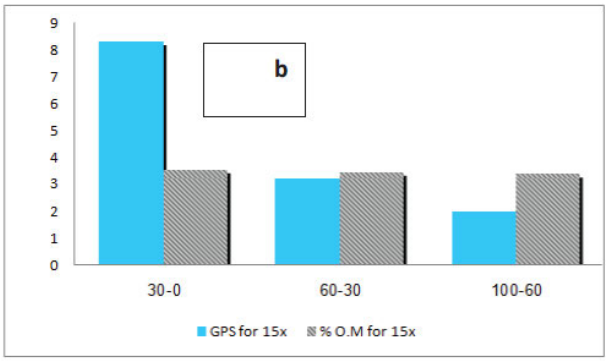
- Raw plant residues and microorganisms (1 to 10 %).
- "Active" organic traction (10 to 40 %).
- Resistant or stable organic matter (40 to 60 %) also referred to as humus.

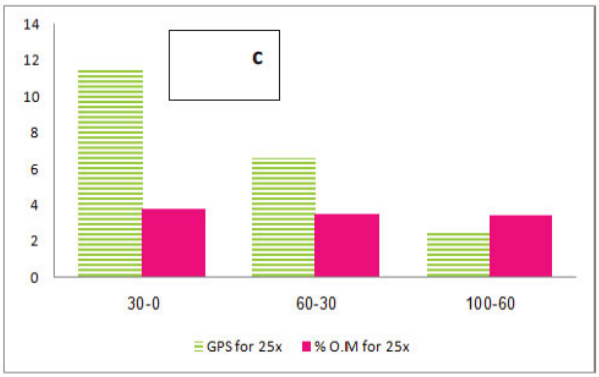
Table 8.1.2.1-92 shows that organic matter content of the soil at different depths ranges between 2–3.8 % which is considered as a moderate organic matter soil. Organic matter content of the soil at different depths for each column nearly the same as shown in Figure 8.1.2.1-53. It is indicated that organic matter only may not affect the adsorption of glyphosate at different depths and it could affect sorption in two ways:

- Reducing glyphosate sorption by blocking sorption sites.
- Increasing glyphosate sorption because poorly ordered aluminium and iron oxides with high sorption capacity are favored at higher soil organic matter content.

Figure 8.1.2.1-53: Organic matter content in 5 X (a), 15 X (b) and 25 X (c) column and concentrations of glyphosate at certain depths







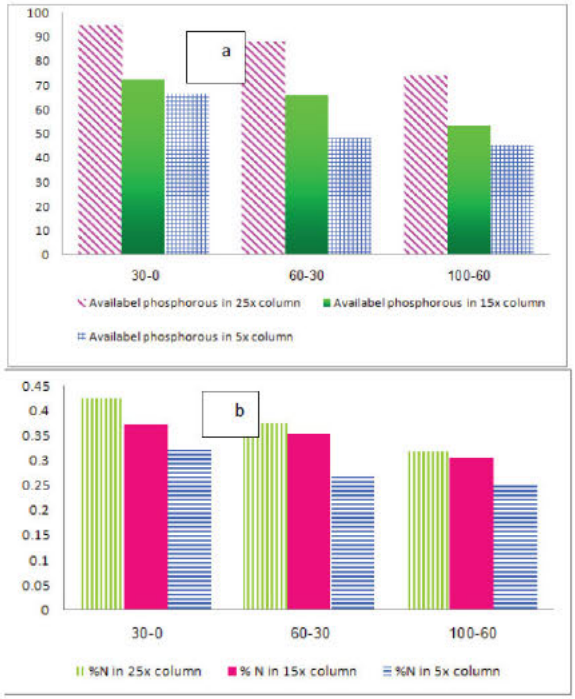
The effect of soil metals

The high sorption values for glyphosate can be in part due to the pH values of soils and to the presence of iron oxides, copper and other metals that can form stable complexes with glyphosate. Glyphosate coordinates strongly to Cu, and Cu–glyphosate complexes formed seem to have higher ability to be adsorbed on the soil than free glyphosate. Copper acts as a bridge between the soil and glyphosate. At these pH values glyphosate is a di–anion and both the carboxylate and the phosphonate functional groups in its molecule are deprotonated, being able to compete for the surface adsorption sites on the metal oxides.

Available phosphorous after glyphosate application

Figure 8.1.2.1-54 shows that the amount of phosphorous in soil columns after application of glyphosate increased this indicates degradation of glyphosate to its components where phosphorous is one of the degradation products. Glyphosate could be source of phosphorous, nitrogen and carbon in soil as it is shown in Figure 8.1.2.1-54 and Table 8.1.2.1-91. The nitrogen content of soil has been increased after glyphosate application to soil columns due to biodegradation of glyphosate.

Figure 8.1.2.1-54: Phosphorous content (a) and nitrogen content (b) in soil columns after application of glyphosate



Conclusion

Adsorption is an important process in determining the fate of glyphosate in soil. The texture for soil used has been found to be silty clay and the total organic matter (TOM) close to 4 %. Batch equilibrium technique was used to evaluate the extent of glyphosate adsorption on soil as adsorbent. Isotherm is in accord with the Freundlich adsorption equation with R2 value 0.98; the parameters of this isotherm have been calculated. The adsorption isotherm was fit the S–type isotherm according to Giles. The values of "n" in Freundlich equitation was more than one indicating good adsorption for glyphosate with the soil used. Freundlich constant "k" indicates the tendency of glyphosate in this study to be adsorbed on soil particles. k increases with increasing the soil minerals and decreases with increasing the depth of soil where the main binding mechanism for glyphosate is the covalent bond between the herbicide and the metals from soil oxides, and so the adsorption decreasing due to decreasing the organic matter content as depth increases. Many factors affect the adsorption of glyphosate as phosphorous content, pH, and temperature. The high sorption values for glyphosate can be in part due to the presence of metal oxides that can form stable complexes with glyphosate.

Assessment and conclusion by applicant:

The article describes a column leaching and adsorption tests with non-labelled glyphosate with a Palestinian agricultural soil.

Due to analytical method insensitivity, the lowest rate examined in the column leaching experiment was 5 times the yearly application rate. In addition, some essential information necessary for assessment of validity of both experiments is not reported (i.e. mass balances, equilibration solution not specified).

The article is classified as not reliable for the column leaching experiment and as reliable with restrictions for the adsorption experiment, *i.e.* it was not used in risk assessment.

Assessment and conclusion by RMS:

This article provides information regarding column leaching and batch adsorption test. For the two tests, the validity of the article cannot be established due to the lack of information such as the mass

balances, concentrations used, stability of the tested compound, efficacy of the analytical method, *etc.*

The article provides supportive information on the adsorption and mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Rampoldi, 2014

Data point:	CA 7.1.3.1.1/028
Report author	Rampoldi, E., et al.
Report year	2014
Report title	Carbon-14-Glyphosate Behavior in Relationship to Pedoclimatic Conditions and Crop Sequence
Report No	DOI 10.2134/jeq2013.09.0362 E-ISSN 1537-2537
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:

The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/014). The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Bergsröm, 2011

Data point:	CA 7.1.3.1.1/028
Report author	Bergström, L. et al.
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Report No	DOI 10.2134/jeq2010.0179 E-ISSN 1537-2537
Guidelines followed in study	OECD Guideline 106 Guideline
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:

The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/017). The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Maqueda et al. 2017

Data point:	CA 7.1.3.1.1, CA 7.2.1.3
Report author	Maqueda C. *et al.*
Report year	2017
Report title	Behaviour of glyphosate in a reservoir and the surrounding agricultural soils

Report No	Science of the Total Environment, (2017) Vol. 593-594, pp. 787-795 http://dx.doi.org/10.1016/j.scitotenv.2017.03.202
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate (glyphosate) is an herbicide currently used on olive crops in Spain. It can be transported to the nearby reservoirs currently used for human consumption. The purpose of this work was to study the behaviour and environmental fate of glyphosate in water and sediments of the Vibora Reservoir, its tributary river, and the surrounding agricultural soils to assess the risk of water pollution of this reservoir. The adsorption of glyphosate by different matrices was as follows: heading of the reservoir sediment (Cabecera) > tail sediment (Cola) > soils > Vibora sediment. The highest amount of oxides (especially Fe oxides) was observed in sediments from Cabecera and Cola whereas the lowest values were recorded on Vibora sediment. Results indicate that the highest glyphosate adsorption is due to the amorphous oxides and the edge sites of the clay minerals. Glyphosate adsorption increased with decreasing pH from 8 to 7. The desorption percentage of glyphosate from the four soils studied ranged only from 0.40 % to 1.22 %. Desorption was almost irreversible for Cabecera and Cola sediments, with values between 0 % and 1.1 %. Conversely, Vibora sediment presented about 20 % desorption, probably due to its coarse texture and lower levels of amorphous oxides. Hockey-stick first-order kinetics was the best descriptor for water glyphosate dissipation at the Cabecera and Cola locations, and simple first-order kinetic for the water from the tributary Vibora River. The half-lives (DT_{50}) were between 6.3 and 11.0 days. The rapid degradation of glyphosate in surface waters and its practically irreversible sorption on these soils and sediments implies that glyphosate use in similar agricultural areas is of very low environmental risk. This study also outlines the importance of the presence of photo-sensitizers in waters in the degradation routes of glyphosate in reservoirs.

Materials and methods

Pesticide

High purity glyphosate (98 % purity) was used in adsorption and dissipation experiments. The herbicide was purchase from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Sampling site

Vibora Reservoir is located at the Vibora riverbed in the province of Jaen, in the south of Spain (37°38′8″N 3°59′36″W). It is a water reservoir for drinking water and fishing and has a capacity of 19 hm^3. It has a tributary river with the same name, Vibora. The province of Jaen is the region with the highest olive production concentration in the world.

Sampling soils, sediments and water

Four agricultural soils (olive trees) near the Vibora Reservoir were selected for this study. The soils were randomly sampled from the 0 – 15 cm layer, air dried, and crushed to pass through a 2 mm sieve before their use in the experiments. Three different sediments were also taken Cabecera and Cola (belonging to the head-board and tail of the Víbora Reservoir, respectively) and other one named Vibora, corresponding to the tributary river of the reservoir with the same name. Samples were frozen and lyophilized before carrying out the different determinations. Soils and sediments were analysed for pH in a water:soil extract at the ratio 2.5:1, total carbonate content, particle size distribution and organic matter (OM) content. The clay fraction of soil was further characterised. The amorphous and organically bound iron, manganese, and aluminium oxides were determined using ammonium oxalate-oxalic acid.

Water samples were collected at the head of the reservoir (Cabecera), the tail of the reservoir (Cola), and the tributary river Vibora at a depth of 50 cm. Samples were stored in amber bottles in the dark at 4 °C during transport to the laboratory and then frozen at −18 °C until use. The sampling was performed in the beginning of April 2015 before glyphosate application on the olive orchards. The presence of

glyphosate and AMPA were determined in soils, sediments and waters before application of the herbicide, and no residues were found.

Adsorption-desorption of glyphosate on soils and sediments
Before performing the batch adsorption experiments, preliminary kinetics studies were carried out. It was found that 24 h was long enough to reach glyphosate adsorption pseudo-equilibrium in soils and sediments. Triplicate adsorption experiments were performed by mixing 5 g of the different soils with 10 mL solution containing various concentrations (1–10 mg/L) of glyphosate, in 50 mL polypropylene centrifuge tubes. The samples were shaken on a platform shaker for 24 h at 20 ± 1 °C. After shaking, the dispersions were centrifuged and the concentration of glyphosate in the supernatant was determined. All the experiments were carried out in triplicate. The differences between initial and final herbicide concentrations were assumed to be due to adsorption. The isotherms were obtained representing the amount adsorbed versus the amount remaining in solution.

Desorption experiments were performed after adsorption equilibrium was achieved by removing half of the supernatant after centrifugation, then replacing it with distilled water and allowing equilibration for an additional 24-h period. The experiment then proceeded according to the protocol used for the adsorption experiment. This process was repeated twice more. The equilibrium pH was maintained both in adsorption and desorption isotherms at pH 8 due to the high carbonate content of soils and sediments samples that buffered the medium. Desorption isotherms were obtained representing the amount that remained adsorbed versus the concentration for each desorption process. Adsorption isotherms were fitted to the Freundlich equation.

The normalized distribution coefficient (K_{fOC}) of organic carbon (OC) was calculated from the K_f values. The adsorption distribution coefficients (K_d) were also determined at 0.01 µmol/L. K_f and K_d values were used to compare the adsorption capacity of the different matrices. For sediments, the adsorption experiments were carried out as in soils but using 1 g and 20 mL solution containing various glyphosate concentrations (0.1 to 1.0 mg/L). Glyphosate adsorption experiments were performed at controlled pH values (7 and 8) by adding aliquots to the different initial solutions of sodium hydroxide or hydrochloric acid such that the final pH was maintained at that desired. These pH values were selected because are those usually found in the reservoir along the different seasons. Desorption experiments were carried out only at pH 8 for comparison with the soils.

Glyphosate dissipation in water under aerobic conditions
Laboratory experiments were carried out to elucidate the glyphosate dissipation kinetics in natural water collected from the reservoir and the Vibora tributary river under simulated light exposition. Experimental conditions were selected as closely possible to the natural aquatic environment. Water samples were premixed before dissipation experiments. Portions of 100 mL of this natural water were distributed in glass containers and glyphosate was added to obtain a concentration of 2.5 mg/L. The water samples were placed in a climatic chamber at 25 ± 1 °C with a 16 h light photoperiod at an intensity of 11 µE/m^2s. Over 20 days, samples were collected from the containers at different time intervals, the suspensions were filtered through a 0.22-µm Millipore glass fibre membrane and the concentration of glyphosate in the filtrate determined by HPLC-MS. All experiments were carried out in triplicate. A parallel experiment was performed to test potential sorption of glyphosate to glass surfaces. The operational conditions were identical but using distilled water instead and the containers covered by aluminium foil to prevent photodegradation. No glyphosate adsorption on the glass container was noticed.

The amount of dissipated glyphosate was plotted versus time. For the calculation of the kinetic parameters, dissipation curves were modelled according to the instructions of the FOCUS guide, using the least squares method with the SOLVER from the Microsoft Office Excel 2007 mathematical program. Dissipation kinetics were fitted to two models: a simple first-order (SFO) model and a first order sequential model (Hockey-Stick, HS). The Chi-square (×2) test with $\alpha = 0.05$ was used to estimate the appropriateness of the model and to assess the accuracy of each resulting fit. The time required for 50 % disappearance of glyphosate (DT_{50}) was determined.

Herbicide analysis
8 mL of the soil supernatant were subjected to solid phase extraction (SPE) on an Oasis HLB 60 mg cartridge, previously conditioned with 2 mL of methanol and 2 mL of acidified water at pH 2.5, and the

extract was collected to an autosampler vial. Recoveries were between 90 % and 97 % for solutions of glyphosate and AMPA of 5 and 10 µg/L. The analysis of glyphosate was carried out by liquid chromatography-tandem mass spectrometry in an Agilent HPLC with a triple quadrupole mass spectrometer (AB Sciex) under the following conditions: Eluent A, 1 % Acetic acid in Water +5 % MeOH; eluent B, 1 % Acetic acid in MeOH; Column Hypercarb 2.1 × 100 mm 5 µm at 40 °C; ionization mode, ESI negative; injection volume, 5 µl; acquired mass transitions (m/z) for glyphosate, 168/63, 168/124, 168/150, 168/81; for AMPA, 110/63, 110/79, 110/81; retention times: Glyphosate, 3 min; AMPA, 1.86 min. The percentage of the eluent A was changed linearly in the time-programmed gradient used as follows: 0 min, 100 %; 10 min, 70 %; and 12 min, 100 %. The flow rate was constant at 0.2 mL/min.

The limits of quantification (LOQ) of both glyphosate and AMPA were 10 µg/L, and their limits of detection (LOD) 3 µg/L.

Results and Discussion

Physico-chemical and mineralogical characteristics of the soils and sediments

Table 8.1.2.1-94: Physico-chemical characteristics of the soils and sediments

	Soils				Sediments		
	1	2	3	4	Cola	Cabecera	Víbora
OM (%)	1.69	1.59	1.91	1.60	1.62	1.73	1.52
$CaCO_3$ (%)	34.5	37.6	16.2	31.5	27.7	22.4	43.9
pH	7.65	7.73	7.47	7.48	7.61	7.34	8.36
Clay (%)	33.6	31.3	28.2	36.5	6.0	8.0	4.9
Silt (%)	31.9	30.5	30.2	32.2	51.2	60.4	8.0
Fine sand (%)	6.0	11.1	8.0	10.5	42.8	31.6	6.4
Coarse sand (%)	28.5	27.0	33.5	20.8	–	–	80.9
Amorphous Fe oxides (%)	1.79	1.71	1.71	1.64	7.77	8.15	1.85
Amorphous Al oxides (%)	1.16	1.33	0.83	1.76	1.71	2.16	0.43
Amorphous Mn oxides (%)	0.19	0.21	0.34	0.38	0.13	0.18	0.15
∑ amorphous oxides (%)	3.14	3.25	2.88	3.78	9.61	10.5	2.43

Table 8.1.2.1-95: Semiquantitative estimation (%) of the clay minerals in soils and sediments

	Soils				Sediments		
	1	2	3	4	Cola	Cabecera	Vibora
Illite	70	60	80	70	25	40	10
Kaolinite	10	10	5	5	5	5	<5
Vermiculite	10	15	9	15	5	5	<5
Chlorite	10	15	6	–	–	–	–
Smectite	–	–	–	10	–	–	–
Quartz	≪5	≪5	≪5	≪5	25	15	25
Calcite	*	*	*	*	35	25	50
Feldspars	≪5	≪5	≪5	≪5	10	10	10

*Calcite was not detected in soils because it was previously removed.

Glyphosate adsorption-desorption on soils

The adsorption isotherms of glyphosate in the soils included in this study are shown in the figures. The experimental data were well fitted to the Freundlich equation. The values of Freundlich parameters are listed in the tables. The correlation coefficients were in all cases > 0.98. N values were close to 1, what could be indicative of low heterogeneity among the sites of the soils where glyphosate has been adsorbed. It was probably due to the low concentration of herbicide used in the adsorption isotherms experiments, implying that glyphosate was adsorbed on high affinity sites, which were not totally occupied in the range of concentrations used. Because n values were very similar among the samples, K_f values could be used to compare the adsorption capacity of the different soils. According to the K_f values the order of adsorption was the following: soil 4 > soil 2 > soil 1 > soil 3

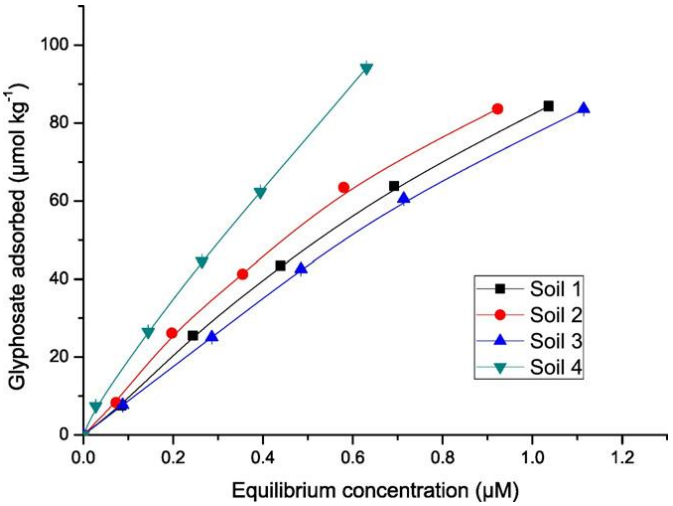


Figure 8.1.2.1-55: Glyphosate adsorption isotherms on soils

Table 8.1.2.1-96: Freundlich adsorption isotherm parameter (K_f and n values), coefficients of determination (R^2) and organic carbon normalized distribution coefficients (KfOC) of glyphosate sorption on the soils. Errors are <2 %

	K_f (L kg^{-1})	n	R^2	K_{foc}
Soil 1	91.09	0.9654	0.987	9,295
Soil 2	98.06	0.8995	09876	10,659
Soil 3	81.39	0.9537	0.9966	7,332
Soil 4	132.6	0.8109	0.991	14,263

K_f values ranged from 81.4 to 133 (L/kg) for soils 3 and 4, respectively. The amount of glyphosate adsorbed in the soil 4 was higher than on the other soils. The physico-chemical characteristics of the four soils surrounding the reservoir are almost similar and the pH values obtained after equilibrating with the four soils were also similar with values about 8, indicating that pH was not responsible for the different adsorption behaviour. The four soils had high amount of clay fraction, with presence of phyllosilicates and medium content of organic matter. The mineralogical difference between soil 4 and the others was the presence of smectite, amounting at about 10 %, which also would increase the cation exchange capacity (CEC).

The role of hydrophobic bonds in the adsorption of non-polar hydrophobic herbicides to soils can be compared by normalizing the Freundlich adsorption parameter to the percentage of organic carbon (K_{foc}) in each sample. However, the values obtained in this study are quite different, ranged from 7332 to 14,263, indicating that the adsorption mechanism is related to some other soil properties in addition to the OC content. Notably, soil 4 presented the greatest value of amorphous oxides of the four studied soils, which could be a factor worthwhile considering the greatest adsorption of glyphosate in this soil. The fact that glyphosate adsorption follows the same pattern as total amorphous oxide content in these soils seems to indicate that the main soil adsorption sites are found on the variable-charge surfaces of such amorphous oxides.

Glyphosate desorption from soils was very little. Total percentages of glyphosate desorbed (after three cycles of desorption) from the soil samples treated with glyphosate 3, 5 and 10 mg/L are shown in the following table. The adsorption was almost irreversible in the four soils, indicating a strong hysteresis. The values of desorption were very similar for the different points of adsorption although a little higher for the points with higher adsorption. The percentage of glyphosate desorbed for the 4 studied soils ranged between 0.40 % and 1.22 %.

Table 8.1.2.1-97: Percentages of glyphosate desorbed (%) from the studied soils. Errors are <2 %

	GPS initial conc. ($mg\ L^{-1}$)	GPS adsorbed ($\mu mol\ kg^{-1}$)	GPS desorbed (%)
Soil 1	3	25.54	0
	5	43.46	0.40
	10	84.33	0.45
Soil 2	3	26.17	0.52
	5	41.27	1.04
	10	84.0	1.22
Soil 3	3	25.1	0.62
	5	42.56	1.07
	10	83.59	1.18
Soil 4	3	26.49	0.32
	5	44.57	0.40
	10	94.16	0.49

The presence of AMPA was not detected in these adsorption-desorption studies. The complete sorption-desorption experiments were performed in shorter time (4 days) than the half-life times usually recorded (15–23.8 days, IUPAC Pesticide Properties Data Base).

Glyphosate adsorption-desorption on sediments

The adsorption experiments of glyphosate in the three studied sediments were carried out at controlled pH values of 7 and 8 by addition of NaOH or HCl aliquots to the different initial concentrations in order to obtain equilibrium pH values similar to pH levels found in the reservoir throughout the year. The adsorption isotherms of Cabecera, Cola and Vibora sediments at pH values 7 and 8 are shown in the figures. The adsorption isotherms of the sediments are presented in separated figures due to the large difference in the amount of glyphosate adsorbed by Cabecera and Cola sediments in comparison with Vibora sediment. Glyphosate adsorption in Cabecera and Cola sediments were not well described by the linearized Freundlich equation. In the case of Vibora, the K_f values obtained were 212 (n, 0.9558; R^2 0.9762) and 46.2 (n, 0.9376; R^2 0.9098) for pH values 7 and 8, respectively. These values are similar to those obtained for the studied soils.

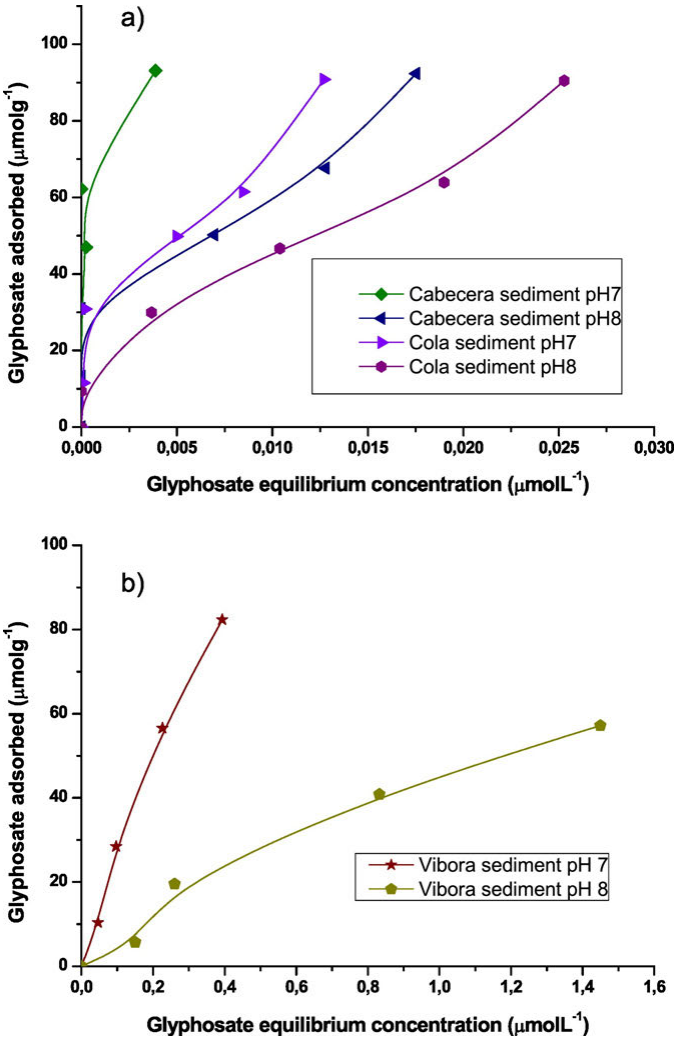


Figure 8.1.2.1-56: Glyphosate adsorption isotherms on sediments: a) Cabecera and Cola sediments; b) Vibora sediment.

As equilibrium concentrations reached in Cabecera and Cola sediments were very low, distribution coefficient (K_d) for the three sediments were calculated at an equilibrium concentration of 0.01 µmol/L to compare the capacity of the different sediments to adsorb glyphosate . The glyphosate adsorption by these sediments was almost complete at an equilibrium pH of 7. The K_d values for glyphosate adsorption to sediments ranged from 67.3 (Vibora pH 8) to 11,800 (Cabecera pH 7). According with K_d values the order of glyphosate adsorption to sediments was as follows:

Cabecera pH 7 > Cola pH 7 > Cabecera pH 8 > Cola pH 8 > > Vibora pH 7 > Vibora pH 8

Glyphosate adsorption to Cabecera and Cola sediments show extremely high K_d values (in the range 4810 - 11800) in comparison with Vibora sediment (67.3 and 404). This behaviour could be related with several physicochemical properties. First of all, these two sediments have higher amorphous oxides values in comparison with Vibora (almost four times higher) and also with the soils previously studied (about three times higher). The possible adsorption mechanism of the glyphosate to the oxides and hydroxides of soils and sediments is through the formation of bonds adsorbent-metal-phosphonic group of glyphosate. Secondly, the clay minerals content is very low in Vibora in comparison to the other two sediments providing lower amount of charge variable sites located on the edge broken bonds. And finally, Vibora sediment presented a loamy sand texture, and Cabecera and Cola sediments present a silt loam texture, providing much higher surfaces for adsorption.

K_d values were also calculated for glyphosate adsorption to the soils under study (carried out at pH 8) at an equilibrium concentration of 0.01 µmol/L, in order to compare their adsorption capacity with that of the sediments. Soil K_d values ranged from 197 to 213, indicating that adsorption was more similar to that observed in Vibora sediment as compared to Cabecera and Cola sediments.

Table 8.1.2.1-98: Distribution coefficients (K_d, L/kg) of glyphosate sorption on the soils and sediments. Errors are <2 %

	pH 7	pH 8
Soil 1	–	197
Soil 2	–	200
Soil 3	–	192
Soil 4	–	213
Vibora sediment	404	67.3
Cabecera sediment	11,800	6225
Cola sediment	7961	4810

Glyphosate adsorption increased with decreasing pH from 8 to 7. The effect of pH may be due to the influence on the charge of the glyphosate molecule and the surface charge of the adsorbent. A decrease in pH facilitates the adsorption of glyphosate in sediments which pose a high content of oxides, because when pH decreases the variable charge surface of the oxides is more protonated. Therefore, the adsorption of negatively charged species of glyphosate will be favoured. In addition, there is also a reduction in the amount of ionized acid functional groups over the surface of the organic matter, enhancing glyphosate sorption. Moreover, there is a slight difference in glyphosate species in solution at pH 7 and 8 attending to glyphosate pKa values that drives larger sorption at the lower pH.

Glyphosate desorption behaviour varied substantially among Vibora and the other two sediments. It is almost irreversible for Cabecera and Cola sediments, with values from 0 % to 1.1 %, while in Vibora about 15–20 % glyphosate was desorbed. It could be related with its coarse texture and the much lower amount of amorphous oxides in Vibora sediment. There is also difference between Vibora glyphosate desorption and that observed previously for soils (about 1 %), due to their clay loam texture in comparison to the loamy sand texture of Vibora sediment, with lower surfaces and, therefore, less adsorption sites.

Table 8.1.2.1-99: Percentages of glyphosate desorbed (%) for the studied sediments at pH 8

	GPS initial conc. ($mg\ L^{-1}$)	GPS adsorbed ($\mu mol\ kg^{-1}$)	GPS desorbed (%)
Cabecera	0.25	30.83	0
	1	96.96	0.97
Cola	0.25	29.90	0
	1	95.02	0.60
Vibora	0.25	19.54	14.71
	1	57.16	19.53

Dissipation of glyphosate in water under aerobic conditions

In these studies, the degradation of glyphosate to its major metabolite AMPA was monitored but the remaining amount in water was below the LOQ of the analytical technique, and after 11 days under the LOD. DT_{50} values obtained for Cabecera and Cola water dissipation were 6.3 and 6.4 days, respectively, and 11.0 for Vibora water. The more rapid dissipation observed in the case of Cabecera and Cola water in relation to Vibora could be due to indirect photolysis through photosensitizers. The suspended solids in these natural waters which are the finer fraction of the sediments will have a high content of iron oxides acting as a photosensitizer and explaining the rapid glyphosate dissipation.

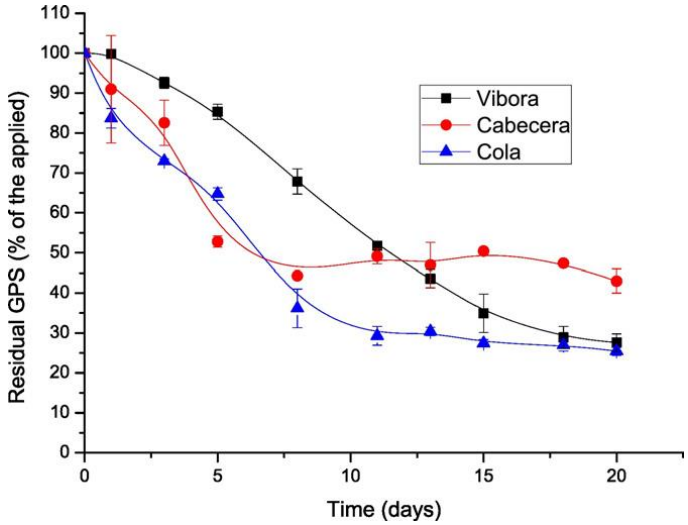


Figure 8.1.2.1-57: Glyphosate dissipation profiles in waters from the Vibora river and from the heading and tail of the reservoir (Cabecera and Cola, respectively).

Table 8.1.2.1-100:

Water	Kinetic model	K1 ($days^{-1}$)	K2 ($days^{-1}$)	tb ($days^{-1}$)	DT50 (days)
Vibora	SFO	0.06713	–	–	10.3
Cabecera	HS	0.11012	0.00131	7.01	6.3
Cola	HS	0.10932	0.00637	11.0	6.4

Conclusions

The results of this study indicate high glyphosate adsorption capacity of the soils surrounding the Vibora Reservoir due to their high amorphous oxides and clay mineral content. Glyphosate adsorption follows the same sequence as total amorphous oxide content. Those sediments taken from the heading and tile of the reservoir showed extremely high adsorption capacity, with K_d values in the range 4810–11800 in comparison to the adsorption to sediments from the tributary river (Vibora, K_d values 67.3 and 404), very similar to those values observed in the surrounding soils. The adsorption of glyphosate to the different matrices was as follows: heading of the reservoir sediments (Cabecera) > tile sediments (Cola) ≫ soils N Vibora sediment. The high K_d values are due especially to the amount of oxides (especially Fe oxides) present in the sediments, which was about three-fold higher for Cabecera and Cola sediments than for the soils and Vibora sediment. In addition, Cabecera and Cola sediments presented a fine texture (silt loam) providing much higher surfaces for adsorption. Glyphosate adsorption to sediments increased with decreasing pH from 8 to 7, due to an increasing positive charge on the sediment surfaces and to the formation of glyphosate species with lower negative charge, which are adsorbed more easily to the negatively charged surfaces of the sediments.

Glyphosate desorption for the studied soils and sediments were almost irreversible, percentages ranging from 0 % to 1.22 %, except in Vibora sediment, which presented 15–20 % desorption, probably due to its coarse texture (loamy sand) and much lower level of amorphous oxides.

The dissipation of glyphosate in the water from the same places where the different studied sediments were taken was also studied. The DT_{50} values obtained for water from Cabecera and Cola were 6.3 and 6.4 days, respectively; for Vibora, this value was 11 days. The more rapid dissipation observed in the case of Cabecera and Cola water could be due to indirect photolysis through photosensitizers. The suspended solids in these natural waters coming from the finer fraction of the sediments are likely to have high levels of iron oxides acting as photosensitizers and explaining the rapid glyphosate dissipation. The rate of glyphosate degradation in the water from Vibora Reservoir was very rapid, decreasing the potential risk impact on the aquatic ecosystem. This behaviour together with the almost irreversible adsorption of glyphosate from its sediments and the surrounding soils indicate the low toxicity risk of glyphosate in this zone, where this herbicide is widely used for olive crops.

Assessment and conclusion by applicant:

Confirmatory data on sorption and water/sediment behaviour and natural water photolysis of glyphosate.

Assessment and conclusion by RMS:

The tests presented in this study were an adsorption test on 4 soils and 2 sediments and a dissipation test in natural water. Few information on the tested substance are reported and the article lacks information to check the validity against current guidelines (*e.g.* raw residues, information on the substance stability, amount of concentration found in the supernatant, efficacy of the analytical method…).

The article provides supportive information on the adsorption of glyphosate and dissipation in natural water, but no reliable endpoints can be derived for use in risk assessment.

Rampazzo et al. 2013

Data point:	CA 7.1.2.2.1
Report author	Rampazzo N. *et al.*
Report year	2013
Report title	Adsorption of glyphosate and aminomethylphosphonic acid in soils.
Report No	International Agrophysics, (2013) Vol. 27, pp. 203-209 doi: 10.2478/v10247-012-0086-7
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The results showed that glyphosate is initially adsorbed mostly in the upper 2 cm. It is then transported and adsorbed after few days in deeper soil horizons with concomitant increasing content of its metabolite aminomethylphosphonic acid (AMPA). Moreover, Fe-oxides seem to be a key parameter for glyphosate and AMPA adsorption in soils. The study showed lower contents of dithionite-soluble and Fe-oxides for the Chernozem, with consequently lower adsorption of glyphosate and AMPA as compared with the Cambisol and the Stagnosol.

Materials and methods

The aim of this study was to investigate the behaviour of glyphosate and AMPA at different soils and time intervals after field application. The experiments were carried out at agricultural experimental fields, where different tillage systems: no-tillage (NT), direct drill, no plough, with a winter green vegetation cover and maize crop in spring, and conventional tillage (CT), plough with or without a winter green vegetation cover in 3 field replications are tested since 2007 (Kirchberg, Styria), 1999 (Pyhra and Pixendorf, Lower Austria).

Three soils formed under different climatic conditions and featuring different physic-mineral composition were investigated: a sandy stagnic Cambisol at Kirchberg (Styria) from tertiary carbonate free sediments, a loamy Stagnosol from carbonate free sediments (flysch, sandstone) at Pyhra (Lower Austria) and a Chernozem from loess at Pixendorf (Lower Austria). Moreover, these three soil types were selected because of their contrasting physic-chemic-mineralogical parameters e.g. texture, carbonate content, pH-value, and Fe-oxides for a better understanding of their influence on the glyphosate behaviour and extraction from soils. All three sites were under comparable tillage systems (no-tillage and conventional tillage) in long-term experiments.

The Roundup Max application was performed at all three sites according to the common agricultural practice i.e. 4 L Roundup Max (450 g glyphosate/L Roundup Max) were dissolved in 200 L of water and applied per ha (2 % herbicide solution). This corresponds to an application of 1,800 g glyphosate/ha or 180 mg glyphosate/m^2.

Soil bulk samples from all plots (NT and CT) were taken for physic-chemic-mineralogical analysis at each site at two soil depths (0-5 and 5-20 cm). The samples were air-dried and sieved at 2 mm size (fine earth). Moreover, for further physical analysis undisturbed samples (cylinders with 200 cm^3) were taken separated from each NT and CT field replication at 5-15 cm soil depth each in 5 repetitions.

In order to investigate the fate of glyphosate and AMPA in depth and time after Roundup Max application, soil bulk samples were taken at different time intervals after application at 10 points within each NT-field replication (pooled to one sample per site) as follows:

Kirchberg:

- immediately after the Roundup Max application, at 0-2 cm soil depth;
- 3 days after application at 0-2 and 2-5 cm soil depth;
- 12 days after application at 0-2, 2-5, and 5-10 cm soil depth.

Pyhra:

- immediately after the Roundup Max application, at 0-2 cm soil depth;
- 28 days after application at 0-2, 2-5, and 5-10 cm soil depth.

Pixendorf:

- immediately after the Roundup Max application, at 0-2 cm soil depth;
- 3 days after application at 0-2 and 2-5 cm soil depth;
- 10 days after application at 0-2, 2-5, and 5-10 cm soil depth.

After each soil sampling, soil samples were immediately transported to the laboratory in cooling boxes. In the laboratory all samples were stored at -18 °C until measurements. All physical, chemical and mineralogical analyses were carried out according to the standard methods.

Results

The results of the investigations of the Chernozem (pH: 7.3; OC: 1.25 % – 1.86 % in 0-20 cm) are shown in the following figure. At the first sampling after field application of Roundup Max about 30 % of the applied glyphosate amount was detected in the upper 0 – 2 cm. The main part of the herbicide adheres at the green plant cover and at first does not enter the soil surface.

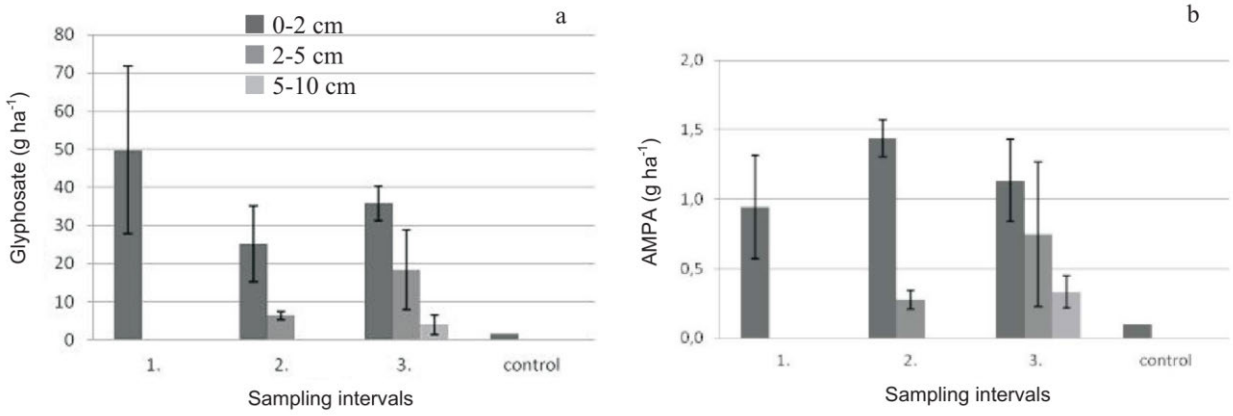


Figure 8.1.2.1-58: Content of: a – glyphosate and b – AMPA in Chernozem at different time intervals and soil depths, sampling: 1. – immediately after application; 2. – 3 days after application; 3. – 10 days after application; control – residues before application

After 3 days the glyphosate content decreased in the topsoil and was transported and adsorbed in the next horizon (2-5 cm) with concomitant increase of the AMPA content. After 10 days the glyphosate content was higher than immediately after application. For this behaviour following hypothesis can be possible:

- plant-adsorbed glyphosate is released to the topsoil after partly decomposition of the weeds;
- during the time between second and third soil sampling about 10 mm precipitation fell down, this may have washed glyphosate from plant leaves out.

The increase of AMPA 3 days after application of Roundup Max shows the very quick degradation of glyphosate to AMPA. This degradation could probably take place already in the Roundup Max package, this would explain the fact that AMPA was detected immediately after the Roundup Max application.

The results of the investigations of the Stagnosol (pH: 5.7 – 5.8; OC: 1.51 % - 1.73 % in 0-20 cm) are shown below. Most of the applied glyphosate was transported and adsorbed in deeper horizons after 28 days. The reference glyphosate and AMPA values refer to the amount of both substances before application e.g. the residues of the previous application (normally 2 years before). That means that in the Stagnosol glyphosate is transported downwards within 2 years and probably bound to deeper soil layers.

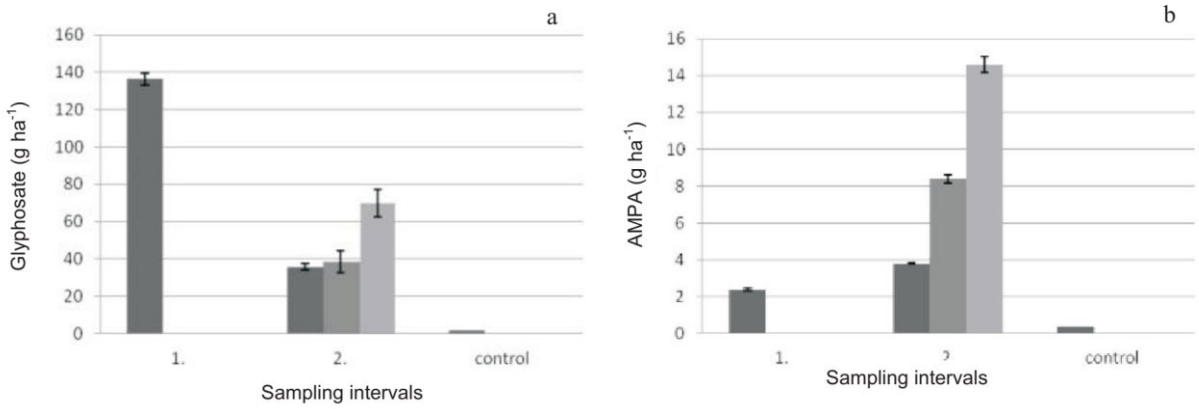


Figure 8.1.2.1-59: Content of: a – glyphosate and b – AMPA in Stagnosol at different time intervals and soil depths, sampling: 1. – immediately after application; 2. - 28 days after application; control – residues before application

The results of the investigations of the Cambisol (pH: 5.6 – 5.7; OC: 1.17 % - 1.61 % in 0 – 20 cm) are shown in below. The Cambisol features the best potential adsorption capacity for glyphosate with about 16,000 mg Fe_d/kg soil and about 3,500 mg Fe_o/kg soil. However, site can be strongly influenced by erosion processes if the infiltration rate for rainfall is reduced by soil crusting. This is the reason why glyphosate strongly decrease in the upper soil horizons but does not accumulate in deeper horizon. A considerable amount of the applied glyphosate may be transported downslope with runoff. Moreover, the degradation from glyphosate to its metabolite AMPA is visible by the increase of AMPA with time.

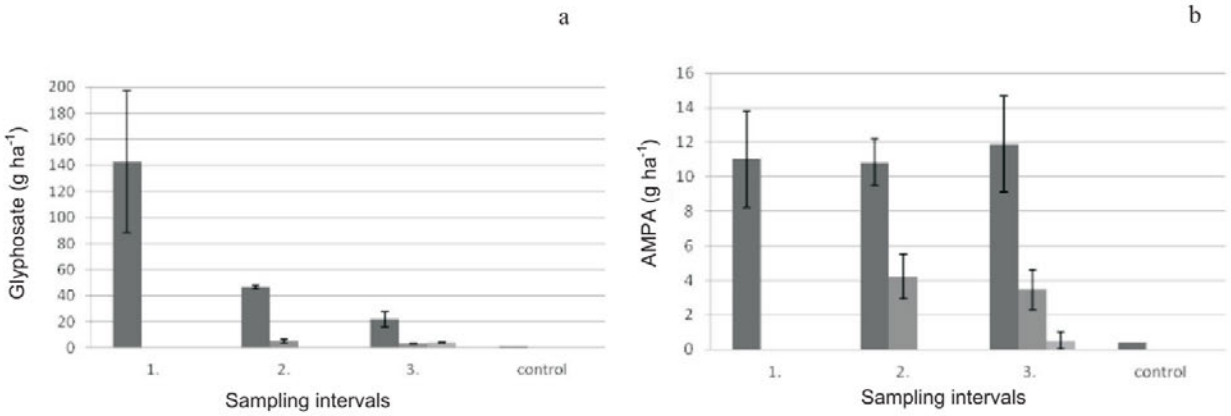


Figure 8.1.2.1-60: Content of: a – glyphosate and b – AMPA in Stagnosol at different time intervals and soil depths, sampling: 1. – immediately after application; 2. – 3 days after application; 3. – 12 days after application; control – residues before application

The results presented in the figure below show distinguished contents of glyphosate and AMPA in the upper horizon of the three different soils according to their chemical-mineralogical adsorption properties. Both the Cambisol and the Stagnosol, with a higher pedogenic Fe-oxide content, 15,000 and 10,000 mg Fe_d/kg soil, respectively, adsorbed a distinctly higher quantity of glyphosate and AMPA than the Chernozem which had a distinctly lower Fe-oxide content (7,900 mg Fe_d/kg soil).

Table 8.1.2.1-101: Fe-oxide distribution in the investigated soil

Site	Soil type (WRB)	Depth (cm)	Fe_p	Fe_o	Fe_d	Fe_o/Fe_d
			(mg kg^{-1})			
Pixendorf	Chernozem	0-5	37	983	7 970	0.12
		5-20	39	1 040	8 378	0.12
Kirchberg	Cambisol	0-5	530	3 422	14 843	0.23
		5-20	569	3 726	15 032	0.25
Pyhra	Stagnosol	0-5	550	3 241	9 959	0.32
		5-20	538	3 215	9 918	0.32

Fe_p – organic bound Fe-oxides, pyrophosphate-soluble, Fe_o – 'amorphous' (weakly crystallized) Fe-oxides, oxalate-soluble, Fe_d – well crystallized Fe-oxides, dithionite-soluble.

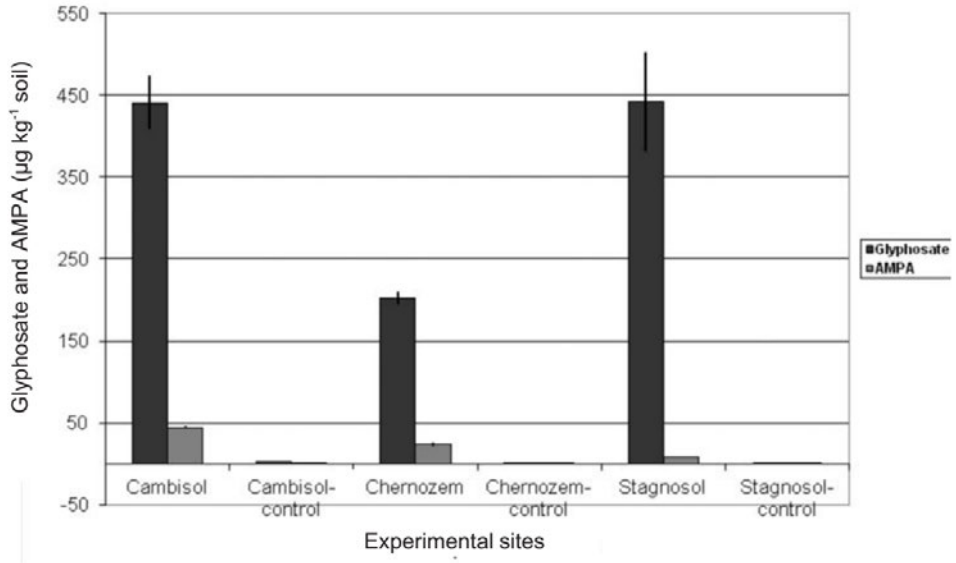


Figure 8.1.2.1-61: Glyphosate and AMPA contents in the investigated soils at 0 – 2 cm soil depth. The control values show the glyphosate contents before application (e.g. the residual traces, next to zero, from the previous application).

Moreover, the weakly weathered Chernozem consequently shows a low content of amorphous (Fe_o) Fe-oxides (973 mg Fe_o/kg soil) with respect to the more highly weathered Cambisol (3,402 mg Fe_o/kg soil) and Stagnosol (3,279 mg Fe_o/kg soil). Higher content of pedogenic Fe-oxides (Fe_d) and even higher contents of amorphous Fe-oxides (Fe_o) lead to higher sorption of glyphosate and AMPA, probably due to a larger and more reactive surface area of amorphous Fe-oxides. Thus, Fe-oxides in general seem to be a key parameter for glyphosate and AMPA adsorption in soils. This study showed lower contents of Fe_d and Fe_o for the Chernozem, with consequently lower adsorption of glyphosate and AMPA compared with the Cambisol and the Stagnosol.

Conclusions

Shortly after Roundup Max application only a part of the applied glyphosate amount enter the upper 0 – 2 cm and is then transported and adsorbed in deeper horizons with time with concomitant increase of the AMPA content. The results showed distinguished contents of glyphosate and AMPA in different soils at the same soil depth, according to their chemical-mineralogical adsorption properties, especially Fe-oxides (Fe_d and Fe_o). Thus, iron-oxides in general seem to be a key parameter for glyphosate and AMPA adsorption in soils.

Assessment and conclusion by applicant:

The study investigates glyphosate and AMPA adsorption to 3 different soils. Iron-oxides appear to play an important role in adsorption of glyphosate and AMPA in these soils.

Assessment and conclusion by RMS:

The adsorption of glyphosate was studied in this article. Information on the soil used is succinct, the stability of the test item is not provided and no detail on the analytical method is provided. Additionally no raw data are available.

The article provides supportive information on the adsorption of glyphosate but no reliable endpoints can be derived for use in risk assessment.

Tush et al. 2018

Data point:	CA 7.1.2.1.1
Report author	Tush D. *et al.*
Report year	2018
Report title	Dissipation of polyoxyethylene tallow amine (POEA) and glyphosate in an agricultural field and their co-occurrence on streambed sediments
Report No	The Science of the total environment (2018), Vol. 636, pp. 212-219
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:

The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.3. The article provides supportive information on the adsorption of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Paradelo M. et al.

Data point:	CA 7.1.2.1.1

Report author	Paradelo M. *et al.*
Report year	2015
Report title	Prediction of the glyphosate sorption coefficient across two loamy agricultural fields
Report No	Geoderma, (2015) Vol. 259-260, pp. 224-232 http://dx.doi.org/10.1016/j.geoderma.2015.06.011
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Sorption is considered one of the most important processes controlling pesticide mobility in agricultural soils. Accurate predictions of sorption coefficients are needed for reliable risk assessments of groundwater contamination from pesticides. In this work, we aim to estimate the glyphosate sorption coefficient, K_d, from easily measurable soil properties in two loamy, agricultural fields in Denmark: Estrup and Silstrup. Forty-five soil samples in Estrup and 65 in Silstrup were collected from the surface in a rectangular grid of 15 × 15-m from each field, and selected soil properties and glyphosate sorption coefficients were determined. Multiple linear regression (MLR) analyses were performed using nine geo-referenced soil properties as variables to identify the parameters related with glyphosate sorption. Scenarios considered in the analyses included: (i) each field separately, (ii) both fields together, and (iii) northern and southern sections of the field in Silstrup. Considering correlations with all possible sets of the same nine geo-referenced properties, a best-four set of parameters was identified for each model scenario. The best-four set for the field in Estrup included clay, oxalate-extractable Fe, Olsen P and pH, while the best-four set for Silstrup included clay, organic carbon (OC), Olsen P and electric conductivity (EC). When the field in Silstrup was separated in a northern and southern section, the northern section included EC, and oxalate-extractable Fe, Al and P, whereas the southern part included pH, clay, OC and Olsen P. The best-four set for both fields together included clay, sand, pH and EC. Thus, the most common parameters repeated in the best-four sets included clay and pH as also reported previously in the literature, but in general, the composition of the best-four set differed for each scenario, suggesting that different properties control glyphosate sorption in different locations and at different scales of analysis. Better predictions were obtained for the best-four set for the field in Estrup ($R^2 = 0.87$) and for both fields ($R^2 = 0.70$), while the field in Silstrup showed a lower predictability ($R^2 = 0.36$). Possibly, the low predictability for the field in Silstrup originated from opposing gradients in clay and oxalate-extractable Fe across the field. Also, whereas a lower clay content in Estrup may be the limiting variable for glyphosate sorption, the field in Silstrup has a higher clay content not limiting the sorption, but introducing more variability in K_d due to changes in other soil properties.

Materials and methods

Field sites

Sampling was carried out at two agricultural loamy fields (Silstrup and Estrup) in Denmark, which belong to the Danish Pesticide Leaching Assessment Program. Silstrup is located in northwestern Jutland (56°55′56.16″N, 8°38′43.91″E) and covers 1.69 ha of loamy agricultural land and slopes gently 1–2°. Two pedological profiles classified the soil as Alfic Argiudoll and Typic Hapludoll according to the USDA classification. The field site in Estrup is located in southern Jutland (55°29′09.96″N, 9°04′09.37″E) and covers 1.26 ha of loamy agricultural land. The field site is virtually flat and the complex geological structure comprises a clay till core with deposits of different age and composition. Three pedological profiles classified the soil as Aquic Argiudoll, Abruptic Argiudoll, and Fragiaquic Glossudalf. Both fields were managed conventionally with regard to crop rotation, fertilization, and soil tillage.

Soil sampling and characterization

Bulk soil was collected from the top 20-cm in a rectangular grid of 15 × 15-m covering the cultivated area of each field. This sampling grid was chosen to represent the spatial variations across the fields, and still keep the sampling intensity at a reasonable level. Sixty-five samples were collected from

Silstrup and 45 from Estrup. Air dried and 2-mm sieved soil samples were used for subsequent soil analysis and sorption experiments. Texture was determined by a combined sieve/hydrometer method. OC was determined on a LECO analyzer coupled with an infrared CO_2 detector (Thermo Fisher Scientific Inc., MA). The pH was measured in a soil/water solution of 8 mL soil suspended in 30 mL of demineralized water and EC was measured in a 1:9 (v/v) soil/water extract. Oxalate-extractable Al, Fe and P (Al_{OX}, Fe_{OX} and P_{OX}) were measured using the procedure for determination of the degree of phosphate saturation in non-calcareous soils described by Shoumans. Available soil phosphorous was determined by the Olsen method.

Glyphosate solution

The sorption studies were performed with ^{14}C-labeled glyphosate ([glycine-2-14C] glyphosate, N-(phosphonomethyl)glycine). Radiolabelled ^{14}C-labeled glyphosate was purchased from Perkin Elmer (Boston, USA). Stock solutions were prepared by dissolving ^{14}C-labeled glyphosate in a 0.01 M $CaCl_2$ solution to an initial glyphosate concentration of 0.23 mg/L. Sodium azide (1.00 g/L) was added to prevent microbial degradation.

Sorption experiments

The glyphosate sorption coefficients were determined by batch equilibrium experiments with three replicates. Air dry soil aliquots (0.5 g) were equilibrated with 0.5 mL of 0.01 M $CaCl_2$ for 24 h in glass centrifuge tubes closed with Teflon caps. Nine milliliters of the 0.01 M $CaCl_2$ containing the desired concentration of glyphosate were added and the samples were rotated end-over-end (30 rpm) for 24 h at 20 °C followed by centrifugation at 5000 rpm for 1 h. Samples of 3 mL supernatant were mixed with 17 mL of scintillation cocktail (Packard Ultima Gold, PerkinElmer, MA). The glyphosate concentration was quantified using a liquid scintillation analyzer (Packard Tri-carb 2250CA, Packard Instrument Co., IL). The stopping criterion was set to 1 %, with a maximum counting time of 1 h. The amount of glyphosate sorbed was calculated as the difference between the solution concentration at the equilibrium and the concentration in vials without soil. Controls were included without soil material but were otherwise treated similarly. The sorption coefficient, K_d was calculated.

Multiple linear regression analysis

To examine the interactions among parameters, a multiple linear regression (MLR) analysis was performed to relate K_d to every combination of nine measured soil properties (pH, EC, clay, sand, OC, Al_{OX} Fe_{OX}, P_{OX}, and Olsen P). The simplest assumption of a linear dependence of K_d on the (combinations of) measured properties was made. The adopted approach with MLR does not necessarily suggest that the underlying processes are inherently linear; some of these processes may exhibit nonlinear, or even discontinuous, dependence on soil properties. However, the aim of the study was merely to determine whether a small set of easily measureable properties showed a strong enough correlation with the glyphosate sorption coefficient for them to be used for field-scale screening. The combinations of properties in sets of varying size best able (defined by maximum R^2) to explain sorption of glyphosate in different scenarios: each field separately and both fields together. In addition, Silstrup was split into a northern and southern section, because this site showed marked differences between North and South in solute transport behaviour and colloid dispersibility and leaching.

Results and discussion

Glyphosate sorption

The glyphosate sorption coefficients were higher in Silstrup (344 – 667 L/kg) than in Estrup (161 – 536 L/kg). Glyphosate was strongly sorbed in the northwestern part of the field in Estrup following the gradients in clay and Fe_{OX} Silstrup showed a maximum sorption capacity in the northern part of the field, with a sorption "hotspot" towards the eastern part. There was no evident spatial correlation of *Kd* with the soil properties in Silstrup.

Single linear regression analysis

The best single predictor of K_d differed across the selected geographical scenarios. In Estrup, Fe_{OX} was the parameter that explained most of the observed variation in K_d (R^2 = 0.73), and clay also provided a good correlation with K_d (R^2 = 0.52). In Silstrup, the inverse correlation with P_{OX} gave the best R^2 (R^2 = 0.20). Dividing Silstrup in northern and southern sections, P_{OX} (R^2 = 0.17) was selected in the North and Olsen P (R^2 = 0.25) in the South. When K_d was predicted from both fields together, clay was the best predictor (R^2 = 0.62). From these results, Fe_{OX} or clay content could be considered as single predictors of K_d explaining more than 50 % of the sorption variability; however, the uncertainty would

be relatively high. The negative correlation with both P_{OX} and Olsen P in Silstrup field suggested that P competes with glyphosate for the sorption sites in the soil, reducing glyphosate sorption

Multiple linear regression analysis
Multiple linear regression analysis was used to examine which set of measured soil properties could explain K_d best using as few assumptions as possible. The number of combinations, c, studied can be calculated by the binomial coefficient:

$$c = \frac{m!}{k!(m-k)!}, 0 < k \leq m \quad (1)$$

where *m* is the number of measurements and *k* is the number of parameters selected. The same predictor properties were used for all the analyses. The number of unique parameter sets obtained from *k* ranging from 1 to 9 was 511 for each of the five geographical scenarios considered: Estrup; Silstrup; Silstrup North; Silstrup South; and both fields together. For each subset of parameters considered, the goodness of the fit (R^2) and the significance level (p for significance, $\alpha = 0.05$) were calculated. The R^2 for each subset based on the MLR to K_d is shown in the figures. The best performances for each *k* value were significant (*p*-values ≤ 0.05) in all the sets. The improvement achieved when multiple parameters were included compared to the best single parameter was dependent on the geographical scenario; in general, the increment in R^2 from using one to nine parameters was between 0.2 and 0.3, with little increase for both fields together, and considerably improvement in Silstrup South. For all of the geographical scenarios, the increasing rate of R^2 with increasing parameter set size decreased after around four parameters suggesting that only four parameters need to be considered in defining a screening set. All of the best-four sets were significant, with a *p*-value ≤ 0.05. The selection of four parameters did not reduce substantially the R^2 compared to using all 10 parameters. The best-four set for each MLR analysis is shown in the following table. Positive signs are assigned to parameters which are positively correlated to K_d and vice versa. The best-four set predicting K_d in Estrup included clay, Fe_{OX}, Olsen P (all positively correlated) and pH (negatively correlated), $R^2 = 0.87$. The best-four set in Silstrup included a positive correlation with clay, and a negative correlation with EC, OC, and Olsen P ($R^2 = 0.36$). P_{OX}, the best single predictor in Silstrup, was not included in the best-four subset. Only for $k = 4$, P_{OX} is out of the best predictors, while it was selected for the other eight sets of k values. This variability in the composition of the parameter sets together with the low performance achieved (less than 50 % of the variability explained) suggest that other factors not measured possibly exerted a strong control on the sorption within this field site. Compared to Estrup, where the low clay content may result in clay being a limiting factor for glyphosate sorption, the field in Silstrup has a higher clay content where more properties are likely to influence glyphosate K_d and the predictability of the best-four set model. Furthermore, the opposite gradients of clay and Fe_{OX} in Silstrup could interfere in their effects on the glyphosate sorption.

Dividing Silstrup in two geographical areas, the selected parameters differed from the whole field. For the northern part, only the negative correlation with EC is common with the results for the whole field. Also, there is a positive correlation between K_d and the amorphous oxides (Fe_{OX} and Al_{OX}) and a negative correlation with P_{OX} for the northern part. For the southern part, the best-four set comprised a negative correlation with pH, clay, OC and Olsen P. Because clay is not the strongest predictor in the set, the negative sign may indicate interactions among clay, OC and Olsen P in controlling K_d.

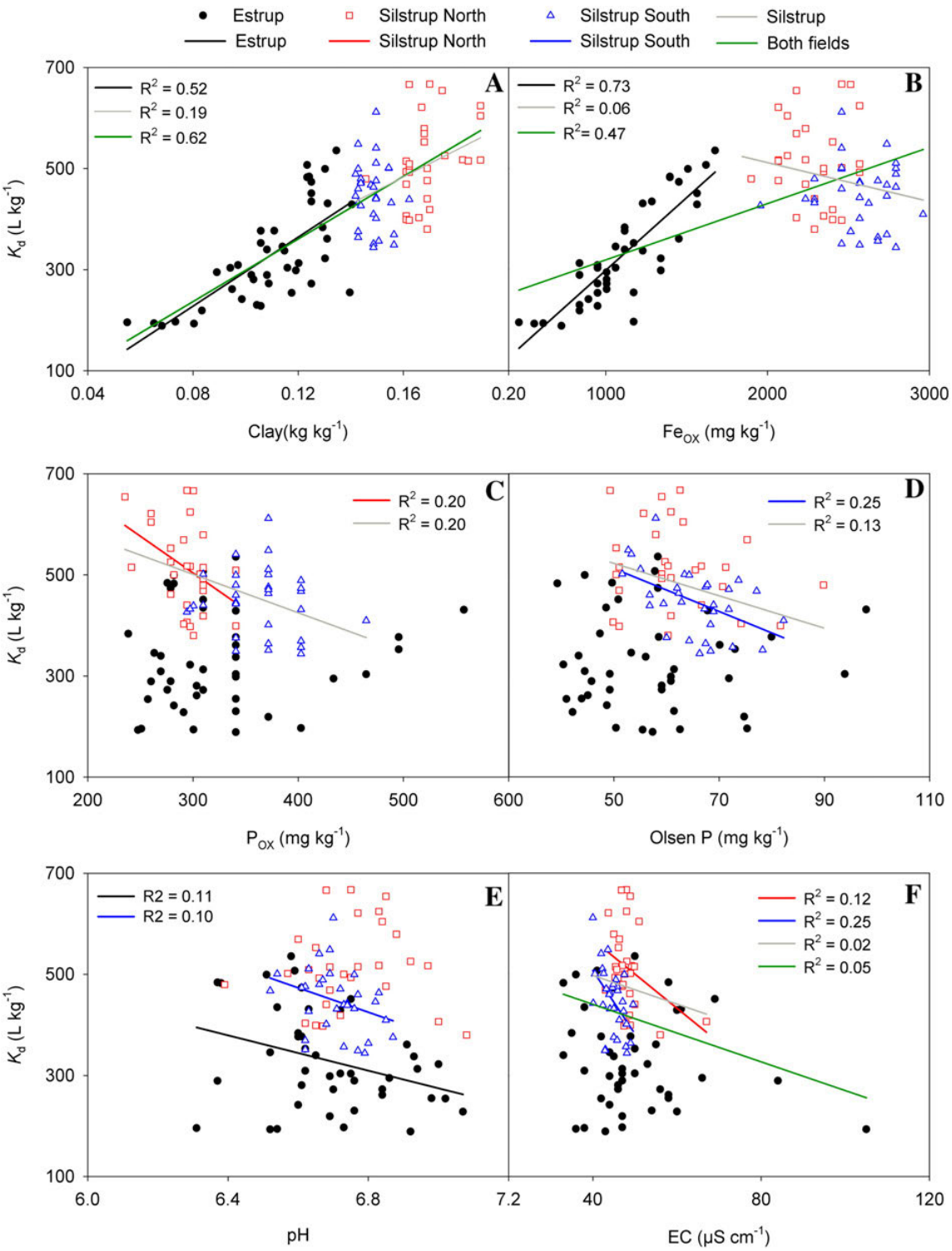


Figure 8.1.2.1-62: The glyphosate sorption coefficient as a function of (A) clay content, (B) oxalate extractable iron, Fe_{OX}, (C) oxalate extracted P, P_{OX} (D) Olsen P, (E) pH and (F) EC for the two fields studied. Notice that Silstrup was divided in northern and southern sections

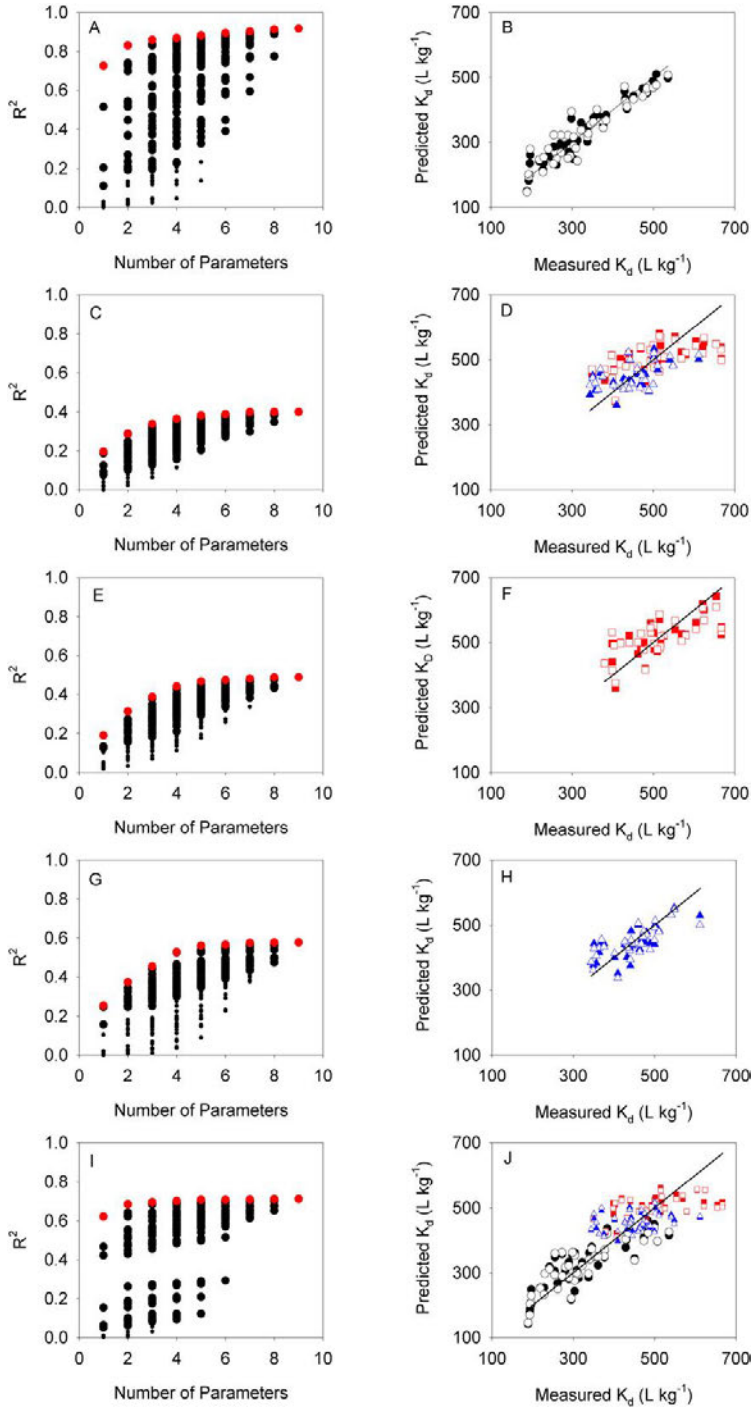


Figure 8.1.2.1-63: Prediction of the glyphosate sorption coefficient, K_d, with the multiple linear regression (MLR) model. The panels A, C, E, G and I show the increase in R^2 as a function of the number of parameters included in the analysis (k = 9), for Estrup, Silstrup, Silstrup North, Silstrup South, and Both fields. Sets with a p value > 0.05 are shown as small dots; sets with a p value ≤ 0.05 have larger dots. The best prediction set (highest R^2 and $p \leq 0.05$) for a particular number of parameters included is marked with a red dot. Panels B, D, F, H and J show the predicted K_d versus the measured K_d for Estrup, Silstrup, Silstrup North, Silstrup South, and both fields, respectively. Filled symbols show the prediction sets using 9 parameters; open symbols show the prediction sets using only four parameters. Black circles represent Estrup points, red squares represent Silstrup North points, and blue triangles represent Silstrup South points. The solid lines represent the 1:1 line.

Table 8.1.2.1-102: The best-four sets from the multiple regression analysis (MLR) predicting the glyphosate sorption coefficient (K_d) for the different geographic scenarios: Estrup, Silstrup, Silstrup North, Silstrup South and both fields. A "–" symbol indicates that the parameter is inversely correlated with K_d, and a "+" symbol indicates that the parameter is positively correlated with K_d. The R^2 values

using a single parameter, the best four sets and all parameters are presented in the first three rows.

	Estrup	Silstrup	Silstrup N	Silstrup S	Both fields
R^2 single	0.73	0.20	0.17	0.25	0.62
R^2 best four set	0.87	0.36	0.43	0.53	0.70
R^2 all parameters	0.93	0.45	0.50	0.69	0.72
pH	−			−	−
EC		−	−		−
Clay	+	+		−	+
Sand					−
OC		−		−	
Al_{ox}			+		
Fe_{ox}	+		+		
P_{ox}			−		
Olsen P	+	−		−	

EC: electrical conductivity; OC: Organic carbon content; Al_{ox}, Fe_{ox}, P_{ox}: Oxalate extractable aluminum, iron and phosphorous; and Olsen P: Available phosphorous.

The best-four set for both fields together was positively correlated with clay and negatively correlated with pH, EC and sand ($R^2 = 0.70$). The selection of clay suggested that the finest fraction of the soil controlled glyphosate sorption across both fields together. The range of EC and pH is similar in both field sites. Increasing pH causes an increase in the negative charges of the soil surfaces as well as on glyphosate molecules. This phenomenon enhances the repulsion between the pesticide and the soil surfaces

Decreasing K_d with increasing EC suggested that cations in the solution complexed glyphosate molecules reducing sorption on the soil surfaces.

For a better understanding of the differences in R^2 in the analyses the predicted values were plotted as a function of the measured values. As expected, using all parameters reduced deviations of the predicted values from the 1:1 line. In general, residuals were well distributed along the 1:1 line for the different geographical scenarios. A slight deviation from the 1:1 line, however, was observed for $K_d > 550$ L/kg that corresponds to the northern Silstrup section. For the northern section, the bias of the predicted K_d from the 1:1 line was reduced. However, the low predictive power suggests that other, unmeasured properties exert a strong control on glyphosate sorption in Silstrup.

Conclusions

The influence of soil physical and chemical properties on glyphosate sorption was studied across two agricultural fields. The best-four set of parameters was considered to give an acceptable prediction of K_d compared to using the nine parameters measured in Estrup and with both fields together, while in Silstrup the performance was low. The most commonly selected parameters for predicting glyphosate sorption across the geographic scenarios studied included clay, pH, Fe_{ox}, EC and P. However, the four most predictive parameters varied depending on the field site. The proposed analysis could explain most of the variability in Estrup but less than half of the variation in Silstrup suggesting that potential factors controlling glyphosate sorption in Silstrup were not determined.

Assessment and conclusion by applicant:

Study of 9 soil factors influencing glyphosate sorption in 2 different fields. Not related to an efate guideline, but supplementary information.

Assessment and conclusion by RMS:

The adsorption of glyphosate was studied in this article. Information on the soil used is succinct, the stability of the test item is not provided nor any mass balance. Additionally no raw data are available.

The article provides supportive information on the adsorption of glyphosate but no reliable endpoints can be derived for use in risk assessment.

B.8.1.2.2. *Adsorption and desorption of metabolite*

B.8.1.2.2.1. Laboratory studies

The adsorption and desorption behaviour in soil of AMPA was investigated in various soils in 6 batch equilibrium studies. Five studies are existing ones and were previously evaluated in DAR (2001) or in RAR (2015). One new study was submitted by the task force in this renewal dossier.

Table 8.1.2.2-1: List of existing and new batch adsorption studies on AMPA

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.1.3.1.2/001 CA 7.1.3.1.2/009	[redacted], 2020 (& amendment)	New study	Acceptable
CA 7.1.3.1.2/006	[redacted], 1993	Acceptable	Acceptable (2 soils)
CA 7.1.3.1.2/002	[redacted], 2003	Acceptable	Not acceptable
CA 7.1.3.1.2/003	[redacted], 2002	Acceptable	Not acceptable
CA 7.1.3.1.2/004	[redacted], 1996	Acceptable	Not acceptable
CA 7.1.3.1.2/005	[redacted], 1993	Not mentioned in RAR (2015) but not accepted in DAR (2001)	Not acceptable

[redacted], 2020

Data point:	CA 7.1.3.1.2/001 + /009
Report author	[redacted]
Report year	2020
Report title	Adsorption/Desorption of ^{14}C-AMPA in Six Soils + Report Amendment 1 to Final Report Adsorption/Desorption of [^{14}C]AMPA in Six Soils
Report No	S19-23618
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	OECD Guideline 106 (January 2000): - Adsorption percentage < 20% for some soils at some concentrations - K_D*(soil/solution) ratio < 0.3 for some soils at some concentrations
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C] BCS-AB47424 (AMPA)

Batch No.	MXM 20136
Specific activity	4.97 MBq/mg
Radiochemical purity	97.7 %

2. Test Soils

The soils were sampled from the upper soil layer. The soils have not been treated with any pesticide for at least five years, except for soil RefeSol 02-A which was treated with glyphosate (amongst other products) about two years prior to sampling. After sampling, the soils were air-dried, sieved through a 2-mm sieve and stored at ambient temperature for up to 30 months. A description of the soils used is summarised in the table below.

Table 8.1.2.2-2: Physico-chemical properties of test soils

Parameter	Results					
Soil	RefeSol 02-A	LUFA 2.2	LUFA 2.3	LUFA 6S	Bourgfelden	Wurmwiese
Horizon (cm)	0-30	0-20	0-20	0-20	0-20	0-20

Geographic Location						
City	Schmallenberg	Hanhofen	Offenbach	Siebeldingen	Bourgfelden	Monheim am Rhein
State	North Rhine-Westphalia	Rhineland-Palatinate	Rhineland-Palatinate	Rhineland-Palatinate	Haut-Rhin	North Rhine-Westphalia
Country	Germany	Germany	Germany	Germany	France	Germany
Textural Class (USDA)	Silt	Sandy loam	Sandy loam	Clay loam	Silt loam	Sandy loam
Sand (5 µm – 2 mm) (%)	7.98	75.44	63.44	24.63	20.32	57
Silt (2 µm – 5 µm) (%)	86.55	17.41	30.08	40.48	63.85	30
Clay (< 2 µm) (%)	5.48	7.15	6.48	34.88	15.83	13
pH						
- in 0.01 M $CaCl_2$	6.6	5.7	6.2	7.3	7.5	5.0
- in water	7.25	6.33	7.01	7.89	8.41	5.2
Organic Carbon (%)	1.18	1.48	0.61	2.07	1.15	2.0
Organic Matter (%) [1]	2.0	2.6	1.1	3.6	2.0	3.4
Cation Exchange Capacity (meq/100 g)	12.1	9.2	5.9	13.7	16.8	10.0

USDA: United States Department of Agriculture
[1] calculated as OC * 1.724

B. STUDY DESIGN

1. Experimental Conditions

Sealed glass bottles were used as test systems. The experiments were performed with duplicate soil samples. All experiments were performed at 20 ± 2 °C in the dark. The test vessels were shaken to keep the soil in homogeneous suspension.

Soil samples were pre-equilibrated with 0.01 M $CaCl_2$ overnight at 20 ± 2 °C prior to application of the test item. Preliminary and definitive phase was performed with duplicate samples and non-sterile soils.

Preliminary Test 1: Extraction efficiency

The test was performed at a test concentration of 5 mg/L by applying various extraction methods. The non-sterile soil samples had been pre-equilibrated at soil-to-solution ratio of 1:30. Following removal of the aqueous phase after centrifugation, a solution of the test item was applied. The work-up was performed after less than about 10 min of contact time with the soil. Three methods were tested and the chosen method had mean values of radioactivity recovered following extraction of 96.4-104.4 % AR while non extracted radioactivity was below 4%.

Preliminary Test 2: Stability of the test item at a concentration of 5 mg/L

The stability of the test item was investigated for duplicates at a test concentration of 5 mg/L and a soil-to-solution ratio of 1:30 following contact with non-sterile soil for 48 hours. Sampling and analysis were performed for the supernatant and soil extracts. Parental mass balances were determined at this stage, by HPLC analysis of supernatants and soil extracts.

This preliminary test resulted in a mean radioactivity adsorbed to soil of 40.4 to 90.0 %. Mean overall recoveries of radioactivity (including NER) were 98.4 to 109.1 % AR. The stability of the test item could not be unequivocally confirmed.

The adequate adsorption equilibration times were determined at a soil-to-solution ratio of 1:100 by preliminary tests considering stability of the test item in contact with non-sterile soil under the test conditions. While stability of the test item could be clearly demonstrated after 4 hours contact time with soil, analytical problems increased for samples that had a contact time of 48 hours as indicated, for example, by double peak formation. With adsorption being a fast process, a contact time of 4 hours with soil was chosen for preliminary test 3 and for definitive test.

The stability of the test item in aqueous 0.01 M CaCl2 solution (in absence of soil) after 48 hours was confirmed by LSC/HLC analysis. Adsorption of the test item to the surface of the test vessels was checked by comparison of radioactivity in control samples after 0 hours and 48 hours.

Preliminary Test 3: Adsorption to and stability at test item concentration of 5 mg/L and 0.05 mg/L

Duplicate samples were investigated for stability of the test item at a soil-to-solution ratio of 1:100 and the highest (5 mg/L) and lowest test concentration (0.05 mg/L) following contact with non-sterile soils for 4 hours. After extraction of soils, the evaluation of adsorption followed the indirect method.

This preliminary test resulted in a mean radioactivity adsorbed to soil of 20.7 to 45.1 % for the test item concentration of 5 mg/L. Mean overall recoveries of radioactivity (including NER) were 91.0 to 103.7 % AR. For the lowest concentration of 0.05 mg/L, mean radioactivity adsorbed to soil ranged 44.5 to 81.4 % and mean overall recoveries of radioactivity (including NER) were 90.1 to 103.3 % AR. HPLC analysis confirmed that all radioactivity in supernatant and soil extracts was assigned to AMPA.

Definitive Test

For the adsorption step of the definitive test, the evaluation followed the indirect method according to OECD Guideline 106. The test was performed with non-sterile soils at a soil to-solution ratio of 1:100 and at the five nominal test concentrations of 0.05, 0.25, 0.5, 2.5 and 5.0 mg/L, therefore covering two orders of magnitude. Two replicates per soil and concentration were used for a contact time with soil of 4 hours. The definitive adsorption step was carried out in the dark at 20 ± 2 °C under continuous agitation. No desorption steps were performed. Overall mass balances of radioactivity per sample were determined for all soils.

Test item stability was investigated by HPLC analysis in aqueous supernatants and soil extracts for 5.0 and 0.05 mg/L concentrations for all soils.

The stability of the test item in aqueous 0.01 M $CaCl_2$ solution (in absence of soil) after 48 hours was confirmed by LSC/HLC analysis. Adsorption of the test item to the surface of the test vessels was checked by comparison of radioactivity in control samples after 0 hours and 48 hours.

2. Analytical Procedures

After the adsorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity in the supernatant was analysed by liquid scintillation counting (LSC) at each concentration.

For determination of material balance and parental mass balance, soil samples of the definitive phase for the test concentrations of 5.0 and 0.05 mg/L of all soils were sequentially extracted at ambient temperature using 1 N NaOH followed by 1 N NaOH/methanol (1/1, v/v) and finally acetone. The extracts were combined for analysis. Aqueous $CaCl_2$ solutions and combined soil extracts were analysed by LSC and HPLC/radiodetection. Extracted soil samples were dried, combusted and analysed by LSC to determine non-extractable radioactivity.

AMPA was shown to be stable under the test conditions with no need for correction of the adsorption results.

Verification of the Determination of Radiocarbon

Liquid scintillation counting (LSC) was used as method to determine the radioactivity. With stability of the test item demonstrated, the latter was, in turn, used to quantify the test item. The LSC limit of detection (LOD) was set to 40 dpm per aliquot, based on two times background count rate criteria (20 dpm), and the limit of quantitation (LOQ) to 60 dpm per aliquot, based on three times background count rate criteria. Within the definitive tests, the lowest measured value determined in an aliquot (500 µL) of adsorption supernatants was 2811 dpm (soil 02A, lowest test concentration K5 of 0.05 mg/L, first replicate), which is approximately 46 times higher than the LOQ. Therefore, the LSC method was suitable for quantification at the lowest test item concentration K5.

Verification of Sample Processing Method

The overall 14C-recovery of applied radioactivity (AR) in the definitve test was between 95.2 and 104.1 % AR after 4 h of adsorption for all soils at the highest test concentration of 5 mg/L and between 94.4 to 105.5 % AR for the lowest test concentration of 0.05 mg/L. With recoveries for the predominant number of samples well beyond 90% AR, the results demonstrate that the sample processing method was adequate to quantitatively recover the radioactivity from the test systems.

Verification of Chromatographic Procedures

The primary analytical method (HPLC/radiodetection) was suitable to determine the test item at the highest test concentration K1 as demonstrated by a HPLC column recovery of 102.1 %. The LOD of the HPLC method was determined as 2946 dpm for a peak, representing 0.98 % of the highest test concentration using detection by Mira. For the lowest test concentration the LOD was determined as 204 dpm for a peak, representing 1.8% using MicroBeta detection (see Appendix 9 for a representative chromatogram).

3. Calculations

Calculation of the Freundlich constant and related K_{FOC} was performed by application of the indirect method. The radioactivity contents in the supernatants after adsorption was used to calculate the adsorption isotherms as well as the related distribution coefficient referenced to organic carbon content K_{Foc} values.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

For the definitive phase, mean material balances of radioactivity including NER were 100.3% (RefeSol 02-A), 96.2% (LUFA 2.2), 100.5% (LUFA 2.3), 103.8% (LUFA 6S), 101.6% (Bourgfelden) and 95.2% AR (Wurmwiese) after 4 hours of adsorption.

B. STABILITY OF TEST ITEM

Stability of the test item was demonstrated during the definitive test by HPLC analysis for the highest (5.0 mg/L) and lowest (0.05 mg/L) test concentrations. At both concentrations, all radioactivity in supernatant and soil extracts was assigned to AMPA. The mean recovery in terms of parental mass balances at a test concentration of 5.0 mg/L was 102.1 % (RefeSol 02-A), 97.8 % (LUFA 2.2), 103.9 % (LUFA 2.3), 102.9 % (LUFA 6S), 101.5 % (Bourgfelden) and 95.0 % AR (Wurmwiese) following 4 hours of adsorption. At a test concentration of 0.05 mg/L, the recovery in terms of parental mass balances was 97.4 % (RefeSol 02-A), 93.9 % (LUFA 2.2), 98.0 % (LUFA 2.3), 104.1 % (LUFA 6S), 103.2 % (Bourgfelden) and 98.6 % AR (Wurmwiese) following 4 hours of adsorption.

C. FINDINGS

The percentage of radioactivity adsorbed to the soil in the definitive tests is given in the table below.

Table 8.1.2.2-3: [^{14}C]AMPA: Percentage adsorbed to soil (mean values)

Soil	Test Concentration [mg/L]				
	5.009	2.552	0.482	0.242	0.046
RefeSol 02-A (silt)	21.3	26.4	34.2	34.8	58.6
LUFA 2.2 (sandy loam)	22.3	25.8	43.5	37.8	49.8
LUFA 2.3 (sandy loam)	13.9	20.9	32.6	31.1	41.9
LUFA 6S (clay loam)	20.9	30.5	27.3	28.3	43.9
Bourgfelden (silt loam)	11.6	18.0	25.8	25.9	38.3
Wurmwiese (sandy loam)	18.4	26.9	29.4	32.4	30.1

The concentration in supernatant and soil extracts for each concentration are presented below.

Table 8.1.2.2-4: Definitive Phase – Adsorption: Concentration of radioactivity in the water phase and soil for Soil 02A

C_0 [µg/mL]	Sample	C_{aq}^{ads} [µg/mL]	C_s^{ads} [µg/g]	log C_{aq}^{ads} [µg/mL]	log C_s^{ads} [µg/g]	Adsorption [%]
5.009	02A_T3-1/100-k1-1	3.8710	114.3475	0.5878	2.0582	22.7
	02A_T3-1/100-k1-2	4.0148	99.0835	0.6037	1.9960	19.9
	mean	**3.9429**	**106.7155**	**0.5957**	**2.0271**	**21.3**
2.552	02A_T3-1/100-k2-1	1.8859	67.1218	0.2755	1.8269	26.1
	02A_T3-1/100-k2-2	1.8742	68.5222	0.2728	1.8358	26.7
	mean	**1.8801**	**67.8220**	**0.2742**	**1.8313**	**26.4**
0.482	02A_T3-1/100-k3-1	0.3103	17.1293	-0.5083	1.2337	35.7
	02A_T3-1/100-k3-2	0.3243	15.5453	-0.4890	1.1916	32.7
	mean	**0.3173**	**16.3373**	**-0.4986**	**1.2127**	**34.2**
0.242	02A_T3-1/100-k4-1	0.1787	6.4884	-0.7479	0.8121	26.1
	02A_T3-1/100-k4-2	0.1370	10.7693	-0.8633	1.0322	43.4
	mean	**0.1578**	**8.6289**	**-0.8056**	**0.9222**	**34.8**
0.046	02A_T3-1/100-k5-1	0.0189	2.8304	-1.7246	0.4519	59.4
	02A_T3-1/100-k5-2	0.0196	2.6445	-1.7072	0.4223	57.7
	mean	**0.0192**	**2.7375**	**-1.7159**	**0.4371**	**58.6**

Table 8.1.2.2-5: Definitive Phase – Adsorption: Concentration of radioactivity in the water phase and soil for Soil 2.2

C_0 [µg/mL]	Sample	C_{aq}^{ads} [µg/mL]	C_s^{ads} [µg/g]	log C_{aq}^{ads} [µg/mL]	log C_s^{ads} [µg/g]	Adsorption [%]
5.009	2.2_T3-1/100-k1-1	3.7897	122.0380	0.5786	2.0865	24.3
	2.2_T3-1/100-k1-2	3.9966	100.6103	0.6017	2.0026	20.2
	mean	**3.8932**	**111.3241**	**0.5901**	**2.0446**	**22.3**
2.552	2.2_T3-1/100-k2-1	2.0042	54.3422	0.3019	1.7351	21.6
	2.2_T3-1/100-k2-2	1.7905	76.6062	0.2530	1.8843	29.9
	mean	**1.8974**	**65.4742**	**0.2775**	**1.8097**	**25.8**
0.482	2.2_T3-1/100-k3-1	0.2608	21.9221	-0.5837	1.3409	45.8
	2.2_T3-1/100-k3-2	0.2830	19.7634	-0.5482	1.2959	41.3
	mean	**0.2719**	**20.8428**	**-0.5659**	**1.3184**	**43.5**
0.242	2.2_T3-1/100-k4-1	0.1509	9.1415	-0.8214	0.9610	37.7
	2.2_T3-1/100-k4-2	0.1506	9.0909	-0.8222	0.9586	37.8
	mean	**0.1507**	**9.1162**	**-0.8218**	**0.9598**	**37.8**
0.046	2.2_T3-1/100-k5-1	0.0227	2.3853	-1.6447	0.3776	51.2
	2.2_T3-1/100-k5-2	0.0240	2.2106	-1.6205	0.3445	48.4
	mean	**0.0233**	**2.2980**	**-1.6326**	**0.3610**	**49.8**

Table 8.1.2.2-6: Definitive Phase – Adsorption: Concentration of radioactivity in the water phase and soil for Soil 2.3

C_0 [µg/mL]	Sample	C_{aq}^{ads} [µg/mL]	C_s^{ads} [µg/g]	log C_{aq}^{ads} [µg/mL]	log C_s^{ads} [µg/g]	Adsorption [%]
5.009	2.3_T3-1/100-k1-1	4.2492	75.6998	0.6283	1.8791	15.2
	2.3_T3-1/100-k1-2	4.3726	62.8692	0.6407	1.7984	12.6
	mean	**4.3109**	**69.2845**	**0.6345**	**1.8388**	**13.9**
2.552	2.3_T3-1/100-k2-1	2.0042	54.6701	0.3019	1.7377	21.5
	2.3_T3-1/100-k2-2	2.0348	51.6894	0.3085	1.7134	20.4
	mean	**2.0195**	**53.1797**	**0.3052**	**1.7256**	**20.9**
0.482	2.3_T3-1/100-k3-1	0.3270	15.5155	-0.4855	1.1908	32.0
	2.3_T3-1/100-k3-2	0.3229	15.8690	-0.4909	1.2006	33.1
	mean	**0.3249**	**15.6923**	**-0.4882**	**1.1957**	**32.6**
0.242	2.3_T3-1/100-k4-1	0.1685	7.3752	-0.7733	0.8678	30.4
	2.3_T3-1/100-k4-2	0.1654	7.7505	-0.7815	0.8893	31.9
	mean	**0.1670**	**7.5629**	**-0.7774**	**0.8786**	**31.1**
0.046	2.3_T3-1/100-k5-1	0.0278	1.8410	-1.5561	0.2651	40.2
	2.3_T3-1/100-k5-2	0.0262	2.0352	-1.5824	0.3086	43.6
	mean	**0.0270**	**1.9381**	**-1.5692**	**0.2868**	**41.9**

Table 8.1.2.2-7: Definitive Phase – Adsorption: Concentration of radioactivity in the water phase and soil for Soil 6S

C_0 [µg/mL]	Sample	C_{aq}^{ads} [µg/mL]	C_s^{ads} [µg/g]	log C_{aq}^{ads} [µg/mL]	log C_s^{ads} [µg/g]	Adsorption [%]
5.009	6S_T3-1/100-k1-1	3.7771	120.3996	0.5772	2.0806	23.9
	6S_T3-1/100-k1-2	4.1847	91.2345	0.6217	1.9602	17.9
	mean	**3.9809**	**105.8170**	**0.5994**	**2.0204**	**20.9**
2.552	6S_T3-1/100-k2-1	1.7671	79.9317	0.2473	1.9027	30.8
	6S_T3-1/100-k2-2	1.7794	77.0856	0.2503	1.8870	30.3
	mean	**1.7733**	**78.5086**	**0.2488**	**1.8948**	**30.5**
0.482	6S_T3-1/100-k3-1	0.3483	13.6041	-0.4581	1.1337	27.7
	6S_T3-1/100-k3-2	0.3545	13.1825	-0.4504	1.1200	26.9
	mean	**0.3514**	**13.3933**	**-0.4542**	**1.1268**	**27.3**
0.242	6S_T3-1/100-k4-1	0.1669	7.6385	-0.7774	0.8830	31.0
	6S_T3-1/100-k4-2	0.1776	6.3315	-0.7506	0.8015	25.5
	mean	**0.1723**	**6.9850**	**-0.7640**	**0.8423**	**28.3**
0.046	6S_T3-1/100-k5-1	0.0271	1.9892	-1.5672	0.2987	41.7
	6S_T3-1/100-k5-2	0.0250	2.2036	-1.6017	0.3431	46.2
	mean	**0.0261**	**2.0964**	**-1.5844**	**0.3209**	**43.9**

Table 8.1.2.2-8: Definitive Phase – Adsorption: Concentration of radioactivity in the water phase and soil for Soil BF

C_0 [µg/mL]	Sample	C_{aq}^{ads} [µg/mL]	C_s^{ads} [µg/g]	log C_{aq}^{ads} [µg/mL]	log C_s^{ads} [µg/g]	Adsorption [%]
5.009	BF_T3-1/100-k1-1	4.5322	48.5097	0.6563	1.6858	10.1
	BF_T3-1/100-k1-2	4.3551	68.3083	0.6390	1.8345	13.1
	mean	**4.4436**	**58.4090**	**0.6477**	**1.7602**	**11.6**
2.552	BF_T3-1/100-k2-1	2.0767	47.8854	0.3174	1.6802	18.7
	BF_T3-1/100-k2-2	2.1121	44.5275	0.3247	1.6486	17.3
	mean	**2.0944**	**46.2065**	**0.3210**	**1.6644**	**18.0**
0.482	BF_T3-1/100-k3-1	0.3572	12.5367	-0.4471	1.0982	26.0
	BF_T3-1/100-k3-2	0.3581	12.7615	-0.4459	1.1059	25.7
	mean	**0.3577**	**12.6491**	**-0.4465**	**1.1020**	**25.8**
0.242	BF_T3-1/100-k4-1	0.1774	6.5716	-0.7509	0.8177	26.6
	BF_T3-1/100-k4-2	0.1811	6.1559	-0.7420	0.7893	25.2
	mean	**0.1793**	**6.3638**	**-0.7465**	**0.8035**	**25.9**
0.046	BF_T3-1/100-k5-1	0.0295	1.7078	-1.5296	0.2324	36.4
	BF_T3-1/100-k5-2	0.0278	1.8668	-1.5565	0.2711	40.2
	mean	**0.0287**	**1.7873**	**-1.5431**	**0.2518**	**38.3**

Table 8.1.2.2-9: Definitive Phase – Adsorption: Concentration of radioactivity in the water phase and soil for Soil WW

C_0 [µg/mL]	Sample	C_{aq}^{ads} [µg/mL]	C_s^{ads} [µg/g]	log C_{aq}^{ads} [µg/mL]	log C_s^{ads} [µg/g]	Adsorption [%]
5.009	WW_T3-1/100-k1-1	3.9359	106.0355	0.5950	2.0255	21.4
	WW_T3-1/100-k1-2	4.2315	77.5334	0.6265	1.8895	15.5
	mean	**4.0837**	**91.7845**	**0.6108**	**1.9575**	**18.4**
2.552	WW_T3-1/100-k2-1	1.8932	67.4042	0.2772	1.8287	25.9
	WW_T3-1/100-k2-2	1.8235	2395.5003	0.2609	3.3794	27.9
	mean	**1.8583**	**1231.4523**	**0.2690**	**2.6040**	**26.9**
0.482	WW_T3-1/100-k3-1	0.3367	14.3843	-0.4728	1.1579	30.2
	WW_T3-1/100-k3-2	0.3435	13.8155	-0.4641	1.1404	28.7
	mean	**0.3401**	**14.0999**	**-0.4684**	**1.1491**	**29.4**
0.242	WW_T3-1/100-k4-1	0.1668	7.5094	-0.7779	0.8756	31.1
	WW_T3-1/100-k4-2	0.1587	8.0776	-0.7994	0.9073	33.7
	mean	**0.1627**	**7.7935**	**-0.7886**	**0.8914**	**32.4**
0.046	WW_T3-1/100-k5-1	0.0335	1.3549	-1.4756	0.1319	28.3
	WW_T3-1/100-k5-2	0.0314	1.4838	-1.5028	0.1714	32.0
	mean	**0.0324**	**1.4194**	**-1.4892**	**0.1517**	**30.1**

The adsorption coefficients $K_{F(ads)}$ of AMPA ranged from 23.365 to 41.931 mL/g for all soils. The Freundlich exponents 1/n were in the range of 0.707 to 0.875. The corresponding, calculated $K_{F, OC(ads)}$ values varied from 1675.1 to 4708.6 mL/g. For details see table below.

Table 8.1.2.2-10: [^{14}C]AMPA: Adsorption parameters in soil at 20 °C

Soil	Adsorption			
	$K_{F(ads)}$	1/n	R^2	$K_{F, OC(ads)}$
RefeSol 02-A (silt)	38.933	0.707	0.976	3299.4
LUFA 2.2 (sandy loam)	41.931	0.752	0.982	2833.1

LUFA 2.3 (sandy loam)	28.722	0.721	0.987	4708.6
LUFA 6S (clay loam)	36.617	0.825	0.979	1768.9
Bourgfelden (silt loam)	23.365	0.713	0.986	2031.8
Wurmwiese (sandy loam)	33.503	0.875	0.984	1675.1

Evaluation of result according to EU OECD 106 Evaluators Checklist

All relevant quality checks following OECD 106 Evaluators Checklist were performed. Parental mass balances of were from 95.0 to 103.9 % (5.0 mg/L) and percentage adsorption was from 10.1 to 59.4 % in the definitive test (see table below). Estimated K_{FE}/K_F values ranged from 0.76 to 1.48. The chromatographic method was verified over the entire range of concentrations measured (LOD = 0.98% AR for the highest concentration and 1.8 % AR for the lowest concentration). K_D x soil/solution ratios were between 0.11 and 1.46 in all soils. The graphical fits of the Freundlich equation are presented in the figures below based on the standard linear regression form using log-log transformed data alongside the associated residual plots. The R^2 of the standard linear regressions ranged from 0.975 to 0.987.

Table 8.1.2.2-11: AMPA - Evaluation of result according to EU OECD 106 Evaluators Checklist

Soil	RefeSol 02-A	LUFA 2.2	LUFA 2.3	LUFA 6S	Bourgfelden	Wurmwiese
Adsorption method (direct/indirect)	indirect	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio (g dw/mL)	1:100	1:100	1:100	1:100	1:100	1:100
Parental Mass Balance (highest test concentration, definitive test)	102.1	97.8	103.9	102.9	101.5	95.0
Adsorbed percentage (%)	19.9-59.4	20.2-51.2	12.6-43.6	17.9-46.2 *(RMS*: 16.5-46.1)*	10.1-40.2 *(RMS*: 9.5-40.2)*	15.5-33.7 *(RMS*: 15.5-34.4)*
K_D x (soil:solution ratio)	0.25-1.46	0.25-1.05	0.15-0.78	0.20-0.86	0.11-0.67	0.18-0.48 *(RMS: 0.18-0.52)*
$^{ads}K_F$ (95 % confidence interval)	38.914 (31.981-47.351)	41.829 (35.298-49.569)	28.675 (25.075-32.793)	36.438 (29.587-44.875)	23.085 (20.150-26.447)	33.682 (28.040-40.460)
$^{ads}1/n$ (95 % confidence interval)	0.7086 (0.616-0.801)	0.7513 (0.670-0.833)	0.7215 (0.655-0.788)	0.8239 (0.719-0.929)	0.7100 (0.641-0.779)	0.8777 (0.783-0.973)
$^{ads}R^2$	0.9752	0.9826	0.9873	0.9762	0.9860	0.9826
$^{ads}K_{F,OC}$	3297.8	2826.3	4700.9	1760.3	2007.4	1684.1
K_{fe}/K_f**	0.91-0.97	1.05-1.12	0.76-0.92	0.85-0.94	0.86-0.96	1.17-1.48

*based on the EFSA OECD 106 excel tool provided by the applicant

** range for all concentrations, based on parental mass balance for the highest concentration

Figure 8.1.2.2-1: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for RefeSol 02-A (EU OECD 106 Evaluators Checklist)

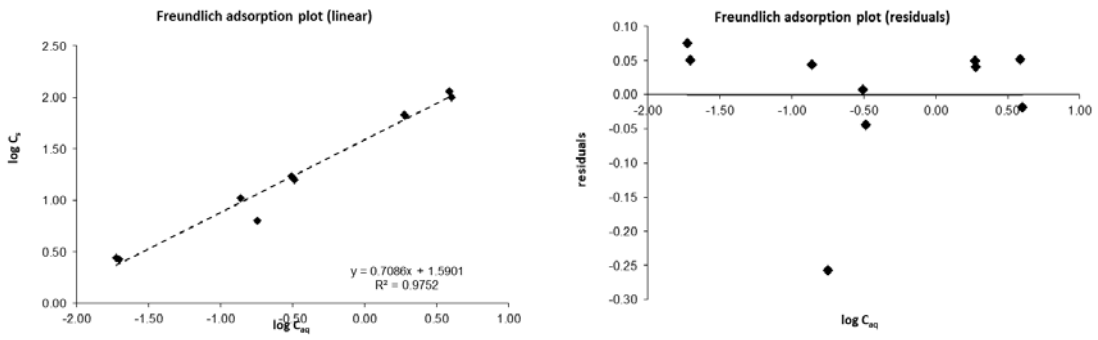


Figure 8.1.2.2-2: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for LUFA 2.2 (EU OECD 106 Evaluators Checklist)

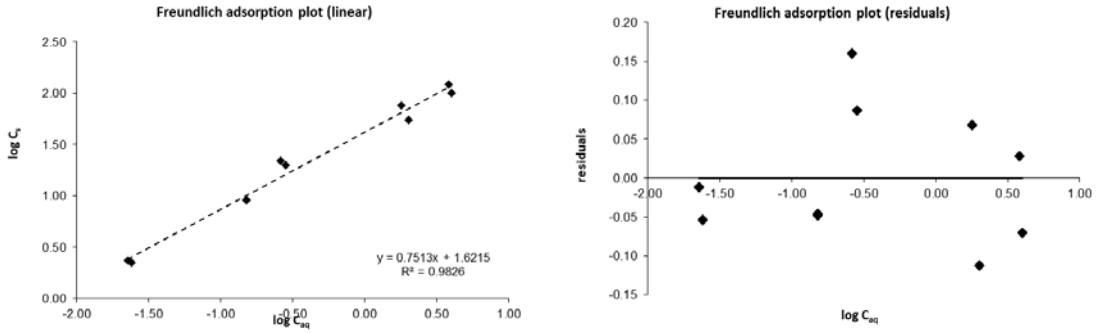


Figure 8.1.2.2-3: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for LUFA 2.3 (EU OECD 106 Evaluators Checklist)

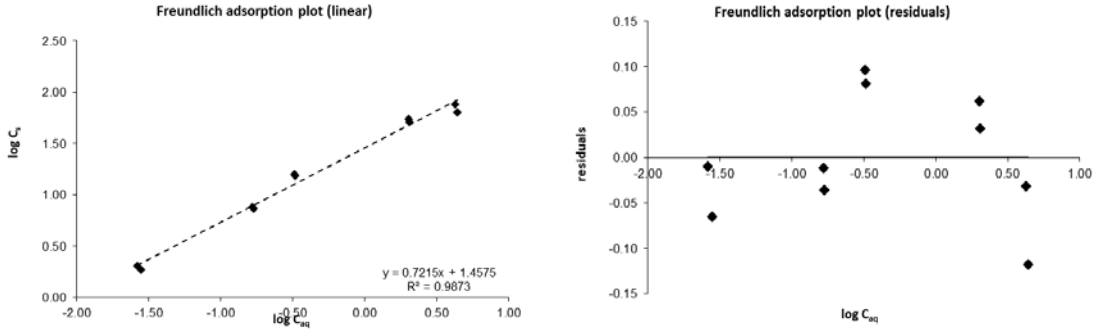


Figure 8.1.2.2-4: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for LUFA 6S (EU OECD 106 Evaluators Checklist)

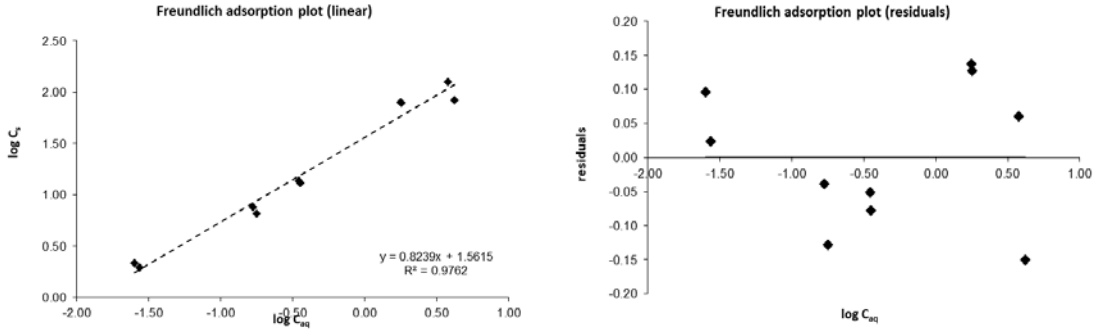


Figure 8.1.2.2-5: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for Bourgfelden (EU OECD 106 Evaluators Checklist)

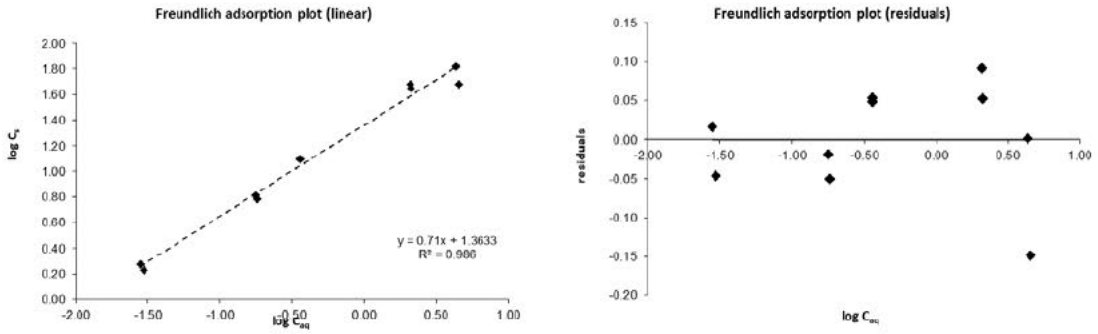
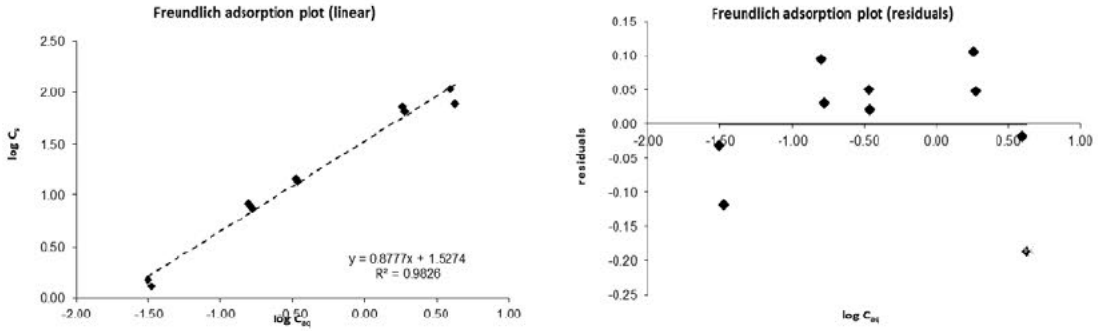


Figure 8.1.2.2-6: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for Wurmwiese (EU OECD 106 Evaluators Checklist)



Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

III. CONCLUSIONS

The Freundlich adsorption coefficients KF(ads) of AMPA as investigated for six soils ranged from 23.368 to 41.947 mL/g. The corresponding values normalised for organic carbon content of soil $K_{F,oc}$(ads) varied between 1675.5 to 4709.0 mL/g.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing no deviations.

The study is considered acceptable to address this data point.

Assessment and conclusion by RMS:

The study is well conducted.

OECD 106 recommends the preliminary steps to be performed, among other reasons, to determine the best soil/solution ratios and equilibrium time. The recommended approach is to test the substance at different ratios and equilibrium times. This was not strictly respected in this study, as for the first preliminary test a soil/solution ratio of 1:30 was used along with an equilibrium time of 48h and, considering the lack of stability of AMPA, preliminary test 3 was performed with the soil/solution ratio of 1:100 along with an equilibrium time of 4h. The soil/solution ratio in preliminary test 3 did provide acceptable results regarding the adsorption percentage. Therefore its use for definitive test can be accepted.

The quality checks according to EU OECD 106 as presented in the study report were checked by RMS. RMS highlights that the f (%) losses indicated under appendix 5 of the study as input data used for evaluation

according to OECD 106 checklist are not those effectively entered in the excelsheet provided by the applicant. However results indicated were obtained with the correct f losses values, obtained at the highest concentration (calculated as 100% - parental mass balance at the highest tested concentration during the definitive phase).

As indicated in OECD 106 and EFSA OECD 106 checklist, the LOQ should be at least two orders of magnitude below the lowest nominal concentration tested. In this study, it is reported that the lowest measured value is 46 times higher than the LOQ. It is therefore expected that LOQ is at least two orders of magnitude below the lowest nominal concentration tested, but this should be confirmed by the applicant. This is identified as a data gap.

RMS highlights that results in Table 8.1.2.2-11 are based on individual replicates. When considering mean of replicates, RMS notes that some criteria are not met.

For LUFA 2.3 and Bourgfelden soils at the highest concentration and for Wurmewiese soil at the 2 highest concentrations, the mean adsorption percentage is below 20%. For RefeSol 02-A, LUFA 2.2, LUFA 6S and Wurmwiese soils at the highest concentration and for LUFA 2.3 and Bourgfelden soils at the 2 highest concentrations, the mean K_D * (soil/solution ratio) are below 0.3. This indicates that the soil:solution ratio of 1:100 may not be optimised.

The Kfe/Kf ratio is above 1.2 for Wurmwiese soil only, but it is significantly above 1.2 for the highest concentration only.

Considering that parental mass balance was quite good and Kfe/Kf are acceptable in most cases, RMS considers that these deviations do not invalidate the results of the study. Overall, the study design is considered as good.

The study is considered acceptable.

[redacted], *1993*

Data point:	CA 7.1.3.1.2/006
Report author	[redacted]
Report year	1993
Report title	Aminomethylphosphonic acid – Determination of the sorption and desorption properties
Report No	92-8-4390
Guidelines followed in study	OECD Guideline 106 U.S. EPA. 1982. Sediment and Soil Adsorption Isotherm CG-1710.
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Soils were not pre-equilibrated - Only 4 concentrations tested - Material balance < 90% for soils #1, #9 and #11 - Parental mass balance < 90% for soils #1, #2, #9 and #11 - Suitability of the LOQ to be confirmed
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes, only for soils #4 and #5

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]Aminomethylphosphonic acid

Lot No.	C-1105.9, C-1521.1, C-1521.2, C-1521.7
Specific activity	3 batches with 23.8 and 1 batch with 26.8 mCi/mmol
Radiochemical purity	≥ 98.7 %

2. Test Soils

The soils were sieved to a particle size of ≤2 mm. The soils were air-dried before application. SLI Soil #1 had a previous history indicating the use of phenoxy herbicides within the 12 months prior to collection. The remaining five soils had no pesticides applied in two or more years. The characterisation of test soils used is summarised in the table below.

Table 8.1.2.2-12: Physico-chemical properties of test soils

Parameter	Results					
Soil Designation	SLI Soil #1	SLI Soil #2	SLI Soil #4	SLI Soil #5	SLI Soil #9	SLI Soil #11
Country	Netherlands	Netherlands	Netherlands	Netherlands	Netherlands	Netherlands
Textural Class (USDA)	Clay loam	Sand	Sand	Clay loam	Loamy sand	Sand
Sand [%] (50 µm – 2 mm)	20.0	88.0	92.0	22.0	76.0	98.0
Silt [%] (2 µm – 50 µm)	45.3	11.3	5.30	49.3	19.3	1.30
Clay [%] (<2 µm)	34.7	0.70	2.70	28.7	4.70	0.700
pH in 1:1 Soil:Water Suspension	7.70	4.70	7.40	7.60	6.30	4.60
pH $CaCl_2$ [2]	-	-	6.9	7.1	5.7	-
Organic Matter (%)	3.60	32.2	2.30	1.60	2.70	0.500
Organic Carbon [1] (%)	2.09	18.72	1.34	0.93	1.57	0.29
Cation Exchange Capacity (meq/100 g)	32.8	28.3	12.0	31.0	10.2	4.80
Moisture at 1/2 bar (%)	36.9	61.5	9.1	36.6	18.5	7.6

1 Calculated as : OC [%] = OM [%] / 1.72
[2] calculated
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Glass centrifuge tubes (50 or 200 mL) with Teflon®-lined caps were used as test systems. The experiments for the definitive test were performed in triplicate.

Preliminary tests

The absence of adsorption of the test item to the test vessel and the stability of the test item in 0.01 M CaCl2 solution were confirmed.

A screening test was performed for each soil at a soil to solution ratio of 1:5, a concentration of 5 mg/L and an equilibrium time of 16 hours. The percentage of adsorption was 92.3, 98.3, 64.2, 87.6, 91.9 and 86.6 % for soils #1, #2, #4, #5, #9 and #11, respectively.

The equilibrium time was determined for each soil, at a concentration of 5 mg/L and a ratio 1:5 for all soils except for soil #2 (ratio 1:100). Equilibrum times of 2 to 72 hours were tested. Equilibrium time was determined to be 16 h for #2, #9 and #11, 24h for #4 and #5, and 48h for #1.

Stability of test item was checked in each soil, at a concentration of 5 mg/L, soil:solution ratio of 1:20 (except for #2, 1:100), for the equilibrium time determined above. HPLC analysis of supernatant and soil extracts was performed. Parental mass balance was 78.93, 15.03, 96.99, 101.8, 68.83 and 87.89% AR for #1, #2, #4, #5, #9 and #11, respectively.

Definitive test

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M CaCl2 solution with a soil-to-solution ratio of 1:20 (1:100 for SLI Soil #2). AMPA was applied at approximate nominal solution concentrations of 5.0, 1.0, 0.2, and 0.04 mg/L in aqueous 0.01 M CaCl2 solution. The adsorption step was carried out for 16 hours in SLI Soil #2, SLI Soil #9, and SLI Soil #11, for 24 hours in SLI Soil #4 and SLI Soil #5, and 48 hours in SLI Soil #1 in the dark at 20 ± 2 °C under continuous agitation.

2. Analytical Procedures

For stability and determination of the parental mass balance test soil samples (nominal concentration of 4.97 mg/L) were extracted up to two times by shaking at ambient temperature using 0.5 N NH4OH after the adsorption step. Supernatant and soil extract were separated by centrifugation and the pH of the soil extracts was adjusted to pH 3 using phosphoric acid. Aqueous supernatants and soil extracts were analysed by HPLC-radiodetection.

After the adsorption step of the definitive test, the aqueous supernatant was separated from the soil by centrifugation and radioactivity in the supernatants was determined by liquid scintillation counting (LSC). Soil samples from the 5.00 or 1.02 mg/L samples, of each soil type from the advanced isotherm test were combusted followed by quantitation using radioassay. This data was used to calculate material balance during the advanced isotherm phase.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation, using the indirect approach.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances during the definitive test were 86.7 % of applied radioactivity (AR) for SLI Soil #1, 92.3 % for SLI Soil #2, 90.8 % for SLI Soil #4, 96.6 % for SLI Soil #5, 83.1 % for SLI Soil #9 and 84.6 % for SLI Soil #11.

B. STABILITY OF TEST ITEM

During the preliminary test, parental mass balances were 78.93 % of applied test item (in aq. supernatant and soil extracts) for SLI Soil #1, 15.03 % for SLI Soil #2, 96.99 % for SLI Soil #4, 101.80 % for SLI Soil #5, 68.83 % for SLI Soil #9 and 87.89 % for SLI Soil #11.

Parental mass balance was not checked during the definitive test.

C. FINDINGS

Concentrations in supernatant and soil are presented below.

Table 8.1.2.2-13: Concentrations in supernatant and soil and percentage of adsorption

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)	Concentration in soil (µg/g)
Soil #1	0.0394	0.00294	0.731
	0.0394	0.00307	0.729
	0.0394	0.00299	0.730
	0.212	0.0203	3.97
	0.212	0.0208	3.96
	0.212	0.0212	3.96
	0.970	0.131	17.1
	0.970	0.132	17.1
	0.970	0.126	17.2
	4.870	1.130	77.4
	4.870	1.150	77.0
	4.870	1.110	77.8
Soil #2	0.0394	0.00144	4.01
	0.0394	0.00144	4.01
	0.0394	0.00158	3.99
	0.212	0.00746	21.7
	0.212	0.00787	21.6
	0.212	0.00856	21.5
	0.970	0.0414	96.9
	0.970	0.0431	96.7
	0.970	0.0418	96.8
	4.870	0.2950	477
	4.870	0.2840	479
	4.870	0.2980	478
Soil #4	0.0488	0.0162	0.656

	0.0488	0.0155	0.670
	0.0488	0.0139	0.702
	0.218	0.0838	2.58
	0.218	0.0867	2.53
	0.218	0.0925	2.41
	1.090	0.5630	9.54
	1.090	0.5460	9.88
	1.090	0.5310	10.2
	5.250	3.0300	39.6
	5.250	3.3000	34.2
	5.250	3.0900	38.4
Soil #5	0.0488	0.00530	0.896
	0.0488	0.00548	0.893
	0.0488	0.00562	0.890
	0.218	0.0400	3.42
	0.218	0.0363	3.50
	0.218	0.0321	3.58
	1.09	0.216	17.5
	1.09	0.219	17.4
	1.09	0.213	17.5
	5.25	1.48	73.4
	5.25	1.66	69.8
	5.25	1.46	73.8
Soil #9	0.0394	0.00237	0.725
	0.0394	0.00218	0.728
	0.0394	0.00213	0.729
	0.212	0.00921	4.02
	0.212	0.0145	3.91
	0.212	0.0119	3.96
	0.970	0.0478	19.2
	0.970	0.0531	19.1
	0.970	0.0516	19.2
	4.87	1.130	81.2
	4.87	0.994	83.9
	4.87	0.946	84.9
Soil #11	0.0394	0.00311	0.716
	0.0394	0.00364	0.705
	0.0394	0.00323	0.713
	0.212	0.0242	3.82
	0.212	0.0182	3.94
	0.212	0.0269	3.76
	0.970	0.1040	19.5
	0.970	0.1180	19.2
	0.970	0.1500	18.6
	4.87	1.37	70.8
	4.87	1.26	73.0
	4.87	1.19	74.4

The adsorption coefficients KF(ads) of AMPA were derived on the basis of the indirect method to result in Freundlich isotherms for the six test soils and ranged from 15.7 to 1570 mL/g. The Freundlich exponents 1/n were in the range of 0.752 to 0.904. The corresponding, calculated $K_{F, OC}$(ads) values varied between 1160 and 24800 mL/g.

Table 8.1.2.2-14: Adsorption parameters of AMPA in soil at 20 °C

Soil	Adsorption			
	$K_{F(ads)}$ [mL/g]	1/n	R^2	$K_{F, OC(ads)}$ [mL/g]
SLI Soil #1	77.1	0.786	0.997	3640
SLI Soil #2	1570	0.904	0.998	8310
SLI Soil #4	15.7	0.752	1.00	1160

SLI Soil #5	53.9	0.791	0.998	5650
SLI Soil #9	110	0.769	0.960	6920
SLI Soil #11	73.0	0.788	0.988	24800

III. CONCLUSIONS

The adsorption coefficients KF(ads) of AMPA for the tested soils calculated based on the Freundlich isotherms ranged from 15.7 to 1570 mL/g. The respective $K_{F, OC}$(ads) values ranged from 1160 to 24800 mL/g.

Assessment and conclusion by applicant:

The study is considered to be valid for the two soils SLI soil #4 and SLI soil #5.

During review for AIR2, Soil SLI Soil #2 was excluded by the RMS due to its high OC content (18.7 %).

For the three soils SLI #1, #9 and #11, overall balances of radioactivity including parental mass balances were below 90 %.

As a conservative assessment the data of the three soils were not included in the actual risk assessment.

All relevant quality checks as part of confirming the acceptability of the study and of the reported endpoints were performed.

For soils SLI Soil #4 and #5, parental mass balances were 97.0-101.8 %, and percentage adsorption was 37.1-89.1 % . Systematic errors estimated via KFE/KF were calculated as low (i.e. ≤1.1). The analytical method covered the entire range of test concentrations (lowest test concentration equivalent to approx. 570 Bq per aliquot which is at least 100 fold higher than the typical instrumental LOD of LSC measurements. Furthermore, the lowest test concentration was approx. 300 fold higher than the highest background reported). The use of the indirect method was appropriate based on a KD x soil/solution ratio >0.3 in all soils. The graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots. The R2 of the standard linear regressions ranged from 0.997 to 0.998 and the visual fit of the standard regression were acceptable.

Table 8.1.2.2-15: Results of evaluation according to EU OECD 106 Evaluators Checklist for AMPA

	Units	**SLI Soil #1**	**SLI Soil #2** [1]	**SLI Soil #4**	**SLI Soil #5**	**SLI Soil #9**	**SLI Soil #11**
Adsorption method	-	indirect	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	(g dw/mL)	1:20	1:100	1:20	1:20	1:20	1:20
Parental mass balance (at highest conc.)	%	78.9	15.0	97.0	101.8	68.8	87.9
Adsorbed percentage	%	76.4-92.5	93.9-96.5	37.1-71.5	68.4-89.1	76.8-95.7	71.9-92.1
K_D x (soil:solution ratio)		3.4-12.4	16.0-27.9	0.5-2.5	2.1-8.5	3.6-21.8	2.6-11.5
$^{ads}K_F$ (95% confidence interval)	L/kg dw	77.027 (69.097-85.867)	1564.904 (1359.514-1801.325)	15.668 (14.720-16.677)	53.185 (48.185-58.704)	109.942 (69.335-174.331)	72.676 (56.036-94.256)
$^{ads}1/n$ (95% confidence interval)	-	0.786 (0.756-0.816)	0.903 (0.871-0.935)	0.751 (0.726-0.776)	0.790 (0.759-0.821)	0.767 (0.653-0.880)	0.785 (0.712-0.857)
$^{ads}R^2$	-	0.997	0.997	0.998	0.997	0.958	0.983
$^{ads}K_{F,OC}$	L/kg OC	3668	8369	1205	5910	6871	24225
K_{FE} / K_F	-	1.4	10	1.1	1.0	1.6	1.2

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

[1] Soil excluded during previous evaluation due to OC of 18.68%.

Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil #1

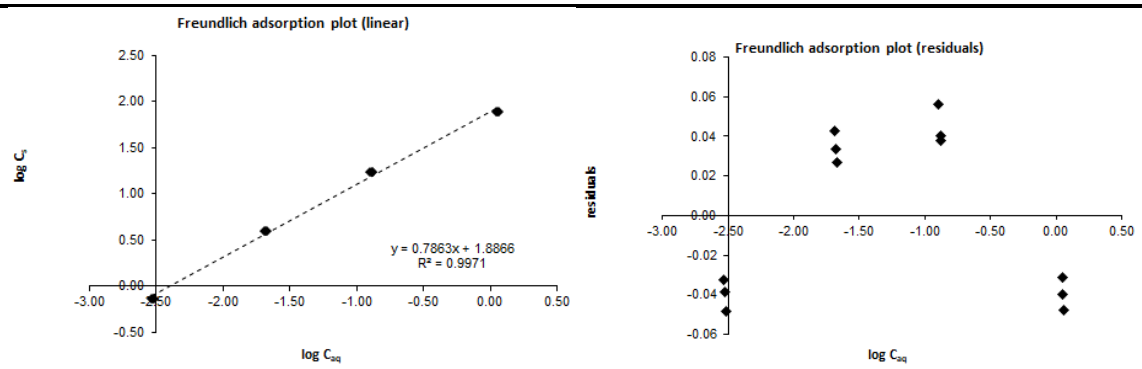
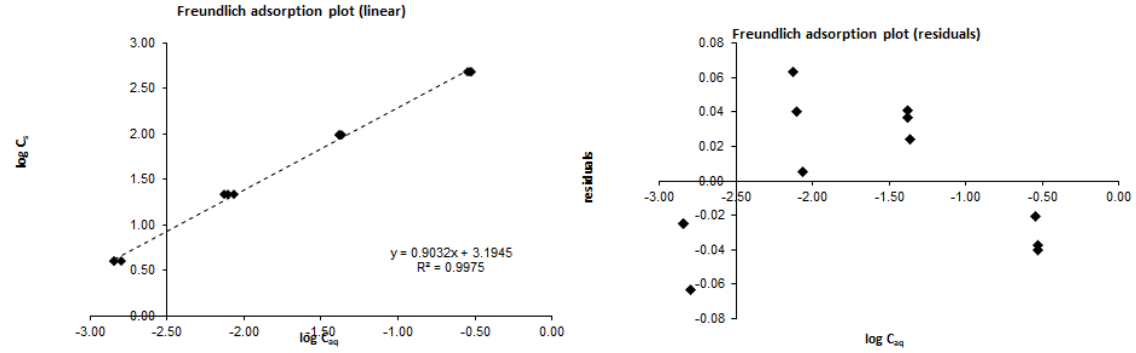
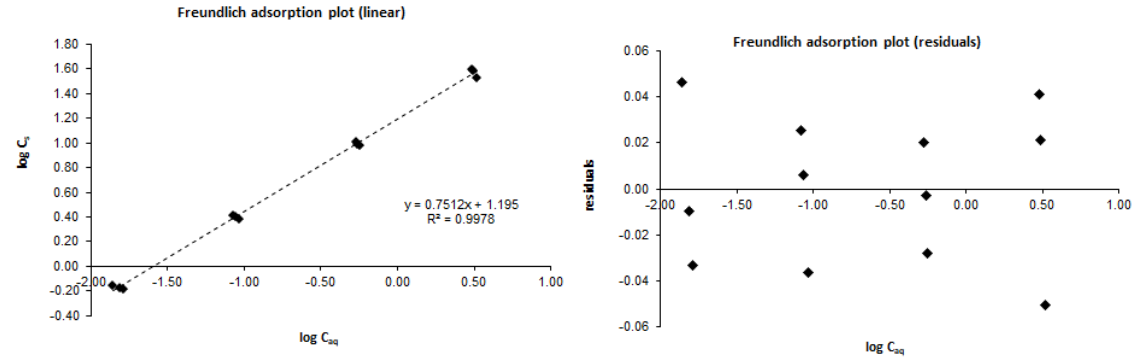


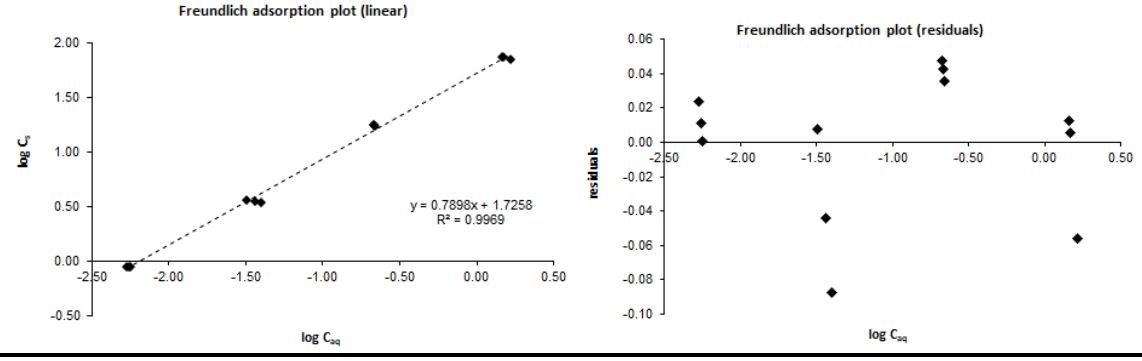
Fig Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil #2



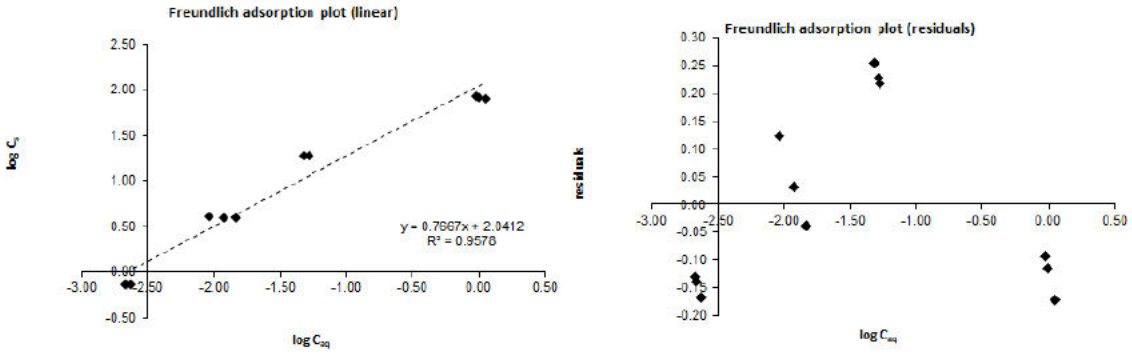
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil #4



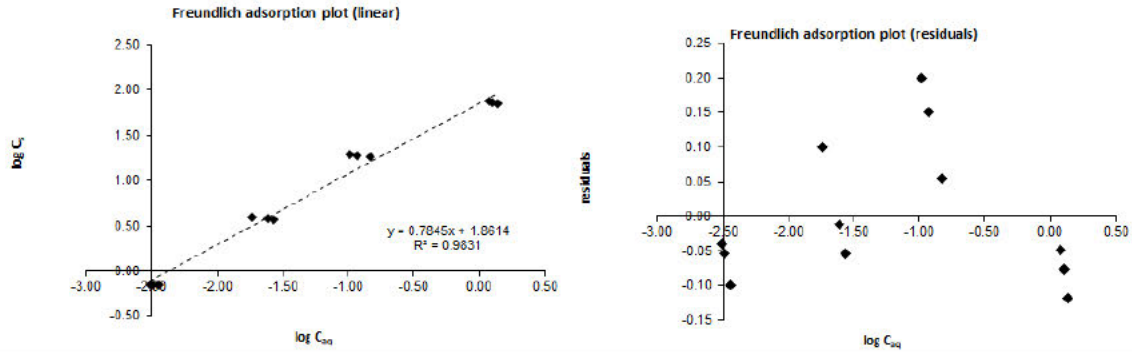
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil #5



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil #9



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil #11



Assessment and conclusion by RMS:

Some deviations are observed from the OECD 106 guidance. Soils were not pre-equilibrated, only 4 concentrations were tested instead of 5, total recovery is below 90% for soils #1, #9 and #11, and parental mass balance is below 90% for soils #1, #2, #9 and #11.

Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was AMPA, as proposed by the applicant results cannot be considered as reliable for soils #1, #2, #9 and #11.

It is also noted that soil #2 has a very high organic matter content (32.2% OM) and was excluded for that reason in the previous assessment. RMS agrees that this is not representative of agricultural soils in Europe.

The applicant indicates that "The analytical method covered the entire range of test concentrations (lowest test concentration equivalent to approx. 570 Bq per aliquot which is at least 100 fold higher than the typical instrumental LOD of LSC measurements. Furthermore, the lowest test concentration was approx. 300 fold higher than the highest background reported)". The lowest nominal test concentration is 0.0394 µg/mL, but it is not clear to which dpm value this corresponds. The background value given in the report is 37-85 dpm. A data gap is set for the applicant to indicate whether the LOQ is at least two orders of magnitude below the lowest nominal concentration tested.

The applicant did not use correctly the Excel tool from the EU OECD 106 Evaluators Checklist: both concentrations in supernatant and soil extracts were directly entered in the tool whereas the indirect method was used in the study. Results obtained by RMS when entering only the concentrations in supernatant are reported below.

Table 8.1.2.2-16: Results of evaluation according to EU OECD 106 Evaluators Checklist for AMPA - RMS

	Units	SLI Soil #4	SLI Soil #5

Adsorption method	-	indirect	indirect
Soil:solution ratio	(g dw/mL)	1:20	1:20
Parental mass balance (at highest conc.)	%	97.0	101.8
Adsorbed percentage	%	37.1-71.5	68.4-89.1
K_D x (soil:solution ratio)		0.6-2.5	2.2-8.2
$^{ads}K_F$ (95% confidence interval)	L/kg dw	17.32 (16.30-18.40)	54.62 (50.17-59.46)
$^{ads}1/n$ (95% confidence interval)	-	0.776 (0.752-0.800)	0.797 (0.770-0.824)
$^{ads}R^2$	-	0.998	0.998
$^{ads}K_{F,OC}$	L/kg OC	1292	5873
K_{FE} / K_F (at highest concentration)	-	1.1	0.98

Note: Values derived from the EFSA evaluators checklist may vary from those in the study report due to rounding errors.

All quality criteria are met for these 2 soils.

The study is considered partly acceptable; only results from soils #4 and #5 are considered reliable. Results from other soils should not be considered further.

[redacted], *2003*

Data point:	CA 7.1.3.1.2/002
Report author	[redacted]
Report year	2003
Report title	Aminomethylphosphonic acid: adsorption-desorption
Report No	IF-02/00005220
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Soil to solution ratio not optimal (very high adsorption) - Parental mass balance <90 %
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C]AMPA

Code	CFQ12959
Specific activity	55 mCi/mmol
Radiochemical purity	98.6 % by HPLC, 98.3 % by TLC

2. Test Soils

The soils were collected fresh from the field before study start (upper horizon of 0 to 20 cm), sieved to a particle size of ≤2 mm and stored at ambient conditions in the laboratory. The soils were air-dried before application. The locations of soil collection were of no agricultural use and no plant protection products were used for several years. The characteristics of test soils is summarised in the table below.

Table 8.1.2.2-17: Physico-chemical properties of test soils

Parameter	Results

Soil Designation	Schwalbach	Hofheim	Bergen-Enkheim
Geographic Location			
City	North-east of Schwalbach/Limes	North of Hofheim and south of Kelkheim	South-east of Bergen and north-east of Enkheim
Country	Germany	Germany	Germany
Textural Class (USDA)	Silt loam	Silt loam	Silty clay
Sand (50 µm – 2 mm)	10.9	29.9	16.7
Silt (2 µm – 50 µm)	68.2	52.3	41.4
Clay (< 2 µm)	20.9	17.8	41.9
pH			
- in CaCl2	5.13	5.10	7.43
- in water	6.09	6.06	8.30
Organic Carbon	1.59	1.24	2.25
Organic Matter [1]	2.74	2.14	3.88
Cation Exchange Capacity (meq/100 g)	14.6	13.5	28.9
MWHC (%)	48.5	43.0	49.4
Bulk Density (disturbed) (g/cm³)	1.00	1.12	1.06

1calculated as: OM [%] = OC [%] x 1.724
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Plastic centrifuge tubes (750 mL) were used as test systems. The experiments were performed in duplicate.

In preliminary tests, the optimal soil-to-solution ratio and the stability of the test item in 0.01 M CaCl2 solution were determined. The stability of AMPA (parental mass balance) was investigated in the course of the definitive test following the desorption phase (no extraction performed following the adsorption phase).

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M CaCl2 solution with a soil-to-solution ratio of 1:5 (10 g soil (dry weight equivalents)/50 mL solution). AMPA was applied at nominal concentrations of 0.05, 0.3, 1, 2.5 and 5 mg/L in aqueous 0.01 M CaCl2 solution. The adsorption step was carried out for 24 hours in the dark at 20 ± 2 °C under continuous agitation. For the desorption phase of the study, the volume of the aqueous solution removed after the adsorption step was replaced by an equal volume of 0.01 mol/L CaCl2 without test item. The mixture was agitated 24 h and centrifuged as in the adsorption step. The whole desorption procedure was repeated on the solid phase with a further quantity of 0.01 mol/L CaCl2 without test item.

2. Analytical Procedures

Following each adsorption or desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of radioactivity in the supernatants was analysed by liquid scintillation counting (LSC). One dimensional TLC was used for the separation of specimen aliquots throughout the study

Following the desorption steps, the remaining adsorbed test item based on the highest test concentration used was extracted two times from soil using 1 M NH3 at ambient temperature. The ratio of extraction solvent and soil was approximately 1:1 (volume:soil dry weight). Specimen agitation was performed for 1 hour. After shaking, the extraction solvent was removed from the slurry by centrifugation. The residual radioactivity in soils was determined by combustion/LSC. Soil extracts were analysed by LSC and TLC-radiodetection to determine the stability of the test item and to establish the parental mass balance.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation using the indirect method.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Material balances were 96.65 to 100.45 % AR for soil Schwalbach, 96.72 to 99.22 % AR for soil Hofheim and 96.55 to 99.89 % AR for soil Bergen-Enkheim.

B. STABILITY OF TEST ITEM

AMPA was sufficiently stable in aqueous 0.01 mol/L CaCl2 solution. Furthermore, recovery of AMPA in aqueous 0.01 mol/L CaCl2 solution, which was agitated with soil following separation by centrifugation and application to the clear supernatants, ranged from 89.8 to 98.1 % AR.

Within the parental mass balance test 65.28, 69.12 and 27.13 % AR could be extracted after the desorption steps for soils Schwalbach, Hofheim and Bergen Enkheim, respectively. Considering residues in aqueous adsorption and desorption supernatants non-extractable residues amounted to approx. 29.5, 22.4 and 50.3 % AR for soils Schwalbach, Hofheim and Bergen Enkheim. In aqueous supernatants and soil extracts, the radioactivity was only assigned to AMPA (95 % of the radioactivity in each phase).

C. FINDINGS

At the end of the adsorption phase 94.94 to 97.85 %, 94.13 to 97.02 % and 86.40 to 92.82 % AR were adsorbed to soils Schwalbach, Hofheim, and Bergen-Enkheim, respectively. The adsorption coefficients KF(ads) of test item calculated based on the Freundlich isotherms of the four test soils ranged from 33.9 to 137.4 mL/g (mean: 86.4 mL/g) and the normalized adsorption coefficients $K_{F, OC}$(ads) (normalized to organic carbon content) ranged from 1507 to 8642 mL/g (mean: 5746 mL/g). The Freundlich exponents 1/n were in the range of 0.907390 to 0.982426 (mean: 0.937734).

At the end of the desorption phase, 1.41 to 2.27 %, 1.61 to 5.22 % and 6.07 to 10.58 % of the initially adsorbed amount was found desorbed from soils Schwalbach, Hofheim and Bergen-Enkheim, respectively.

Table 8.1.2.2-18: [^{14}C]AMPA: Percentage of adsorbed/desorbed in soils (mean values)

Soil	Test Concentration (nominal) [mg/L]									
	Adsorption [1]					Desorption [2]				
	0.05	0.3	1.0	2.5	5.0	0.05	0.3	1.0	2.5	5.0
Schwalbach	97.03	95.44	97.41	96.12	97.00	1.44	2.22	1.72	1.58	2.21
Hofheim	96.69	95.46	95.61	96.48	94.39	1.70	3.30	3.57	4.36	2.92
Bergen-Enkheim	92.66	86.96	90.83	88.85	87.49	6.15	11.10	8.43	9.70	10.08

[1] End of adsorption phase, values expressed as percentage of applied radioactivity
[2] Sum of steps one and two of desorption phase, values expressed as percentage of applied radioactivity
Values calculated by the applicant in the course of writing this summary are given in *italics*

Table 8.1.2.2-19: [^{14}C]AMPA: Adsorption parameters in soil at 20 °C

Soil	Adsorption			
	K_F [mL/g]	1/n	R^2	$K_{F, OC}$ [mL/g]
Schwalbach	137.4	0.982426	0.978367	8642
Hofheim	87.9	0.923385	0.989020	7089
Bergen-Enkheim	33.9	0.907390	0.989228	1507

III. CONCLUSIONS

The individual results of the adsorption coefficients on basis of soil organic carbon ($K_{F, OC(ads)}$), assessed with the aid of the Freundlich adsorption isotherm were: Schwalbach test system 8642 mL/g with 1/n of 0.982426; Hofheim test system 7089 mL/g with 1/n of 0.923385; Bergen-Enkheim test system 1507 mL/g with 1/n of 0.907390.

Assessment and conclusion by applicant:

The test was performed using the indirect method for determination of adsorption following the decrease of the test item in aqueous supernatant. This is allowed for the definitive phase following the current EU OECD 106

Evaluators Checklist in case the stability of the test item had been demonstrated in terms of the parental mass balance (PMB). The parental mass balances (PMB) were below 90 % AR to result in NER >10 % for all soils.

The results of the study are thus considered as invalid.

Results of the parental mass balance are not reported in detail, hence no f-factor can be specified and the check for system error cannot be performed. Therefore, the results of the study are considered as not reliable and an evaluation following the EFSA OECD 106 Evaluators Checklist is considered not necessary. However, for the sake of completeness results of the evaluation according to EFSA OECD 106 Evaluators Checklist are provided below.

Results of evaluation according to EFSA OECD 106 Evaluators Checklist for AMPA

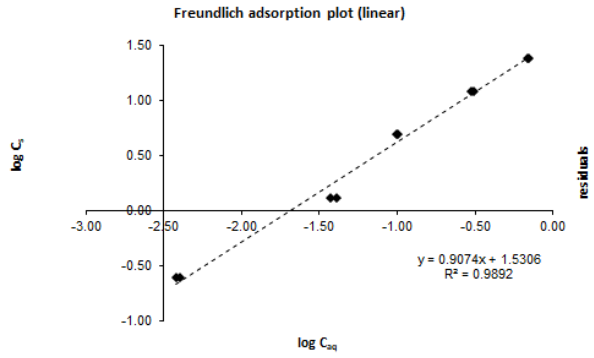
	Units	**Bergen-Enkheim**	**Schwalbach**	**Hofheim**
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	<90 [1]	<90 [1]	<90 [1]
Adsorbed percentage	%	86.4-92.8	94.9-97.9	94.1-97.0
K_D x (soil:solution ratio)		6.4-13.0	18.8-45.6	16.0-32.6
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	33.929 (26.699-43.116)	137.371 (84.902-222.264)	87.913 (64.660-119.528)
$^{ads}1/n$ (95 % confidence interval)	-	0.907 (0.830-0.985)	0.982 (0.863-1.102)	0.923 (0.844-1.003)
$^{ads}R^2$	-	0.989	0.978	0.989
$^{ads}K_{F,OC}$	L/kg OC	1508	8640	7090
K_{FE} / K_F	-	- [2]	- [2]	- [2]

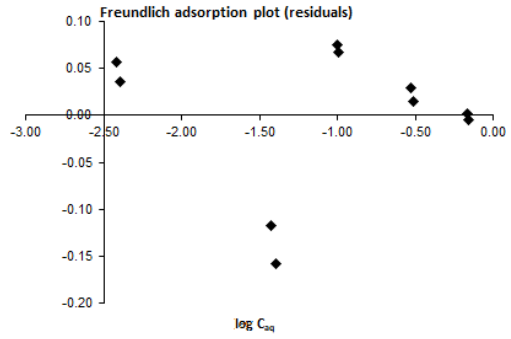
Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

[1] Extraction performed was not exhaustive (NER >10 %) resulting in a PMB <90 %.

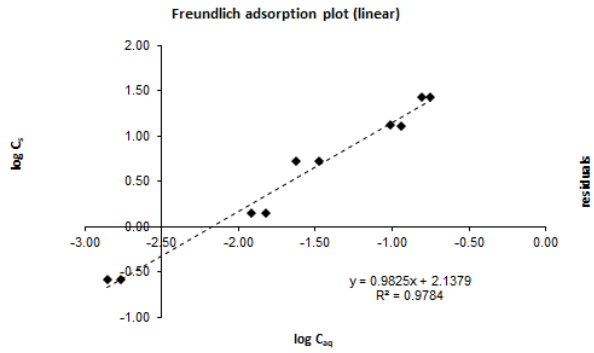
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to missing results of parental mass balance test providing the f-factor necessary for the calculations.

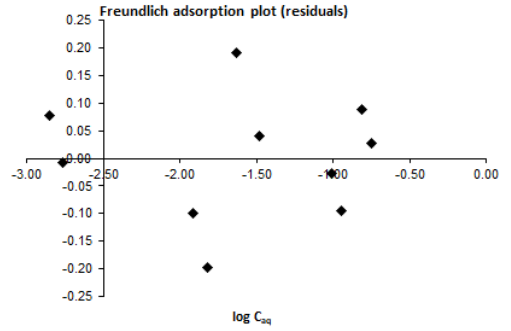
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Bergen-Enkheim



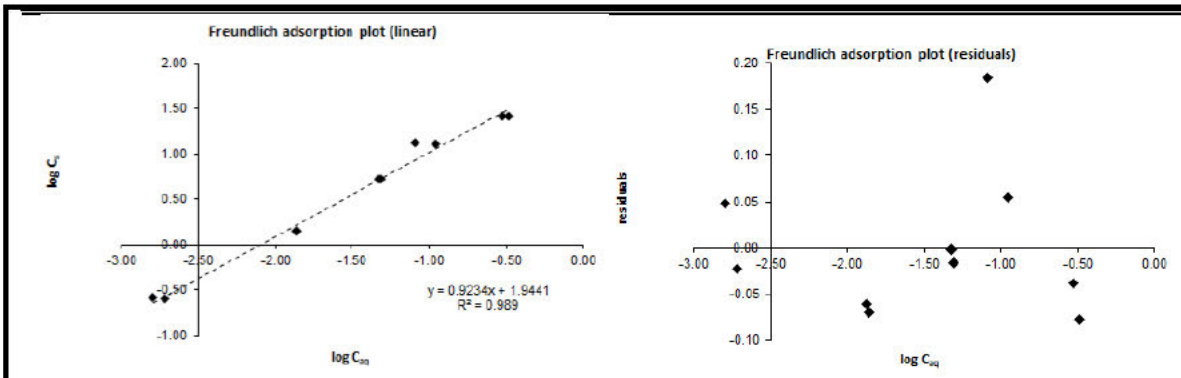


Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Schwalbach





Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Hofheim



Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Some deviations to OECD 106 are identified. Selected soil to solution ratio is not optimal (very high adsorption percentage measured), mass balance performed at the end of the desorption phase indicates significant formation of non extractable residues in the 3 soils (22-50% AR). This indicates that parental mass balance is necessarily significantly below 90% AR.

Since adsorption parameters were calculated based on the indirect method, considering that 100% of recovered radioactivity in the supernatant was AMPA results cannot be considered as reliable.

The study is not considered acceptable.

[redacted], *2002*

Data point:	CA 7.1.3.1.2/003
Report author	[redacted]
Report year	2002
Report title	Adsorption/desorption behaviour of AMPA on soil according OECD Guideline 106 (adopted January 2000)
Report No	PR02/007
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Preliminary test for determination of optimal soil to solution ratio and equilibrium time performed on 1 soil only - LOD not reported, the LOQ for analysis of radioactivity in supernatant is not 2 orders of magnitude below the lowest nominal concentration tested - In soil LUFA 2.2, in the definitive test only 3 concentrations in the supernatant are above the LOQ - Analytical method not validated for analysis in supernatant
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[15N]Aminomethylphosphonic acid (stable labelled)

Lot No.	UCL01/95
Specific activity	Not provided
Purity	98.8 %

2. Test Soils

The standard soils were air-dried at ambient temperature before application. The characterisation of test soils used is summarised in the table below.

Table 8.1.2.2-20: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	Lufa 2.1	Lufa 2.2	Lufa 3A
Country	Germany	Germany	Germany
Textural Class (USDA)	Sand	Loamy sand	Sandy Silt Loam
Sand (50 µm – 2 mm)	87.2	75.3 ± 2.0	47.3 ± 2.3
Silt (2 µm – 50 µm)	9.0	16.6 ± 1.4	35.9 ± 2.2
Clay (< 2 µm)	3.8	8.1 ± 1.2	16.9 ± 0.1
pH - in $CaCl_2$	5.2	5.6 ± 0.4	7.1 ± 0.0
pH in H_2O [2]	5.8	6.1	7.6
Organic Carbon (%)	0.9	2.3 ± 0.2	2.6 ± 0.7
Organic Matter (%) [1]	1.5	4.0	4.5
Cation Exchange Capacity (mval/100 g)	6	11 ± 2	19 ± 5
MWHC (g H2O ad 100 g soil DW)	30	50 ± 5	50 ± 7
Bulk Density (disturbed) (g/cm3)	1.42	1.15 ± 0.038	1.1 ± 0.12

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72
[2] Calculated with equation reported in EFSA guidance 2017[11]: $pH_{H2O}=0.982pH_{CaCl2} + 0.648$.
DW: dry weight, USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

The test system for batch equilibrium experiments consisted of 250 mL glass bottles with polymer screw caps. All experiments were performed in duplicates.

Preliminary tests:

The absence of adsorption of the test item to the test vessel and the stability of the test item in 0.01 M CaCl2 solution were confirmed.

The tests to determine the optimal soil-to-solution ratio (3 ratios tested: 1:25, 1:50 and 1:100) and the appropriate adsorption and desorption equilibration times (equilibrium time between 0 and 48 hours) were performed for soil Lufa 2.2 only. They indicate that the optimal ratio derived was 1:50, with an equilibrium time of 48 h.

Additional tests were performed on the 3 soils, using a ratio of 1:50 and a concentration of 10 mg/L to determine mass balance and to check the stability of the test item. Mass balances were above 90% and stability of the test item was confirmed.

Screening test on the 3 soils to determine the adsorption kinetics was also performed, using a ratio of 1:50 and a concentration of 10 mg/L.

From mass balance and screening test, it was concluded that a ratio of 1:25 would be more appropriate for soil LUFA 2.1.

Definitive phase

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M CaCl2 solution with a soil-to-solution ratio of 1:50 (2 g soil (dry weight equivalents)/ 100 mL solution) for soils Lufa 3A and Lufa 2.2 and 1:25 (4 g soil (dry weight equivalents)/ 100 mL solution)for

11 EFSA (European Food Safety Authority), 2017. EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2017;15(10):4982, 115 pp. https://doi.org/10.2903/j.efsa.2017.4982

soil Lufa 2.1. Test item was applied at nominal test concentrations of 10.0, 3.0, 1.0, 0.30 and 0.10 mg/L in aqueous 0.01 M CaCl2 solution. The adsorption step was carried out for 48 hours at 22 ± 2 °C under continuous agitation.

The desorption phase was performed by supplying pre-adsorbed soil samples with fresh aqueous 0.01 M CaCl2 solution for each test concentration. The resultant samples were re-equilibrated for 48 hours at 22 ± 2 °C under continuous agitation.

2. Analytical Procedures

The aqueous supernatant after each adsorption and desorption step was separated by centrifugation and the AMPA residues in the supernatant were analysed by gas chromatography-mass spectrometry (GC-MS). The chromatographic method was a validated method from a water/sediment study. Applicability of the method on determination of AMPA in 0.01 M CaCl2 supernatants was demonstrated within the current study. The limit of quantitation (LOQ) was 0.029 µg/mL.

In the preliminary mass balance test, the soils were extracted for 15 minutes at ambient temperature in an ultrasonic bath using aqueous NaOH solution (4 NaOH pellets dissolved in 10 mL water) after the adsorption step. The soil extracts were analysed by GC-MS following centrifugation.

3. Calculations

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation by the indirect method.

II. RESULTS AND DISCUSSION

A. MASS BALANCE AND STABILITY OF TEST ITEM

Mean mass balances during the preliminary test after 48 h of equilibration were 95.8, 92.3, and 91.1 % AR for soil Lufa 2.1, Lufa 2.2, and Lufa 3A, respectively. All radioactivity was assigned to AMPA.

Mass balance was not checked during the definitive phase.

B. FINDINGS

At the end of the adsorption phase 25.4-70.0 %, 69.0-96.8 %, and 22.9-63.9 % of the applied test material (mean of replicates) were adsorbed to soils Lufa 2.1, Lufa 2.2, and Lufa 3A, respectively.

Table 8.1.2.2-21: Concentrations in supernatant and soil and percentage of adsorption

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)	Concentration in soil (µg/g)	Adsorption percentage (%)
LUFA 2.1	0.100	0.0293	1.78	70.7
	0.100	0.0307	1.74	69.3
	0.301	0.1359	4.15	54.8
	0.301	0.1531	3.72	49.1
	1.000	0.5286	11.84	47.1
	1.000	0.5287	11.84	47.1
	3.009	1.959	26.40	34.9
	3.009	1.964	26.27	34.7
	10.04	7.463	64.71	25.7
	10.04	7.516	63.38	25.1
LUFA 2.2	0.100	nd	-	-
	0.100	nd	-	-
	0.301	0.0109*	15.43	96.4
	0.301	0.0086*	15.55	97.1
	0.999	0.0932	48.23	90.7
	0.999	0.0961	48.07	90.4
	3.006	0.520	132.41	82.7
	3.006	0.538	131.46	82.1
	10.028	3.113	368.26	69.0
	10.028	3.117	368.05	68.9
LUFA 3A	0.100	0.0376	3.18	62.4

	0.100	0.0347	3.33	65.3
	0.301	0.1475	7.82	51.0
	0.301	0.1493	7.73	50.4
	1.000	0.5974	20.51	40.2
	1.000	0.6050	20.12	39.5
	3.009	2.070	47.87	31.2
	3.009	2.085	47.10	30.7
	10.036	7.718	118.19	23.1
	10.036	7.760	116.05	22.7

nd: not detected

* < LOQ

At the end of the desorption phase, 24.33-49.04 %, 4.1-18.7 % and 19.55-48.05 % of the initially adsorbed amount was desorbed in soils LUFA 2.1, LUFA 2.2 and LUFA 3A, respectively.

Table 8.1.2.2-22: [^{15}N]AMPA: Percentage of adsorbed and desorbed in soils (mean values)

Soil	Test Concentration [mg/L]									
	Adsorption [1]					Desorption [2]				
	10.0	3.0	1.0	0.30	0.10	10.0	3.0	1.0	0.30	0.10
Lufa 2.1	25.4	34.8	47.1	52.0	70.0	49.04	45.08	37.13	31.92	24.33
Lufa 2.2	69.0	82.4	90.6	96.8	n.d.	18.7	13.6	8.25	4.1	n.d.
Lufa 3A	22.9	31.0	39.9	50.7	63.9	48.05	42.85	39.25	31.1	19.55

[1] end of adsorption phase, mean values expressed as percentage of applied radioactivity

[2] end of desorption phase, mean values expressed as percentage of applied radioactivity

n.d.: not detected

The adsorption constants KF(ads) of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 16.746 to 189.714 mL/g (arithmetic mean: 78.52 mL/g). The Freundlich exponents 1/n were in the range of 0.5506 to 0.6710 (arithmetic mean: 0.629) indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. The corresponding, calculated $K_{F, OC}$(ads) values varied between 1119 and 8248 mL/g (arithmetic mean: 3743 mL/g).

The desorption coefficients KF(des) of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 21.38 to 49.48 mL/g. The Freundlich exponents 1/n were in the range of 0.9729 to 0.9894. The corresponding, calculated $K_{F, OC}$(des) values varied between 1607 and 2376 mL/g.

Table 8.1.2.2-23: [^{15}N]AMPA: Freundlich adsorption/desorption parameters in soil at 22 °C

Soil	Adsorption				Desorption			
	K_F [mL/g]	1/n	R^2	$K_{F, OC}$ [mL/g]	K_F [mL/g]	1/n	R^2	$K_{F, OC}$ [mL/g]
Lufa 2.1	16.746	0.6650	0.9953	1861	21.38	0.9747	0.9999	2376
Lufa 2.2	189.714	0.5506	0.9983	8248	49.48	0.9894	1.0000	2151
Lufa 3A	29.087	0.6710	0.9995	1119	41.78	0.9729	0.9995	1607

III. CONCLUSIONS

The adsorption coefficients KF(ads) of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 16.746 to 189.714 mL/g. The corresponding, calculated $K_{F, OC}$(ads) values varied between 1119 and 8248 mL/g.

Assessment and conclusion by applicant:

The study is considered valid for soils Lufa 2.1 and 2.2. Although relatively low 1/n values were obtained the results are considered acceptable since all relevant quality checks confirmed the reliability of the results, and the study was performed using a validated analytical method.

Results for soil Lufa 3A are considered as supportive due to a high K_{FE}/K_F of 1.6 indicating potential systemic errors resulting from loss of test item. Therefore, Freundlich coefficients $K_{F(ads)}$ of soil Lufa 3A should be

excluded from risk assessment. However, in general it could be possible to derive single concentration K_D values from the parental mass balance test for soil Lufa 3A.

All relevant quality checks as part of confirming the acceptability of the study and of the reported endpoints were performed. These checks confirmed that overall mass balance of 91.1-95.8 %, and % adsorption of 22.8-97.1 % were all acceptable for all soils. Systematic errors estimated via KFE/KF were shown to be low (i.e. ≤1.2) for soils 2.1 and 2.2. For soil 3A systemic errors were shown to be high with K_{FE}/K_F of 1.6. The validity of the analytical method was confirmed over the entire range of concentrations measured (LOQ at least two orders of magnitude lower than lowest test concentration). In general, the use of the indirect method was appropriate based on a K_D x soil/solution ratio >0.3 in all soils. The graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots. The R2 of the standard linear regressions ranged from 0.994 to 0.999 and the visual fit of the standard regression were acceptable.

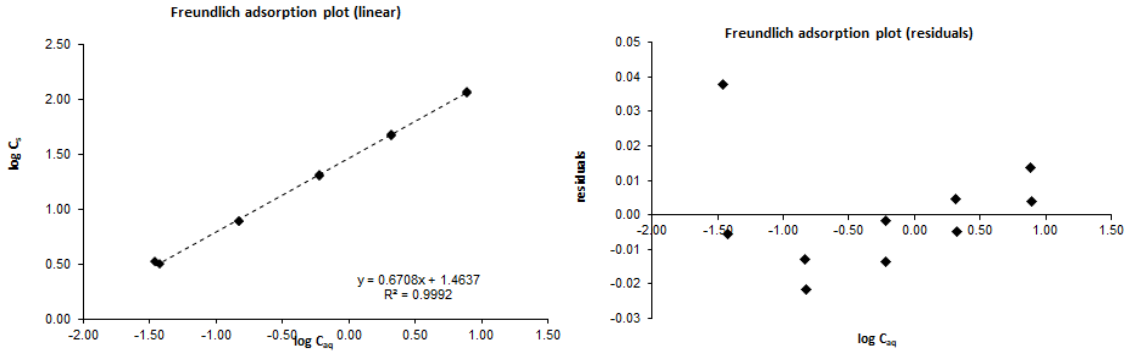
AMPA: Evaluation of result according to EU OECD 106 Evaluators Checklist

	Units	3A	2.1	2.2
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:50	1:25	1:50
Parental mass balance (at highest conc.)	%	91.1	95.8 [1]	92.3
Adsorbed percentage	%	22.7-65.4	25.1-70.8	69.0-97.1
K_D x (soil:solution ratio)		0.3-1.9	0.3-2.4	2.4-36.2
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	29.086 (28.191-30.010)	16.744 (15.380-18.229)	189.555 (175.875-204.299)
$^{ads}1/n$ (95 % confidence interval)	-	0.671 (0.655-0.687)	0.664 (0.623-0.706)	0.550 (0.522-0.578)
$^{ads}R^2$	-	0.999	0.994	0.997
$^{ads}K_{F,OC}$	L/kg OC	1119	1861	8242
K_{FE} / K_F	-	1.6	1.2	1.1

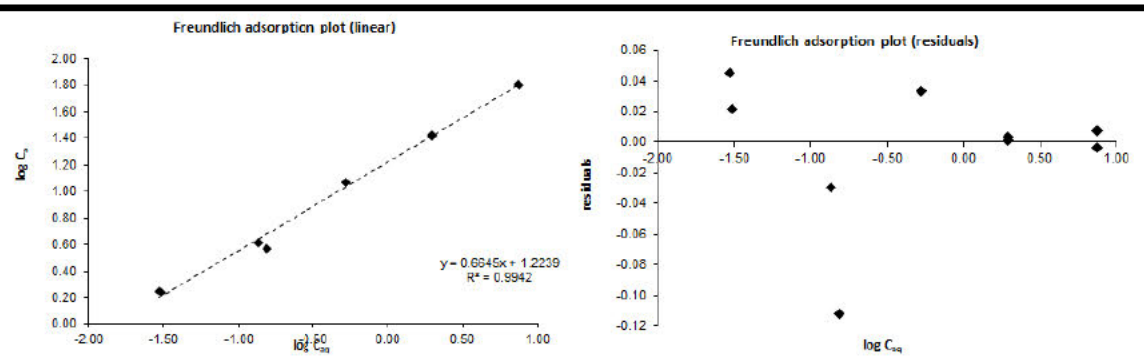
Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

[1] Parental mass balance established at a soil:solution ratio of 1:50.

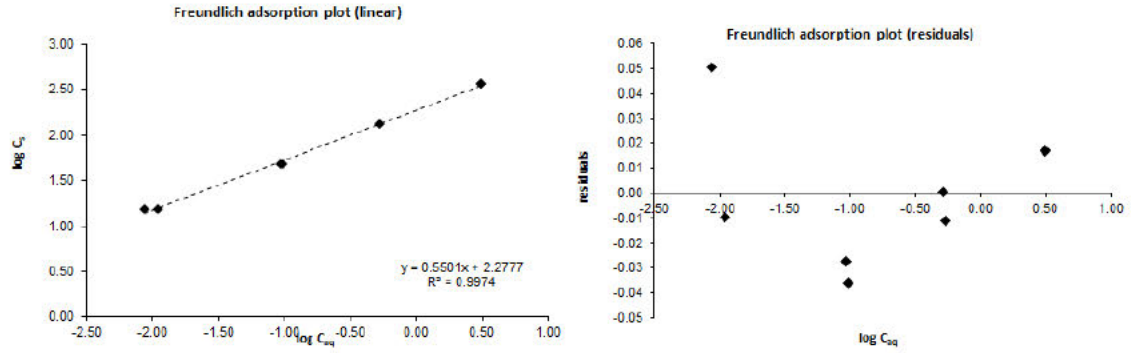
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 3A



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 2.1



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 2.2



Assessment and conclusion by RMS:

According to section B5, the analytical method used in this study is not considered acceptable for analysis of AMPA in the aqueous phase. As a consequence, results cannot be validated and the study is not acceptable.

The following additional deviations were also identified.

RMS notes that the preliminary tests to determine the optimal soil to solution ratio and equilibrium time was performed on one soil only (LUFA 2.2).

Despite the indications from the preliminary test performed on LUFA 2.2, the percentage of adsorption in the definitive test for this soil is very high at some concentrations (100% at 0.1 µg/mL, 96.8% at 0.301 µg/mL and 90.6% at 1.004 µg/mL), indicating that the soil/solution ratio may not be optimal. The OECD 106 guidance recommends that "the percentage adsorbed is above 20%, and preferably >50%, while care should be taken to keep the test substance concentration in the aqueous phase high enough to be measured accurately. This is particularly important in the case of high adsorption percentages". The concentration in the supernatants for soil LUFA 2.2 is below the LOQ for 2 concentrations (not detected at 0.1 µg/mL and 0.0086-0.0109 µg/mL at 0.301 µg/mL).

For other soils, contrary to the applicant statement, RMS notes that the LOQ of the analytical method is not 2 orders of magnitude below the lowest nominal concentration tested.

The study is not acceptable.

[redacted], *1996*

Data point: CA 7.1.3.1.2/004

Report author [redacted]

Report year	1996
Report title	Glyphosate acid: adsorption and desorption properties of the major metabolite, AMPA, in soil
Report No	RJ2129B
Guidelines followed in study	OECD Guideline 106 U.S. EPA Series 163-1, Leaching and Adsorption/Desorption Studies
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Preliminary test for determination of equilibration time performed for soil Visalia only - No preliminary test for determination of soil-to-solution ratio and adsorption to test vessel - Soil to solution ratio not optimal for 4 soils (very high adsorption) - Test concentrations do not cover 2 orders of magnitude - Detailed parental mass balance not available
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C]Aminomethylphosphonic acid

Lot No.	Not provided
Specific activity	1.828 GBq/mmol
Radiochemical purity	97 %

2. Test Soils

The soils were air-dried and sieved to a particle size of ≤2 mm. The soils were gamma irradiated with between 25 and 40 kGy to eliminate any living organisms within the soil. The characterisation of test soils used is summarised in the table below.

Table 8.1.2.2-24: Physico-chemical properties of test soils

Parameter	Results				
Soil Designation	Lillyfield	Visalia	Wisborough Green	Champaign	18 Acres
Geographic Location					
City	Churt	Visalia	Wisborough Green	Champaign	Bracknell
State	Surrey	California	Sussex	Illinois	Berkshire
Country	England	USA	England	USA	England
Textural Class (USDA)	Sand	Sandy loam	Silty clay loam	Silty clay loam	Sandy loam
Sand (50 µm – 2 mm)	92 %	69 %	8 %	12 %	58 %
Silt (2 µm – 50 µm)	4 %	18 %	60 %	52 %	23 %
Clay (< 2 µm)	4 %	13 %	32 %	36 %	19 %
pH (in 1:2 soil:water suspension)	5.7 %	8.4 %	5.7 %	6.2 %	7.4 %
Organic Matter	0.5 %	1.0 %	3.9 %	3.7 %	3.1 %
Organic Carbon 1	0.29 %	0.58 %	2.27 %	2.15 %	1.80 %
Cation Exchange Capacity (meq/100 g)	1.8	7.3	11.9	28.3	14.4
Water Holding Capacity					
at 1/3 bar (%)	3.11	10.4	30.9	22.7	17.1
at 15 bar (%)	1.11	4.80	19.8	13.5	10.4

1 Calculated as : OC [%] = OM [%] / 1.72
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes (50 mL) were used as test systems. The experiments were performed in duplicate.

In a preliminary test, the appropriate adsorption equilibration time was determined for soil Visalia, only. The stability of AMPA (parental mass balance) was investigated in the course of the definitive test.

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M CaCl2 solution with a soil-to-solution ratio of 1:10 (2.0 g soil (dry weight equivalents) / 20 mL solution). Test item was applied at nominal concentrations of 2.0, 1.0, 0.2, 0.1 and 0.05 mg/L in aqueous 0.01 M CaCl2 solution. The adsorption step was carried out for 21 hours at 20 ± 2 °C under continuous agitation.

In the desorption phase, pre-adsorbed soil prepared separately for the desorption phase was supplied with fresh aqueous 0.01 M CaCl2 solution. The resultant samples were re-equilibrated for 21 hours at 20 ± 2 °C under continuous agitation.

2. Analytical Procedures

After each adsorption and desorption step, the aqueous supernatant was separated from the soil by centrifugation and radioactivity in the supernatants was determined by liquid scintillation counting (LSC). Aqueous supernatants were analysed by thin layer chromatography (TLC).

Following the adsorption and desorption phase soils were extracted twice by shaking at ambient temperature using ammonium phosphate buffer. Soil extracts were analysed by TLC-radiodetection. The extracted soils were dried and radioactivity was determined by combustion and LSC.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation, using the indirect method.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances ranged from 101 to 105 % AR for soil Lillyfield, from 99 to 106 % AR for Visalia soil, from 99 to 104 % AR for Wisborough Green soil, from 95 to 104 % AR for Champaign soil, and from 97 to 102 % AR for 18 Acres soil.

B. STABILITY OF TEST ITEM

Analysis of aqueous supernatants and soil extracts showed that more than 90 % of the applied radioactivity (% AR) could be assigned to AMPA. Mean amounts of non-extractable residues (NER) were 3.8, 7.7, 11.3, 7.6 and 4.6 % AR for soils Lillyfield, Visalia, Wisborough Green, Champaign and 18 Acres, respectively.

C. FINDINGS

At the end of the adsorption phase, an average of 96.1 % of the applied test material were adsorbed to soil Lillyfield, 61.9 % to soil Visalia, 98.8 % to soil Wisborough Green, 98.0 % to soil Champaign and 93.0 % to soil 18 Acres. The adsorption coefficients KF(ads) of AMPA calculated based on the Freundlich isotherms of the five test soils ranged from 9.97 to 509 mL/g. The Freundlich exponents 1/n were in the range of 0.78 to 0.91, demonstrating a small decrease in adsorption with increasing rate of application, however, there was not saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC}$(ads) values varied between 1720 and 45900.

During the single desorption step, calculated $K_{F, OC}$(des) values varied between 2080 and 71500 indicating that the adsorption of AMPA is not very reversible. At the end of the desorption phase, 2-3 % of the initially adsorbed amount was desorbed in soil Lillyfield, 17-36 % in soil Visalia, 0-1 % in soil Wisborough Green, 1-2 % in soil Champaign and 2-6 % in soil 18 Acres.

Table 8.1.2.2-25: [^{14}C]AMPA: Percentage adsorbed and desorbed in soil (mean values)

	Test Concentration [mg/L]	
	Adsorption [1]	Desorption [2]

Soil	0.05	0.1	0.2	1.0	2.0	0.05	0.1	0.2	1.0	2.0
Lillyfield	97.2	96.5	96.5	95.7	94.6	2.0	2.0	2.0	3.0	3.0
Visalia	70.2	68.8	67.5	53.2	49.7	20.0	17.0	20.0	29.0	36.0
Wisborough Green	99.0	98.9	99.0	98.7	98.6	0.0	1.0	0.0	1.0	1.0
Champaign	98.4	98.3	98.3	97.9	96.9	1.0	1.0	1.0	1.0	2.0
18 Acres	94.5	94.3	94.3	92.3	89.7	2.0	4.0	4.0	4.0	6.0

[1] End of adsorption phase, mean values expressed as percentage of applied radioactivity
[2] End of first desorption phase, mean values expressed as percentage of the initially adsorbed amount

Table 8.1.2.2-26: [^{14}C]AMPA: Adsorption / desorption parameters in soil at 20 °C

Soil	Adsorption				Desorption
	$K_{F(ads)}$ [mL/g]	1/n	R^2	$K_{F, OC(ads)}$ [mL/g]	$K_{F, OC(des)}$ [mL/g]
Lillyfield	133	0.86	1.00	45900	71500
Visalia	9.97	0.78	1.00	1720	2080
Wisborough Green	509	0.91	1.00	22500	29600
Champaign	237	0.86	1.00	11100	15000
18 Acres	74.2	0.84	1.00	4130	5130

III. CONCLUSIONS

The adsorption coefficients KF(ads) of AMPA calculated based on the Freundlich isotherms of the five test soils ranged from 9.97 to 509 mL/g. The corresponding, calculated $K_{F, OC}$(ads) values varied between 1720 and 45900 mL/g. During the single desorption step, calculated $K_{F, OC}$(des) values varied between 2080 and 71500 mL/g indicating that the adsorption of AMPA is not very reversible.

Assessment and conclusion by applicant:

On the basis of information provided in the report the results of the study are considered as supportive. The results of the parental mass balance test were not presented in detail while it is stated that >90% of applied radioactivity was recovered as AMPA in aqueous supernatant and soil extracts for all soils. However, the raw data of the study possibly could provide more detailed information on the results of the parental mass balance in order to evaluate the results according to OECD Guideline 106 and its respective EU Evaluators Checklist.

The evaluation according to EFSA OECD 106 Evaluators Checklist using all available data are provided in the table and figures below for information.

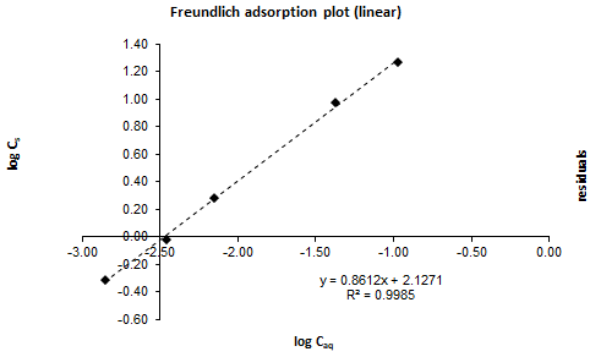
Table 8.1.2.2-27: Metabolite AMPA: Results of evaluation according to EU OECD 106 Evaluators Checklist

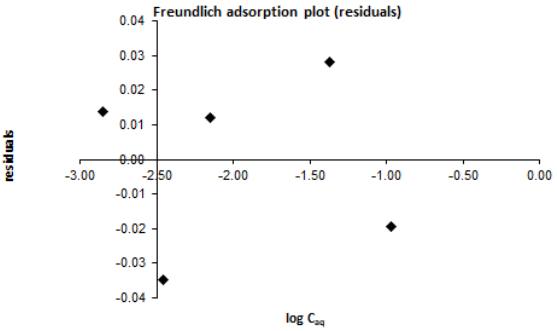
	Units	Lillyfield	Visalia	Wisborough Green	Champaign	18 Acres
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:10	1:10	1:10	1:10	1:10
Parental mass balance (at 2nd highest conc.)	%	- [1]	- [1]	<90 [2]	- [1]	- [1]
Adsorbed percentage	%	94.7-97.2	50.2-70.6	98.6-99.0	96.9-98.4	89.8-94.6
K_D x (soil:solution ratio)		17.5-34.4	1.0-2.4	70.7-98.5	31.3-60.9	8.7-17.3
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	134.004 (99.736-180.047)	9.974 (8.005-12.428)	531.319 (363.191-777.277)	240.014 (131.592-437.767)	74.216 (52.072-105.778)
$^{ads}1/n$ (95 % confidence interval)	-	0.861 (0.800-0.923)	0.776 (0.696-0.855)	0.917 (0.852-0.981)	0.860 (0.749-0.971)	0.844 (0.761-0.927)
$^{ads}R^2$	-	0.998	0.997	0.999	0.995	0.997
$^{ads}K_{F,OC}$	L/kg OC	44668	1662	23101	11429	4123
K_{FE} / K_F [3]	-	-	-	-	-	-

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Results of parental mass balance test not reported.
[2] Formation of NER >10 %.

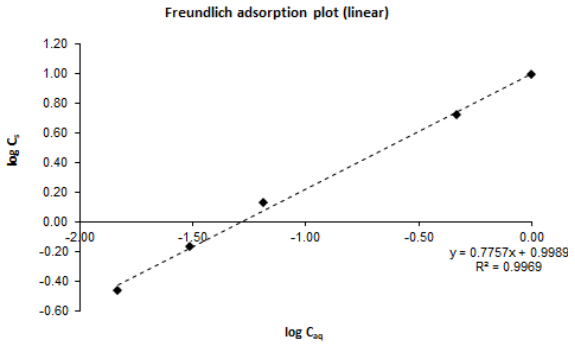
[3] Cannot be calculated since the f-factor cannot be specified due to missing data of the parental mass balance test.

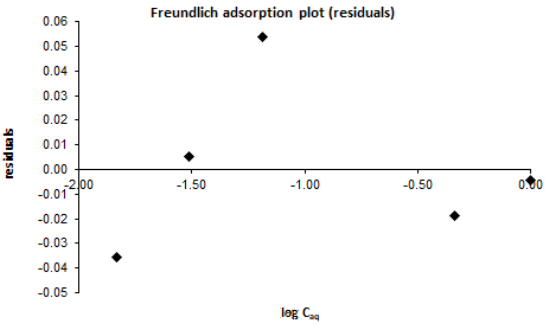
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Lillyfield



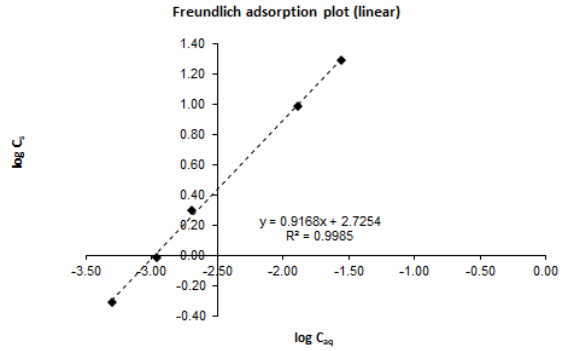


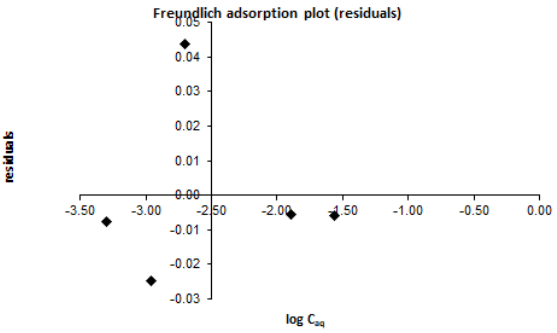
Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Visalia



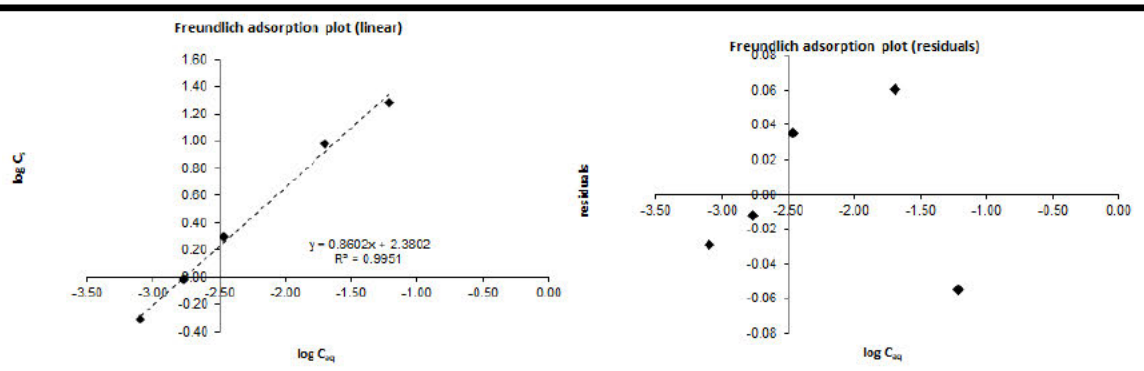


Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Wisborough Green

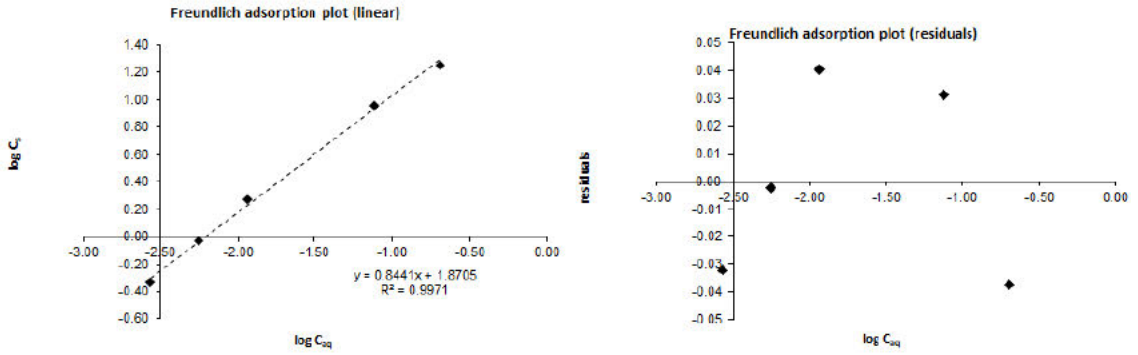




Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil Champaign



Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression) and the corresponding plot of the residuals for soil 18 Acres



Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable.

Several deviations from OECD 106 are identified. Preliminary test for determination of equilibrium time was performed on 1 soil only, no preliminary test to determine optimal soil to solution ratio and to check adsorption to test vessel was done, test concentrations do not cover 2 orders of magnitude, selected soil to solution ratio is not optimal (very high adsorption measured in all soils except Visalia) and detailed parental mass balance is not available.

It is indicated in the study that test item stability was checked, however detailed results are not available and RMS cannot confirm that test item is stable.

The study is not considered acceptable.

[redacted], *1993*

Data point:	CA 7.1.3.1.2/005
Report author	[redacted]
Report year	1993
Report title	Adsorption of aminomethylphosphonic acid to soil particles in six soil types
Report No	IMW-R93/056
Guidelines followed in study	Dutch Guideline For Registration Pesticides, Part G1.2
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-equilibration of soil samples - No preliminary tests on soil:solution ratio, equilibration time or stability of test item - Aqueous $CaSO_4$ solution used instead of $CaCl_2$ - Only 4 concentrations tested, not covering 2 orders of magnitude

	- LOQ of 0.5 µg/mL is not 2 orders of magnitude below the lowest nominal concentration - Total recovery and parental mass balance were not established - For soils A, B and C, concentrations in supernatants are below LOQ for 2 or 3 tested concentrations
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentioned in RAR (2015), but not accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Aminomethylphosphonic acid (non-labelled)
Lot No. 108 F 3811
Chemical Purity 99 %

2. Test Soils

The soils were sieved to a particle size of ≤2 mm and stored at 3 ± 2 °C prior to use. The characterisation of test soils used is summarised in the table below.

Table 8.1.2.2-28: Physico-chemical properties of test soils

Parameter		Results					
Soil Designation		Sandy loam	Sand Low humic-content (lhc)	Gray brown podzol	Sandy (A)	Sandy (B)	Sandy (C)
Geographic Location							
City		Heerewaarden	Lisse	Caen	Zeist	Zeist	Maarn
Country		Netherlands	Netherlands	France	Netherlands	Netherlands	Netherlands
Textural Class (USDA)							
Sand	(50 µm – 2 mm)	64.1	97.0	6.7	89.2	91.4	88.9
Silt	(2 µm – 50 µm)	23.1	0.5	74.0	7.0	4.8	7.0
Clay	(<2 µm)	12.8	2.5	19.3	3.8	3.8	4.1
pH							
- in KCl 1		7.5	7.2	6.5	4.3	4.4	4.3
- in KCl 2		7.8	8.0	6.4	4.4	4.7	4.4
Organic Carbon 3		1.22 %	0.58 %	1.51 %	3.26 %	2.09 %	2.44 %
Organic Matter		2.1 %	1.0 %	2.6 %	5.6 %	3.6 %	4.2 %
CaCO3 (%)		8.0	1.5	0.2	0.1	0.1	0.1

1 pH values measured at Bedrijfslaboratorium voor Grond- en Gewasonderzoek
2 pH values measured at IMW-TNO
3 Calculated as OM / 1.72

B. STUDY DESIGN

1. Experimental Conditions

Scintillation vials (20 mL volume) with screw caps were used as test systems. The experiments were performed in duplicate. A stock solution of 0.2517 g test substance in 500 mL of 0.01 M CaSO4 solution was prepared and used for equilibration. The vials were shaken for 20 h at 20 ± 2 °C in a temperature controlled room.

The adsorption step was carried out at a soil to solution ratio of 1:10 for 20 hours by shaking non-pre-equilibrated samples of air-dried soils with a 0.01 M aqueous calcium sulfate solution of AMPA. Nominal concentrations of AMPA were 50.0, 20.0, 10.0 and 5.0 mg/L.

2. Analytical Procedures

The aqueous supernatant after adsorption was separated by centrifugation. AMPA residues in the supernatant were analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 254 and 313 nm.

The limit of detection (LOD) and limit of quantitation (LOQ) for HPLC analysis were 0.l µg/mL (0.1 mg/L) and 0.5 µg/mL (0.5 mg/L), respectively.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation, using the indirect method.

II. RESULTS AND DISCUSSION

The stability of the test item during the adsorption phase was not investigated. No preliminary tests to establish the appropriate soil:solution ratio, equilibration time, and sorption of test item to test vessel surface were performed.

Freundlich adsorption coefficients for aminomethylphosphonic acid ranged from 5.8 to 351 mL/g for the six tested soils. 1/n values were in the range of 0.44 to 0.60. The corresponding, calculated KF, OM(ads) values varied between 586 and 7979 mL/g. A summary of the results of the adsorption isotherms tests is presented in the table below.

Table 8.1.2.2-29: AMPA: Distribution between solution and soil (mean values)

Soil	Fraction	Test concentration [mg/L]			
		5	10	20	50
Sandy loam	Solution (µg/mL)	1.65	3.49	8.91	33.51
	Adsorbed (µg/g)	34.60	79.10	112.15	151.55
Sand lhc	Solution (µg/mL)	4.19	8.03	16.59	44.08
	Adsorbed (µg/g)	9.15	33.65	35.40	45.90
Gray brown podzol	Solution (µg/mL)	0.55	1.35	3.74	15.96
	Adsorbed (µg/g)	45.60	100.45	163.85	327.10
Sandy (A)	Solution (µg/mL)	<0.1	<0.1	0.30	1.83
	Adsorbed (µg/g)	51.05 [1]	113.95 [1]	198.25	468.40
Sandy (B)	Solution (µg/mL)	<0.1	<0.1	0.49	2.44
	Adsorbed (µg/g)	51.05 [1]	113.95 [1]	196.35	462.25
Sandy (C)	Solution (µg/mL)	<0.1	0.15	0.74	3.58
	Adsorbed (µg/g)	51.05 [1]	112.45	193.90	450.85

[1] Assumed to be completely adsorbed to the soil.

Table 8.1.2.2-30: AMPA: Adsorption parameters in different soils at 20 °C

Soil	Adsorption			
	K_F [mL/g]	1/n	r	$K_{F, OM}$ [mL/g]
Sandy loam	35	0.46	0.93	1678
lhc sand	5.8	0.60	0.83	586
Gray brown podzol	73	0.57	0.99	2812
Sandy (A)	351	0.48	1.0 [1]	6275
Sandy (B)	287	0.53	1.0 [1]	7979
Sandy (C)	245	0.44	0.99 [2]	5835

[1] Two data points

[2] Three data points

III. CONCLUSION

The adsorption coefficients KF(ads) of AMPA acid for the tested soils calculated based on the Freundlich isotherms ranged from 5.8 to 351 mg/L. The respective KF, OM(ads) values ranged from 586 to 7979 mg/L.

Assessment and conclusion by applicant:

The study was considered as not valid during review for AIR2 by the RMS.

There were multiple deviations from OECD Guideline 106 including the use of calcium sulfate solution instead of calcium chloride as aqueous phase. In addition, soil samples were not pre-equilibrated. No preliminary tests

were performed to establish the appropriate soil:solution ratio, equilibration time, and the stability of the test item. Finally, limits of detection (LOD, 0.1 mg/L) and quantitation (LOQ, 0.5 mg/L) do not fulfil the criterion of 1% as set by the guideline.

The study and its results were not considered for environmental risk assessment.

Assessment and conclusion by RMS:

The study summary above was not completed by RMS since the study is not considered acceptable. In addition, the study report is very brief, with no sufficient details to check the design and the quality of the study.

Based on the few information available in the report, several deviations from OECD 106 are identified. Soil samples were not pre-equilibrated, no preliminary tests were performed, aqueous CaSO4 solution was used instead of CaCl2 solution, only 4 concentrations not covering 2 orders of magnitude were used, LOQ is not 2 orders of magnitude below the lowest nominal concentration, concentrations in supernatant are below the LOQ for soils A, B and C for 2 or 3 tested concentrations, total recovery and parental mass balance were not established. The stability of the test item was not checked.

This study is not considered acceptable.

B.8.1.2.2.2. Relevant articles from literature search

Within the actual review of scientific literature for glyphosate (2010-2020), 2 articles were identified in total to potentially provide relevant information to the data point. They are already summarized under the literature search on adsorption for glyphosate presented above.

Table 8.1.2.2-31: Adsorption/desorption – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.3.1.2/007	Skeff *et al.*, 2018	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions	Summary under B.8.1.2.1
CA 7.1.3.1.2/008	Sidoli *et al.*, 2016	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions	Summary under B.8.1.2.1

B.8.1.2.3. *Summary on adsorption desorption*

Glyphosate

The adsorption of glyphosate was investigated in 11 batch adsorption studies. Reliable results were obtained on 10 soils. The calculated adsorption coefficients normalised to organic carbon content, $K_{F,OC(ads)}$, range from 1031 to 9615 mL/g (geometric mean: 4348 mL/g). The Freundlich exponents expressed as 1/n are in the range of 0.546 to 0.777 (arithmetic mean: 0.682). Glyphosate is considered as low mobile to immobile in soil according to McCall classification. Adsorption of glyphosate was found to be not dependent on pH of soil (see below).

Table 8.1.2.3-1: Soil adsorption glyphosate (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.3.1.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.2.1)

Soil Type	OC (%)	pH ($CaCl_2$)	pH (H_2O)	K_D (mL/g)	$K_{D, OC}$ (mL/g)	K_F (mL/g)	$K_{F, OC}$ (mL/g)	1/n
Speyer 2.2, sandy loam	1.71	5.6	5.21	-	-	59.44	3476	0.546
RefeSol 01-A, loamy sand	0.8	5.33	6.11	-	-	59.80	7476	0.704
18 Acres, sandy clay loam	1.9	6.2	6.11	-	-	166.4	8755	0.579
M-SL-PF (Mutchler, US), sandy clay loam	1.9	6.1	6.44	-	-	152.4	8024	0.546
Speyer 2.3, sandy loam	0.67	5.9	7.02	-	-	52.9	7892	0.751

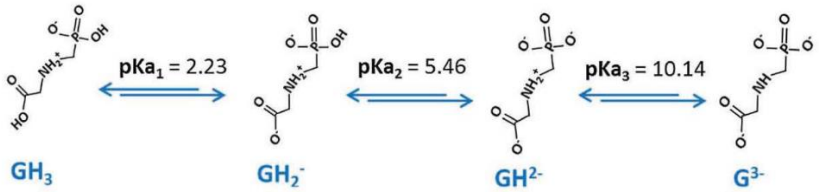
Soil Type	OC (%)	pH ($CaCl_2$)	pH (H_2O)	K_D (mL/g)	$K_{D, OC}$ (mL/g)	K_F (mL/g)	$K_{F, OC}$ (mL/g)	1/n
RefeSol 02-A, silt loam	0.92	6.19	6.98	-	-	88.46	9615	0.658
Gartenacker, loam	2.1	7.1	7.16	-	-	21.6	1031	0.757
Speyer 6S, clay	1.78	7.2	7.32	-	-	70.52	3962	0.736
Speyer 5M, sandy loam	0.92	7.4	7.56	-	-	18.9	2049	0.770
LAD-SL-PF (Pavillion, US), sandy loam	0.87	8.1	8.11	-	-	18.1	2082	0.777
Geometric mean (if not pH dependent) (n = 10)						54.23	4348	-
Arithmetic mean (if not pH dependent) (n = 10)						-	-	0.682
pH dependence							No	

Assessment of pH dependency of adsorption parameters of glyphosate

The adsorption of glyphosate was investigated in several literature studies. Although results from these studies cannot be used to derive reliable endpoints for risk assessment, the studies bring supportive information regarding the adsorption of glyphosate.

Glyphosate comprises of one basic amino function and three ionizable acidic sites. It has a number of pKa (2.23, 5.46 and 10.14 according to Dollinger *et al.* 2015; values consistent with validated pKa of 2.34 and 5.73 reported for glyphosate acid in the LoEP Phys-Chem section) and therefore exists as multiple species depending on pH, as presented below (Dollinger *et al.* 2015). At typical soil pH 5-9, the main species are GH_2^- and GH^{2-}, corresponding to net negative charges of one and two, respectively.

Speciation of glyphosate through the entire soil pH range (Dollinger *et al* (2015))



Based on its structure, some pH-dependency of adsorption could be expected.

Results from the literature review seem to indicate a pH dependency between Kf and pH_{CaCl2}, but no dependency between Kd or Kf and pH_{KCl} or pH_{H2O}. It is however indicated that various other soil parameters (*e.g.* CEC, clay content, phosphate amount, amorphous iron and aluminium oxide contents) also impact the adsorption of glyphosate. Correlation between Kfoc and pH was not investigated in the available literature review.

The pH dependency of the adsorption parameters $K_{F(ads)}$ and $K_{F, OC(ads)}$ of glyphosate from the applicant's studies was assessed by the applicant using the German Input Decision Tool 3.3 (Holdt, G. *et al.* (2012)), based on pH_{H20}. Additional test was performed by RMS based on pH_{CaCl2}, due to the observations from studies of the literature review.

Table 8.1.2.3-2: Glyphosate: Results from Kendall test for $K_{F(ads)}$ and $K_{F, OC(ads)}$ values and pH values

Compound	Parameter	pH_{H20}		pHC_{CaCl2}	
		Kendall tau (stringency of the correlation)	**p (level of significance)**	**Kendall tau (stringency of the correlation)**	**p (level of significance)**
Glyphosate	$K_{F(ads)}$	-0.539	0.039	-0.333	0.210
	$K_{F, OC(ads)}$	-0.315	0.243	-0.200	0.474

Based on Kendall test, there is a significant correlation between the pH_{H20}-value and the adsorption coefficient $K_{F(ads)}$, but no correlation between pH_{CaCl2}-value and $K_{F(ads)}$. There is no significant correlation between the pH_{H20}-value or pH_{CaCl2}-value and the adsorption coefficient $K_{F, OC(ads)}$.

In addition, RMS provides below plots presenting Kf or Kfoc against pH.

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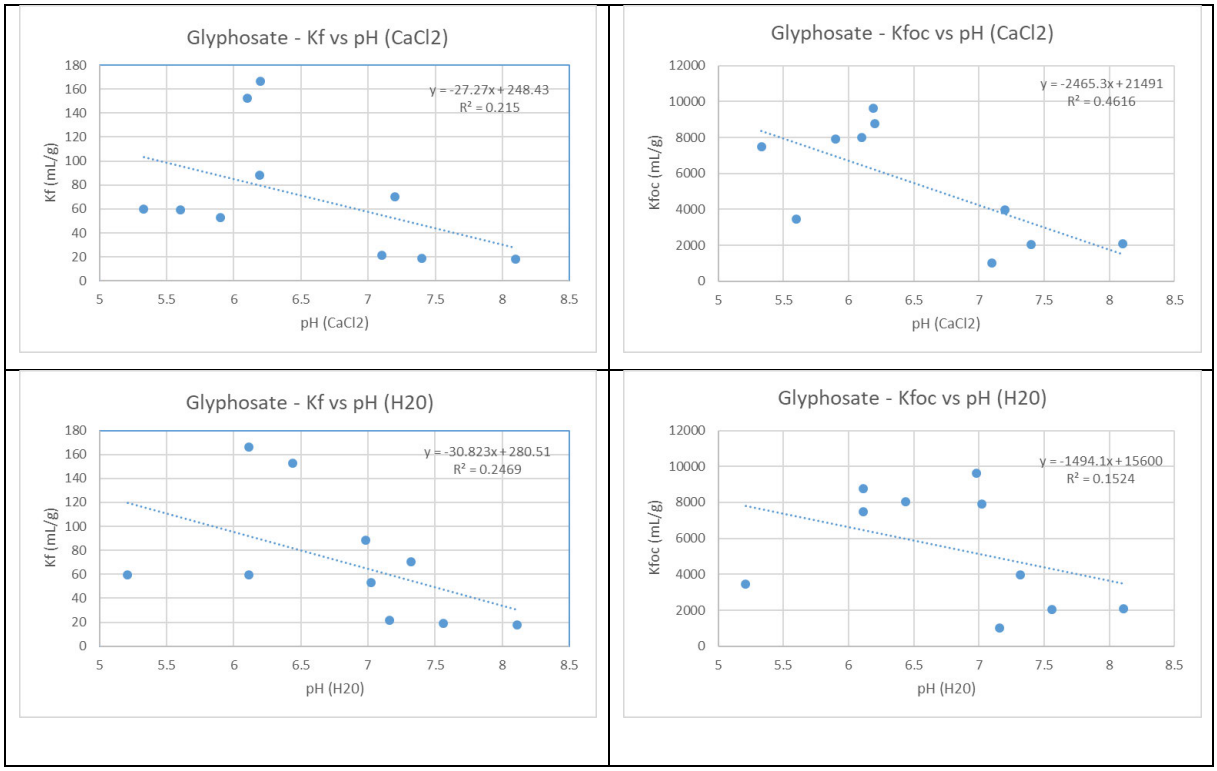


Figure 8.1.2.3-1: Glyphosate: Relation between $K_{F(ads)}$ values and pH (left) as well as $K_{F, OC(ads)}$ and pH (right)

Based on the available dataset, RMS considers that pH-dependency does not need to be taken into account for modelling.

AMPA

The adsorption of AMPA was investigated in 6 batch adsorption studies. Reliable results were obtained on 8 soils. The calculated adsorption coefficients $K_{F, OC(ads)}$ (normalised to organic carbon content) range from 1160 to 5650 mL/g (geometric mean: 2541 mL/g). The Freundlich exponents 1/n are in the range of 0.707 to 0.875 (arithmetic mean: 0.767). AMPA is considered as low mobile to immobile in soil according to McCall classification. Adsorption of AMPA was found to be not dependent on soil pH.

Table 8.1.2.3-3: Soil adsorption AMPA (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.3.1.2 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.2.1)

Soil Type	OC (%)	pH ($CaCl_2$)	pH (H_2O)	K_D (mL/g)	$K_{D, OC}$ (mL/g)	K_F (mL/g)	$K_{F, OC}$ (mL/g)	1/n

RefeSol 02-A Silt	1.18	6.60	7.25	-	-	38.9	3299	0.707
LUFA 2.2 Sandy loam	1.48	5.70	6.33	-	-	41.9	2833	0.752
LUFA 2.3 Sandy loam	0.61	6.20	7.01	-	-	28.7	4709	0.721
LUFA 6S Clay loam	2.07	7.30	7.89	-	-	36.6	1769	0.825
Bourgfelden Silt loam	1.15	7.50	8.41	-	-	23.3	2032	0.713
Wurmwiese Sandy loam	2.00	5.00	5.20	-	-	33.5	1675	0.875
SLI Soil #4, sand	1.34	6.9[1]	7.4	-	-	15.7	1160	0.752
SLI Soil #5, clay loam	0.93	7.1[1]	7.6	-	-	53.9	5650	0.791
Geometric mean (if not pH dependent) (n = 8)						29.8	2541	-
Arithmetic mean (if not pH dependent) (n = 8)						-	-	0.767
pH dependence							No	

[1] Calculated with equation reported in EFSA guidance 2017: pH_{H2O}=0.982pH_{CaCl2} + 0.648.

Assessment of pH dependency of adsorption parameters of AMPA

The pH dependency of the adsorption parameters $K_{F(ads)}$ and $K_{F, OC(ads)}$ of AMPA was re-assessed by the RMS using the German Input Decision Tool 3.3 (Holdt, G. *et al.* (2012)), based on the reliable adsorption endpoints. Similarily to glyphosate, a pKa for AMPA is available and presented in the literature section: AMPA pKa_3 = 5.4. Results from the literature review did not seem to indicate a pH dependency from Kf of AMPA.

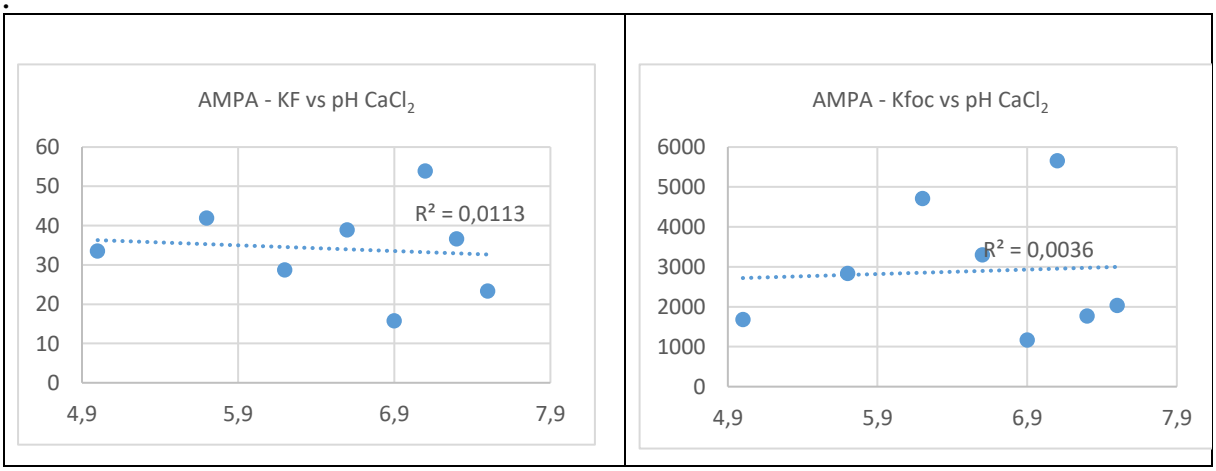
Table 8.1.2.3-4: AMPA: Results from Kendall test for $K_{F(ads)}$ and $K_{F, OC(ads)}$ values and pH values

Compound	Parameter	pH_{H20}		pHC_{CaCl2}	
		Kendall tau (stringency of the correlation)	p (level of significance)	Kendall tau (stringency of the correlation)	p (level of significance)
AMPA	$K_{F(ads)}$	-0.143	0.711	-0.143	0.711
	$K_{F, OC(ads)}$	0.071	0.902	0.071	0.902

Based on Kendall test, there is no significant correlation between the pH_{H20}-value or pH_{CaCl2}-value and the adsorption coefficients $K_{F(ads)}$ and $K_{F, OC(ads)}$.

In addition, RMS provides below plots presenting Kf or Kfoc against pH.

.



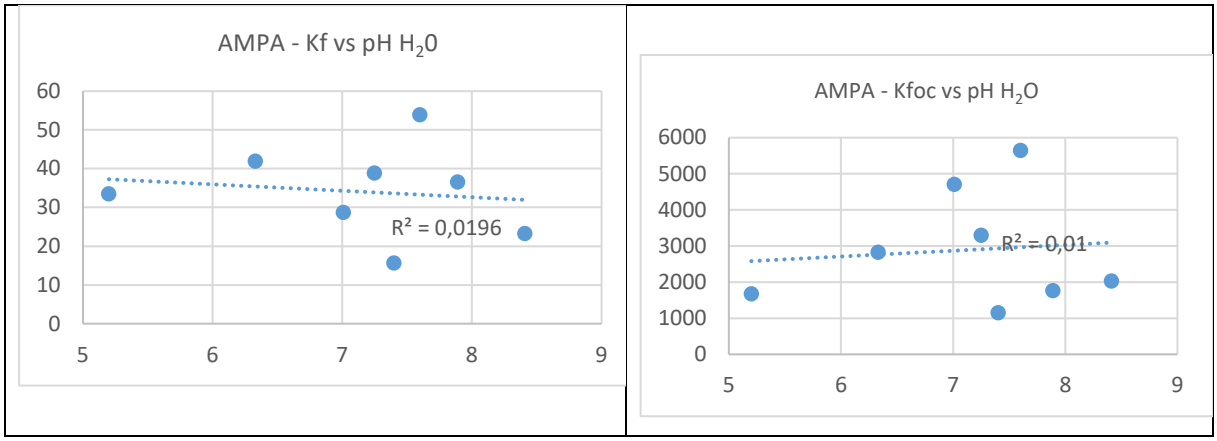


Figure 8.1.2.3-2: AMPA: Relation between $K_{F(ads)}$ values and pH (left) as well as $K_{F, OC(ads)}$ and pH (right)

RMS considers that the adsorption behaviour of AMPA is not pH-dependent.

B.8.1.2.4. *Aged sorption*

A study on aged sorption is not required and was not conducted.

B.8.1.3. Mobility in soil

B.8.1.3.1. *Column leaching studies*

Reliable adsorption coefficients of the active substance were obtained by adsorption/desorption studies and, consequently, column leaching studies are not strictly required. However, five column leaching studies and two aged column leaching studies with glyphosate or glyphosate-trimesium are available. For studies performed with glyphosate-trimesium only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment.

No study is available on AMPA and none is required since reliable adsorption coefficients are available.

Table 8.1.3.1-1: List of existing column leaching studies on glyphosate

Annex point	Study	Study type	**Previous evaluation in RAR (2015) / DAR (2001)**	**Status in RAR (2021)**
CA 7.1.4.1.1/001	[redacted], 1996	Aged column Leaching	Accepted in RAR (2015)	Acceptable
CA 7.1.4.1.1/002	[redacted], 1992	Column Leaching	Accepted in RAR (2015)	Supportive
CA 7.1.4.1.1/003	[redacted], 1992	Column Leaching	Accepted in RAR (2015)	Supportive
CA 7.1.4.1.1/004	[redacted], 1992	Aged column Leaching	Accepted in DAR (2001)	Supportive
CA 7.1.4.1.1/005	[redacted], 1991	Column Leaching	Accepted in RAR (2015)	Not acceptable
CA 7.1.4.1.1/006	[redacted], 1978	Column Leaching	Conisdered as additional information in RAR 2015	Not acceptable
CA 7.1.4.1.1/007	[redacted], 1972	Column Leaching	Not considered on RAR 2015, summarised in DAR 2001 but no conclusion on reliability	Not acceptable

In the scientific literature research for glyphosate (2010-2020), two articles were identified to provide further information relevant to the data point.

Table 8.1.3.1-2: Column leaching – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status

CA 7.1.4.1.1/008	Gjettermann et al., 2011	Column Leaching	Glyphosate	Reliable with restrictions
CA 7.1.4.1.1/009	Gjettermann et al., 2011	Column Leaching	Glyphosate	Reliable with restrictions

B.8.1.3.1.1. Column leaching of the active substance

[redacted], *1996*

Data point:	CA 7.1.4.1.1/001
Report author	[redacted]
Report year	1996
Report title	[14C]-Glyphosate: Determination of the mobility of aged residues in one soil
Report No	96-121-1020
Guidelines followed in study	SETAC procedures for assessing the environmental fate and ecotoxicity of pesticides, Annex of FAO revised guideline on environmental criteria for the registration of pesticides, BBA Guideline Part IV, 4-2
Deviations from current test guideline	From OECD 312 - LOD/LOQ not reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]glyphosate
Lot No.: Not indicated
Specific activity: 316 µCi/mg
Radiochemical purity: 99.6 %

2. Soil:

The tested soil was collected at a depth of 20 cm and did not receive any pesticides for 4 years. After receipt at Springborn Laboratories (Europe) AG, Horn, Switzerland on 23 May, 1995, the soil was placed outside in the Springborn holding area and kept in a wooden box seeded with Phacelia and irrigated if necessary to provide natural conditions. An amount of the test soil was collected from the Springborn soil holding area located outside of the facility on 15 October 1995 and sieved to 2 mm. The soil moisture content was determined and adjusted to the approximate incubation moisture. Thereafter, the soil was stored under test conditions in closed plastic boxes. During storage, the soil was moistened, if necessary and thoroughly mixed daily to provide aerobic conditions for the soil microflora.

Table 8.1.3.1-3: Soil physicochemical properties

Parameter	Results
Soil type	Sand
Common name	Speyer 2.1
Batch number	F 12095
Country	Germany
Sand (50 µm – 2 mm) (%)	88.4
Silt (2 µm – 50µmm) (%)	9.8
Clay (< 2 µm) (%)	1.9
pH (CaCl2)	5.9
Organic carbon (%)	0.62
Cation exchange capacity (meq/100 g)	5.0
Maximum Water Holding Capacity (%)	31
Bulk Density (disturbed) (g/cm3)	Assumed: 1.5
Microbial biomass (mg C/100g)	Study start: 46 Study end: 71

B. STUDY DESIGN

1. Experimental conditions

The application rate was calculated as 3.33 mg/kg dry soil corresponding to a field application rate of 2.5 kg/ha (based on a bulk density of 1.5 g/cm^3 and a depth of 5cm). The radiolabelled test compound was isotopically diluted with the analytical standard of the test compound, yielding a specific activity of the application solution of 80.1 µCi/mg.

Prior to application, the soil moisture was adjusted to the approximate target moisture of 45% MWHC. Thereafter, a 200 µL aliquot of the application solution containing 0.335 mg of the diluted 14C-test substance was added drop by drop to each 100 g (equivalent dry weight) soil sample by means of a Hamilton syringe.

The control soil samples were adjusted with deionised water to the target moisture of the respective soils. The aerobic incubation part of the study was carried out in all-glass metabolism flasks equipped with a trapping system, at 20°C and 45% MWHC. Ethylene glycol was used to trap organic volatiles, 0.5 M sodium hydroxide was used to trap 14CO2.The metabolism flasks were continuously ventilated with CO2 free and moistened air.

The aged leaching part of the study was conducted with 40 cm long all-glass column equipped with a porous glass-filter plate at the bottom. The inner diameter of the column was 4.8 cm. The water supply to the column was by means of a peristaltic pump. The leaching experiment was performed in duplicate. The columns were packed with untreated, pre-weighed, air-dried soil up to 28 cm. Thereafter, the soil columns were saturated with 0.01 M CaCl2 (approximately 236 mL). Aliquots of the dried treated soil were then packed on top of the untreated soil columns. Leaching was performed with a total of 380 mL of 0.01 M CaCl2 solution per column over 2 days. This corresponds to an irrigation rate of 200 mm per 48 hours.

2. Sampling

Samples were taken immediately after dosing and after 5 and 8 days of aerobic incubation. A total of 10 samples were incubated aerobically. Five samples were used to monitor the degradation of the test compound up to its DT50. Aliquots of 3 aged samples were used to confirm the DT50 and to conduct the aged leaching experiment. Aliquots from volatility traps for organic compounds and 14CO2 were collected on days 1, 2, 4, 5, 7 and 8 post-treatment. Trapped radioactivity was measured by LSC.

Leachates were collected after 24 and 48 h from soil columns. After the percolation period, soil columns were sacrificed and sectioned in 5 soil layers of 6 cm each.

3. Analytical procedures

Radioactivity in traps for volatiles and leachates were measured by LSC. Soils of segment 1were extracted exhaustively with total 125 mL of 0.35 M H3PO4/0.09 M CaCl2 per 50 g dry weight soil. Soils were extracted three times at room temperature using the soil to solvent ratio of approximately 1:2.5 (w:v). This procedure was done by shaking the samples on an overhead shaker. After centrifugation of each individual extract, the radioactivity in extracts was determined by LSC.

Non-extractable radioactivity of extracted wet or air dried soil was measured by post-extraction combustion followed by radio assay.

Extractable radioactivity of glyphosate and its radioactive degradation products was qualitatively and quantitatively analysed by HPLC without any further clean-up (direct injection, column: Nucleosil 5 SB 20 cm x 0.4 cm id; flow rate: 1 mL/min) with radiometric detection (RAM). One dimensional, radio-TLC (Thin-Layer Chromatography on silica gel 60 F 254, 0.25 mm Merck) plates with selected samples helped to tentatively characterise AMPA using solvent system consisting of 40 mL methanol, 20 mL water, and 3 mL of 25 % aqueous ammonia.

II. RESULTS AND DISCUSSION

A. DATA

The material balance and degradation product pattern of [14C]-glyphosate in soil Speyer 2.1 is presented in the table below. The values are presented in % of AR, at start of the ageing period and after 5 and 8 days of incubation.

Table 8.1.3.1-4: Material balance of [14C]glyphosate in soil Speyer 2.1 during 8 days of incubation

Radioactive residues (%)	Incubation time (days)					
	0	5	8 (mean)	8 (1) [a]	8 (2) [a]	8 (3) [a]
Volatiles						
Carbon dioxide	n.d.	12.0	19.5	19.7	19.3	19.6
Organic volatiles	n.d.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Glyphosate	92.1	63.9	50.7	55.9	51.9	51.2
AMPA	4.1	19.7	21.7	16.0	20.6	21.6
Total Extractables	96.3	83.6	72.7	71.9	72.5	72.8
Non-extractables	0.3	0.8	1.4	1.2	1.4	1.5
Recovery	96.6	96.5	93.3	92.8	93.3	93.9

[a] 8(1) and 8(2) stand for the aged samples of which aliquots were applied on top of untreated Speyer 2.1 columns A and B, respectively. 8(3) was applied on top of column C (reserve).
n.d. = not determined

The vertical distribution of aged soil residues of [14C]-glyphosate in Speyer 2.1 sand after percolation of 200 mm artificial rain and the radioactive residues in soil columns are presented in Table 8.1.3.1-5 and Table 8.1.3.1-6, respectively.

Table 8.1.3.1-5: Vertical distribution of aged soil residues of [14C]glyphosate in Speyer 2.1 soil (sand)

	Speyer 2.1 Column A		Speyer 2.1 Column B	
	(%)[1)]	(%)[2)]	(%)[1)]	(%)[2)]
Leachate Day 1	< 0.1	< 0.1	< 0.1	< 0.1
Leachate Day 2	< 0.1	< 0.1	< 0.1	< 0.1
Total leachate	< 0.1	< 0.1	< 0.1	< 0.1
CO2 Headspace	3.2	2.4	3.1	2.3
Organic volatiles headspace	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles headspace	3.2	2.4	3.1	2.3
Column segment 1 (top)	99.1	72.4	97.9	72.4
Column segment 2	2.0	1.5	3.3	2.5
Column segment 3	< 0.1	< 0.1	< 0.1	< 0.1
Column segment 4	< 0.1	< 0.1	< 0.1	< 0.1
Column segment 5 (bottom)	< 0.1	< 0.1	< 0.1	< 0.1
Total column segments	101.2	73.9	101.3	74.9
Recovery	104.4	76.3	104.4	77.2

1) Values were calculated in percent of radioactivity applied to each column.
2) Values were calculated in percent of radioactivity applied to each soil sample prior to aging and leaching

Table 8.1.3.1-6: Radioactive residues in the top segment of the duplicate soil columns

Radioactive residues (%)		Column A Segment 1 (Top)			Column B Segment 1 (Top)		
		(%)[1)]	(%)[2)]	(%)[3)]	(%)[1)]	(%)[2)]	(%)[3)]
Extractables	Glyphosate	72.5	71.8	52.5	71.0	69.6	44.7
	AMPA	25.2	24.9	18.2	24.7	24.2	24.7
	Total	97.6	96.7	70.7	95.7	93.7	69.3
Non-extractables		2.4	2.4	1.7	2.2	2.2	1.6
Recovery		100.0	99.1	72.4	97.9	95.9	70.9

1) Radioactive residues related to extractable and non-extractable radioactivity per sample.
2) Values were calculated in percent of radioactivity applied to each column.
3) Values were calculated in percent of radioactivity applied to each soil sample prior to aging and leaching.

B. MASS BALANCE

The overall recovery over the incubation period amounted to 94.4 % AR. Regarding the leaching experiment, the results demonstrated that 101.2 and 101.3 % of the applied radioactivity applied onto the duplicate soil columns was retained by the column. The majority (99.1 % and 97.9 %) of radioactivity was found in the 0 to 6 cm segment. Significantly less radioactivity was found in the 6 to

12 cm soil layer: 2.0 % and 3.3 % of total column A and B radioactivity, respectively. Leached radioactivity did not exceed 0.1% of the total column radioactivity. Head volatiles contributed 3.2 and 3.1 %. The total recovery for both columns A and B amounted to 104.4 %. This value corresponds to 76.3 % and 77.2 % of the radioactivity which had been applied to each individual metabolism flask.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

During aerobic incubation almost complete extraction of radioactivity was observed, since 96.6 % of the applied radioactivity was found in the extracts and 0.3 % in the extracted soil. Thereafter a constant and significant decrease of extractable radioactivity was seen during the eight days of incubation: Extractables accounted for a range of 71.9 % to 72.8 % which corresponds to a mean of 72.7 % of the applied radioactivity. At the same time, non-extractable radioactivity accounted on average at 1.5 %.

Following leaching, 97.6 % and 95.7 % of the 0 to 6 cm soil segment radioactivity (column A and B, respectively) was extractable. Non-extractable radioactivity amounted to 2.4 % (column A) and 2.2 % (column B).

D. VOLATILE RADIOACTIVITY

Volatiles increased constantly and significantly during aerobic incubation. By far, most of the volatile radioactivity was 14CO2. Organic volatiles contributed less than 0.1 % of the applied radioactivity. The total amount of volatiles during sample incubation was between 19.3 % and 19.7 % which corresponds to a mean value of 19.5 %.

E. TRANSFORMATION OF THE TEST ITEM

In the 0 to 6 cm segment 72.5 % and 71.0 % of the extractable segment radioactivity was characterised as glyphosate. These values correspond to 52.5 % and 44.7 % of total applied radioactivity to one metabolism system. In the extracts from the top segment AMPA appeared at 25.2 % and 24.7 % of the column layer radioactivity corresponding to 18.2 and 24.7 % of the total applied radioactivity. The dpm level of the 6 to 12 cm layers of both columns was very low. As a consequence, a characterization was not feasible.

III. CONCLUSIONS

The results indicate that glyphosate and its major soil metabolite are immobile in the representative sandy soil used in this study. No residues penetrated deeper than 12 cm into the soil column and radioactivity in the leachates did not exceed 0.1 % of the applied radioactivity.

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed via aged column leaching experiments. Only small amounts of radioactivity were found in the leachate, while the majority of the test substance remained in the topmost soil segments or was mineralised during aging. The results demonstrate that glyphosate is not prone to leaching in soil. The study is considered as supportive information.

Assessment and conclusion by RMS:

This aged column leaching study is well performed. The LOD/LOQ are not provided in the report, but results seem to indicate that the LOD was sufficient.

The study is acceptable.

[redacted], *1992*

Data point:	CA 7.1.4.1.1/002
Report author	[redacted]
Report year	1992
Report title	Leaching characteristics of formulated [14C]glyphosate in three soils
Report No	281430
Guidelines followed in study	Biologische Bundesanstalt Deutschland (BBA) Richtlinien Teil IV, 4.2 Dezember 1986

Deviations from current test guideline	From OECD 312: - LOD/LOQ not reported - No determination of mass balance - Soil column segments not analysed
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Identification: Formulated [14C]glyphosate
Lot No.: CFA.745 C5
Specific activity: 11.1 MBq/mg (299 µCi/mg)
Radiochemical purity: 99.0 % and 98.3 % as determined before and after conduct of the test

and

Identification: Glyphosate
Lot No.: 185-ff-131
Chemical purity: 99.5 % (0.1 %NaCl, 0.1 %H2O)

2. Soil

The study was performed with three German standard soils: Speyer 2.1, 2.2 and 2.3, sampled from the top 20 cm of each soil profile. They had not been subjected to any pesticide, organic nor inorganic fertilizer treatments for the past 2 years. The soils were air dried and sieved through a 1 mm sieve. The moisture content of the soils was adjusted to the field capacity.

Characteristics of the test soils are presented in the table below.

Table 8.1.3.1-7: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Textural Class	Loamy sand	Sandy loam	Sandy loam
Sand (> 0.2 mm)	67.6	48.4	44.5
Sand/Silt (20 µm – 200 µm) [2] (%)	23.3	39.6	31.2
Silt (2 µm – 20 µm) [2] (%)	3.8	7.1	13.4
Clay (< 2 µm) [2] (%)	5.3	4.9	10.9
pH [1]	6.0	6.0	6.6
Organic carbon (%)	0.48	2.55	0.74
Cation exchange capacity (meq/100 g)	3.6	7.2	4.5
Bulk density (g/cm3) [3]	1.65/1.66	1.45/1.43	1.46/1.43/1.41

[1] medium not indicated
[2] 2-20 µm corresponding to fine and medium silt, 20-200 µm corresponding to coarse silt and fine sand and > 200 µm corresponding to medium and coarse sand according to German DIN 4022
[3] determined for each column separately

B. STUDY DESIGN

1. Experimental conditions

Glass columns (5.0 cm inner diameter, 40.0 cm length), corresponding to a cross-sectional area of 19.6 cm^2, were filled with the air-dried untreated soils up to 30 cm, a paper filter was placed on top of the soil and thereafter saturated with water overnight. The bulk density in the soil columns ranged from 1.41 to 1.66 g/cm3, depending on the soil type. Two replicate soil columns were treated per soil type. Additionally, one column was filled with Speyer 2.3 soil and not treated. Leachates obtained from this column served as control samples.

A stock solution was prepared from radio-labelled and un-labelled material, and determined to have a concentration of 1.42 mg/ml. The formulation solution, consisting of isopropylamine, Berol, ethylene glycol and bi-distilled water, and the stock solution were used to prepare the application solution with a content of 0.680 mg/ml. A total volume of 1000 µl was applied, which corresponded to 0.680 mg a.s./column or a field rate of 3.47 kg a.s./ha. The applied amount was slightly lower than the target value of 3.60 kg a.s./ha. The test article was applied onto the top layer of the saturated soils in an aqueous formulation solution, dropwise following a spiral movement about 0.5 cm away from the column walls, to avoid preferred paths of flow.

Thereafter, a paper filter was placed on top of the column and then the rain-simulation was started using bi-distilled water. Artificial rain of about 200 mm (= 196 ml daily or 393 ml total as target volume) was delivered within 48 hours by means of a peristaltic pump at a flow rate of about 0.14 ml/min. The leaching study was performed at room temperature in the dark for two days.

2. Sampling

The total leachate was collected in Erlenmeyer flasks from 0-24 hours and 24-48 hours. After completion of the leaching period, the columns were sectioned into 6 cm segments and stored at -20 °C.

3. Analytical procedures

The radioactivity was determined on a Packard scintillation counter.

For the characterisation of radioactivity in leachate one-dimensional TLC was performed on pre-coated plates of cellulose with a layer thickness of 0.5 mm and on RP-18 F 254 plates with a layer thickness of 0.25 mm. 150 ml of leachates obtained were concentrated by lyophilisation. The residues were suspended in 3 ml of bi-distilled water, centrifuged and chromatographed. SS 11 (n-Propanol/water/acetic acid/ammonia solution 25 % (40+20+10+5)) and SS 16 (Methanol/water/ammonia solution 25 % (40+10+0.5)) were used as solvent systems. Co-chromatography was performed by mixing the solutions containing the radioactive material 1:1 with a solution containing the analytical standard (6 mg/mL).

The radioactive zones on TLC-plates were detected by scanning with an Automatic TLC Linear Analyser. Un-labelled parent compound was visualised by spraying with ammonium molybdate (1 % in water) followed by spraying 1 % tin(II)-chloride (dissolved in 10 % HCl) and heated for 5 minutes at 100 °C.

The radiochemical purity of the test article was determined by TLC in two solvent systems SS 11 and SS 16. The results obtained indicated that the purity of 99.0 % agreed well with that given by the sponsor, e.g. 99 % using a HPLC method. A further purity check performed on September 19, 1991, using a HPLC method confirmed the radiochemical stability of the test article resulting in 99.3 % (RCC-Project 271618). Concentrations and stability of stock and application solutions was confirmed via LSC.

II. RESULTS AND DISCUSSION

A. DATA

The radioactivity levels found in the leachates is presented for both columns in the following tables.

Table 8.1.3.1-8: Leached water (ml) and radioactivity levels (in % of AR) in the leachate from soil Speyer 2.1 treated with formulated [14C]glyphosate

Parameter	Time interval: 0 – 24 h		Time interval: 24 – 48 h		Total: Time interval: 0 – 48 h	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Leached water (ml)	190.9	193.1	201.0	201.3	391.9	394.4
Mean	192.0		201.2		393.2	

Radiocativity (%)	0.16	0.10	0.81	1.82	0.97	1.92
Mean (%)	0.13		1.32		1.45	

Table 8.1.3.1-9: Leached water (ml) and radioactivity levels (in % of AR) in the leachate from soil Speyer 2.2 treated with formulated [14C]-glyphosate

Parameter	Time interval: 0 – 24 h		Time interval: 24 – 48 h		Total: Time interval: 0 – 48 h	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Leached water (ml)	193.5	177.1	201.3	194.6	394.8	371.7
Mean	185.3		198.0		383.3	
Radiocativity found (%)	< 0.01	0.01	0.07	0.16	0.07	0.17
Mean (%)	0.01		0.11		0.12	

Table 8.1.3.1-10: Leached water (ml) and radioactivity levels (in % of AR) in the leachate from soil Speyer 2.3 treated with formulated [14C]-glyphosate

Parameter	Time interval: 0 – 24 h		Time interval: 24 – 48 h		Total: Time interval: 0 – 48 h	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Leached water (ml)	195.4	190.4	202.5	205.5	397.9	395.9
Mean	192.9		204.0		396.9	
Radiocativity found (%)	0.01	0.04	0.75	0.47	0.76	0.51
Mean (%)	0.02		0.61		0.64	

Table 8.1.3.1-11: Total concentration of radioactivity (mg a.s./kg) found in the leachate 1 of soils Speyer 2.1, 2.2 and 2.3 treated with formulated [14C]-glyphosate (0 - 48 h)

Parameter	Speyer 2.1		Speyer 2.1		Speyer 2.1	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Concentration (mg/kg)	0.017	0.033	0.001	0.003	0.013	0.009
Mean	0.025		0.002		0.011	
1 1 liter of leachate was taken as equivalent to 1 kg						

B. CHARACTERISATION OF LEACHATES

The mean total leached volume per column amounted to 393.2 mL, 383.3 mL and 396.9 mL for soils Speyer 2.1, 2.2 and 2.3, respectively. These values compared well with the target value of 393 ml per column.

In soil Speyer 2.1, the leachates from two columns for 0-24 hours contained 0.13 % of the applied radioactivity, whereas the second fraction (24-48 hours) contained 1.32 %. The mean total radioactivity detected (0-48 hours) was 1.45 % of the total applied radioactivity. In terms of mg a.s./kg the highest concentration of parent equivalents found was 0.033 mg/kg. A mean total of 0.025 mg/kg was obtained for these two columns.

Soil Speyer 2.2 contained a higher amount of organic carbon and thus its adsorption capacity was larger. For the 0-24 hour interval only 0.01 % of the applied radioactivity was found in the two columns. In the 24-48 hour interval, this value increased to 0.11 %. The mean total radioactivity in the 0-48 hour interval amounted to 0.12 % of the total applied radioactivity. In terms of mg a.s./kg, for each column, not more than 0.003 mg/kg were detected.

In soil Speyer 2.3 also low levels of radioactivity were found in the leachates of the two columns used. In the time interval from 0 to 24 hours, only 0.02 % of the applied radioactivity was found. In the following 24 hours, an increase to 0.61 % took place. Hence, in the 0-48 hour period, a mean total radioactivity of 0.63 % was found.

The highest total amount of parent equivalents per column was 0.013 mg/kg. Thus, a mean total of 0.011 mg/kg was obtained for these two soil columns in the 0-48 hour leaching period.

For soil Speyer 2.1 at least three radioactive fractions could be detected by TLC, whereby the presence of parent molecule besides two unknown polar fractions seems probable. However, the total concentration of [14C]-glyphosate equivalents in the leachate of soil Speyer 2.1 did not exceed 0.011 mg/kg. Similar results were obtained for the other two soils.

III. CONCLUSIONS

The radioactivity levels found in the leachates from soils Speyer 2.1, 2.2 and 2.3 amounted to 1.45 %, 0.12 % and 0.63 % of the total applied radioactivity, respectively. These levels represented 0.025 mg/kg, 0.002 mg/kg and 0.011 mg/kg in the leachates from soils Speyer 2.1, Speyer 2.2 and Speyer 2.3, respectively.

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed via column leaching experiments. Only negligible amounts of applied radioactivity were encountered in the leachate. Soil columns were not analysed. The results confirmed the low leaching potential of glyphosate in soil. Due to the limited information provided in the study report and in view of the fact that adequate batch equilibrium data is available, the study is considered as supportive information.

Assessment and conclusion by RMS:

Some deviations from OECD 312 are identified. The study only focused on the radioactivity recovered in the leachates. No analysis of the radioactivity recovered in soil column was done, and no mass balance is provided. LOD/LOQ are not mentioned in the study report.

This study is considered as supportive.

[redacted], *1992*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation (TMS) are not reported below for easiness of reading.

Data point:	CA 7.1.4.1.1/003
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Leaching of formulated material in soil column
Report No	RJ1247B
Guidelines followed in study	Guidelines for the Official Testing of Plant Protection Products Part IV, December 1986 4-2. Seepage Behaviour of Plant Protection Products (formerly BBA Memorandum No. 37). Federal Biological Research Centre for Agriculture and Forestry Federal Republic.
Deviations from current test guideline	From OECD 312: - Soil history not reported - No determination of mass balance - Soil column segments not analysed
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate-trimesium in concentrated solution
Formulated product:	YF7712A
Nominal concentration:	480 g/L glyphosate-trimesium
Measured concentration:	97 %

2. Soil

Three German standard soils, Speyer 2.1, Speyer 2.2 and Speyer 2.3, were used. The physical and chemical characteristics of the soils were determined by Natural Resource Management Ltd, Jealott's Hill Research Station, Jealott's Hill, Bracknell, Berkshire. RG12 6EY as presented in the table below.

Table 8.1.3.1-12: Soil physicochemical properties

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Textural Class (USDA)	Sand	Loamy sand	Sandy Loam
Sand (50 µm – 2 mm) (%)	89	84	71
Silt (2 µm – 50µmm) (%)	7	11	18
Clay (< 2 µm) (%)	4	5	11
pH [1]	5.4	5.7	6.7
Organic matter (%)	1.4	5.1	2.5
Organic carbon (%) [2]	0.81	2.96	1.45
Cation exchange capacity (meq/100 g)	3.5	8.2	8.3

[1] Medium not indicated
[2] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

The soil columns were made of glass tubing (35 cm length) with an internal diameter of ≤ 5 cm. A glass funnel of internal diameter 5.2 cm was attached to the bottom of the column by glass fusion. The funnel stem was plugged with glass wool and the funnel filled with acid-washed quartz sand. The columns were uniformly packed with air-dried 1 mm sieved soil to a depth of 30 cm. The soil was added in small increments (approximately 1 cm depth). The initial 5 cm soil added was weighed and used to determine the total weight of soil required to fill the column (30 cm). This was used as an additional check to ensure a uniform density was achieved. The average air dried weight of Speyer 2.1, 2.2 and 2.3 soils added to the columns was 1000 g, 923 g and 904 g, respectively. The top 5 cm of the glass column contained no soil and a glass wool pad was placed on top of the soil to assist uniform distribution of water to the soil surface. Triplicate columns containing each soil type were prepared, two of which were to be treated with glyphosate-trimesium and a third to be used as an untreated control.

The columns were clamped in a vertical position and a flask placed under each to collect the leachate. Before application of the pesticide, the soil columns were maintained at a constant temp 22 ± 5 °C and were leached for between 40 and 45 hours with deionised water applied at a rate of 0.15 – 0.17 cm3/min using a peristaltic pump. After this time, during which the soil in the columns had become saturated, the water flow was stopped and the columns left for 1-3 hours to allow excess water to drain off.

The rate of application to the soil surface of each column was 4 kg a.s./ha concentrated solution. Formulated glyphosate-trimesium (10 cm^3) was diluted in 100 cm^3 of ultra-pure water. An aliquot (10 cm^3) of this solution was further diluted to 100 cm^3 with ultra-pure water. An aliquot of this diluted suspension (170 µL) was evenly applied to the soil surface of each column (except untreated controls) using a syringe. A 5 mm band of soil was placed around the circumference of the column to minimise the risk of leaching between the soil and glass interface. After treating the columns, the glass wool pad was replaced on top of the soil and the water flow re-started. A total of 393 cm^3 of deionised water (equivalent to 200 mm rain) was applied dropwise to the top of each column within a period of 48 hours using a peristaltic pump.

2. Analytical procedures

After completion of leaching, the observed volume, odour and colour of each leachate was recorded. Each leachate was analysed by two analytical procedures, one to determine the concentration of glyphosate (N-phosphonomethylglycine) and one to determine trimesium (trimethylsulphonium ion (TMS+)) concentration.

For glyphosate (PMG) analysis, the method involved an aliquot of the leachate was diluted 1:10 with deionised water, and percolated through cation exchange resin. An aliquot was evaporated to dryness, dissolved in 0.1 M disodium-hydrogen borate and derivatised with 9-fluorenylmethyl chloroformate.

Final quantitative determination of the derivative was by high performance liquid chromatography (HPLC) using fluorescence detection.

Residues were quantified using external standards and corrected for recovery values generated by analysis of fortified control samples if < 100 %.

The mean recovery value for trimesium in spiked leachate was found to be 103 % (coefficient of variation = 13 %, n = 6).

The mean recovery value for glyphosate in spiked leachate was found to be 93 % (coefficient of variation = 14 %, n = 6).

The limit of determination in leachate was set at 25 µg/L.

II. RESULTS AND DISCUSSION

A. DATA

Residues of glyphosate in the leachate obtained from soil columns treated with the formulation YF7712A, are given in the following table, together with the volume, colour and odour of the leachate.

Table 8.1.3.1-13: Results of leachate analysis of glyphosate (PMG)

Application Rate (kg a.s./ha)	Volume (ml)	Odour	Colour	Residues PMG (µg/L)
Leachate Type : Speyer 2.1				
Control	387	Rich Earth	Clear light, amber	< 25
4.0	395	Rich Earth	Clear light, amber	< 25
4.0	397	Rich Earth	Clear light, amber	< 25
Leachate Type : Speyer 2.2				
Control	395	Damp Earth	Amber	< 25
4.0	397	Damp Earth	Amber	< 25
4.0	400	Oamp Earth	Amber	< 25
Leachate Type : Speyer 2.3				
Control	180 [1]	Fresh Earth	Very light, amber clear	< 25
4.0	390	Fresh Earth	Very light, amber clear	< 25
4.0	385	Fresh Earth	Very light, amber clear	< 25

[1] After 48 hours only 180 ml of leachate had passed through the column containing Speyer 2.3 control. This was due to the soil being packed so tightly that the flow of water was impeded.

B. CHARACTERISATION OF LEACHATES

From an application of glyphosate-trimesium at 4 kg a.s./ha onto coarse sand, loamy sand and sandy loam soils, residues of glyphosate were below the limit of determination (i.e. < 25 µg/L). The total amount of glyphosate-trimesium in the leachate was thus less than 2 % of that applied.

III. CONCLUSIONS

Normal agricultural use of glyphosate-trimesium is unlikely to result in any contamination of ground water.

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed via column leaching experiments. While column soils were not analysed for the test substance, only small amounts were found in the leachate. The results demonstrate that glyphosate is not prone to leaching in soil. The study is considered as supportive information.

Assessment and conclusion by RMS:

Some deviations from OECD 312 are identified. Soil history was not reported. The study only focused on the radioactivity recovered in the leachates. No analysis of the radioactivity recovered in soil column was done, and no mass balance is provided.

The study is considered as supportive.

[redacted], *1992*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation (TMS) are not reported below for easiness of reading.

Data point:	CA 7.1.4.1.1/004
Report author	[redacted]
Report year	1992
Report title	(14C)-Glyphosate-Trimesium: Aged soil Leaching
Report No	7113-38/172
Guidelines followed in study	OECD 312
Deviations from current test guideline	From OECD 312: - Soil history not reported - Incubation temperature fell to 14 °C on three days during the 1st week of incubation; temperature was not recorded for two days - No determination of mass balance in the leaching part of the study - Soil column segments not analysed
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]glyphosate-trimesium, anion labelled
Lot No.: 91-J19
Specific activity: 2.07 GBq/mmol
Radiochemical purity: > 99 %

Identification: [14C]glyphosate-trimesium, cation labelled
Lot No.: 91-70
Specific activity: 2.02 GBq/mmol)
Radiochemical purity: 98 %

2. Soil:

A mildly humus sand (Speyer standard soil 2.1) was supplied by ICI Agrochemicals, Jealott's Hill, Bracknell, Berkshire. The soil was stored outside undercover prior to use. Deionised water was added regularly to prevent dehydration.

After sieving (2 mm) the soil was characterised for organic matter content, particle size distribution, cation exchange capacity, moisture holding capacity at 1/3 and 15 bar, and pH (H2O) by ICI Agrochemicals, Jealott's Hill, Bracknell, Berkshire. Maximum water holding capacity (MWHC) was determined at the Soil Survey and Land Research Centre, Shardlow, Derbyshire.

Characteristics of the test soils are presented in the table below.

Table 8.1.3.1-14: Soil physicochemical properties

Parameter	Results
Soil	Speyer 2.1

Textural Class (USDA)	Sand
Sand (50 µm – 2 mm) (%)	89
Silt (2 µm – 50µmm) (%)	8
Clay (< 2 µm) (%)	3
pH (water)	6.9
Organic matter (%)	1.8
Organic carbon (%) [1]	1.04
Cation exchange capacity (meq/100 g)	2.6
Maximum Water Holding Capacity (%)	30.44
Water Holding Capacity at 0.33 bar (%)	4.16
Water Holding Capacity at 15 bar (%)	2.98

[1] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

Eighteen portions of 2 mm sieved Speyer 2.1 soil (100 g dry weight equivalent) were weighed into Erlenmeyer flasks (250 mL) and adjusted to 40 % of the MWHC. Moistened carbon dioxide-free air was drawn over the surface of each sample except when condensation within the units caused the moisture content of the soil to rise. When this occurred, un-moistened carbon dioxide free air was drawn through the units until the soil moisture content returned to the correct level. The moisture content of the soil samples was determined every two to three days and any moisture loss was replaced with deionized water. The units were incubated in the dark at 20 ± 2 °C in a thermostatically controlled water bath. Flasks were pre-incubated for 36 days prior to test article application to permit the soils to equilibrate.

[14C]anion labelled glyphosate-trimesium (0.394 mg/mL; 1 mL) and [14C]cation labelled glyphosate-trimesium (0.366 mg/mL; 1 mL) in HPLC grade water were each applied to eight soil samples dropwise using a glass pipette. Two units were not treated with test article. After test article application the flasks were shaken to ensure thorough mixing of the samples.

Following test article addition, air drawn through the units was passed through a series of three traps, the first empty trap acting as a security trap and the second and third containing ethanolamine to trap liberated 14CO2. The ethanolamine was changed 7, 14, 23 and 30 days after test article application.

For the preparation of the soil columns six glass columns (ca. 35 cm length x 5 cm inner diameter) were used. The column outlets were plugged with glass wool and acid washed sand was placed in the conical part of the columns. Air dried soil, 1 mm sieved, was added to the columns with mechanical shaking to a depth of 28 cm. Shaking was continued until the surface of the column had settled. A Whatman GFA glass fibre filter paper disc was placed on the top of each column. The soil was then saturated by adding water dropwise to the surface of the column until seepage water percolated through the foot of the column. The application of the treated, aged soils took place within seven hours of saturation.

After 30 days of incubation two samples were transferred to the top of separate saturated soil columns after removing the glass fibre filter paper. Two samples of untreated incubated soil were transferred to the top of the remaining two columns. Leachate from these columns was used as blank material for liquid scintillation counting (LSC). A quantitative transfer was achieved using a small volume of water. The added soil was pressed down and a glass fibre filter paper placed on the top of each soil column. Light was excluded from the columns and collecting vessels by surrounding them with aluminium foil. The leaching was conducted at room temperature.

Each column was eluted with the equivalent of 200 mm of deionised water (ca. 393 mL) over a period of 48 h. Steady leaching rates were achieved using a calibrated multichannel peristaltic pump. An additional volume of water (18.3 mL), equivalent to the quantity of water required to raise the moisture content of 100 g dry weight equivalent of Speyer 2.1 soil from 40 % MWHC to 100 % MWHC, was then applied to each column.

2. Sampling

Duplicate soil samples treated with each form of test article were sampled immediately after application and after 30 days of incubation.

Leachates were collected over the entire 48 h period forming one merged sample. Additional leachates collected after the 48 h period were assayed radioactivity separately.

3. Analytical procedures

The total soil sample in each incubate was extracted, on the day of sampling, three times with ammonia solution (0.5 M; 100 mL) for glyphosate analysisfor 30 minutes with mechanical shaking. Extracts were separated from soil by centrifugation and were kept cool and dark between successive extractions. The total weight of extract was determined and a weighed subsample (ca. 10 g) was taken for further analysis. The remaining extract was stored at ca. -18 °C. Following centrifugation subsamples of extract were passed through a series of filters. The filters were rinsed with small volumes of ammonia solution (0.5 M) and formic acid (1 M). The rinsings were pooled with the filtrate. The filtered extracts were neutralised with concentrated formic acid, total weights determined and weighed aliquots were radio-counted. Combined filtered and neutralised extracts were freeze-dried and re-suspended in formic acid (1 M, 5 to 10 mL). The suspensions were transferred to vials, to provide samples for chromatography. The original flasks were rinsed with formic acid (1 M, 20 mL) and the rinsings were weighed and counted. Prior to TLC, the reconstituted extracts were basified with ammonia (about 1 to 2 mL) and thoroughly mixed to produce a very fine suspension. Aliquots (100 µl) of this suspension were radio-counted to determine recovery. Prolonged storage before chromatography of the basified extracts was avoided.

The following solvents and plates were used for thin layer chromatography (TLC).

Table 8.1.3.1-15: Solvents and plates used for TLC

Compound	No.	Solvent	Plate
[14C]anion labeled Glyphosate-trimesium	1	Methanol : Ammonia (10 %) : Trichloroacetic acid solution :Water 12:3:1:6 (v/v/v/v)	Analtech Silica HLF
	5 [1]	Methanol : Ethanol : Ammonia (s.g.880) : water 3:3:2:2	Analtech Silica HLF
[1] Solvent 2 was replaced by solvent 5 (amendment to protocol and deviations)			

For determination of glyphosate, aliquots (ca. 15 µL) of appropriate extracts were chromatographed with non-radiolabelled glyphosate-trimesium and AMPA. Radiolabelled compounds were detected and quantified by linear analysis. Non-radiolabelled glyphosate and AMPA were detected on each TLC plate by spraying with ninhydrin solution.

For determination of radioactivity weights or volumes of all samples were measured where appropriate in duplicate and determined by LSC.

Triplicate portions of air dried, extracted soil samples (ca. 0.1 g) were combusted and radioactivity was determined by LSC.

II. RESULTS AND DISCUSSION

A. DATA

The recovery of applied radioactivity during the ageing period in the extracts, in the combusted soil and in ethanolamine traps for [14C]glyphosate and its degradation products determined in different solvents is presented in the tables below.

Table 8.1.3.1-16: Percent of applied radioactivity in soil extracts, combusted soil and volatiles from [^{14}C]glyphosate (PMG) during ageing period

		Day 0 [1]			Day 30 [2]		
		Rep.1	Rep.2	Mean	Rep.1	Rep.2	Mean
	Glyphosate	75.10	69.54	72.32	14.01	12.30	13.16

		Day 0 [1]			Day 30 [2]		
		Rep.1	Rep.2	Mean	Rep.1	Rep.2	Mean
[14C]anion and degradates in Extracts	AMPA	2.49	2.79	2.64	25.43	27.09	26.26
	Other	1.46	1.60	1.53	1.51	2.57	2.04
	Origin material	2.52	1.80	2.16	0.81	1.21	1.01
	Unresolved background	0.99	1.07	1.03	1.98	1.59	1.79
	Procedural loss	14.68	18.90	16.79	8.89	6.42	7.66
Total		97.24	95.70	96.47	52.63	51.18	51.91
[^{14}C]anion and degradates in Soil residues (combusted)		3.46	3.61	3.54	12.00	12.87	12.44
[^{14}C]anion and degradates in Ethanolamine traps		-	-	-	30.95	35.43	33.19
Total		100.70	99.31	100.01	95.58	99.48	97.53

[1] Values from solvent system 5
[2] Values from solvent system 1

The radioactivity leached from Speyer 2.1 soil aged for 30 days treated with [14C]anion labelled Glyphosate-trimesium is presented in the table below.

Table 8.1.3.1-17: Percent of applied radioactivity in the leachate

Column Identification		Percent of radioactivity applied to soil prior to ageing present in :			Leachate Volume (mL)	Additional Leachate Volume (mL)	Concen-tration in total Leachate (µg/mL)
		Initial. Leachate	Additional Leachate	Total Leachate			
[^{14}C]Anion	Rep.1	0.045	0.003	0.048	392	29.2	< 0.001
	Rep.2	0.136	0.009	0.145	384	24.9	0.001
	Mean	0.091	0.006	0.097	388	27.1	< 0.001

B. MASS BALANCE

Thirty days after test article application overall recoveries of applied radioactivity ranged from 96 to 99 % for anion labelled glyphosate-trimesium.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Immediately after test article application, the majority of applied radioactivity (> 95 %) was extractable. After 30 days the percentage of applied radioactivity recovered in the soil extract declined to about 52 for anion labelled test article.

D. VOLATILE RADIOACTIVITY

Significant quantities of 14CO2 were formed, namely about 33% of applied radioactivity. Levels of unextracted radioactivity increased to about 12 % after 30 days.

E. TRANSFORMATION OF THE TEST ITEM

Glyphosate was the only major components detected when day 0 soil extracts were analysed by TLC. Small quantities (each less than 4 % of the applied radioactivity) of AMPA, polar material and unidentified degradates (observed on TLC) were also present in extracts.

After 30 days of incubation, AMPA was the major component in [14C]anion test article treated soil extracts accounting for about 26 % of applied radioactivity. Glyphosate, polar material and unidentified degradates comprised about 13, 1 and 2 %, respectively.

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed via aged column leaching experiments. The mean percentages of the applied radioactivity recovered in the leachates were 0.1 for anion labelled glyphosate-trimesium. This is considerably less than the 2 % of applied radioactivity that would trigger analysis of the leachates. The results illustrate that neither glyphosate, nor its degradation product AMPA are likely to leach into groundwater. The study is considered as supportive information.

Assessment and conclusion by RMS:

Some deviations from OECD 312 are identified.

Soil history was not reported. Microbial biomass was not measured, however considering the the ageing period lasted only 30 days, this is not considered as a significant deviation.

The study only focused on the radioactivity recovered in the leachates. No analysis of the radioactivity recovered in soil column was done, and no mass balance for the leaching part of the study is provided. Finally, according to the study report, incubation temperature fell do 14 °C on three days during the 1st week of incubation, due to failure of the water bath heater, and temperature was not recorded for two days as the thermometer bulb had been displaced above the water surface.

The study is considered as supportive.

[redacted], *1991*

Data point:	CA 7.1.4.1.1/005
Report author	[redacted]
Report year	1991
Report title	Behavior of glyphosate in water and soil, Part 4 Leaching behaviour, second performance
Report No	PR90/002
Guidelines followed in study	BA-guideline for testing of pesticides Part IV 4-2
Deviations from current test guideline	From OECD 312: - Soil history not reported - Water instead of artificial rain ($CaCl_2$) was used - No determination of mass balance - Soil column segments not analysed - Report is lacking important information on the study design
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate
Formulated product: Taifun 360
Nominal concentration: 360 g/L Glyphosate
Sample No.: 10/08/90

2. Soil:

Soils were received from landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), Speyer. Characteristics of the test soils are presented in the table below.

Table 8.1.3.1-18: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Textural Class (DIN)	Sand	Loamy sand	Sandy loam
Sand (0.63 – 2.0 mm)	4.5 ± 0.6	2.0 ± 0.6	3.2 ± 0.6
Medium sand (0.2 – 0.63 mm)	62.9 ± 2.4	52.6 ± 3.3	32.5 ± 3.2
Fine sand (0.063 – 0.2 mm)	20.0 ± 2.8	27.4 ± 5.0	28.4 ± 2.9
Coarse silt (0.02 – 0.063 mm)	4.7 ± 2.0	7.4 ± 3.5	16.4 ± 3.3
Medium silt (0.006 – 0.02 mm)	2.5 ± 0.7	3.5 ± 1.4	7.5 ± 1.2
Fine silt (0.002 – 0.006 mm)	1.9 ± 0.8	2.1 ± 0.7	3.9 ± 0.5
Clay (< 2 µm) (%)	3.5 ± 1.6	5.1 ± 1.4	8.3 ± 1.4
pH [1)]	5.7	5.6	6.4
Organic carbon (%)	0.70 ± 0.07	2.29 ± 0.37	1.34 ± 0.14
Cation exchange capacity (meq/100 g)	4.9 ± 0.8	9.7 ± 0.3	9.5 ± 0.9
Maximum Water Holding Capacity (%)	31.9 ± 0.6	44.3 ± 1.1	34.9 ± 1.6

1 Medium not indicated

B. STUDY DESIGN

1. Experimental conditions

Soil columns containing the standard soils of LUFA, Speyer, 2.1, Speyer 2.2 and Speyer 2.3 were saturated with water. Then 50 µL (equivalent of 360 µg glyphosate / 20 cm2 = 1.8 kg a.s./ha) of the solution was distributed on top of each soil column. The soil columns were leached with water for about 48 hours.

2. Sampling and analytical procedure

Leachates were collected and analysed using a GC-ECD method.

The test was performed twice. In a first test, only leachate from Speyer soil 2.2 was analysed, in a repeat test leachates all three Speyer soils were analysed.

II. RESULTS AND DISCUSSION

A. DATA

Residues for glyphosate and AMPA are presented in the table below.

Table 8.1.3.1-19: Residues (µg/L) in leachates

Test	Soil	Leachate (ml)	Residues (µg/L)	
			Glyphosate	AMPA
1	Speyer 2.1	396	n.a.	n.a.
	Speyer 2.2	401	< 1.0	< 1.0
	Speyer 2.3	400	n.a.	n.a.
2	Speyer 2.1	407	< 1.0	n.a.
	Speyer 2.2	396	2.6	n.a.
	Speyer 2.3	422	< 1.0	n.a.
n.a. = not analysed				

B. CHARACTERISATION OF LEACHATES

In the first test, measured concentrations of glyphosate and AMPA in the Speyer 2.2 soil were < 1.0 µg/L, in the second test glyphosate measured concentrations were < 1.0 µg/L in Speyer 2.1 and 2.3 soil and 2.6 µg/L in Speyer 2.2 soil.

III. CONCLUSIONS

For all investigated cases the quantity of active ingredient on drainage water was < 2 % of the original amount given on top of the columns. Glyphosate does not show any significant leaching behavior.

Assessment and conclusion by applicant:

The mobility of glyphosate and AMPA was assessed via column leaching experiments. Results confirm the low leaching potential of glyphosate and AMPA. Due to the fact that some residues in soil columns were not analysed and in view of the limited information given in the report, the study is considered as supportive information.

Assessment and conclusion by RMS:

The study report is very brief. The limited information available in the study report prevents RMS to validate the study.

From the available information, the following deviations from OECD 312 are identified. Soil history was not reported. Water instead of artificial rain ($CaCl_2$) was used. The study only focused on the radioactivity recovered in the leachates. No analysis of the radioactivity recovered in soil column was done, and no mass balance is provided.

This study is not considered acceptable.

[redacted], *1978*

Data point:	CA 7.1.4.1.1/006
Report author	[redacted]
Report year	1978
Report title	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 1010
Report No	MSL-0207
Guidelines followed in study	None
Deviations from current test guideline	From OECD 312: - Hilo and Molokai soils not representative of EU agricultural soils - Leon, Hilo and Molokai soils from areas where high use of glyphosate is expected; soil history not reported for any soil - all three soils used for aged residue test have sand content < 70% and were air dried - Inner diameter of columns below 4 cm (3.8 cm) - Water instead of artificial rain ($CaCl_2$) was used - conditions of ageing not reported - more than 500mm of eq rainfall was applied on the leaching column, over a time not indicated - the aged column received artificial rain over 2 weeks
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled Test Material:
Identification: [14C]glyphosate (MON-0573)
Specific activity: 10.12 mCi/mM
Radiochemical purity: 94.0 %

Non-radiolabelled test compound
Identification: [14C]sodium sesquiglyphosate (MON-0101)

Specific activity: not indicated
Chemical purity: not indicated

2. Soil:

All soils were air dried and sieved to 2 mm. Characteristics of the test soils are presented in the table below.

Table 8.1.3.1-20: Characteristics of test soils

Parameter	Results						
Soil	Ray	Drummer	Spinks	Lintonia	Leon	Hilo	Molokai
Textural Class (USDA)	Silt loam	Silty clay loam	Sandy loam	Sandy loam	Fine sand	Volcanic ash	Lava
Sand (50 µm – 2 mm) (%)	4.6	2.4	75.1	86.0	94.0	54.0	18.0
Silt (2 µm – 50 µm) (%)	84.2	68.8	17.8	11.0	5.0	20.0	30.0
Clay (< 2 µm) (%)	10.0	25.3	4.8	1.8	1.0	26.0	52.0
pH [1]	8.1	6.2	4.7	6.5	4.8	5.7	7.0
Organic carbon [2] (%)	0.70	1.97	1.39	0.41	0.58	5.51	1.74
Organic matter (%)	1.2	3.4	2.4	0.7	1.0	9.5	3.0
Cation exchange capacity (meq/ 100 g)	10.4	24.6	11.3	5.1	7.2	60.0	20.0
Maximum Water Holding Capacity (%)	23.9	28.8	17.9	15.6	-	-	-

USDA: United States Department for Agriculture
[1] medium not indicated
[2] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

Glass columns of 3.8 cm inner diameter (1.5 inches) were constructed from 15 segments of 2 cm length and an upper segment of 10 cm length. The bottom segment was packed with glass wool and placed in a Coors funnel (4.3 cm inner diameter). The columns were uniformly packed with air-dried soil. The total weight of soil used for each column was recorded. Water was added to the soil columns that were aged before leaching so the moisture content of these columns was 15 to 20 % at the time chemical was added. An aqueous solution of glyphosate (MON-0573) or sodium sesquiglyphosate (MON-0101) diluted with [14C]-MON-0573 equal to 1.2×10^7 to 2.6×10^7 dpm, equivalent to 8.97 kg a.s./ha (8 lbs/acre) was applied to the surface of the soil columns.

The following table presents the soils and compounds used for the rapid and the aged leaching part of the study.

Table 8.1.3.1-21: Overview of soils and compounds used for rapid and aged leaching

Soil column	Study type	Compound applied
Ray	aged	Sodium sesquiglyphosate (MON-0101)
Ray	aged	
Hilo	aged	Glyphosate (MON-0573)
Molokai	aged	
Ray	rapid	
Lintonia	rapid	
Drummer	rapid	
Spinks	rapid	Glyphosate (MON-0573)
Florida	rapid	
Hilo	rapid	
Molokai	rapid	

For the rapid leaching, test duplicate columns were set up for each soil with the exception of Hilo and Molokai. After application of the chemical, the soil columns were allowed to stand for 30 minutes before water was added.

Those columns that were set up to evaluate leaching of chemical aged on soil were immediately topped with a sidearm and coupled with an Ascarite trap. 14CO2 evolution was measured throughout the entire ageing period. Duplicate columns of Ray, Molokai, and Hilo soils were treated with [14C]-glyphosate for this study. Leaching of [14C]-sodium sesquiglyphosate was determined only on Ray soil as there has been no indication that glyphosate and sodium sesquiglyphosate were significantly different except for their solubility.

In both types of columns water was added at a rate slower than the infiltration capacity of the soil. The columns that were leached rapidly required 540 ml H20 corresponding to 508 mm (20 inches) of rainfall. The aged columns were allowed to stand 30 days before biweekly leaching with the equivalent of 13 mm (½ inch) of rainfall per day.

2. Sampling

The eluants from the rapid leaching columns were measured and aliquoted for LSC.

The eluants from the aged columns were pooled and stored for analysis after completion of the 45 day leaching period. Then they were concentrated, filtered, and submitted to analyses. The Ascarite towers were changed periodically and analysed tor 14C02.

The 2 cm soil segments were separated, immediately after leaching was complete, frozen, lyophilized, and analysed for 14C-content.

3. Analytical procedures

The eluants, which varied in volume from 360 to 415 ml, were analysed by LSC and TLC. The soil segments were separated immediately after leaching was completed; subsequently each soil segment was frozen, lyophilized, and analysed by combustion and LSC. An aliquot of 2.0 g, of the uppermost segment of all columns was extracted 2 times with 10 ml of 0.5 N NH40H. The extract was concentrated and analysed by TLC. The total recovery of 14C-activity applied was calculated, and the distribution was recalculated based on 100 % recovery. The distribution (% of AR) of glyphosate and AMPA was determined in the eluants and in the extracts from the uppermost segments from all columns.

II. RESULTS AND DISCUSSION

A. DATA

The total recovery and the distribution of 14C-activity (combusted segments) from rapidly leached soil columns and from soil columns after ageing is presented in the tables below.

Table 8.1.3.1-22: Distribution of ^{14}C-activity in rapidly leached soil columns (combusted segments) (% AR)

Soil / TLC Segment	Lintonia	Ray	Spinks	Florida	Drummer	Hilo	Molokai
1	33.29	24.53	72.12	21.18	80.03	99.47	98.57
2	25.28	24.30	24.65	19.79	14.26	0.17	0.93
3	17.30	17.98	1.85	15.47	2.35	0.15	0.13
4	10.44	14.48	0.38	15.41	0.85	0.05	0.30
5	4.84	6.84	0.21	10.40	0.42	0.04	0.01
6	2.27	2.37	0.13	6.67	0.28	0.02	0.01
7	0.80	1.35	0.09	4.10	0.20	0.02	-
8	0.44	0.74	0.07	2.39	0.12	0.01	-
9	0.19	0.31	0.05	1.90	0.31	0.01	-
10	0.14	0.14	0.04	0.75	0.06	0.01	-
11	0.11	0.10	0.03	0.34	0.06	-	-
12	0.11	0.09	0.03	0.27	0.05	-	-
13	0.11	0.07	0.16	0.15	0.03	-	-
14	0.11	0.07	0.04	0.13	0.06	0.01	-

Table 8.1.3.1-22: Distribution of ^{14}C-activity in rapidly leached soil columns (combusted segments) (% AR)

Soil / TLC Segment	Lintonia	Ray	Spinks	Florida	Drummer	Hilo	Molokai
15	0.09	0.07	0.10	0.05	0.04	0.01	-
Eluant	4.38	6.56	0.10	1.00	0.88	0.03	0.05
Totally recovered	78.71	90.53	95.48	99.87	88.95	98.68	101.66

Table 8.1.3.1-23: Distribution of 14C-activity in soil columns (combusted soil segments) after ageing (% AR)

Soil	Ray	Ray	Hilo	Molokai
TLC Segment	MON 0101 applied	MON 0573 applied	MON 0573 applied	MON 0573 applied
1	30.38	30.30	40.39	97.53
2	0.87	1.07	0.20	0.03
3	0.47	0.49	0.05	0.03
4	0.25	0.27	0.07	0.01
5	0.22	0.24	0.07	0.01
6	0.19	0.17	0.04	0.01
7	0.36.	0.10	0.04	0.01
8	0.13	0.12	0.02	-
9	0.11	0.09	0.02	-
10	0.08	0.11	0.02	-
11	0.14	0.08	0.02	0.01
12	0.18	0.05	0.02	0.01
13	0.03	0.06	0.02	-
14	0.14	0.08	0.01	-
15	0.07	0.04	0.01	0.01
Total in soil	33.67	33.26	41.00	97.66
Total in eluent	1.16	1.56	0.22	0.02
14CO2, evolved	65.17	65.18	58.97	2.12
Total recovery	95.75	98.40	84.62	98.94

The following tables summarise the analysis of the leachates and the analysis of the extracts from the uppermost soil segments.

Table 8.1.3.1-24: Analysis of glyphosate and AMPA in the leachates

Soil column	Study type	Compound applied	Radioactivity in the leachates (% AR)	% of radioactivitiy in leachates	
				Glyphosate	AMPA
Ray	aged	Sodium sesquiglyphosate (MON-0101)	1.2	1.0	0.1
Ray	aged	Glyphosate (MON-0573)	1.5	0.8	0.7
Hilo	aged		0.1	-	-
Molokai	aged		0.2	-	-
Ray	rapid	Glyphosate (MON-0573)	6.6	5.8	0.8
Lintonia	rapid		4.4	3.9	0.5
Drummer	rapid		0.9	0.6	0.3
Spinks	rapid		0.1	-	-
Florida	rapid		1.0	0.6	0.4
Hilo	rapid		0.1	-	-
Molokai	rapid		0.1	-	-

Table 8.1.3.1-25: Analysis of glyphosate and AMPA in the extracts from the uppermost soil segments

Soil column	Study type	Compound applied	Extractables (% AR)	% of total radioactivity in extracts	
				Glyphosate	AMPA
Ray	aged	Sodium sesquiglyphosate (MON-0101)	51.1	34	66
Ray	aged	Glyphosate (MON-0573)	52.4	26	84
Hilo	aged		8.4	94	6
Molokai	aged		47.0	22	78
Ray	rapid	Glyphosate (MON-0573)	72.3	76	24
Lintonia	rapid		78.9	80	20
Drummer	rapid		77.8	86	14
Spinks	rapid		95.7	90	10
Florida	rapid		99.1	93	7
Hilo	rapid		15.1	94	6
Molokai	rapid		50.3	86	14

B. MASS BALANCE

The rate of leaching varied with the soil; e.g. Leon fine sand was leached in 8 hours while Drummer silty clay loam was leached in 44 hours. The total recovery of applied 14C-activity is less than 100 % in those soils which required longer to leach and in those soils in which degradation of glyphosate (MON-0573) to 14C02 occurred rapidly (Drummer, Ray and Lintonia). Total recovery of the 14C-activity applied was 95 % or greater on all of the aged soil columns.

The leachate contained 1.0 %, or less, of the applied 14C-activity with the exception of Ray and Lintonia which contained 6.6 and 4.4 % of the 14C-activity applied, respectively. Glyphosate (MON-0573) showed very little mobility on any of the soils after 508 ml (20 inches) of water was applied immediately. The greatest mobility observed was on Leon fine sand, and even in this case only 20 % of the 14C-activity applied, leached more than 10 cm.

Only 0.1 to 1.5 % [14C] of applied radioactivity was found in the eluents. In leachates [14C]glyphosate ranged from 0.8 to 1.0 and [14C]AMPA ranged from 0.1 to 0.7 % of applied AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Extractable residues in the top segment reached from in the rapid leaching study ranged from 15.1 % to 99.1 % (Ray) of applied radioactivity. For the aged columns 8.4 to 52.4 % (Molokai) to 1.56 % were extracted from the uppermost soil segment.

D. VOLATILE RADIOACTIVITY

Degradation of [14C]glyphosate to 14CO2 was negligible on Hilo volcanic ash (2.12 % of AR), but rapid degradation occurred on Molokai and Ray soils (58.97 and 65.10 % of AR, respectively).

E. TRANSFORMATION OF THE TEST ITEM

TLC analysis of the NH40H extract of the uppermost segment showed 14 to 24 % degradation of [14C]glyphosate to AMPA in these same soils. Analysis of the extracts of the uppermost segment resulted in 85 % AMPA (MON-0453) in Ray soil and 78 % AMPA in Molokai soil, as would be expected based on the degradation to 14CO2.

The data from the aged soil columns indicated that there was no leaching of AMPA (MON-0435), the degradation product of glyphosate and sodium sesquiglyphosate, or the compounds themselves.

Assessment and conclusion by applicant:

Column leaching experiments with several different soils were conducted to assess the leaching behavior of aged and freshly applied glyphosate. Only small amounts of AR were found in the leachate, while the majority of the test substance was encountered in the soil or in CO2 traps in case

of aged substance, demonstrating the low leaching potential of glyphosate. In view of the irrigation regime used, i.e. freshly applied columns received 540 ml at a rate slower than the infiltration capacity and the aged columns received 13 mm of rainfall daily over a two weeks period, the study is not fit for the purpose to describe the leaching behavior of glyphosate. Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

Several deviations from OECD 312 are identified:

Soil history is not reported; Leon, Hilo and Molokai soils were sampled from areas where high use of glyphosate is expected; Hilo (volcanic ash) and Molokai (lava) soils are not representative of EU agricultural soils. All three soils used for aged column leaching test have sand content < 70%. Microbial biomass was not measured, although this is not considered a major deviation considering the limited time of ageing.

The conditions of ageing of the soils are not reported.

The diameter of the soil columns is slightly below 4 cm. Water was used instead of artificial rainfall (CaCl2). The non aged leaching column received more then 200 mm rainfall, over a time not indicated. The aged leaching columns received slightly less than 200 mm rainfall over 2 weeks instead of 48 hours.

The study is not acceptable.

[redacted], *1972*

Data point:	CA 7.1.4.1.1/007
Report author	[redacted]
Report year	1972
Report title	MON-0573, Residue and Metabolism, Part 2: The photolysis, run-off and leaching of MON-0573 on or in soil
Report No	258
Guidelines followed in study	United States Department of Agriculture's guidelines for studies to determine the impact of pesticides on the environment as stated in PR Notice 70-15, June 23, 1970
Deviations from current test guideline	From OECD 312: - Soil thin layer chromatography study is not in line with pertinent guideline
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not mentioned in RAR (2015) but summarised in DAR (2001) (no conclusion on reliability was reported)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [14C]MON-0573 (N-(phosphonomethyl-14C)-glycine)
Specific activity: 8.03 mCi/mmol
Radiochemical purity: 97.0 %

2. Soil:

Characteristics of the three test soils are presented in the table below.

Table 8.1.3.1-26: Characteristics of test soils

Parameter	Results		
Soil	Ray	Drummer	Norfolk
Textural Class (USDA)	Silt loam	Silty clay loam	Sandy loam
Sand (50 µm – 2 mm) (%)	6.0	2.0	86.0
Silt (2 µm – 50µmm) (%)	83.2	55.4	11.0
Clay (< 2 µm) (%)	9.6	36.8	2.3
pH [1]	6.5	7.0	5.7
Organic carbon (%) [2]	0.58	3.48	0.58
Organic matter (%)	1.0	6.0	1.0

[1] Medium not indicated
[2] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

Soil thin layer chromatography (TLC) was utilized to investigate the vertical mobility of glyphosate in soil. Separate soil TLC plates (20 cm x 20 cm) with a soil thickness of 0.76 mm were prepared using a light (Norfolk sandy loam), medium (Ray silt loam) and heavy soil type (Drummer silty clay loam).

10 ml of a solution of 46.75 mg of [14C]glyphosate dissolved in 46.75 ml of 0.1 M NH4CO3 was applied to a 2 cm band located 3 cm from the bottom of each soil TLC plate (origin). The soil TLC plates were developed with distilled water in a horizontal position in a water saturated chamber. TLC plates were connected to the development by a paper-towel wick. The development time for the solvent front to migrate 16 cm beyond the origin was 9, 0.7 and 1.3 hours for the sandy loam, silt loam and silty clay loam soils, respectively. Following development, the plates were dried and the distribution of radioactivity between the origin and final solvent front was determined for each band by autoradiography. After evaluation of the first development, the plates were developed a second time with water and analysed as before. The mobility of radioactivity (Rf) was calculated as the distance of the leading edge of radioactivity from the origin divided by the distance of the solvent front from the origin following each development.

The total 14C-activity present in soil samples was determined by combustion of homogenised and lyophilised samples. Combustion analysis was performed using a Peterson automatic combustion apparatus followed by liquid scintillation counting of the resulting 14CO2 (PACA/LSC). The total 14C-activity present in aqueous samples was determined by liquid scintillation counting using Packard Insta-Gel scintillation fluid.

II. RESULTS AND DISCUSSION

Glyphosate was so strongly adsorbed by all three soils used to investigate its vertical mobility by TLC so that 97-100 % of the applied 14C-activity had an Rf of less than 0.09. Similarly, 95-99 % of the applied 14C-activity remained at an Rf of less than 0.09 after the second development. In no case any of the radioactivity showed an Rf value greater than 0.18.

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed using soil thin layer chromatography. The test substance was almost immobile, in line with the low leaching potential of glyphosate. As the methodology used in the study is not in line with current requirements, the study is not considered fit for the purpose to describe the mobility of glyphosate in soil.

Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

The methodology used in the study is not in line with current requirements..

The study is not acceptable.

B.8.1.3.1.2. Relevant articles from literature search

Gjettermann et al., 2011

Data point:	CA 7.1.4.1.1/008
Report author	Gjettermann, B. et al.
Report year	2011
Report title	Kinetics of Glyphosate Desorption from Mobilized Soil Particles
Document No	DOI 10.2316/sssaj2010.0198 ISSN 1435-0661
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Desorption kinetics of chemical compounds can be important both for their mobility in soil and for the significance of particle–facilitated transport. We studied desorption of glyphosate [N–(phosphonomethyl) glycine] on mobilized particles from two soil columns (50–cm height, 30–cm diameter), i.e., particles leached by free drainage from the bottom and particles mobilized by splash erosion and collected next to the top of the column. Leaching and splash erosion were driven by three, 30–mm irrigation events following surface application of 14C–labeled glyphosate.

Fresh leachate samples were investigated within 30 min of sampling, and desorption from splash–eroded particles in suspension (100 mg solid/L) was followed for 48 h (starting 2.0 min after immersion). Glyphosate concentrations were determined by measuring the 14C activity using liquid scintillation counting. Similar fractional amounts of glyphosate (on average, 10–20 % in 20 min) desorbed from leached and from splash–eroded particles (>20 nm) shortly after leaching or immersion, respectively, indicating that the processes of desorption from the different sources of particles were similar. In leachate, about 45 to 79 % remained particle bound after 20 min, while calculated values at equilibrium were 20 % or less. Equilibrium was established after about 5 to 10 h in suspensions with splash–eroded particles, except for one sample. These direct observations, supported by estimated values of the Damköhler number, lead to the conclusion that desorption kinetics are important for evaluating the significance of dissolved and particle–facilitated transport of glyphosate. To quantify particle–facilitated glyphosate transport, the water and solid phases in the leachate should consequently be separated within a few minutes after leaching.

Materials and methods

Experimental Setup

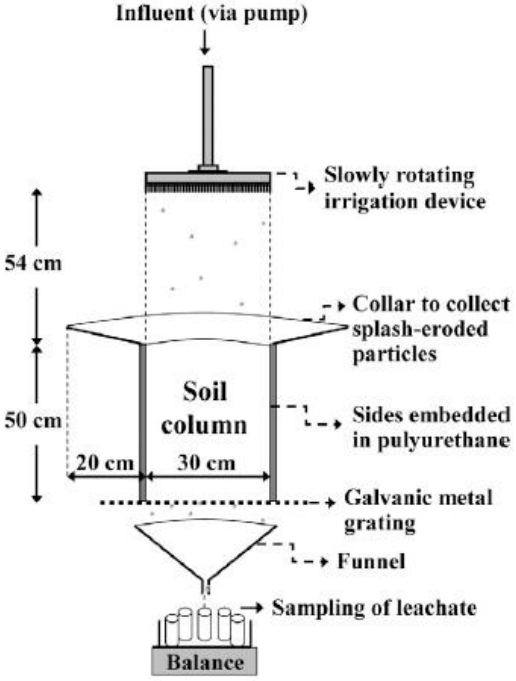
The investigated sandy loam soil and parts of the experimental setup have previously been described in detail by Gjettermann et al. (2009), who focused on the influence of soil structure on particle–facilitated pesticide leaching. Two undisturbed cylindrical soil columns (B1 and B2) were carefully excavated from a recently tilled (plowed and drilled) experimental plot, encapsulated with polyurethane foam, and trimmed at the bottom end (50–cm cylinder length, 30–cm diameter). As reference, two columns (A1 and A2) were excavated from an untilled plot. The coding of the columns used in the glyphosate experiments by Gjettermann et al. (2009) has been kept to facilitate comparison. Particular care was observed not to disturb the surfaces of the columns or to block large macropores while trimming the bottom ends. The investigated sandy loam soil (an Agrudalf) is located at the University of Copenhagen

research farm Roerrendegaard at Taastrup, Denmark, and has previously been described by Petersen et al. (2001).

The contents of coarse sand (200–2000 μm), fine sand (20–200 μm), silt (2–20 μm), clay (<2 μm), and organic C in the upper 30 cm were 29, 40, 18.5, 12.5, and 1.2 %, respectively.

A schematic presentation of the experimental setup is given in Figure 8.1.3.1-1. All irrigation water applied to the columns (influent) had a composition similar to rainwater (Miljøstyrelsen, 1996) containing 0.017 mmol/L CaCO3, 0.018 mmol/L KNO3, 0.021 mmol/L MgSO4, 0.126 mmol/L NaCl, and 0.94 mmol/L NH4Cl. The pH was 6.32 and the electrical conductivity was 0.047 mS/cm. Water was applied to the column with a pump (FMI Pump QG 150, Fluid Metering Inc., Syosset, NY) through a motor–driven, slowly rotating sprinkling device with 90 syringe needles (Trumo, 25G), to ensure a uniform application rate of 15.0 mm/h (1.06 L/h). The sprinkling device was placed 54 cm above the surface of the column. The drop size was determined at the used intensity by sampling and weighing about 20 drops (10 repetitions). The mass of a drop was 6.4 ± 0.5 mg, corresponding to a (spherical) drop diameter of 2.3 ± 0.9 mm.

Figure 8.1.3.1-1: Schematic illustration of the experimental setup for the leaching experiments and for the collection of splash–eroded soil particles



The columns were rewetted at the start of the experiment by irrigation, which was stopped 35 min after the first appearance of leachate. One day later, 14C–labeled glyphosate mixed with the commercial glyphosate product Roundup Bio (Monsanto Europe, Antwerp, Belgium) was applied uniformly to the surface. The glyphosate stock solution had a specific concentration activity of 0.80 MBq/mg and contained 4.4 % 14C–labeled glyphosate, 93.5 % unlabeled glyphosate, and 2.1 % aminomethylphosphonic acid (AMPA). The applied dose of glyphosate (12.5 mg/column) was comparable to current agricultural practice. A total of 13 mL of solution (stock solution and rinse water) was applied during the glyphosate application.

Three irrigations were applied to the soil columns 5, 8, and 12 d after rewetting, respectively. Each event lasted 2.0 h and had a constant intensity of 15.0 mm/h. Water drained freely from the column during and after each irrigation event. The mass of drainage water (leachate) was measured continuously. The leachate was sampled continuously, yielding a total of 21 samples, each containing 30 to 50 mL of leachate. The top ends of the columns were covered with plastic whenever possible to minimize evaporation.

A new plastic collar was mounted around the tops of the columns before each irrigation event to collect water splashes and soil particles (splash–eroded particles) that were eroded by drops and thrown over the sides (height up to 1 cm above the soil surface) with the droplets. All of these water droplets were collected on the collar. The water droplets generally evaporated within a few hours. The particles left were scraped off the collar 24 h after the irrigation event, allowed to air dry, and sieved through a 100-µm sieve. The mass of air–dry particles <100 µm was determined. This material was used for the desorption experiments.

Measurements

The 14C activity of unfiltered and filtered leachate samples was measured with a Wallac 1414 (Perkin Elmer Corp., Waltham, MA) liquid scintillation counter (LSC) using 10 mL of scintillation cocktail (InstaGel, PerkinElmer) to a 9–mL sample. Using 14C–labeled pesticides has the consequence that also metabolites, for example the major metabolite of 14C–glyphosate (14C–AMPA) were measured by LSC.

The detection limit of the 14C LSC analysis was 16.4 disintegrations min−1 (0.038 µg 14C–glyphosate/L) and the method had trueness for quantification on 14C standard buttons (PerkinElmer) of 100.2 ± 0.8 %.

The effect of quenching was automatically adjusted by the LSC, and increasing quench induced by increasing particle concentration was accurately measured. Gjettermann et al. (2009) found good agreement between these determinations and direct chemical measurements of glyphosate plus AMPA, and they showed that AMPA constituted only a minor part (up to 17.5 %) in leachate samples from the investigated columns.

Particle concentration in the leachate was determined indirectly from the measured turbidity. Turbidity was measured with a turbidity meter (Tintometer GmbH, Dortmund, Germany). Samples were shaken and immediately transferred to glass vials. Turbidity was then measured after exactly 60 s. With the chosen procedure, isolated soil particles were <30 to 50 µm (equivalent spherical diameter), assuming a particle density of 1600 to 2650 kg/m3. The mass of particles was estimated in 70 randomly selected leachate samples of known volume to establish a relationship between turbidity and concentration of particles. The samples were centrifuged (30 min at 4100 × g) and washed twice with deionized water. Finally, the particles were dried at 105°C before determining the mass. The correlation between turbidity T (in nephelometric turbidity units [NTU]) and the concentration of soil particles in the leachate was used to calculate the concentration of particles: concentration of particles (mg/L) = 110 ln(T) − 241 (R2 = 0.74, 70 samples). For turbidity <20 NTU, equivalent to particle concentrations of less than approximately 88 mg/L, the relationship was poor and this limit was therefore used as the detection limit.

Desorption was investigated in five leachate samples from each of the tilled soil columns (Samples 2, 8, and 14 from the first irrigation event and Sample 21 from the other two events; see Table 8.1.3.1-27). The samples were selected to illustrate the development in glyphosate levels during the three irrigation events. Only one leachate sample (Sample 2, first irrigation) from each of the untilled columns was investigated, the amount of sediment being too small (considerably below the detection limit) and the uncertainty of the determination on individual samples too high during the later phases of the drainage events (Gjettermann et al., 2009). Approximately 40 mL of leachate sample was collected and a stopwatch was activated. A 10–mL sample was immediately filtered (0.02–µm inorganic, anopore filter, Frisenette, Knebel, Denmark) into a clean glass and the time (about 1.5 min) was recorded. Nine milliliters of the filtrate was later extracted for 14C–activity measurement. After 5 min, another 10 mL of leachate was extracted and filtered for activity measurement. This was repeated after another 5 to 10 min and, if the amount of the original sample allowed it, after approximately 30 min. The so–called reaction time associated with each filtration, tr, was assigned as the time span from the midpoint [(tbeg + tend)/2] of the sampling interval to when filtration had just been performed.

The concentrations of soil particles in the leachate used for the desorption experiments were not measured but estimated as the average of measured concentrations in the directly preceding and succeeding samples (first irrigation) or as the concentration measured in the directly preceding sample (second and third irrigations). The sample size did not allow combined determination of both particle concentration and desorption, and larger samples would have compromised the need for fast separation

of colloids from the water phase. The uncertainty associated with this procedure was estimated from concentration difference between consecutive samples measured by Gjettermann et al. (2009), the absolute average concentration difference being assigned as D.

Detectable splash erosion occurred from both of the tilled columns (B) in all events, but not from the untilled columns (A). Sieved and air–dried, splash–eroded particles generated during each irrigation event from the tilled columns were immersed (at time zero) in stirred artificial rainwater (irrigation water) yielding a suspended particle concentration of Cparticle = 100 mg/L. Samples of 10 mL were extracted and filtered, and the 14C activity of the filtered samples was determined five or six times, typically 2.0, 10.0, 60, 120, 1440, and 2880 min after immersion (equivalent to tr) as described above for the leachate.

Table 8.1.3.1-27: Overview of analyses conducted on leachate samples from each of the three irrigation events

Sample no.	First irrigation	Second and third irrigation
1	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
2	sorption and desorption kinetics of pesticide	total concentration of pesticide, colloidal and soluble organic c, ph, conductivity and turbidity.
3–7	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
8	sorption and desorption kinetics of pesticide	total concentration of pesticide, colloidal and soluble organic c, ph, conductivity and turbidity.
9–13	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
14	sorption and desorption kinetics of pesticide	total concentration of pesticide, colloidal and soluble organic c, ph, conductivity and turbidity.
15–20	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
21		sorption and desorption kinetics of pesticide

Data Analyses

The specific activity of the applied glyphosate stock solution was checked by LSC analysis at each application time. The measured specific activity was used to convert the measured 14C activity into glyphosate concentration units. The concentration of particle–bound glyphosate (Cp) in the leachate was defined as the difference between the measured concentration in the suspension (total concentration, Cs) and the measured concentration in the filtrate (dissolved glyphosate, Cd). The particle–bound fraction of leached glyphosate was calculated as Cp/Cs. Rates of change of the particle–bound fraction were estimated by least squares linear regression, i.e., from fitting experimental data to the simple approach presented by

[1]

$$\frac{100C_p}{C_s} = -\alpha t_r + \beta$$

where the rate α (% min−1) and β (%) are constants (positive α values indicate desorption) and tr is the time of reaction (min).

Equation [1] was also fitted to the first data points from experiments with splash–eroded particles in an attempt to obtain similar time scales of desorption from the different sampling types of particles (leached and splash eroded). In this analysis, Cs was obtained as the sum of particle–bound and dissolved concentrations at equilibrium (Cp,eq and Cd,eq, respectively), and Cp was calculated as the difference between Cs and Cd.

At long experimental periods (0 <tr ≤48 h), however, the desorption from splash–eroded particles was not linear and the points were therefore also described according to

[2]

$$\frac{dC_d}{dt} = -k\left(C_d - C_{d,eq}\right)$$

where Cd (µg/L) is the dissolved concentration (<20 nm), t is the time (h), Cd,eq (µg/L) is the "equilibrium" dissolved concentration, and k is a rate constant (h–1). This approach has been termed a linear driving force approximation (LeVan et al., 1997) or a first–order mass transfer model (Lick et al., 1997). Assuming that Cd at time t = 0 h when the particles were suspended [Cd(0)] = 0, Eq. [2] can be integrated into

[3]

$$C_d = C_{d,eq}\left[1 - \exp\left(-kt_r\right)\right]$$

where tr is the time of reaction. The Solver function in Excel (Wraith and Or, 1998) was used to adjust the model parameters Cd,eq and k by minimizing the difference between predicted and measured Cd values (maximizing R2). Because the chemical or physical processes involved in the desorption are expected to be similar in the two data sets, the choice of a linear vs. an exponential model is based solely on the number of data points available and the time scale used to observe the different particles.

For the splash–eroded particles, the total content of glyphosate in the sample was not measured (the results had to be discarded due to an error in the laboratory). It therefore had to be estimated. Gjettermann et al. (2009) reported a Kd value of 503 L/kg for the bulk topsoil (and 496 L/kg for AMPA). It has previously been shown that particles larger than about 0.1 mm are not present in drainage from the investigated field site (Holm et al., 2003), indicating that coarse sand and parts of the fi ne sand fraction either are not mobile or are immobilized on the way through the soil column. For this soil, it may generally be expected that the Fe and Al oxides that sorb glyphosate is mainly present in the fraction <20 µm. Hence, Kd for the investigated leached particles will be larger than that for the bulk soil. Based on the texture of the topsoil, 40 % of the constituents were >0.100 mm. Hence, an estimate of Kd was obtained as 503 L/kg / 0.60 = 8.4 × 102 L/kg. This is a conservative estimate because it assumes no sorting of particles below the 0.1–mm limit within the soil columns. Estimates of the concentration of particle–bound glyphosate at equilibrium, Cp,eq (µg/L) were obtained from the fitted Cd,eq, the soil/water ratio (particle concentration Cparticle, kg/L), and Kd as Cp,eq = CparticleKdCd,eq. Hence, in the absence of direct measurements, the total glyphosate concentration was calculated as

[4]

$$\begin{aligned} C_s &= C_{d,eq} + C_{p,eq} \\ &= C_{d,eq}\left(1 + C_{particle}K_d\right) \end{aligned}$$

The Damköhler number, Da, is a measure of the relative importance of kinetics to equilibrium processes in transport (Bold et al., 2003). The Da is defined as the ratio between the transport and the reaction time scales, and can be calculated as

[5]

$$D_a = \frac{k}{\left(U/L\right)}$$

where L (cm) is the transport distance (e.g., length of column, cm) and U (cm/h) is the water velocity in the soil.

Results and Discussion

Desorption in Leachate from Tilled Soil

Measured dissolved glyphosate concentrations in the leachate from the tilled soil generally increased with time (Figure 8.1.3.1-2), and the particle–bound fraction decreased (Figure 8.1.3.1-3). Thus, one immediate finding of the experiment is that considerable amounts of glyphosate desorbed from leached soil particles (>20 nm) during the investigated period (about 20 min). Desorption was particularly large for the first irrigation on Column B1 (Figure 8.1.3.1-2), probably reflecting leaching of highly pesticide–enriched particles. Thus, the initially (about 1.5 min after sampling) measured concentration of

glyphosate on particles was 19 to 24 mg/kg for this irrigation event, while it was between 7.7 and 3.5 mg/kg for the other irrigations on Column B1 and all irrigations on Column B2. The data do not indicate that concentrations of dissolved glyphosate reached stable levels.

The concentration of leached particles, Cparticle (Table 8.1.3.1-28), could be a critical factor for the α values describing glyphosate desorption. Higher concentrations of particles should result in lower desorption rates due to a higher final equilibrium value (c.f. Eq. [4]). The particle concentrations showed little variation from sample to sample within events (Table 8.1.3.1-28, D ≤24 mg/L), although it often varied significantly from the beginning to the end of an irrigation event (Gjettermann et al., 2009). Thus, the uncertainty associated with the estimated Cparticle values in Table 8.1.3.1-28 is probably on the order of 24 mg/L or less. The concentrations ranged between 123 and 292 mg/L and were higher for Column B1 than for Column B2. The expected dissolved mass fraction at equilibrium, Cd,eq/Cs can be estimated by rearranging Eq. [4] and inserting the measured particle concentrations from Table 8.1.3.1-28. According to this calculation, the mass of sorbed glyphosate at equilibrium in the leached samples will account for 20 % or less of the mass in solution. Hence, with the investigated range of particle concentrations and the high initial fractions of particle–bound glyphosate (Figure 8.1.3.1-3), the samples are far from equilibrium and particle concentrations should not be important for the relative amount of desorbed pesticide or the desorption rates.

Figure 8.1.3.1-2: Concentration of dissolved glyphosate (Cd) in leachates from two soil columns, B1 (left) and B2 (right), at different reaction times (tr, 0–30 min): (a) and (b) data for the first irrigation event (Samples 2, 8, and 14); (c) and (d) data for the second and third irrigation events (Sample 21)

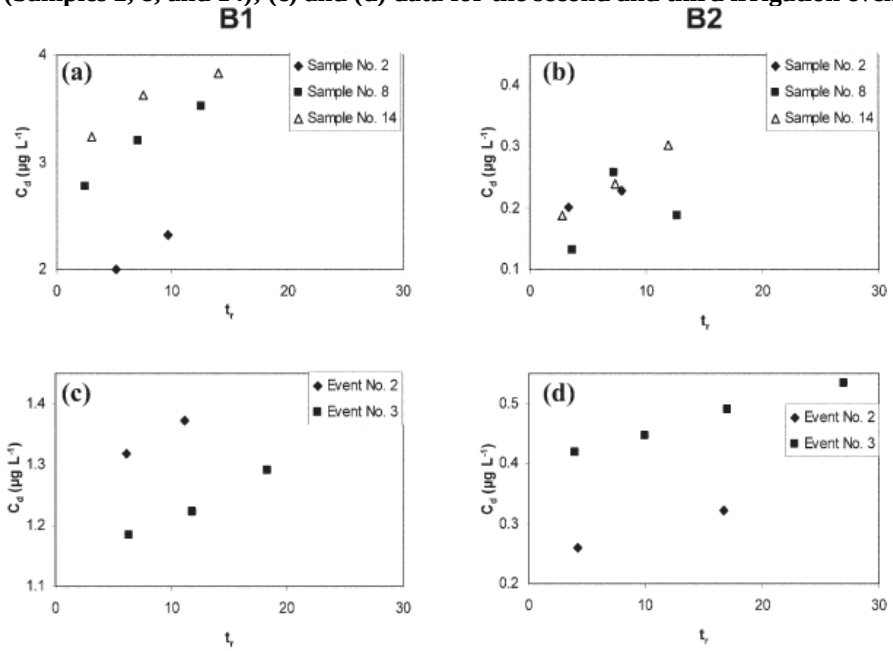


Table 8.1.3.1-28: Relative desorption rate (α), intercept parameter (β), concentration of soil particles (Cparticle) in the investigated leachate, and average absolute particle concentration difference between consecutive samples (D) derived from experiments on leachates; α, β, and coefficient of determination (R2) obtained by fitting data from different soil columns (B1, B2, A1, and A2), irrigation events (1–3), and samples (2, 8, 14, and 21) to Eq. [1]

Column	Irrigation no.	Sample no.	α	β	R^2†	$C_{particle}$	D
			% min^{-1}	%		— mg L^{-1} —	
B1	1	2	0.94	78		292	
	1	8	0.80	72	0.98	276	9 (6)‡
	1	14	0.56	67	0.92	268	
	2	21	0.41	53		212	13 (19)
	3	21	0.33	58	0.99	263	6 (5)
B2	1	2	0.48	85		199	
	1	8	0.39	87	0.11	134	24 (20)
	1	14	0.98	89	1.00	151	
	2	21	0.69	67		123	14 (13)
	3	21	0.51	60	0.99	166	11 (13)
A1	1	2	0.37	21		264	23(21)
A2	1	2	−1.03	8	1.00	190	21(26)

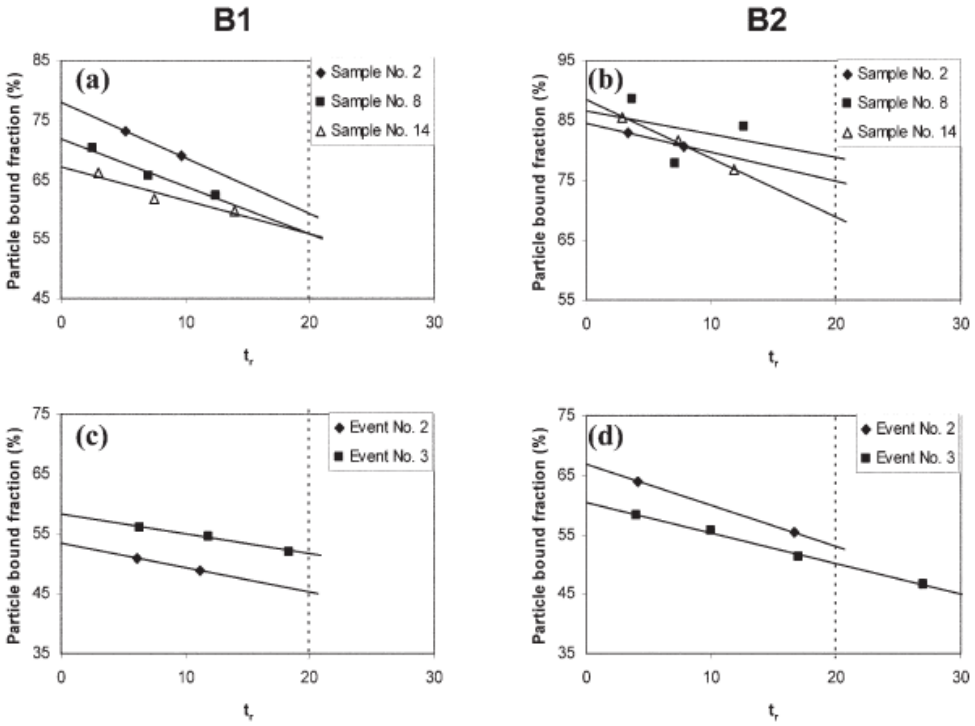
† Not applicable when the number of samples was <3.
‡ Standard deviation in brackets ($14 < n < 21$).

In general, Eq. [1] fitted well to the measured fractions of particle–bound glyphosate (Figure 8.1.3.1-3). The coefficients of determination were high (R2 ≥0.92), except for Sample 8 from the first irrigation on Column B2 (Table 8.1.3.1-28). By using this equation with the parameter values from Table 8.1.3.1-28, it was estimated that 7 to 20 % (on average, 12 %) of the leached glyphosate was desorbed from soil particles (>20 nm) within the first 20 min after sampling, corresponding approximately to the time scale of the observations. The reaction time (tr) associated with the first filtration varied somewhat between events due to differing lengths of the sampling intervals. The 20–min relative desorption may be overestimated if the last measureements were close to the equilibrium concentrations (which was probably not the case according to the above calculations), and it may be underestimated if desorption took place much faster before the first filtrations (1.5 min after sampling).

The reaction time, defined as the time from the midpoint of the sampling interval, can be considered as an estimate of the time span after leaching. Hence, an estimate of the particle–bound fraction of glyphosate at a given time after leaching can be obtained from Eq. [1]. The data indicate that 45 to 79 % of the leached glyphosate was still particle bound 20 min after leaching. Thus, the rates of desorption measured shortly after sampling could not fully account for the amounts of glyphosate being desorbed 20 min after leaching.

The particle–bound fraction measure in leachate from the tilled soil 1.5 min after sampling varied between 51 and 89 % (Figure 8.1.3.1-3). This is in accordance with results reported by Gjettermann et al. (2009). It is probable that such figures depend considerably on the conditions that eventually lead to bypass flow and leaching. The applied methods were chosen to minimize desorption in the leachate before sampling and phase separation.

Figure 8.1.3.1-3: Particle (>20–nm) bound fraction of glyphosate in leachates from two soil columns, B1 (left) and B2 (right), at different reaction times (tr, 0–30 min): (a) and (b) data for the first irrigation event (Samples 2, 8, and 14); (c) and (d) data for the second and third irrigation events (Sample 21)



The particle–bound fractions of glyphosate measured in the leachate from the two untilled soil columns (A1 and A2) 1.5 min after sampling were 19 and 14 %, respectively. This conforms to previously reported results that the fraction of particle–bound glyphosate in recently produced leachate can be much smalller with a minimally disturbed soil structure than with a tilled structure (Gjettermann et al., 2009). The particle–bound fraction decreased with time after sampling in the A1 sample, indicating desorption, whereas it increased in the A2 sample, indicating sorption (Table 8.1.3.1-28). By inserting the estimated Kd (= 8.4 × 102 L/kg) in Eq. [4], the fractions of particle–bound glyphosate at equilibrium were estimated to be 18 and 14 % for the A1 and A2 samples, respectively. Hence, leached glyphosate from the untilled soil columns appears to have been close to equilibrium, which is probably why both sorption and desorption may have occurred, as indicated by the measurements.

The individual desorption rates are relatively uncertain, being based on only two to four measured particle–bound fractions (Figure 8.1.3.1-3). More observations could have been obtained, but only if the sample sizes had been increased correspondingly. This would have increased the time of reaction and hence desorption taking place before the measurements. The trends observed are similar for all samples, however, corroborating the conclusion that the particle–bound glyphosate in the solution leaching from the tilled columns was not in equilibrium with the surrounding water phase.

Desorption from Splash–Eroded Particles

Noticeable splash erosion occurred during all irrigation events involving the tilled columns. The amounts of (air–dry) splash–eroded particles varied between 31 and 70 mg per event independent of irrigation number and column. All fine–earth particle sizes were present, in accordance with earlier findings that eroded material is typically unsorted (Heilig et al., 2001; Hairsine and Rose, 1991; Al–Durrah and Bradford, 1982). Larger particles were removed by using the 100–µm sieve in consequence of the earlier reported finding that particles smaller than about 0.1 mm are not present in drainage water from the investigated field site (Holm et al., 2003). The fine particles released considerable amounts of glyphosate after being suspended. Hence, dissolved glyphosate concentrations increased with time, with gradually decreasing rates (Figure 8.1.3.1-4 a and b). The rates were still relatively high after 1 h. After a few hours, concentrations were high compared with concentrations measured in most leachates (Figure 8.1.3.1-2), except from the first irrigation on Column B1. An equilibrium concentration of dissolved glyphosate appeared to be reached after about 5 to 10 h, except for the first irrigation event on Column

B2; equilibrium was not attained within 48 h in this case. Glyphosate desorption decreased successively with irrigation event number.

Equation [3] fitted well to the measured dissolved concentration as a function of time (0–48 h) (Figure 8.1.3.1-4 a and b; Table 8.1.3.1-29). Coefficients of determination, R2, varied between 0.87 and 0.98. The rate constant of desorption, k, was found to be in the range 0.57 to 1.19 h−1, largest for the first irrigation event on Column B1. The equilibrium concentration, Cd,eq, was in the range 1.56 to 4.1 µg/L, decreasing successively with each additional irrigation event.

Table 8.1.3.1-29: Parameters and key data derived from experiments on splash-eroded particles. Rate constants (k), dissolved concentrations at equilibrium (Cd,eq), and coefficient of determination (R2) obtained by fitting Eq. [3] to all data (reaction time tr 0–48 h) from the two columns (B1 and B2) and three irrigation events. Relative desorption rate (α), intercept parameter (β), and R2 obtained by fitting Eq. [1] to data for relatively short time scales: results based on the first three data points (tr = 2, 10, and 60 min) and desorption rate based on the first two data points (tr = 2 and 10 min)

Column	Irrigation no.	From Eq. [3], 0–48 h			From Eq. [1] 2–60 min			From Eq. [1] 2–10 min
		k	$C_{d,eq}$	R^2	α	β	R^2	α
		h^{-1}	µg L^{-1}		% min^{-1}	%		% min^{-1}
B1	1	1.19	4.0	0.98	0.56	71	0.94	1.62
	2	0.75	2.19	0.91	0.54	79	0.98	1.09
	3	0.59	1.83	0.94	0.48	82	0.98	1.06
B2	1	0.57	4.1	0.92	0.41	81	0.99	0.79
	2	0.58	2.59	0.95	0.55	87	1.00	0.40
	3	0.99	1.56	0.87	0.50	73	0.98	1.10

The equilibrium concentrations, Cd,eq, the previously estimated Kd = 8.4 × 102 L/kg, and the constant particle concentration Cparticle = 100 × 10−6 kg/L were used when calculating total concentrations (Eq. [4]), particle–bound fractions, and relative desorption rates (Eq. [1]). Accordingly, the fitted dissolved concentrations at equilibrium (Table 8.1.3.1-29) represent about 92 % of the total concentrations. The linear relationship Eq. [1] fitted well to the three data points representing the particle-bound fraction vs. time (2 to 60 min) after dissolving the splash–eroded particles, R2 being in the range 0.94 to 1.00 (Figure 8.1.3.1-4 c and d; Table 8.1.3.1-29). The relative desorption rates (α) were estimated to be in the range 0.41 to 0.56 % min−1 (average 0.51 % min−1), with no systematic dependence on irrigation event or column (Table 8.1.3.1-29). Hence, at these rates, 8 to 11 % (average 10 %) would be desorbed during a 20–min time period. The rates tended to be slightly smaller than desorption rates measured in the leachate at a similar or somewhat shorter time scale (α for Columns B1 and B2 in Table 8.1.3.1-28, average value 0.61 % min−1). For the period 2 to 10 min, the measured relative desorption rates were in the range 0.40 to 1.62 % min−1 (Table 8.1.3.1-29; average value 1.01 % min−1), i.e., generally somewhat larger than for the period 2 to 60 min. This was expected also from the good fit of all the data to Eq. [3]. The rates obtained for the 2– to 10–min period were of the same order of magnitude, although generally larger than desorption rates measured in the leachate at a similar or somewhat longer time scale (α for Columns B1 and B2 in Table 8.1.3.1-28). Calculated from rates obtained for the 2– to 10–min period, 8 to 32 % (average 20 %) would desorb in 20 min right after the first fractionation. Overall, similar desorption rates were found for leached and splash–eroded particles when determined at similar time scales. This indicates that similar desorption processes were involved for the two types of particles.

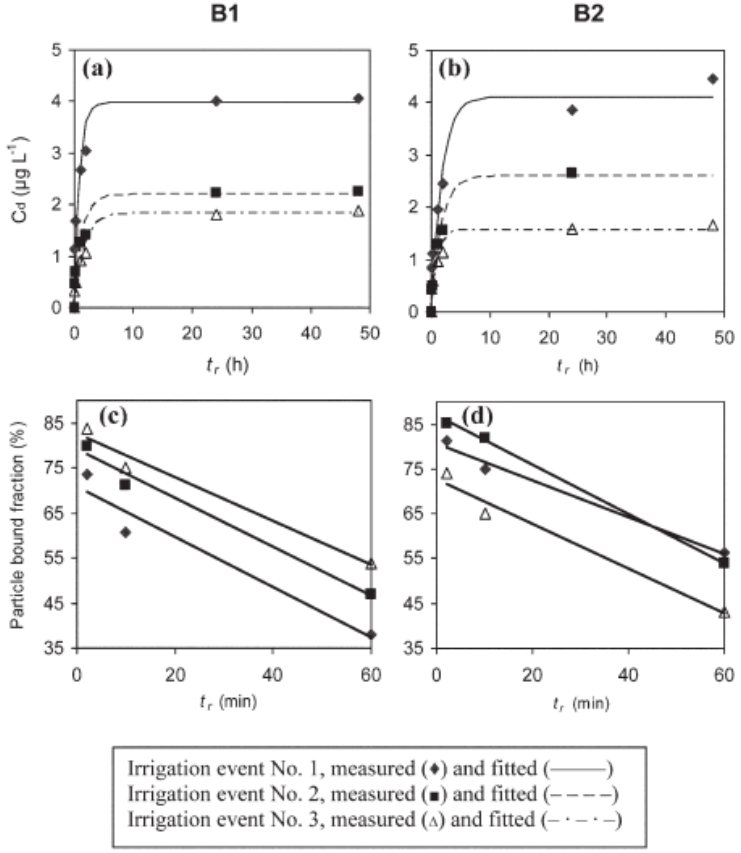
The initial glyphosate concentrations (mg/kg) were somewhat higher on splash–eroded particles than on leached particles. For the first measurements on leached particles made 1.5 min after sampling, the range of concentrations was 4 to 24 mg/kg; for measurements on splash–eroded particles made 2.0 min after immersion, the range of estimated concentrations was 13 to 36 mg/kg. The concentrations decreased systematically with succeeding irrigation event for both types of particles. The splash–eroded particles may have been enriched with glyphosate when the water droplets evaporated on the collar after irrigation; however, concentrations on splash–eroded particles from the first irrigations (about 44 mg/kg according to Table 8.1.3.1-29 and Eq. [4]) were within a realistic range for the uppermost soil layer shortly after spraying. Thus, by assuming that the applied glyphosate was distributed in the uppermost

2– to 5–mm soil layer having a bulk density of 1.6 g/cm3, an expected average glyphosate concentration of 55 to 22 mg/kg can be calculated for the layer.

The splash–eroded particles were air dry, and the particle–bound fraction of the glyphosate must therefore have been close to 100 % right before the particles were immersed in water (at tr = 0). At tr = 2.00 min, however, the particle–bound fraction had already decreased to between 74 and 85 % (Figure 8.1.3.1-4 c and d). It is difficult to model this very rapid decrease as a function of time when seen at the shorter time scales. It indicates that a fraction (up to 26 %) of the glyphosate could have been very weakly bound. Physical effects of the immersion may also have affected the rapid glyphosate release.

From the linear models shown in Figure 8.1.3.1-4 c and d, it can be calculated that about 60 to 76 % of the glyphosate was still particle bound 20 min after immersion of the particles in water. The values are of a similar magnitude as the estimated particle–bound fractions in the leachate 20 min after leaching (45–79 %, cf. above). In the study on leachate, it is probable that desorption had taken place in wet fractions of the soil columns before leaching and from leached particles in the leachate before the first fractionation. This may to some extent have reduced the initially measured fraction of particle–bound glyphosate and the measured desorption rates.

Figure 8.1.3.1-4: Desorption of glyphosate in suspensions containing splash–eroded soil particles from two soil columns, B1 (left) and B2 (right): (a) and (b) concentration of dissolved glyphosate (Cd) monitored at long time scales of reaction (0 <tr ≤48 h), the curves represent least squares fits of Eq. [3] to data points; (c) and (d) particle–bound fraction monitored at short time scales (2–60 min), the lines represent least squares fits of Eq. [1] to data points



Can Desorption Kinetics be Ignored in Glyphosate Transport?

Bold et al. (2003) investigated the significance of kinetics in contaminant transport using an intraparticle diffusion model to account for the kinetic contaminant–particle interaction. They showed by sensitivity analysis that kinetic limitations of contaminant–particle interactions have to be taken into account for 0.01 <Da <100. They also concluded that for Da <0.01, desorption of contaminants from particles is so slow that it can be neglected.

A range of possible outcomes of Da for the present column experiments was estimated based on desorption rate coefficients obtained in the 0– to 48–h experiments on splash–eroded particles (k values). The fluid velocity inside the column depends on whether it is moving through macropores or the matrix. Two extreme boundaries could be: (i) transport exclusively through a water–filled continuous macropore from the surface to the bottom of the column, and (ii) transport exclusively thought the soil matrix. For extreme (i), a continuous macropore with a diameter of 0.6 cm (area = 0.28 cm2), and steady–state condition, the irrigating rate (1060 cm3/h) would give rise to an average fluid velocity of approximately 3700 cm/h. For extreme (ii), a homogeneous soil matrix (column area = 707 cm2), steady-state condition, and a water content equal to field capacity (about 30 %), the irrigation would give rise to an average fluid velocity of approximately 5 cm/h. For extreme (i), the Da would be in the range of 0.01 to 0.02 (cf. Eq. [4]), depending on the value of k. For extreme (ii), the Da would be in the range of 6 to 12. These intervals, even the one for homogeneous matrix flow, are within the critical range estimated by Bold et al. (2003), indicating that kinetic limitations of glyphosate–particle interactions have to be taken into account in describing the transport. In reality, bypass flow and glyphosate transport below the 25–cm depth took place almost exclusively in earthworm channels in the size range 2 to 8 mm (Gjettermann et al., 2009), indicating conditions much closer to extreme (i) than (ii). Although we realize that the measured rates are not necessarily representative of the conditions throughout the soil columns, the results of this analysis indicate that particle mobilization and particle–facilitated transport could play a critical role in pesticide leaching under such conditions.

The interaction between contaminants and mobile particles has, in many studies, been described as an instantaneous equilibrium process (e.g., Prechtel et al., 2002; Villholth et al., 2000). To our knowledge, no study has described the importance of desorption kinetic behavior of contaminants in structured soil with special attention to facilitated transport. Turner et al. (2006), however, revealed that Cs desorption from illite particles was slower than Sr desorption and demonstrated that this difference in desorption kinetics resulted in greater colloid–facilitated transport of Cs in columns packed with a quartz porous medium. They estimated Da to be in the range of 0.00035 to 0.086 for Cs and 0.97 to 2.0 for Sr. Van de Weerd and Leijnse (1997) also found that desorption of Am from humic particles was a slow process that could only be described by taking into account a kinetic interaction between Am and humic particles. These findings combined with the current investigations show that it is important to consider desorption kinetics as an integral part of the transport process when considering particle–facilitated transport of glyphosate and other non-instantaneously desorbing contaminants.

Conclusion

Glyphosate desorbed with similar fractional rates from leached and from splash–eroded particles (>20 nm) when investigated at similar relatively short time scales. Thus, 7 to 20 % of the total amount of leached glyphosate (average 12 %) desorbed in 20 min shortly after leaching, while on average between 10 and 20 % desorbed from splash-eroded soil particles in suspension in 20 min shortly after immersion. The similarities support the view that the particles investigated and the processes of desorption were similar for the two types of material. Concentrations of glyphosate on leached particles were always somewhat lower than concentrations on splash–eroded particles.

Equilibrium concentrations were generally obtained within 5 to 10 h in suspensions containing splash–eroded particles. Hence, depending on the time of fractionation of the collected samples (in the interval 0–10 h), very different relative amounts of particle–bound glyphosate may be found; to quantify particle–facilitated glyphosate transport, the water and solid phases should be separated immediately after leaching. Furthermore, an analysis of the Damköhler number indicates that desorption kinetics is important for glyphosate transport and for the significance of particle–facilitated transport.

Assessment and conclusion by applicant:

The article describes a leaching experiment with glyphosate in soil columns. The desorption of glyphosate from soil particles and its effect on interpretation of leaching experiments was in the focus of the study and desorption kinetics of particle-bound glyphosate are postulated to influence glyphosate transport strongly. Not all necessary information was reported to check the validity of the results (no mass balances, study set-up not clearly described, insufficient information on soil

properties and soil origin, test item not sufficiently described, temperature not provided, molecular identity of desorbed radioactivity not determined).

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

The article essentially focused on the desorption of glyphosate after a column leaching experiment. As noted by the applicant, the article lacks details on the method and experimental set up. In addition, it is noted that the conditions of irrigation of the columns (3 irrigations at 5, 8 and 12 days, each event lasted 2 h with an intensity of 15 mm/h) are not in agreement with the ones recommended in the OECD 312 (200 mm articifical rain over 48 h).

The article provides supportive information on the leaching of glyphosate, but no reliable endpoints can be derived for use in risk assessment

Gjettermann et al., 2011

Data point:	CA 7.1.4.1.1/009
Report author	Gjettermann, B. et al.
Report year	2011
Report title	Evaluation of Sampling Strategies for Pesticides in a Macroporous Sandy Loam Soil
Document No	DOI 10.1080/15320383.2011.620049 E-ISSN 1549-7887
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

It is not straightforward to sample and demonstrate the presence and transport of pesticides in heterogeneous soil. Following leaching experiments with four differently structured 50–cm–long soil columns (tilled and untilled soil), the objective of this study was to investigate the extent that visual tracing of the dye Brilliant Blue could support in soil sampling for two strongly sorbing pesticides (14C–labeled glyphosate and pendimethalin). About 830 samples were collected. No pesticide was found below 10–25 cm depth by random sampling, even though 0.21–0.31 % of the applied amounts were leached, and 0.18 % of the soil volume was sampled. With similar sampling efforts, the pesticides could generally be traced throughout the columns by sampling from stained soil volumes, only. None of the two particular sampling strategies for pesticides produced accurate mass balances or balances that were obviously better than the other. No pesticide was detected outside stained soil volumes, except for glyphosate in one sample. Below 30 cm, stained soil comprized on average 5 % of the total soil volume, leaving 95 % as expectedly pesticide–free. The results suggest that much more efficient sampling for sorbing pesticides can be obtained by using the dye and focusing on stained soil volumes.

Materials and methods

Soil Columns

The macroporous Rorrendegaard sandy loam soil investigated in this experiment is developed on till from the Weichselian glaciation. The contents of coarse sand (200–2000 µm), fine sand (20–200 µm), silt (2–20 µm), and clay (<2 µm) in the upper 30 cm is 29 %, 40 %, 18.5 %, and 12.5 %, respectively,

and the organic C content is 1.2 %. At 50 cm depth, the number of vertically oriented earthworm channels (diameter: 3–8 mm) is typically in the range 200–600 m−2. The soil has previously been described in detail by Petersen et al. (2001) and Gjettermann et al. (2009).

Undisturbed soil columns (diameter: 30 cm; 0–60 cm soil depth) were sampled in late autumn from two experimental plots with different tillage treatments (A and B). For each of the previous nine years the same cereal crop (wheat or barley) had been grown in the plots. Plot A had not been tilled for one year, and it had not been subjected to deep (>4–6 cm), loosening tillage for eight years. Plot B had been under traditional tillage (including annual ploughing) for at least nine years. It had been ploughed and drilled for wheat one month before sampling, and a new wheat crop had just been established. Treatment A (untilled) gave rise to a relatively stable soil structure with vertically oriented earthworm channels from the partially covered surface (old wheat stubble, weeds, and moss) to the bottom of the columns. Treatment B (tilled) did result in a more variable structure with stubbles being heterogeneously incorporated in the plough layer (0–25 cm). Fewer vertically oriented earthworm channels penetrated all the way to the surface. Columns (two per treatment) were manually excavated and encapsulated with polyurethane to stabilize and seal the walls. Care was observed not to disturb the surface structure. The columns were trimmed to 50 cm length from the bottom end, avoiding sealing macropores, and placed on a galvanized metal grating. The columns were sealed with plastic foil at the upper end and stored at 2–3°C whenever not used in the experiments.

Leaching Experiments

The leaching experiments have been described in detail by Gjettermann et al. (2009) and were in brief as follows: Columns were rewetted by irrigation one day before pesticide application. Each pesticide was applied uniformly to the surface of one column per treatment. The pesticides were applied in doses similar to the ones used in agriculture, i.e. 12.51 mg glyphosate/column and 14.24 mg pendimethalin/column. Glyphosate was taken from a stock solution made from the commercial product Roundup Bio, 14C–glyphosate, and blank formulation (all from Monsanto). The stock solution had a specific concentration activity of 0.80 MBq/mg. It contained both glyphosate and its major metabolite, AMPA (2.034 g/L in total) distributed on 14C–glyphosate (4.4 %), unlabeled glyphosate (93.5 %), and AMPA (2.1 %). Pendimethalin was taken from another stock solution made from the commercial product Stomp mixed with 14C–pendimethalin (both from BASF). This stock solution had a specific concentration activity of 4.55 MBq/mg. It contained 14C–pendimethalin (4.2 %) and unlabeled pendimethalin (95.8 %).

Leaching was driven by irrigation water having a composition similar to rain water (Gjettermann et al., 2009). The water was applied uniformly at a fixed intensity (15 mm/h) to the top of the columns through a rotating irrigation device. Each column received three 2.0 hours irrigation events 5, 8, and 12 days after rewetting, respectively. Thus 30 mm of irrigation water was applied in each event, corresponding approximately to 7.6 % of the total soil pore volume. A 15 mm/h rain event in 2 h may be considered as an extreme for Danish conditions expected to occur about once every 10 years, even though short–time rain intensities are frequently much higher (Madsen et al., 2009). Water was allowed to drain freely from the bottom of the columns. Pesticide contents in the leachate were determined by measuring the 14C–activity with liquid scintillation counting. Brilliant Blue was applied to the four columns (one per combination of soil treatment and pesticide) after the pesticide–leaching experiments. The dye was applied in aqueous solution (4.0 g/L) as a standard irrigation (i.e. 15 mm/h in two hours) after rewetting.

Sampling

Samples were obtained from 9 or 10 separate column sections prepared 1–2 days after dye application. Initially, the columns were sectioned into 7 or 8 depth intervals (cylindrical slices). All columns were cut at 15, 20, 25, 30, and 40 cm depth using a steel thread or a narrow–bladed saw to minimize smearing. Two more cross-sections were made at depths below 15 cm in three columns, whereas only one cross–section was obtained in one column representing treatment B. All cross–sections were carefully cleaned for traces of soil materials and dye being smeared during the cutting procedure. They were then subjected to intensive diffuse light and photographed using a 3.0 Mpx camera.

Soil sampling within the slices was conducted according to three different strategies: (1) 5–10 soil samples were taken from different, intensively blue–colored soil volumes in the vicinity of dyed (flow active) macropores; (2) 10 core samples were taken randomly within non–colored areas (as determined

at the top–end); and (3) 10 completely randomized core samples were collected. Thus, typically 25–30 soil samples were taken per slice. However, due to extensive staining making it difficult to avoid blue soil, sampling according to strategy 2 was not performed above 5–10 cm depth. Furthermore, special procedures were followed in the uppermost slice. Following strategy 3, 10 samples were taken randomly in the 0–0.5 cm and the 0.5–1.5 cm depth intervals (the uppermost 1.5 cm was not included when sampling below). With strategy 1, sampling started at 0.5 cm depth (treatment A) or 1.5 cm depth (treatment B) because it was not possible to identify flow active macropores in the uppermost layer. Sampling according to strategy 1 was accompliced by scraping 1–2 mm of stained soil from the inside of biopores or cracks using a spatula. Sampling following strategies 2 and 3 was supported by coordinates generated by a random number generating program. It was done using a drill (diameter: 4.0 mm) throughout the entire soil layer. Hence with 10 samples per layer, roughly 0.18 % of the total soil volume was sampled. A total of 185–240 soil samples were obtained per column. Similar samples, according to the sampling strategy, were pooled within each soil layer.

Analyses

The pooled soil samples were air–dried, grounded using a ball–mill (350 rpm for 1 min), and mixed carefully. 14C–activity was measured by LSC after heating of 250 mg soil to 800°C in a constant flow of oxygen (Packard Sample Oxidizer Model 507) followed by 14C–CO_2 absorption by Carbosorb E (Packard) and Permafluor E+ (Packard). Two replicates were analyzed from each pooled soil sample. The concentrations of pesticides in soil were calculated from the specific concentration activity of the 14C–labeled pesticides and the relationship between labeled and unlabeled pesticide in the applied pesticide solutions. The detection limits for glyphosate and pendimethalin in soil were 0.01 and 0.005 mg/kg, respectively.

All the blue-stained representations of flow patterns appearing on photos of the cross–sections were manually transferred to transparent plastic sheets, and the new binominal representations (images showing either color or no color) were digitized using the procedures described by Petersen et al. (1997). The only distinction made in this process was whether or not blue dye was visible on the photos as evaluated by one person. The photos were handled in systematic order governed by a random serial number assigned to each. The digitized images were then scaled in two mutually perpendicular directions, and the fractional dye–stained area (DC, %) was determined using the image processing program ImageJ (Collins, 2007). The thickness of the uppermost completely dyed in soil layer (maximum depth with DC = 100 %) was measured. Fractional volume of dyed soil in a given soil layer was calculated as the average of DC observed at the top and bottom ends.

Mass balances for the pesticides were established based on sampling strategy 1 and 3, respectively. For strategy 1, measured pesticide concentration in soil was multiplied with fractional volume of dyed soil and by the mass of soil to get the pesticide content of a given soil layer. Concentrations obtained with strategy 3 were applied in the uppermost 0.5 cm (treatment A) or 1.5 cm (treatment B) layers in the lack of strategy 1 observations. For strategy 3, the pesticide content of a given soil layer was obtained by multiplying the measured concentration by the mass of soil. A dry bulk density of 1.60 g/cm3 (average value for all columns) was applied throughout in these calculations.

Results and Discussion

Distribution of Pesticides

Significant pesticide concentrations (above the detection limits) could be traced all the way through the columns by sampling strategy 1, except for glyphosate in treatment B at 25–50 cm depth. The concentrations generally decreased with depth (Figure 8.1.3.1-5). Below 30 cm depth, the pendimethalin concentrations approached the detection limits.

No significant amounts of the pesticides were found using sampling strategy 2, except in one sample (glyphosate in treatment A at 20–25 cm depth, cf. Figure 8.1.3.1-5). Thus, as a rule, pesticides were not detected by sampling outside blue-stained areas, not even at 5–10 cm depth, where considerable amounts of pesticide were found with the other sampling strategies. This is particularly noteworthy because three irrigations were carried out after pesticide application prior to application of the dye solution. Stronger sorption of the pesticides than of the dye may be part of the explanation. For the investigated (top–) soil, Gjettermann et al. (2009) reported soil–water partition coefficients (Kd–values) of 503 L/kg for

glyphosate and 242 L/kg for pendimethalin. Hence, both pesticides sorb strongly to the soil material. For Brilliant Blue, Flury and Flühler (1995) have reported much smaller Kd–values in the range 0.19–5.78 L/kg. Somewhat stronger sorption than measured by Flury and Flühler has been found for soils rich in clay minerals (German–Heins and Flury, 2000; Ketelsen and Meyer–Windel, 1999). It should be noticed that the sampling strategy being based on coring from upper surfaces of the slices does not completely exclude the inclusion of blue-stained soil material.

It was not possible in any case to trace the pesticides all the way through the columns by using strategy 3. With treatment A, glyphosate was not found below 10 cm depth and pendimethalin not below 20 cm. The pesticides tended to be found at slightly greater depths with treatment B. However, no significant concentrations were found below 25 cm depth. Hence with completely randomized sampling, pesticides were not found in significant amounts in the soil below 10–25 cm depth even though significant amounts (0.21–0.31 % of applied) were leached (Table 8.1.3.1-30) and 0.18 % of the soil volume was sampled. It was noticed that the strategy generally led to the inclusion of some blue–colored soil in the pooled samples when applied above 15–20 cm. Pesticide concentrations decreased strongly with depth in the 0–5 cm depth interval (Figure 8.1.3.1-5). By far the highest concentrations were found in the uppermost 5 mm of soil being completely dyed–in for both tillage treatments. Glyphosate concentrations measured in this layer were 8.59 and 10.5 mg/kg for the A and B treatment, respectively, while the corresponding numbers for pendimethalin were 24.6 and 14.1 mg/kg. Significant pesticide concentrations measured according to strategy 1 were always higher than concentrations obtained at the corresponding depths by strategy 3 (Figure 8.1.3.1-5). The differences between the two repeated measurements of pesticide concentrations of the soil samples were negligible (not shown).

Figure 8.1.3.1-5: Glyphosate and pendimethalin concentration (CG and CP, respectively) as a function of soil column depth obtained by the sampling strategies 1 (dyed), 2 (non–dyed), and 3 (random). Data for the two tillage treatments (A and B, average of 2 repeated measurements). Notice the broken 2nd axes

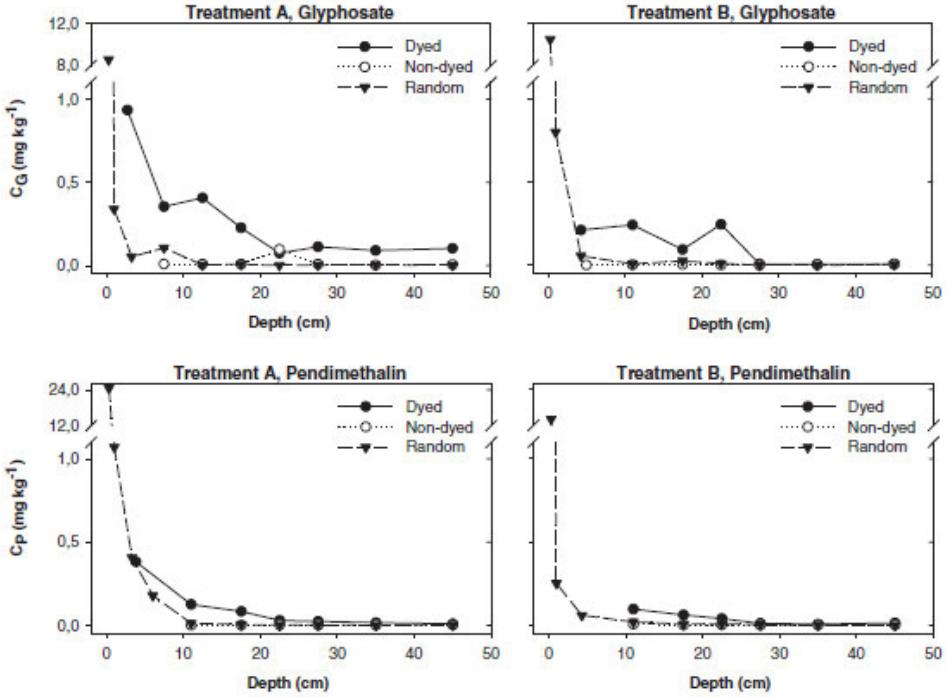


Table 8.1.3.1-30: Amounts of pesticides retrieved in columns estimated from two different column sampling strategies (1 and 3), and amounts lost with leachate (% of applied)

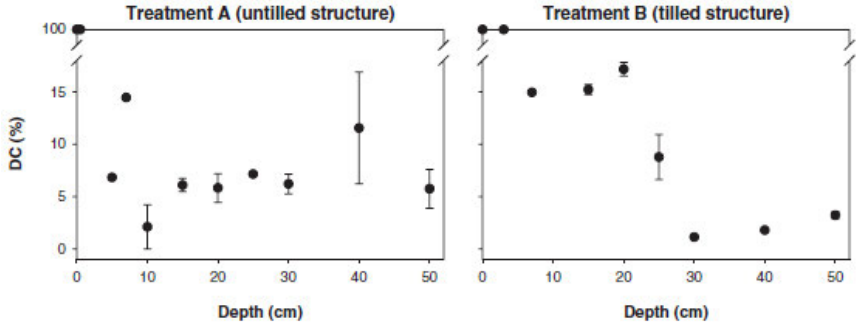
	Treatment A		Treatment B	
	Strategy 1	Strategy 3	Strategy 1	Strategy 3
Glyphosate found in column	63	50	67	61
Glyphosate leached	0.31	0.31	0.21	0.21
Pendimethalin found in column	110	123	65	63
Pendimethalin leached	0.23	0.23	0.21	0.21

Applied amounts per column, Glyphosate: 12.5 mg; Pendimethalin: 14.2 mg.

Dye Patterns

The thickness of the uppermost completely dyed–in soil layer was about 0.5 cm for treatment A and about 3 cm for treatment B. Thus, the fractional volume of dyed soil was 100 % above 0.5–cm depth in columns subjected to treatment A and above 3 cm in columns subjected to treatment B. The fractional area covered with dye (DC) rapidly decreased with depth right below these depths. In the topsoil, DC tended to be larger for treatment B than for treatment A, whereas the opposite trend was observed in the subsoil (Figure 8.1.3.1-6).

Figure 8.1.3.1-6: Fractional area covered with dye (DC) at different soil depths. Average values for each of the two tillage treatments (A and B, n = 2) with the range shown by the bars. Notice the broken 2nd axis



Below 30–cm depth, the stained flow pathways were mainly concentrated around vertically oriented earthworm channels comprising a relatively small fraction of the total soil volume. The dye had typically penetrated less than 1–2 cm into the soil matrix from these flow active macropores (4–10 per column). This is more than previously reported from studies conducted under field conditions (Petersen et al., 1997), probably due to the wet conditions prevailing in the columns with drainage at atmospheric pressure from 50–cm depth. For treatment A, a considerable fraction of the stained soil volume was found at the column walls in connection with large flow–active macropores that were cut during the excavation process. On average for all columns, the fractional volume of dyed soil below 30–cm depth comprized 5 %, leaving about 95 % as unstained and expectedly pesticide free.

Mass Balances

Under typical field conditions, half–lives (DT50 values) for glyphosate and pendimethalin are about 12 and 90 days, respectively (PPDB, 2010). However, under the low temperatures prevailing in the columns, both pesticides are expected to be slowly degradable. Furthermore, any non–volatile metabolites containing the 14C would be included in the measurements. The columns were sealed with plastic foil, except when used in the experiments. Hence, losses due to degradation and evaporation are expected to be very small. Also, the fraction of applied pesticide (14C) being leached was small (0.21–0.31 %) and unimportant for the mass balance (Table 8.1.3.1-30). Consequently, we expected a recovery close to 100 % based on the soil sampling alone. We found between 50 and 123 % with sampling strategy 3, and between 63 and 110 % with strategy 1, respectively (Table 8.1.3.1-30). Hence, none of the sampling strategies resulted in the expected (slightly less than) 100 % recovery in the columns although the balances tended to be better for strategy 1 than 3.

Both methods of constructing a mass balance obviously had large uncertainties. The largest concentrations (and amounts) of pesticide were found in the uppermost 0.5 cm of the profile, and the

major uncertainty appears to be the sampling of this top layer. If, for instance, the actual depth of sampling was 6 mm rather than 5, the error to the mass balance would be 8–19 % for the investigated columns. However, any difference in mass recovery obtained with the two sampling strategies is not related to this uncertainty, since the same sampling of this uppermost thin soil layer was used in both cases. The method based on sampling strategy 1 does correctly include some pesticide from lower parts of the columns. However, it may be biased if sampling for the pesticides did not fully represent the stained soil volumes. The mass recovery was of the same order of magnitude or considerably better than that obtained by Flury et al. (1995) working with a systematic very dense two–dimensional sampling scheme for herbicides in structured field soil.

Tracing Pesticides in Macroporous Soil

The magnitude of preferential flow contributing to the leaching of pesticide is difficult to quantify from studies on soil samples. Prichard et al. (2005) investigated the predominant source of pesticide residues detected in domestic wells located in an area with cracking clay soil. Although preferential flow through macropores within the field was a potential pathway, pesticide residues were retained in the top 15 cm of the soil. They deduced that the contribution of preferential transport to leaching was insignificant, despite the fact that lack of correlation of pesticide data between soil and water samples has previously been documented for soils with preferential flow (e.g. Sanchez et al., 2006; Laabs et al., 2000; Malone et al., 2000). Sanchez et al. (2006) found high concentrations of methidathion in the upper 25 cm of a soil profile but very low concentrations below this depth. They attributed high concentrations found sometimes in leachates from deeper layers to preferential flow processes. Laabs et al. (2000) similarly suggested that absence of pesticide residues in soil at depths below 25 cm combined with observed leaching indicated non–chromatographic transport of these substances in the soil profile. Malone et al. (2000) concluded that a sampling strategy including the mixing of horizontal slices with dimension 3.75 × 30 × 30 cm was not well suited to trace the movement of pesticides in the subsoil of structured soils. The present study support the interpretations made by Laabs et al. (2000), Malone et al. (2000), and Sanchez et al. (2006).

As expected, visible traces of Brilliant Blue were indicators for the occurrence of the pesticides in the soil. The measurements strongly suggest that both pesticides were transported exclusively within some fraction of the stained soil volume. With transport concentrated on a few macropores as in the subsoil of the present study, it should be possible to sample virtually all dye (and pesticide) at a given depth. Further developed, strategy 1 could therefore be used as the basis for better quantification of strongly sorbing pesticides in macroporous subsoil profiles. It is likely that the dye tracer should be applied under similar conditions (e.g. soil structure, soil moisture content, irrigation/precipitation amount and intensity) as the pesticides themselves.

Conclusions

Visible traces of Brilliant Blue transported under similar conditions as the two pesticides indicated the occurrence of both pesticides in the soil. The results suggest that efficient sampling for sorbing pesticides can be obtained by using the dye and focusing on stained soil volumes. None of the investigated sampling strategies led to mass balances that were accurate enough to detect amounts of pesticide leaching.

Assessment and conclusion by applicant:

The article describes a leaching experiment on soil columns with a dye and glyphosate (as well as pendimethalin). Glyphosate was only transported within a fraction of the stained soil volume. Some important information about study conditions are missing: agricultural use of the soil, temperature, soil parameters, details on analytics and on substance identification, sample storage conditions before analysis. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

The laboratory experiment presented in this article is based on the same characteristics as the previous one and the same deviations are observed. No sufficient information is available to check the validity of the results. Additionally, the scope of the study is not on the leaching of glyphosate itself but on evaluating the usage of a dye to improve sampling strategies.

The article provides supportive information on the leaching of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

B.8.1.3.1.3. Column leaching of metabolites, breakdown and reaction products

Reliable adsorption coefficients of soil metabolites were obtained in adsorption/desorption studies and, consequently, column leaching studies are not required (please refer to CA 7.1.3.2) and were not provided.

B.8.1.3.2. *Lysimeter studies*

Lysimeter studies are not considered necessary since reliable adsorption coefficient values could be obtained from the adsorption/desorption studies reported above. No lysimeter study was provided.

In the scientific literature research for glyphosate (2010-2020), four articles were identified to provide further information relevant to the data point.

Table 8.1.3.2-1: Lysimeter experiments – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status
CA 7.1.4.2/001	Napoli *et al.*, 2015	Lysimeter	Glyphosate	Reliable with restrictions
CA 7.1.4.2/002	Al-Rajab & Hakami, 2014	Lysimeter	Glyphosate	Reliable with restrictions
CA 7.1.4.2/003	Bergstrom *et al.*, 2011	Lysimeter	Glyphosate	Reliable with restrictions
CA 7.1.4.2/004	Gros *et al.*, 2020	Lysimeter	Glyphosate	Reliable with restrictions

Napoli et al., 2015

Data point:	CA 7.1.4.2/001
Report author	Napoli *et al*
Report year	2015
Report title	Leaching of Glyphosate and Aminomethylphosphonic Acid through Silty Clay Soil Columns under Outdoor Conditions
Document No	DOI 10.2134/jeq2015.02.0104 E-ISSN 1537-2537
Guidelines followed in study	None
Deviations from current test guideline	- Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate [*N*-(phosphono-methyl)-glycine] is the main herbicide used in the Chianti vineyards. Considering the pollution risk of the water table and that the vineyard tile drain may deliver this pollutant into nearby streams, the objective of the present study was to estimate the leaching losses of glyphosate under natural rainfall conditions in a silty clay soil in the Chianti area. The leaching of glyphosate and its metabolite (aminomethylphosphonic acid [AMPA]) through soils was studied in 1-m-deep soil columns under outdoor conditions over a 3-yr period. Glyphosate was detected in the leachates for up to 26 d after treatments at concentrations ranging between 0.5 and 13.5 µg/L. The final peak (0.28 µg/L) appeared in the leachates approximately 319 d after the first annual treatment. Aminomethylphosphonic

acid first appeared (21.3 µg/L) in the soil leachate 6.8 d after the first annual treatment. Aminomethylphosphonic acid detection frequency and measured concentration in the leachates were more than that observed for the glyphosate. Aminomethylphosphonic acid was detected in 20% of the soil leachates at concentrations ranging from 1 to 24.9 µg/L. No extractable glyphosate was detected in the soil profile. at the end of each year of experimentation, the total amount of AMPA recovered in the soil profiles was about 0.03%, based on the amount of glyphosate applied The AMPA content in the surface soil layer ranged between 0.0013 and 0.0021%, based on the amount of glyphosate applied. Overall, these results indicate that both glyphosate and AMPA leaching through a 1-m soil column may be potential groundwater contaminants.

Materials and methods

Borate buffer (0.05 M) was prepared by dissolving 1.9 g disodium-tetraborate-decahydrate in 100 mL ultra-pure water. The FMOC-Cl solutions (1 g/L) were prepared by dissolving 10 mg FMOC-C1 in 10 mL acetonitrile. Glyphosate and AMPA working standard (30 µg/L) were prepared by dissolving glyphosate and AMPA in ultra-pure water. Working standards were stored at 4°C for no more than 1 wk.

In summer 2006, three lysimeters were installed at a lysimeter station in Montepaldi, San Casciano Val di Pesa, Tuscany, Italy. Each lysimeter consisted of a cube-shape casing (1-m edge) made of 4-mm-thick stainless steel sheet. At the bottom end of each lysimeter, a polyethylene corrugated drainage pipe was installed to collect the leachate. During the summer of 2006, the containers were filled with a silty clay soil collected from a nearby Chianti vineyard that had been mechanically weeded over the previous 3 yr. The soil was taken from the 0- to 100-cm layer of three randomly selected vine interrows. The soil was then taken and placed in the lysimeter, taking care to maintain the profile's natural order of layers. During the monitoring period, hourly temperature and rainfall data were measured by a meteorological station located 300 m from the experimental site. The annual mean temperature and precipitation at the study site were 14.6°C and 914 mm/yr, respectively.

The commercial formulations of glyphosate (360 g/L a.i.) were applied in the study area at a dose of 2 L/ha per application. There were one to two spring applications along each vine row, covering a strip of ~1 m. This implies that along the treated strip, the concentration of the active ingredient ranged from 70 to 150 mg/m^2 depending on the number of spring applications. For the lysimeter study, the concentration data associated with the two spring applications was modeled. Therefore, in the middle of March and in the middle of May, glyphosate was applied to each lysimeters. An aqueous solution of herbicide was sprayed onto the surface of the soils to simulate an application rate of 0.72 kg/ha a.i.

Drainage water was collected after each rainfall event from 1 Mar. 2007 to 28 Feb. 2010. To ensure limited degradation, leachate volumes were determined gravimetrically and then preserved in the dark at −20°C for a maximum of 25 d until analysis. On 26 Feb. 2007 and then at the end of each year (i.e., the last week of February), the soil was sampled in triplicate for each of the lysimeters, which were separated into six layers (0–5, 5–20, 20–40, 40–60, 60–80, and 80–100 cm), air-dried, weighed, and sieved. The chemical and physical analyses were performed on air-dried, 2-mm fractions taken from each layers. The soil characteristics are listed below.

Table 8.1.3.2-2: Principal chemical and physical properties of study soil; organic matter and carbonates in percentage of the weight of the 2-mm sieved soil; soil electric conductivity (EC) and cation-echange capacity (CEC) are reported

Layer	Particle-size distribution (USDA)						Bulk density	Organic matter	Total carbonates	EC	CEC
	Gravel	Fine earth									
		Coarse sand	Fine sand	Coarse silt	Fine silt	Clay					
	%						kg m^{-3}	%		dS m^{-1}	cmol kg^{-1}
0–5 cm	18.2	7.7	8.6	14.7	28.5	40.5	1332	0.81 ± 0.21	14.8 ± 0.2	0.21	23.6 ± 0.5
5–20 cm	19.6	13.1	14.4	13.3	16.2	43.0	1347	0.64 ± 0.22	14.7 ± 0.2	0.2	22.6 ± 0.5
20–40 cm	20.7	15.0	15.2	13.7	14.8	41.3	1410	0.43 ± 0.17	14.7 ± 0.2	0.2	22.1 ± 0.5
40–60 cm	19.9	20.0	18.3	14.5	14.0	33.2	1421	0.37 ± 0.19	14.8 ± 0.2	0.21	20.5 ± 0.5
60–80 cm	22.3	25.0	19.7	14.2	13.4	27.7	1469	0.35 ± 0.07	14.9 ± 0.2	0.2	20.6 ± 0.5
80–100 cm	21.6	26.1	19.5	14.4	13.1	26.9	1488	0.33 ± 0.08	15.1 ± 0.2	0.2	20.2 ± 0.5

Water samples were filtered through 1-mm glass-fiber filters. The liquid was immediately derivatized. The herbicide residues in the sediment, along with the residues in the soil samples, were extracted first by ultrasonic extraction in methanol after which the derivatization procedure was used. To reduce the sorption of glyphosate and AMPA from the methanol-extracted solutions onto glassware surfaces, water and soil samples were dispensed in parallel into plastic vials. Methanol (50 mL) was added to soil samples (50 mg) that had been dried and sieved. The soil suspension was mixed for 60 min and then left at 20°C for 24 h to allow complete solvent evaporation. Then, 15 g of soil was added to 40 mL of solvent and sonicated at 30 to 40 kHz for 30 min. Extracts were filtered through Whatman 40 filter paper, and the filtrate was evaporated on a rotary vacuum evaporator at 40°C to dryness. The residue of herbicide extract was dissolved in 5.0 mL of water and then collected in plastic vials for the derivatization procedure. Sediment extraction was performed as depicted for soil samples.

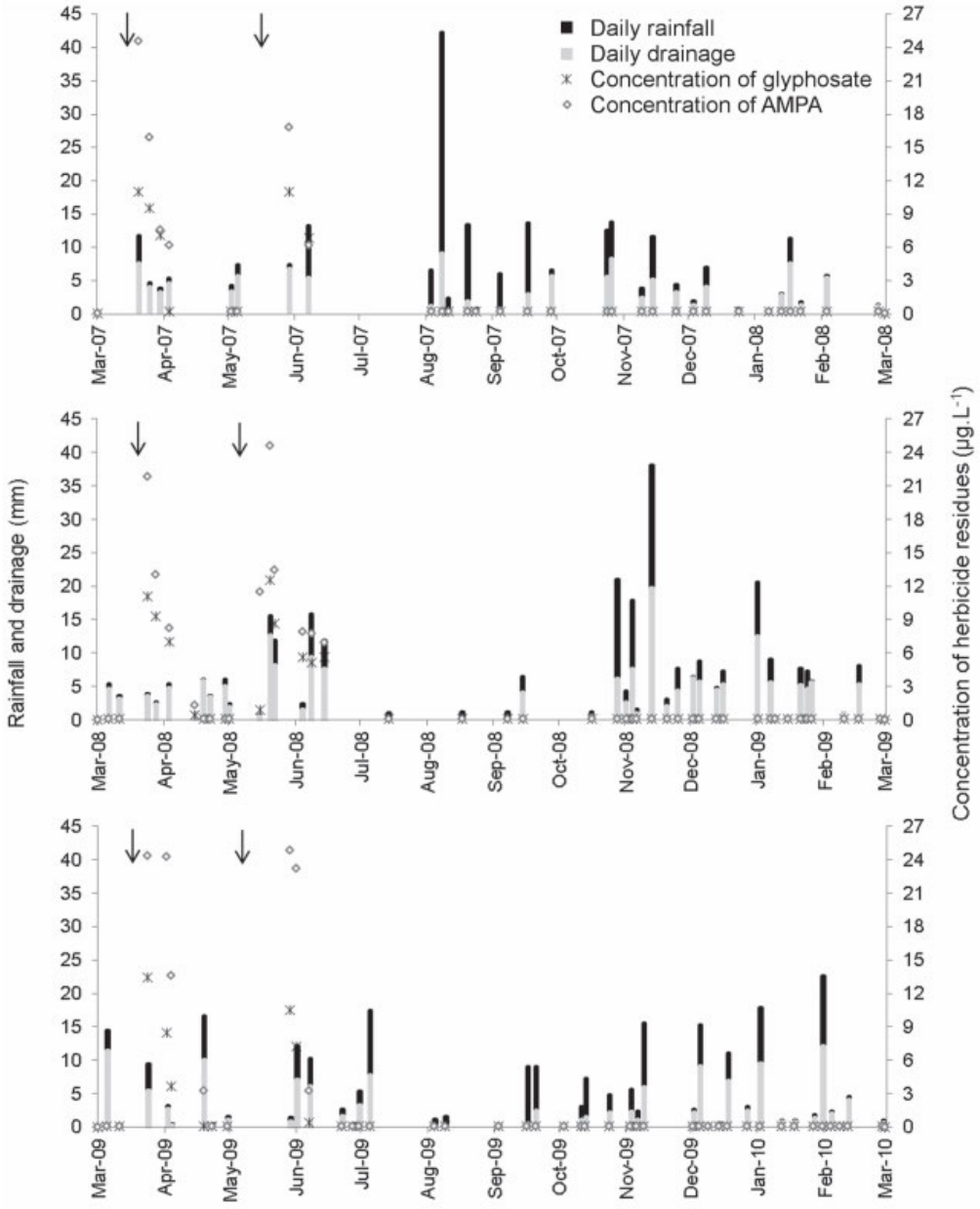
Following Le Bot *et al.* (2002), 3-mL samples were derivatized by adding 0.5 mL borate buffer and, after mixing, 500 µL FMOC-Cl solution. Then, samples were shaken for 1 h and incubated, allowing the reaction to take place for 15 h at room temperature (20°C). Derivatization was performed in the dark. The reaction was stopped by adding formic acid at about pH 3.0. The samples were washed with 2 mL diethyl ether to eliminate excess derivatization reagent.

The solid-phase extraction (SPE) was performed by means of a Dionex AutoTrace 280 SPE autosampler (Thermo Scientific). Glyphosate and AMPA were analyzed by liquid chromatography–electrospray ionization–tandem mass spectrometry (TSQ Vantage triple quadrupole mass spectrometer, Thermo Scientific), which comprise an analytical column (Syncronis C8, 2.1 by 150 mm, 5 mm, Thermo Scientific) and a column guard (Syncronis C8, 2.1 by 10 mm, 5 mm, Thermo Scientific). Each standard and sample (3 mL) were injected onto the analytical column and then eluted in gradient mode using a binary solvent mix comprising 99% 5 mM ammonium acetate and 1% acetonitrile (mobile phase A) and 99% acetonitrile and 1% 5 mM ammonium acetate (mobile phase B). The mobile phase flow rate was 0.3 mL/min. Analyses were performed in negative ionization mode with a spray voltage of 3.5 kV. The source temperature and the ion transfer tube temperature were 325°C and 250°C, respectively. The minimum detectable level (MDL) was 0.1 µg/L for glyphosate and AMPA in leachates and 10 µg/kg in soil.

Results

The daily rainfall and the glyphosate and AMPA concentrations in the leachates are presented in the figure below.

Figure 8.1.3.2-1: Daily rainfall and drainage and concentrations of glyphosate and AMPA measured in the leachates of the vineyard soil from March 2007 to March 2010. Herbicide dates of application are indicated with arrows



The cumulative rainfall amounts for the period from 1 March to 28 February of the subsequent year were 524, 751, and 1429 mm during the first, second, and third year of the experiment, respectively. During the monitoring period, glyphosate was detected in 3 % of the soil leachates at concentrations ranging from 0.2 to 1 µg/L and in 16 % of the leachates at concentrations ranging from 1 to 13.47 µg/L. Glyphosate appeared at high concentrations (12.1 ± 1.3 µg/L) in the soil leachates 9.3 ± 4 d after each treatment.

Glyphosate was detected in the leachates for 25.8 ± 8.3 d after treatments at concentrations exceeding 0.5 µg/L. During the latter, average drainage of 15.5 ± 2.9 mm was measured, corresponding to 22.9 ± 6.7 mm of measured rainfall. Thereafter, the glyphosate concentration in leachates decreased to 0.1 µg/L. At the end of each trial year, the final glyphosate peaks appeared in the leachates between late January and early February (about 318.9 ± 8 d after the first annual treatment) at an average concentration of 0.3 µg/L.

Similar to the results for glyphosate, AMPA first appeared at an average concentration of 21.3 ± 6.2 µg/L in the soil leachate approximately 6.8 ± 1.2 d after each treatment. Aminomethylphosphonic acid was detected more frequently than glyphosate; it was detected in 13% of the leachates from soil at

concentrations ranging from 0.2 to 1 µg/L and in 20 % of the leachates from soil at concentrations ranging from 1 to 24.9 µg/L.

The amounts of water drained from the soil for the period from 1 March to 28 February of the following year, were 113.8, 187.4, and 130.5 mm, respectively, during the first, the second and the third year of the experiment. Approximately 0.19, 0.31, and 0.12% of the amount of glyphosate distributed in the first, second, and third year of the experiment, respectively, were recovered in the leachates as glyphosate, whereas 0.49, 0.78, and 0.48%, respectively, were recovered as AMPA.

On the basis of the analysis, the number of days from the treatment (DN) showed the highest negative correlation with the glyphosate and AMPA concentrations in leachate ($p \leq 0.001$). In contrast, the daily mean temperature (T_{med}) and the daily rainfall (R) showed a positive role in determining the herbicide concentration ($p \leq 0.05$). Since these variables were not autocorrelated, they were selected as independent variables X_1, X_2, and X_3, respectively, for the multiregressive model (Eq. [1]). The multiregression analysis led to the set up of Eq. [2] and [3] for the estimation of glyphosate and AMPA concentration in leachate, respectively:

$$Y_{\text{glyphosate}} = 0.0508T_{\text{med}} - 0.3445\text{DN} - 0.0179R + 13.2308 \quad [2]$$

$$Y_{\text{AMPA}} = 0.1937T_{\text{med}} - 0.6727\text{DN} - 0.1412R + 25.2585 \quad [3]$$

The glyphosate and AMPA concentrations were computed using data measured during the second and the third year of the experiment.

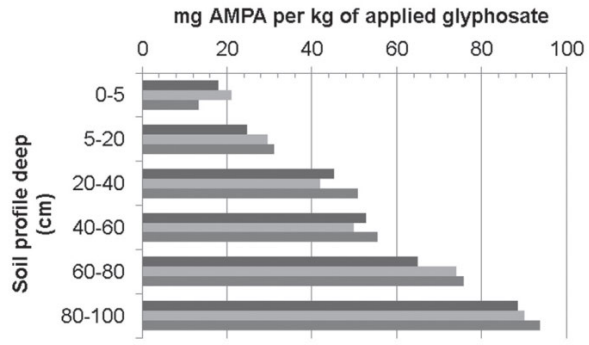
At least for the current study, climatic conditions and for this soil type, Eq. [4] and [5] can be used to determine the number of days free of rain (NR) necessary to ensure a safe threshold for distributing the herbicide.

$$\text{NR}_{\text{glyphosate}} = -2.9028Y_{\text{glyphosate}} + 0.1475T_{\text{med}} - 0.0519R + 38.4058 \quad [4]$$

$$\text{NR}_{\text{AMPA}} = -1.4866Y_{\text{AMPA}} + 0.2879T_{\text{med}} - 0.2099R + 37.5479 \quad [5]$$

No extractable glyphosate was detected in the soil profile. Aminomethylphosphonic acid was found as deep as 100 cm in the soil column. The concentration of AMPA increased with increasing depth, thus indicating a gradual accumulation of AMPA in the lower profile during the 3-yr experimental period. On the contrary, AMPA was distributed throughout the soil columns as shown in the following figure.

Figure 8.1.3.2-2: Distribution profile of aminomethylphosphonic acid (AMPA) in the soil 1 year after the application of glyphosate for the first (dark gray), the second (light gray), and the third (medium gray) year of experiment



The AMPA content in the surface soil layer ranged between 0.0013 and 0.0021%, based on the amount of glyphosate applied. The AMPA content in the lowest layer ranged between 0.0089 and 0.0094%,

based on the amount of glyphosate applied. During the 3 yr, a continuous increase in the concentration of AMPA in the lower layers of the profile was measured; however, there are no statistical data to attribute this to an accumulation effect, but rather to different weather conditions. Finally, at the end of each year of experimentation, the total amount of AMPA recovered in the soil profiles was about 0.03%, based on the amount of glyphosate applied.

The amounts of glyphosate and AMPA, in terms of applied glyphosate, measured in the leachates and in the soil profiles were summed on a yearly basis.

Table 8.1.3.2-3: Mass balance of glyphosate and aminomethylphosphonic acid (AMPA) in leachates and soil profles (in percentage based on the amount of glyphosate applied) for the three experimentation years

Year	Herbicide leachates		Herbicide in soil		Total residue
	Glyphosate	AMPA	Glyphosate	AMPA	
	%				
First year	0.19	0.49	0	0.03	0.70
Second year	0.31	0.78	0	0.03	1.11
Third year	0.12	0.48	0	0.03	0.63

Conclusion

After a 3-yr experimental period under outdoor conditions, the present work has demonstrated that both glyphosate and AMPA may be transported in leachates through 100 cm of soil profile, thus confirming the high mobility of this herbicide. The mean annual percentage of glyphosate and AMPA, as a percentage of applied glyphosate, recovered in leachates were about 0.2 and 0.58%, respectively. Moreover, results suggested that preferential, flow along with rains that occurred within 2 wk after the treatment, can cause the leaching of glyphosate and AMPA in high concentration. At least in this environment and for this soil, a multiregressive equation was found to determine the number of days free of rain necessary to ensure a safe herbicide distribution. Soil analyses indicated that glyphosate was below detection in 1 yr. On the contrary, the total amount of AMPA, based on the amount of glyphosate applied, recovered in the soil profiles was around 0.03% at the end of each year of experimentation. Overall, these results suggest that when applied to shallow soils, herbicides can pose a risk of groundwater contamination, and, when applied to pipe-drained crops, contaminated leachate can be transported by the pipe drain to surface waters.

Assessment and conclusion by applicant:

The article describes a lysimeter study with glyphosate using three lysimeters from the Chianti region in Italy. The study is well described, however, there is some information missing to check the validity of the study against current guidelines. The use of clay soil (low- to non-permeable soil) is considered not appropriate for lysimeter experiments, and from the information given in the article it cannot be excluded that the leaching may have been caused by preferential flow rather than percolation through the soil column. Further, there is no information available whether, apart from application, lysimeters were handled according to normal agricultural practice. From the information reported it is not possible to calculate annual mean concentrations in leachate for glyphosate and AMPA which would however be required in view of risk assessment.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

This article presents the results of lysimeter experiments conducted over three years with a silty clay soil.

Some of the details needed to check the validity of the study are missing (e.g. management of the lysimeters, measurements of soil temperature and soil moisture).

The soil selected is not in line with OECD 22 recommendations, with ca 40% clay in the top 40 cm. As indicated in the guidance, the shrinkage of clay soils can lead to cavities along the walls of the lysimeter and thus to rapid movement of water and chemicals directly to the bottom of the lysimeter. In addition, as concluded in the article, radioactivity recovered in the leachates is expected to be due to preferential flow, whereas lysimeter studies are usually designed to investigate percolation through the soil column.

The annual mean concentrations in leachates, which are the relevant values for regulatory risk assessment, cannot be calculated.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Al-Rajab & Hakami, 2014

Data point:	CA 7.1.4.2/002
Report author	Al-Rajab, A., Hakami, O.M.
Report year	2014
Report title	Behavior of the non-selective herbicide glyphosate in agricultural soil
Document No	DOI 10.3844/ajessp.2014.94.101 E-ISSN 1558-3910
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:
The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/015).
The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Bergström et al., 2011

Data point:	CA 7.1.4.2/003
Report author	Bergström, L. *et al.*
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Document No	DOI 10.2134/jeq2010.0179 E-ISSN 1537-2537
Guidelines followed in study	OECD 106 Guideline
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Assessment and conclusion by RMS:
The article was found relevant for multiple data points. The summary is provided under point B.8.1.1.2.1 (CA 7.1.2.1.1/017).

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Gros et al., 2020

Data point:	CA 7.1.4.2/006
Report author	Gros, P. *et al.*
Report year	2020
Report title	Leaching and degradation of $^{13}C_2$-^{15}N-glyphosate in field lysimeters
Document No	Environmental monitoring and assessment, (2020) Vol. 192, No. 2, pp. 127 DOI 10.1007/s10661-019-8045-4
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Glyphosate (GLYP) may have effects in various compartments of the environment such as soil and water. Although laboratory studies showed fast microbial degradation and a low leaching potential, it is often detected in various environmental compartments, but pathways are unknown. Therefore, the objective was to study GLYP leaching and transformations in a lysimeter field experiment over a study period of one hydrological year using non-radioactive $^{13}C_2$-^{5}N-GLYP labelling and maize cultivation. ^{15}N and ^{13}C were selectively measured using isotopic ratio mass spectrometry (IR-MS) in leachates, soil, and plant material. Additionally, HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) was used for quantitation of GLYP and its main degradation product aminomethylphosphonic acid (AMPA) in different environmental compartments (leachates and soil). Results show low recoveries for GLYP (<3 %) and AMPA (<level of detection) in soil after the study period, whereas recoveries of ^{15}N (11–19 %) and ^{13}C (23–54 %) were higher. Time independent enrichment of ^{15}N and ^{13}C and the absence of GLYP and AMPA in leachates indicated further degradation. ^{15}N was enriched in all compartments of maize plants (roots, shoots, and cobs). ^{13}C was only enriched in roots. Results confirmed rapid degradation to further degradation products, e.g., $^{15}NH_4^+$, which plausibly was taken up as nutrient by plants. Due to the discrepancy of low GLYP and AMPA concentrations in soil, but higher values for ^{15}N and ^{13}C after the study period, it cannot be excluded that non-extractable residues of GLYP remained and accumulated in soil.

Materials and methods

The leaching experiment was set up in two field lysimeters (non-weighing zero tension), which were installed in the Lysimeter Station at the Helmholtz Centre for Environmental Research-UFZ (Falkenberg, Germany; 52°51′ N, 11°48′ E). These lysimeters were constructed in 1981 in sheet steel vessels with cuboid shape of 1 × 1 m surface area and 1.25 m depth. The lysimeters were filled with sandy loam (0–30 cm topsoil: 74 % sand, 14 % silt, 12 % clay, pH 4.8, organic C = 1.1 %; 30-100 cm subsoil: 75 % sand, 17 % silt, 8 % clay, pH 5.6, organic C = 0.2 %) and an additional 25 cm-drainage layer composed of three sublayers (sand, gravel, and coarse gravel) at the bottom. The soil texture is representative for the river Elbe valley in the Federal State Saxony-Anhalt. Conventional agricultural management was oriented according to best management practice. In 2017, maize was planted which was embedded in a regionally typical crop rotation of sugar beets-winter wheat-potatoes-winter barley-maize. The present study investigated a period of one hydrological year starting in the hydrological summer semester in May 2017. Any weeds were removed mechanically, followed by 13C2-15N-GLYP (GLYPi) application (2017/24/04) via spraying as a worst-case scenario. Application rate was equivalent to maximum allowed annual for Germany (3.6 kg/ha/a) with practical concentration

of GLYP formulations (480 g/kg) (360 mL GLYPi, dissolved in 750 mL H2O). Drift by air flow was prevented by temporally fencing the application area with a ring of steel (1 m in height). Three days after GLYPi application, 5 L of the conservative KBr tracer solution was applied at a rate corresponding to 40 kg/KBr/ha to each of the lysimeters to provide information on the movement of water through the soil column. Lysimeters were cultivated with maize (9 plants per lysimeter, equally spaced). No fertilizers or treatments for weeding were executed during the study period.

Sampling of leachates, soil, and plant material

Lysimeter soils were sampled from 0 to 5 cm depth (5 spots equally spaced in each lysimeter) at 4 dates over the study period (before and directly after application, 165 and 360 days after application). Soil sampling before application characterizes the basic level of GLYP*i* concentration, whereas the sample directly after application represents 100 % of initial GLYP*i*. To keep the soil column intact, samples from the whole topsoil (0-30 cm) and the subsoil (30-60 cm) were taken only at the end (day 360 after application) of the study period. Soil samples were air dried and sieved (2 mm). Subsamples of the sieved soils were finely ground for further measurement with IR-MS. Residues of GLYP*i* and AMPA*i* were extracted from 5 g of the sieved soil in 40 mL of a 1 M KOH solution (shaking overnight and centrifugation for 10 min at 1558 g) and stored at -20 °C until quantitation via HPLC coupled to electrospray ionization mass spectrometry (HPLC-ESI-MS/MS).

Leachates were collected weekly in polyethylene canisters and volumes were recorded. Subsamples of 150 mL were taken and stored in a freezer at -20 °C in 3 × 50 mL centrifuge tubes for further measurements with ion chromatography (IC) and HPLC-ESI-MS/MS. A total of 50 mL of each sample were lyophilized to dryness (-50 °C, 0.025 mbar; Christ Alpha 1-4, Martin Christ Gefriertrocknungsanlagen GmbH, D-37250 Osterode, Germany) and solid residue amounts were weighed back and stored for measurements with isotopic ratio mass spectrometry (IR-MS).

Mature maize plants (roots, shoots, and cobs) were harvested in September 2017 from the two treated lysimeters and one untreated neighbouring plot as reference. Subsamples of 3 plants per lysimeter were harvested for further measurements of plant biomass. Moist weight was determined followed by drying at 60 °C and measuring of dry matter weight. Plant compartment samples (root, shoot, and cobs) were shredded and subsequently finely ground separately and stored until further measurements with IR-MS.

Sample analyses

Conservative tracer and isotope ratio analyses

Br^- tracer analysis in the leachate was performed using ion chromatography (column: Metrosep A SUPP 5150 × 4.0 mm, pre-column: Metrosep A SUPP 4/5 Guard, eluent: 0.3 mM Na_2CO_3 and 1.0 mM $NaHCO_3$, flow: 0.7 mL/min, separation mode: isocratic; Metrohm, D-70794 Filderstadt, Germany).

Isotopic ratios for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in soil, plant compartments, and lyophilized leachate samples were measured through the elemental analyser (Eurovector EA, Via F.lli Cuzio 42, 27100 PAVIA, Italy; IR-MS GVIsoprome, Elementar Analysensysteme GmbH, Elementar-Straße 1, 63505 Langenselbold, Germany) in the Institute for Nutritional Sciences, University of Gießen, Germany. For this purpose, finely ground soil and plant samples from the two treated sites and one untreated site (reference) were measured in triplicates. Lyophilized leachate samples from lysimeter leachates were measured in duplicates. Equations 1 and 2 show the calculation of $\delta^{15}N$ and $\delta^{13}C$ derived from isotopic ratios of the sample in relation to defined standard isotopic ratios from air for N and Pee Dee Belemnite (PDB) for C; values are generally given in ‰.

$$\delta^{13}C = \left(\frac{\left(^{13}C/^{12}C\right)_{\text{sample}}}{\left(^{13}C/^{12}C\right)_{\text{PDB}}} - 1 \right) \qquad (1)$$

$$\delta^{15}N = \left(\frac{\left(^{15}N/^{14}N\right)_{\text{sample}}}{\left(^{15}N/^{14}N\right)_{\text{air}}} - 1 \right). \qquad (2)$$

GLYP*i* and AMPA*i* analyses

Soil extracts and leachate samples were analysed for GLYP*i* and AMPA*i* with HPLC-ESI-MS/MS after derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl), as described in Wirth *et al.* (2019). The utilized system was composed of an LC-2040C Nexerai and a triple quadrupole mass spectrometer LCMS8060 (Shimadzu, Duisburg, Germany) equipped with a heated ESI-source. The FMOC derivatives were separated on a Gemini 3 µm NX-C_{18} column (Column 1: 150 × 2 mm, Aschaffenburg, Phenomenex, Germany).

Non-isotope-labelled GLYP (LGC Standards, Wesel, Germany) was used as internal standard for GLYP*i* (Sigma Aldrich, Taufkirchen, Germany) quantitation. Since AMPA*i* is not commercially available as a standard substance, no HPLC-ESI-MS/MS-optimization and, thus, no calibration could be carried out for this compound. Therefore, AMPA*i* was determined only qualitatively. Analytes were detected in the multiple reaction monitoring (MRM) mode. The MRM transitions were determined and optimized utilizing standard compounds. However, as AMPA*i* is not commercially available, instrumental MRM optimization for AMPA*i*-FMOC could not be performed. Therefore, the settings for the MRM transitions for this compound were chosen as follows: optimization was carried out for ^{13}C-^{15}N-AMPA-FMOC and AMPA-FMOC (LGC Standards, Wesel, Germany) and their fragmentation patterns were utilized to derive the expected masses of the precursor and product ions for ^{15}N-AMPA-FMOC (AMPA*i*-FMOC). Further parameters of the MRM transitions were set by averaging values for ^{13}C-^{15}N-AMPA-FMOC and AMPA-FMOC.

To further verify that the targeted and detected compound was the ^{15}N-AMPA-FMOC, a selection of samples was additionally separated on a different LC-column (Column 2: Kinetex 2.6 µm EVO C18 100 Å, 150 × 2.1 mm, Phenomenex, Aschaffenburg, Germany). The proposed AMPA*i*-FMOC was eluted from both columns at similar retention times as AMPA-FMOC which confirms its presence. Due to the lack of an AMPA*i*-FMOC calibration, these data could be evaluated only semi-quantitatively. Quantitation of GLYP*i* was carried out through weighting with the glyphosate internal standard signal.

Table 8.1.3.2-4: Measurement modes for identification and quantitation of 13C2-15N-glyphosate and 15N-aminomethylphosphonic acid using high performance liquid chromatography tandem mass spectrometry (HPLC-ESI-MS/MS)

Component	Measurement mode	Precursor m/z	Product m/z	Collision energy	Retention time column 1(min)	Retention time column 2 (min)
$^{13}C_2$-^{15}N-Glyphosate-FMOC (GLP*i*)	-	392.10	170.15	14	9.10	8.77
			152.20	24		
			63.10	48		
Glyphosate-FMOC	-	390.00	168.15	14	9.10	8.76
			150.20	23		
			63.05	49		
^{15}N-AMPA-FMOC* (AMPA*i*)	+	335.20	179.05	−23	9.47	9.18
			178.15	−48		
			157.05	−10		
			113.05	−15		
AMPA-FMOC	+	334.20	179.05	−23	9.47	9.19
			178.15	−46		
			156.00	−10		
			112.05	−15		
^{13}C-^{15}N-AMPA-FMOC	+	336.20	179.05	−22	9.46	n.a.
			178.10	−50		
			158.15	−10		
			114.10	−15		

*derived from the optimized MRM transitions of ^{13}C-^{15}N-AMPA-FMOC and AMPA-FMOC

Results

Precipitation and leachate analysis

The study period from May 2017 to April 2018 was characterized by overall high amounts of precipitation that exceeded the monthly 30-year mean values (1981-2010) for this region, except for the months May, September, and February. Especially, June and July were characterized by heavy rainfall events that summed up to 123 and 125 mm per month precipitation, greatly exceeding the mean values of 57 ±22 mm (June) and 61 ±32 mm (July). These events resulted in large amounts of leachate in July 2017 (60.4 and 66.3 L). Weekly leachate amounts, collected from May 2017 until July 2017 to December 2017 until April 2018, had a mean volume of 5.1 L per week. For the period from August 2017 to November 2017, no leachates were received although precipitation occurred, most likely because of transpiration and water uptake by plants. Total volumes of leachates for the two lysimeters were 203 and 215 L over the study period. The Br^--breakthrough started in week 10 after application, where 35 and 37 L of leachate were received in the two tested lysimeters. Residues from the conservative tracer KBr were detected later on in all leachates. Due to the occurrence of Br^- in the leachates after 10 weeks and its slowly increasing concentrations over the following weeks along with continually received leachates, the main transport mechanism through the soil column can be assumed as matrix flow for the studied period.

For the natural ^{15}N background representing the ratio of $^{15}N/^{14}N$ of the air nitrogen, the $\delta^{15}N$ has been set to 0. Discrepancies towards higher values indicate an enrichment of ^{15}N. In the first 2 weeks after application, a strong decrease of leachate $\delta^{15}N$ to negative values was detected, indicating an enrichment of ^{14}N. In the following weeks 3 to 10, the $\delta^{15}N$ in the leachate was constant between 0 and 1.7 ‰, and it increased over time from week 11 after GLYP*i* application. After the period with no leachates, the trend of $\delta^{15}N$ had a sigmoidal shape with an assumed maximum limit of about 50 ‰ for the last 10 weeks of the experimental period. This maximum level corresponds to a mass rate of about 10 µg ^{15}N/week of leached GLYP*i* active ingredient equivalent or its N-containing degradation products.

Values for $\delta^{13}C$ started at about -8 ‰ and fluctuated between -12 and -6 ‰ for the first 10 weeks before they strongly increased and reached values of -2.6 and -0.5 ‰ in the two lysimeters. After the period with no leachates, the $\delta^{13}C$ started at lower levels of -8.9 and -2.1 in lysimeters 1 and 2, respectively. The trend of increasing $\delta^{13}C$ values starting at -5.5 ‰ went on and ended at -2.3 ‰ for the remaining 20 weeks of the study, although with a less steep slope than in the first experimental phase.

Trends of ^{13}C and ^{15}N originating from GLYP*i* in the leachate did not correlate, which may be an indication for an independent movement of these isotopes through the soil column. Also, IR-MS cannot distinguish between GLYP*i* and its degradation products, but simultaneous occurrence and parallel trend would be an indication for a displacement of intact GLYP*i*, which appears unlikely from these data. The analyses for GLYP*i* and AMPA*i* using HPLC-ESI-MS/MS in the leachates showed no occurrence of residues of these compounds above detection limits (0.1 µg/L). Therefore, the leached ^{15}N and ^{13}C residues are most likely no constituents of intact GLYP*i* or AMPA*i* but originated from further degradation products.

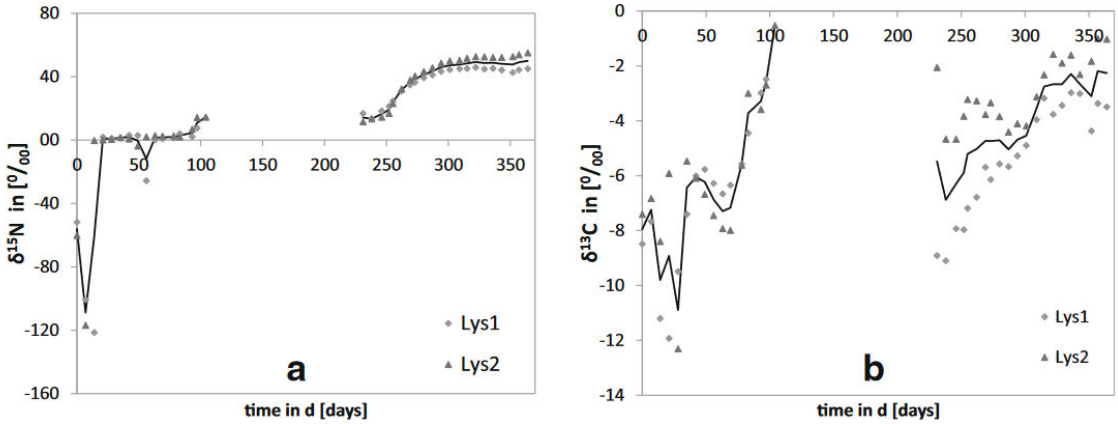


Figure 8.1.3.2-3: ^{15}N (a) and $\delta^{13}C$ (b) values for lyophilized leachates over the one-year study period in lysimeter 1 (Lys1) and lysimeter 2 (Lys2) and mean values (continuous line)

Soil analyses

The concentrations of GLYP*i*, ^{15}N and ^{13}C in the lysimeter soils derived from HPLC-ESI-MS/MS and IR-MS, respectively, were normalized and set to 100 % since GLYP*i* was not detectable in soil extracts sampled before GLYP*i* application (data not shown). The $\delta^{15}N$ decreased within 165 days after application to 24 and 29 % of the initial value and decreased further to 11 and 19 % until the end of the study period. This indicates that amounts of the added artificial ^{15}N isotopes in soil decreased over time. The same was true for $\delta^{13}C$ which decreased down to 30 and 66 % compared with the initial value and ended at 23 and 54 % in the two lysimeters.

Measurement of the GLYP*i* residues through HPLC-ESI-MS/MS showed that about 4 and 6 % of the initial GLYP*i* concentration remained in the soil after 165 days and the recovery decreased further down to 1 and 3 % in the two lysimeters until the end of the study. AMPA*i* was detected in the topsoil extracts of all samples after application, and GLYP*i* and AMPA*i* were not detected in the subsoil (results not shown). This indicates that AMPA*i* had not been formed, and GLYP*i* was already decomposed by microorganisms or scarcely displaced from surface into subsoil. Therefore, leaching of GLYP*i* or AMPA*i* can be considered as insignificant in this experiment and rapid degradation to further products most likely happened. Nevertheless, it is still possible that strongly bound non-extractable, and therefore non-detected residues of GLYP*i* or AMPA*i* could have remained in soil too, partly explaining the higher amounts of ^{13}C and ^{15}N after 165 and 360 days.

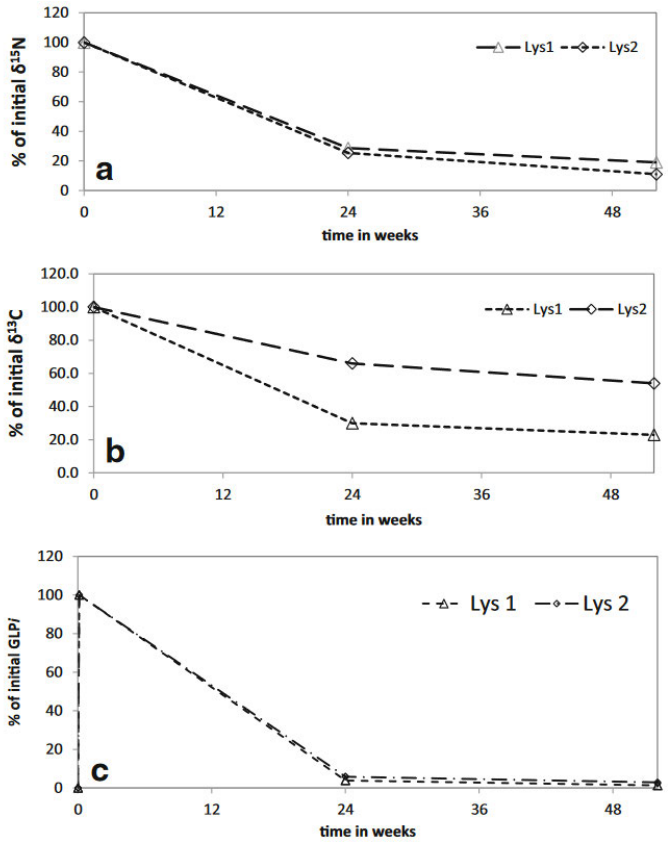


Figure 8.1.3.2-4: Development of $\delta^{15}N$ (a), $\delta^{13}C$ (b), and $^{13}C_2$-^{15}N-glyphosate (c) in topsoil samples compared with initial values (set to 100 %) over the studied period in lysimeter 1 (Lys1) and lysimeter2 (Lys2)

Plant material analyses

^{15}N was enriched highly significantly ($p < 0.01$) in all sampled plant compartments (root, 39 ±10 ‰ and 54 ±16; shoot, 28 ±13 ‰ and 51 ±16 ‰; cob, 34 ±12 ‰ and 51 ±14 ‰) compared with reference plant parts from a lysimeter that was not treated with GLYP*i* (root, 2.5 ±1.6 ‰; shoot, 2.0 ±0.9 ‰; cob, 4.0 ±1.9 ‰). By comparison, ^{13}C was highly significantly enriched only in the plant roots from the two lysimeters treated with GLYP*i* (-12.75 ±0.07 ‰ and -12.84 ±0.06 ‰) compared with maize roots from the lysimeter with no herbicide treatment (-13.03 ±0.08 ‰). In contrast, ^{13}C was significantly depleted ($p < 0.01$) in the cob material of plants from lysimeters with GLYP*i* treatment (-23.24 ±0.18 ‰ and -24.06 ±0.96 ‰) compared with those with no treatment (-22.84 ±0.25 ‰). There was not a significant difference in the shoots between the treated (-13.69 ±0.06 ‰ and -13.58 ±0.05 ‰) and non-treated lysimeters (-13.56 ±0.45 ‰).

The enrichment of ^{15}N in roots, shoots, and cobs can result only from uptake from the soil and distribution through the plant. Since ^{15}N is bound in GLYP*i* or its ^{15}N containing degradation products, those degradation products must have acted as plant nutrients. Furthermore, as plants do not take up organic substances like GLYP*i* or AMPA*i* over the root system, the occurrence of ^{15}N can be plausibly explained only by an uptake of mineral ^{15}N ($^{15}NH_4^+$ and/ or $^{15}NO_3^-$) as mineralized degradation products from GLYP*i*, which are formed by microbial degradation in the rhizosphere (Duke *et al.* 2012).

The enrichment of ^{13}C in the roots compared with plants from the non-treated lysimeter may be explained by attachment, possibly due to mycorrhizal fungi associated with the maize roots (Bott *et al.* 2011) that utilize organic substances as nutrients for growth.

In summary, since (i) ^{15}N has been taken up by the maize roots and distributed into all plant compartments and (ii) ^{13}C is only associated with the plant roots, the interaction of these labelled atoms with the plants most plausibly resulted from the independent interaction of the inorganic degradation

products of GLYP*i*. $^{13}CO_2$ and $^{15}NH_3$ as the inorganic end-products of the degradation process can be emitted via the air path. This was shown for ^{14}C labelled GLYP (Grundmann *et al*. 2008). But since (i) $^{15}NH_3$ is water-soluble and forms $^{15}NH_4^+$ in soil solution and (ii) ^{15}N was taken up by plants, it is rather unlikely for inorganic N to be emitted into the air.

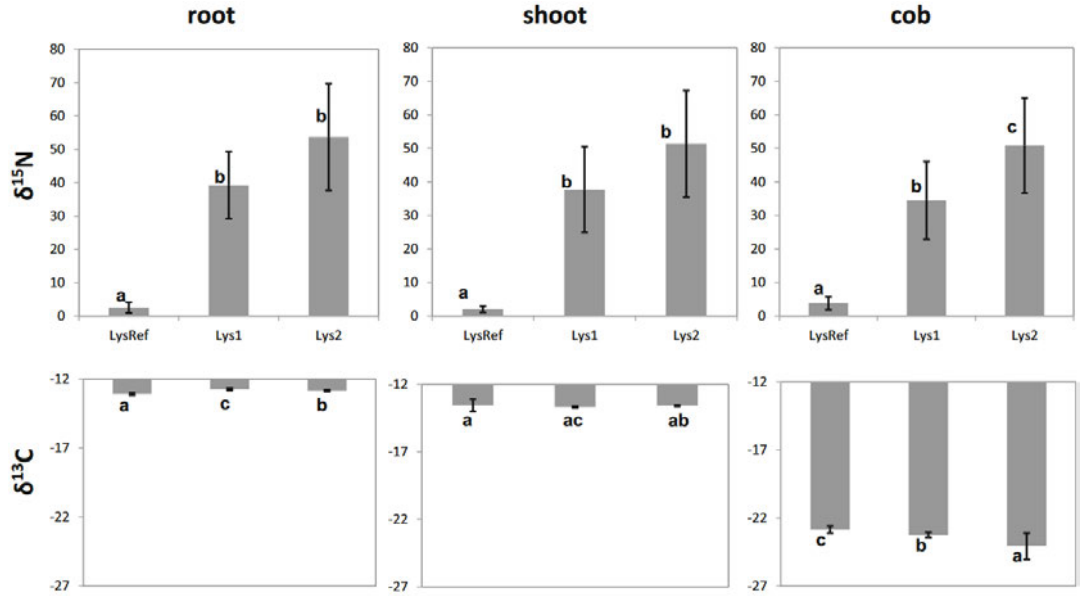


Figure 8.1.3.2-5: $\delta^{15}N$ and $\delta^{13}C$ mean values in plant material of roots, shoots, and cobs of maize plants of the tested lysimeter 1 (Lys1), lysimeter 2 (Lys2), and a reference lysimeter (LysRef)

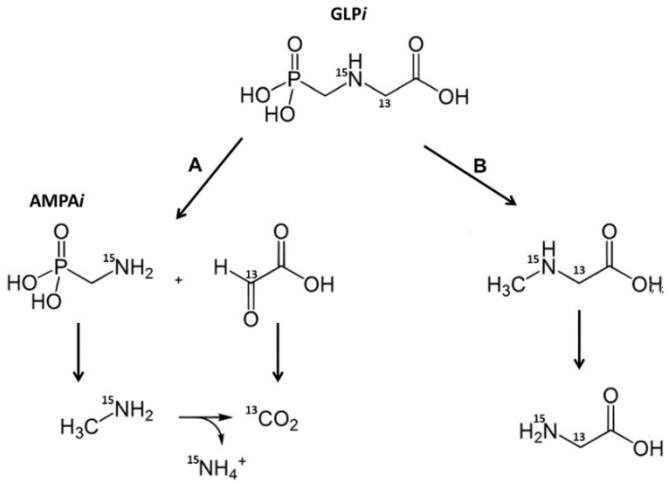


Figure 8.1.3.2-6: Degradation pathways of isotopic labelled $^{13}C_2$-^{15}N-glyphosate (GLP*i*) and its main degradation product ^{15}N-aminomethylphosphonic acid (AMPA*i*) with indicated positions of labelling (modified from Giesy *et al.* 2000)

Discussion

The present study was designed in detail so that field conditions are reflected and also all relevant compartments are considered.

In the present study, concentrations of extracted GLYP*i*-residues were low in soil at the end of the study period compared with the initial concentrations at the beginning. But fractions of ^{15}N and ^{13}C above extracted GLYP*i*-residues indicate that either non-extractable GLYP*i* is still left and/ or further degradation products accumulated in soil.

The ^{13}C and ^{15}N are signals of leachates, but absence or low concentrated (<LOD) residues of GLYP*i* and AMPA*i* indicate that further degradation products have been leached through the soil column. The

noncorrelated appearance of ^{13}C and ^{15}N signals in leachates makes the degradation pathway B rather unlikely. Instead, pathway A is supported by the noncorrelated appearance of ^{13}C and ^{15}N signals in leachates, among which ^{13}C can originate from glyoxylic acid and ^{15}N from detected AMPA*i* or further degradation products, such as methylamine and ammonium ions. Along with small concentrations of extracted GLYP*i* under practically optimal leaching conditions in the very wet hydrological year 2017/ 2018, the present findings indicate that rapid degradation most likely is the best explanation for the absence of concentrations above LOD of GLYP*i* and AMPA*i* in leachates.

Conclusions

Isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ and resulting changes of $\delta^{13}C$ and $\delta^{15}N$ from isotopically labelled glyphosate (GLYP*i*) and its degradation products were successfully quantified using isotopic ratio mass spectrometry (IR-MS) in different compartments (leachates, soil, and plant material) of a field lysimeter. Therefore, this experimental approach was well suited to trace GLYP*i* under practice-near experimental conditions.

Since (i) the great decline of GLYP*i* content down to <3 % of initial amounts in soil during the one-year study period and (ii) a lower decline of ^{13}C (<60 %) and ^{15}N (<20 %), we conclude that either further degradation products had been formed and/ or non-extractable and, therefore, strongly bound GLYP*i* remained in soil and accumulated. The disparate increase of $\delta^{13}C$ and $\delta^{15}N$ values in leachates and plant material is explained plausibly by (i) rapid degradation of GLYP*i* within one vegetation period and, also (ii) the selective uptake of mineralized ^{15}N species from degraded GLYP*i* as plant nutrient, most likely NH_4^+ or NO_3^-. These findings from a wet hydrological year support the assumption that the risk of leaching of applied GLYP to other waterbodies can be considered to be low under central European climatic conditions. Accumulation in soil may enhance the risk of further distribution in the environment by soil erosion.

Assessment and conclusion by applicant:

The article reports results of a lysimeter experiment with $^{13}C_2$-^{5}N-glyphosate in Germany. Besides analysis of lysimeter leachate, also soil and plant (maize) samples were analyzed. Although, the methods and results are well described, no endpoint can be derived due to some deviations from the relevant guideline (OECD 22). For example, it is not clear whether an undisturbed soil monolith has been used, and the origin and storage of soil is not reported. Amounts of precipitation are not reported in sufficient detail (only for 2 months) and amounts of leachate are only given as overall sum and weekly averages. For glyphosate, the sensitivity of the analytical method is not reported (AMPA was analyzed only qualitatively), and the stability of analytes in leachates and extracts during frozen storage was not shown. Results of leachate analysis were not reported in µg/L (only as $\delta^{13}C$ and $\delta^{15}N$), and results of soil analysis were only given in % of initial concentration.

The article is therefore considered as reliable with restrictions.

Assessment and conclusion by RMS:

RMS agrees with the applicant's conclusion.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

B.8.1.3.3. *Field leaching studies*

Field leaching studies were not performed and are not required. A comprehensive set of laboratory data on degradation and adsorption of parent active substance and AMPA allow for an assessment of the leaching potential of glyphosate and AMPA under various environmental conditions by the use of computer simulations as given by the FOCUS scenarios approach.

In the scientific literature research for glyphosate (2010-2020), six articles were identified to provide further information relevant to the data point.

Table 8.1.3.3-1: Field leaching experiments – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status
CA 7.1.4.3/001	Ulen *et al.*, 2014	Field leaching	Glyphosate	Reliable with restrictions
CA 7.1.4.3/002	Ulen *et al.*, 2012	Field leaching	Glyphosate	Reliable with restrictions
CA 7.1.4.3/003	Aronsson *et al.*, 2011	Field leaching	Glyphosate	Reliable with restrictions
CA 7.1.4.3/004	Kjaer *et al.*, 2011	Field leaching	Glyphosate	Reliable with restrictions
CA 7.1.4.3/005	Candela *et al.*, 2010	Field leaching	Glyphosate	Reliable with restrictions
CA 7.1.4.3/001	Rasmussen, S., et al. 2015	Modelling	Glyphosate	Reliable with restrictions

Ulen et al., 2014

Data point:	CA 7.1.4.3/001
Report author	Ulen, B.M. *et al.*
Report year	2014
Report title	Spatial variation in herbicide leaching from a marine clay soil via subsurface drains
Document No	DOI 10.1002/ps.3574 E-ISSN 1526-4998
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Subsurface transport via tile drains can significantly contribute to pesticide contamination of surface waters. The spatial variation in subsurface leaching of normally applied herbicides was examined together with phosphorus losses in 24 experimental plots with water sampled flow-proportionally. The study site was a flat, tile-drained area with 60 % marine clay in the topsoil in southeast Sweden. The objectives were to quantify the leaching of frequently used herbicides from a tile drained cracking clay soil and to evaluate the variation in leaching within the experimental area and relate this to topsoil management practices (tillage method and structure liming).

In summer 2009, 0.14, 0.22 and 1.62 %, respectively, of simultaneously applied amounts of MCPA, fluroxypyr and clopyralid were leached by heavy rain five days after spraying. In summer 2011, on average 0.70 % of applied bentazone was leached by short bursts of intensive rain 12 days after application. Peak flow concentrations for 50 % of the treated area for MCPA and 33 % for bentazone exceeded the Swedish no-effect guideline values for aquatic ecosystems. Approximately 0.08 % of the glyphosate applied was leached in dissolved form in the winters of 2008/2009 and 2010/2011. Based on measurements of glyphosate in particulate form, total glyphosate losses were twice as high (0.16 %) in the second winter. The spatial inter-plot variation was large (72–115 %) for all five herbicides studied, despite small variations (25 %) in water discharge.

The study shows the importance of local scale soil transport properties for herbicide leaching in cracking clay soils.

Materials and Methods

The field site is located in a flat valley with a clay soil of marine origin in eastern Sweden. The experimental field was tile-drained in 2006 to 0.9 m depth. Twenty-four of these plots were used in the present experiment. The plots are situated in two rows of 14 plots at varying distance from an open ditch that acts as the recipient of drainage water from the surrounding valley. Three management practices were randomly assigned to the plots: Conventional autumn ploughing, shallow autumn tillage and structure-liming (i.e. liming carried out to reduce phosphorus leaching and to improve crop yield by improving soil structure). Soil pH and total organic carbon (OC) content are given in Table 8.1.3.3-2. There were no significant differences ($P > 0.05$) in soil pH and OC between treatments.

Table 8.1.3.3-2: Mean values and standard deviations (SD) of soil pH and concentrations (%) of organic carbon (OC) at the start of the project in the autumn of 2007 and five years later in the spring of 2012 after repeated different tillage treatments and after structure liming first year

Mean values and standard deviations (SD) of soil pH and concentrations (%) of organic carbon (OC) at the start of the project in the autumn of 2007 and five years later in the spring of 2012 after repeated different tillage treatments and after structure liming first year

			Shallow tillage		Structure-limed		Conventional ploughing	
Sampling time	Property	Depth (cm)	Mean	SD	Mean	SD	Mean	SD
2007	pH	0–2	6.4	0.2	6.3	0.1	6.4	0.1
2012	pH	0–2	6.1	0.2	6.5	0.5	6.1	0.1
2007	OC (%)	0–23	2.4	0.5	2.5	0.5	2.5	0.5
2007	OC (%)	23–60	1.4	0.3	1.5	0.4	1.5	0.3
2007	OC (%)	60–90	0.6	0.1	0.6	0.1	0.6	0.1
2012	OC (%)	0–2	2.7	0.2	2.7	0.3	2.6	0.3

Pesticide leaching

We studied the leaching of seven different pesticides with contrasting properties (Table 8.1.3.3-3). It should be noted that both pesticide half-lives and adsorption partitioning coefficients are dependent on soil properties and the values presented in the tables may, therefore, not be representative for the clay soil at this site. Pesticide leaching was studied in two different crop rotations (Table 8.1.3.3-4), both with oats and peas during the last two years (2010–2011). In crop rotation I (20 plots), conventional autumn ploughing was compared with shallow autumn tillage and the effects of previous structure-liming in autumn 2007 were examined. Glyphosate was applied before sowing in spring 2008 to control couchgrass in eight shallow-tilled plots in crop rotation I (Table 8.1.3.3-5). In early summer the same year, the low-dose substances thifensulfuron-methyl and tribenuron-methyl were applied in both rotations. In autumn 2008 glyphosate was applied after harvest (four plots) in crop rotation II in order to control couchgrass and volunteer cereals. The three pesticides clopyralid, fluroxypyr and MCPA (all ingredients in the same commercial product) were sprayed for weed control on 9 June 2009 in crop rotation I (20 plots) and on 23 June 2010 in both rotations (24 plots). Glyphosate was applied after harvest in September 2010 and bentazone was applied on 11 June 2011 in both rotations (24 plots). Most applications were made in the evening, with no wind and always in the recommended dose. The total loads of herbicides applied (Table 8.1.3.3-5) were similar to those reported from agricultural catchments within the Swedish National Pesticide Monitoring Programme. Precipitation was measured at the site with unheated tilting bucket equipment and collected in a data logger.

Table 8.1.3.3-3: Herbicide properties and potential data taken from the Pesticide Properties Database (PPDB, 2010) Substance

Herbicide properties and leaching potential data taken from the Pesticide Properties Database (PPDB, 2010)[11] Substance					
	DT_{50}lab[a](days)	DT_{50}field[b](days)	K_{oc}[c] ($cm^3\ g^{-1}$)	GUS[d]	pK_a[e]
Bentazone	13	14	55.3	2.30	3.28
MCPA	24	25	74[f]	2.94	3.73
Fluroxypyr	1	51	195[f]	0	2.94
Clopyralid	34	11	5.0	5.06	2.01
Glyphosate	12	12	1435	−0.49	2.34
Thifensulfuron-methyl	4	4	28.3	1.53	4.4
Tribenuron-methyl	14	14	35	2.88	4.7

[a] Degradation half-life for aerobic conditions measured in the laboratory.
[b] Degradation half-life for aerobic conditions measured in the field.
[c] Adsorption distribution coefficient to organic carbon.
[d] Groundwater ubiquity score.
[e] Acid dissociation constant.
[f] Freundlich adsorption coefficient to organic carbon.

Table 8.1.3.3-4: Year, crop, date and commercial brand name of herbicides applied in 2008-2011 in crop rotations I and II (number of conventionally ploughed plots/total number of treated plots)

Year, crop, date and commercial brand name of herbicides applied in 2008–2011 in crop rotations I and II (number of conventionally ploughed plots/total number of treated plots)						
Year	Rotation I Crop	Date	Herbicide (16/20 plots)	Rotation II Crop	Date	Herbicide (4/4 plots)
2008	Spring barley	24/4	Glypro Bio[a, b]	Winter wheat	26/6	Harmony 50T Plus[c]
	Spring barley	26/6	Harmony 50T Plus[c]	After W wheat	16/8	Glypro Bio[b]
2009	Spring barley	9/6	Ariane S[d]	Winter wheat	6/5	Harmony 50T Plus[c]
2010	Oats	23/6	Ariane S[d]	Oats	23/6	Ariane S[d]
	Oats	22/9	Glypro Bio[b]	After oats	22/9	Glypro Bio[b]
2011	Pea	11/6	Basagran[e]	Pea	11/6	Basagran[e]

[a] Only in eight shallow-tilled plots.
[b] Active ingredient glyphosate (49%).
[c] Active ingredients thifensulfuron-methyl (37%) and tribenuron-methyl (17%).
[d] Active ingredients MCPA (20%), fluroxypyr (4%) and clopyralid (2%).
[e] Active ingredient bentazone (87%).

Table 8.1.3.3-5: Year, date of application, substance anylsed in drainage water, crop and applied dose of detected substance, together with the general dose (in g/ha) applied in Swedish monitored small catchments in 2008-2011

Year, date of application, substance analysed in drainage water, crop and applied dose of detected substance, together with the general dose (in g ha^{-1}) applied in Swedish monitored small catchments in 2008–2011					
Year	Date	Substance	Crop	Dose (g ha^{-1})	General dose (g ha^{-1})
2008	24/4	Glyphosate	Before barley	707	748
2008	26/4	Thifensulfuron-methyl	Spring barley	4	6
		Tribenuron-methyl	Spring barley	2	3
2008	16/8	Glyphosate	After winter wheat	1060	1116
2009	26/5	Thifensulfuron-methyl	Winter wheat	6	6
		Tribenuron-methyl	Winter wheat	3	3
2009	9/6	Clopyralid	Spring barley	52	48
		Fluroxypyr	Spring barley	104	81
		MCPA	Spring barley	520	590
2010	23/6	Fluroxypyr	Oats	104	75
		MCPA	Oats	520	510
		Glyphosate	After oats	1060	1110
2011	11/6	Bentazone	Pea	475	500

Water sampling and analysis

Water discharge from each plot was measured with tilting vessels in an underground basement where sampling of drainage water also took place. The water was sampled flow-proportionally, with every subsample representing 0.003 mm discharge in summer and 0.04 mm discharge in the rest of the year. The bulk samples were collected weekly (or for the first flow events following application more frequently). The concentration of thifensulfuron-methyl and tribenuronmethyl (in 2008) was determined with solid-phase extraction followed by liquid chromatography and mass spectrometry (LCMS) and the concentration of clopyralid, fluroxypyr and MCPA (in 2009) by the same solid-phase extraction and by derivatisation and gas chromatography/mass spectrometry (GC/MS). Fluroxypyr and MCPA (in 2010) and bentazone (in 2011) were analysed by mass spectrometric determination (LC-MS/MS). Dissolved glyphosate (DissGly) and its main metabolite AMPA were analysed in winter 2008/2009 and 2010/2011, which involved ion exchange and derivatisation, followed by final identification and quantification by GC/MS. In winter 2010/2011, glyphosate analysis included particulate glyphosate (PartGly), which was trapped using a cellulose acetate filter with pore size 0.45 µm.

Table 8.1.3.3-6: Monthly precipitation (Prec) and total snow accumulation (Snow acc) in winter periods (October-April current year and January-April following year), water discharge (Flow) and ratio Flow/Prec dfor the experimental years and long-term (1988-2011) average

Monthly precipitation (Prec) and total snow accumulation (Snow acc) in winter periods (October–April current year and January–April following year), water discharge (Flow) and ratio Flow/Prec for the experimental years and long-term (1988–2011) average

Year		May	June	July	August	September	October–April	Snow acc (cm)
2008	Prec (mm)	30	5	44	42	8	385	14
	Flow (mm)	10	1	0	4	5	395	
	Flow/Prec	0.33	0.20	0	0.03	0.63	0.99	
2009	Prec (mm)	45	95[a]	94	54	35	384	50
	Flow /mm)	3	43	3	1	0	370	
	Flow/Prec	0.07	0.45	0.03	0.02	0	0.85	
2010	Prec (mm)	53	39	155[b]	95	51	290	75
	Flow (mm)	16	3	8	87	42	310	
	Flow/Prec	0.30	0.08	0.05	0.92	0.82	0.85	
2011	Prec (mm)	40	70	26	138	72	358	3
	Flow (mm)	3	14	0	0	4	350	
	Flow/Prec	0.08	0.20	0	0	0.06	0.96	
1988–2011	Prec (mm)	40	67	82	69	63	338	32
	Flow (mm)	7	12	2	18	10	360	
	Flow/Prec	0.15	0.18	0.02	0.21	0.16	0.97	

[a] Maximum intensity 46 mm day^{-1} in the middle of the month.
[b] Maximum intensity 79 mm day^{-1} at the end of the month.

Results and Discussion

Concentrations of pesticides in drain water

The sulphonylureas (thifensulfuron-methyl and tribenuronmethyl) were not detected above LOD in 2008. Because of the fast dissipation of these substances, they were not analysed for in subsequent years. Unlike these low-dose substances, detectable levels of all other herbicides were found every year in drain flow in the first 1–2 months after early summer application. Detectable concentrations of fluroxypyr and MCPA were also observed 31 days after application (Table 8.1.3.3-7) in the samples taken after flooding of the measuring station. Detection of pesticides in the first few rainfall/drainage events after application is consistent with the flow was the simultaneous arrival of clopyralid and fluroxypyr on 14–16 June 2009, despite large differences in K_{oc} values (Table 8.1.3.3-3). However, since only five days had passed between application and rainfall, the substances might not have been in equilibrium with the soil solid material due to slow kinetics. Dissolved glyphosate was detected in consecutive events in autumn 2008. Both particle-bound glyphosate and dissolved glyphosate were detected in the discharge from all fast-flow events in autumn 2010. Levels above the $C_{\text{no effect}}$ concentrations were observed in 50 % of the plots for MCPA and in 33 % for bentazone (Table 8.1.3.3-7). Levels of glyphosate, AMPA, clopyralid and fluroxypyr were on all occasions below their $C_{\text{no effect}}$ concentrations. The coefficient of variation in the most important leaching event for the substances studied varied between 72 and 115 % between all different plots (including different

treatments) and increased in the order bentazone < clopyralid < fluroxypyr < PartGly < MCPA < DissGly. These highly variable pesticide concentrations were not significantly correlated to the basic soil factors pH value, clay content and organic matter content in the topsoil, which only showed minor variance (2, 17 and 10 %, respectively).

Table 8.1.3.3-7: Year, date of application of substance (including glyphosate metabolite AMPA) and glyphosate in dissolved (diss) form and total glyphosate, numbers of plots (Plots), number of days (No. days) until major rain event, Swedish guideline values for no effect ($C_{no\ effect}$), maximum (Max) and mean concentration in the main drainage event, ratio of number of plots with concentration exceeding $C_{no\ effect}$ to total number of plots treated (Ratio $C_{no\ effect}$) and total period (days) after application when values exceeding were $C_{no\ effect}$ detected

Year, date of application of substance (including glyphosate metabolite AMPA) and glyphosate in dissolved (diss) form and total glyphosate, numbers of plots (Plots), number of days (No. days) until major rain event, Swedish guideline values for no effect ($C_{no\ effect}$), maximum (Max) and mean concentration in the main drainage event, ratio of number of plots with concentration exceeding $C_{no\ effect}$ to total number of plots treated (Ratio $C_{no\ effect}$) and total period (days) after application when values exceeding $C_{no\ effect}$ were detected

Year	Date	Substance	No. days	$C_{no\ effect}$ (µg L^{-1})	Maximum (µg L^{-1})	Mean (µg L^{-1})	Ratio $C_{no\ effect}$	Period (days)
2008	16/8	Glyphosate diss.	47	100	1.2	0.48	0/4	—
		AMPA	47	500	0.3	—	—	—
2009	9/6	Clopyralid	5	50	5.5	2.2	0/20	—
		Fluroxypyr	5	100	1.7	0.67	0/20	—
		MCPA	5	1	5.5	2.0	10/20	5–14
2010	23/6	Fluroxypyr[a]	31	100	0.3	0.081	0/24	—
		MCPA[b]	31	1	0.04	0.007	0/24	—
2010	22/9	Glyphosate diss.	33	100	3.9	0.58	0/24	—
		Glyphosate total	33	100	9.4	2.2	0/24	—
		AMPA	33	500	0.7	—	—	—
2011	11/6	Bentazone	12	30	63	23.9	8/24	12–16

[a] Generally only analysed in dissolved form.
[b] Late collection of sample, as the measuring station was flooded.

Leaching losses of pesticides

The amount of pesticide leached in summer periods from conventionally ploughed plots sprayed simultaneously with the same herbicide in 2009–2011 varied between 0.2 and 3.3 g/ha (0.1–1.6 % of amount applied) (Table 8.1.3.3-8). Leaching losses above 1 % are generally associated with large rainfall amounts shortly after application. However, for our case the hydrological conditions did not represent 'worst-case' leaching conditions and hence the large leaching losses demonstrate the great potential for preferential transport in this soil. Losses exceeding 0.1 % took place from 22 to 24 plots (92–100 % of the experimental area) for clopyralid and bentazone, while the relative losses of MCPA exceeding 0.1 % represented 42 % of the area. The relative leaching losses of the substances studied here are presented below. Surprisingly, autumn application of glyphosate in 2008 and 2010 resulted in quite similar losses in dissolved form in the following winters (0.9 g/ha corresponding to 0.08 % of applied amounts; Table 8.1.3.3-8), irrespective of whether the main discharge took place after autumn rain followed by a mild winter (2008) or in connection with snowmelt after a cold winter with continuous snow cover (2011). Due to slow degradation during the winter of 2010/2011 owing to long-lasting snow cover, glyphosate was available for leaching during the main snowmelt event, which was fast and probably resulted in preferential transport.

Table 8.1.3.3-8: Year, date, applied substance, including the sum of the three components in the commercial product Ariane S, mean loss from all ploughed plots with standard deviation (SD), mean losses relative to applied amount, range of the relative losses and area with relative losses exceeding 0.1 g/ha. Glyphosate was analysed in both dissolved (diss.) and particulate (part.) form in 2010

Year, date, applied substance, including the sum of the three components in the commercial product Ariane S, mean losses from all ploughed plots with standard deviation (SD), mean losses relative to applied amount, range of the relative losses and area with relative losses exceeding 0.1 g ha $^{-1}$. Glyphosate was analysed in both dissolved (diss.) and particulate (part.) form in 2010

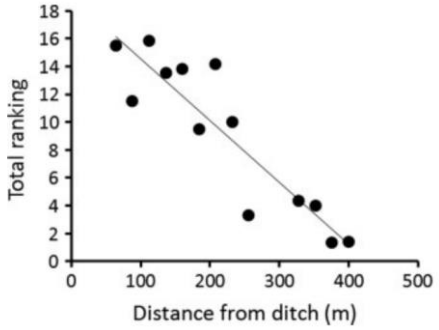
Year	Date	Substance	Mean (g ha^{-1})	SD	Relative losses (%)	Range of relative losses (%)	Area (%)
2008	16/8	Glyphosate diss.	0.89	0.64	0.084	0.02–0.17	25
2009	9/6	Clopyralid	0.84	0.70	1.62[c]	0.09–4.55	92
		Fluroxypyr	0.24	0.36	0.22	0.002–0.96	60
		MCPA	0.71	0.89	0.14	0.003–0.49	42
		Sum	1.81	3.03	0.34	0.02–1.36	60
2010	23/6	Fluroxypyr[a]	> 0.03	0.02	> 0.03	—	—
2010	22/9	Glyphosate diss.[b]	0.90	0.32	0.085	0.05–0.12	—
		Glyphosate part.	0.82	0.25	0.064	0.08–0.10	—
		Glyphosate total	1.72	1.47	0.15	0.12–0.23	100
2011	11/6	Bentazone	3.31	2.92	0.70[b]	0.42–2.16	100

[a] From late sample after flooding of the measuring station.
[b] Period 22 September–15 April and calculated from the same four plots as treated in 2008.
[c] Relative losses of clopyralid were significantly greater ($P < 0.01$) than losses of fluroxypyr applied simultaneously.

Herbicide correlation with particulate phosphorus and plot position

In spite of the small variation in the amounts of water discharge between plots, there was a large variation in herbicide losses for all substances resulting from the highly varying concentrations in drainage water. Similar relationships have previously been reported between total glyphosate and PP for the same field. Due to their strong sorption, both glyphosate and PP are considered to leach mainly through preferential transport in macropores. Our results suggest that preferential transport dominates leaching also for the weakly sorbed substances at this site. In addition, since the pesticides which were applied at the soil surface were leaching with a similar pattern as PP, this suggests that the topsoil was the major source of leached PP. We did not observe any surface runoff in the direction of the Recipient ditch during the experimental period. Lateral flows below the soil surface and e.g. on a plough pan were also unlikely to occur, since there was no distinct plough pan at the site. There was no correlation between the topsoil (0–23cm) pH and the plot position. However, topsoil OC clearly increased with decreasing distance between plot mid-point and the recipient ditch (R^2 = 0.70 %, $P < 0.001$) and pH in the deeper subsoil (60–90 cm) decreased (R^2 = 78 %, $P < 0.001$). The concentration of all pesticides tended to increase with decreasing distance between plot position and the recipient ditch. The relationship was significant for bentazone, and was also significant from a total ranking of all pesticides detected (Figure 8.1.3.3-1).

Figure 8.1.3.3-1 Total ranking of mean concentration of clopyralid, fluroxypyr, MCPA, bentazone, dissolved glyphosate and particulate glyphosate related to the distance between the ditch and the centre of the respective plot. The estimates were made for the observed concentrations in the major event for every substance. The slope of the regression line is significantly different from zero (P < 0.001).



Effects of soil management, soil structure, pH and organic matter

There was a general tendency for larger losses of all substances from shallow-tilled plots than from ploughed plots, with or without previous structure liming (Table 8.1.3.3-9). The apparent differences,

which were not significant for any single substance, increased in the order clopyralid < MCPA < bentazone < fluroxypyr < total glyphosate. However, estimated for all five substances lumped together (paired *t*-test), the difference between shallow-tilled and ploughed structure-limed plots was significant ($P < 0.05$), both before and after adjustment to the effect of plot position in relation to the ditch. From soils where preferential flow and transport are important, ploughing is generally considered to reduce pesticide leaching by interrupting continuous macropores. For our case the larger losses from the shallow-tilled plots may also have been an effect of shallow and uneven accumulation of crop residues in these plots which resulted in uneven infiltration and preferential herbicide transport along straw residues. At the study site, it has been demonstrated that structure liming (quicklime) significantly improves soil aggregate stability measured as a decrease in readily dispersed clay. Improved aggregate stability should influence the transport of glyphosate which adsorbs strongly to clay particles. However, the improved aggregate stability did not result in any significantly smaller losses of glyphosate from structure limed plots compared to conventionally tilled plots.

Table 8.1.3.3-9: Year of application, mean and standard deviation (SD) of transported masses of the applied substances in g/ha) from tilled, structure-limed (+ ploughed) and conventionally ploughed plots

Year of application, mean and standard deviation (SD) of transported masses of the applied substances (in g ha^{-1}) from tilled, structure-limed (+ ploughed) and conventionally ploughed plots

		Shallow tillage Mean			Structure-limed Mean			Conventional ploughing Mean		
Year	Substance	Un-adjusted	Adjusted	SD	Un-adjusted	Adjusted	SD	Un-adjusted	Adjusted	SD
2009	Clopyralid	1.16	1.14	0.77	1.07	1.13	0.95	0.73	0.71	0.59
2009	Fluroxypyr	0.36	0.35	0.29	0.25	0.26	0.28	0.22	0.21	0.25
2009	MCPA	1.11	1.09	1.03	0.86	0.88	1.25	0.63	0.61	0.82
2011	Bentazone	4.78	4.73	2.84	3.52	3.75	3.81	3.40	3.32	2.53
2010	Glyphosate[a]	3.81	3.77	2.58	0.85	0.90	1.13	1.59	1.56	1.56

Note: Mean transported losses are given both as unadjusted values and values adjusted for the distance to the ditch.
[a] Total glyphosate in both particulate and dissolved form in the period 22 September 2010–15 April 2011.

For ionisable pesticides, leaching is also affected by soil pH, with weaker sorption at higher pH. Based on the p*K*a values of the substances studied here and the small differences in pH between treatments (Table 8.1.3.3-9), any pH effects on leaching were probably minor. The topsoil OC content is often higher under long term shallow tillage than under conventional tillage, which has consequences for pesticide sorption and degradation. However, in our case the OC content was not significantly different between treatments and there were no significant differences in subsoil OC between plots with different management regimes. The coefficient of variation in relative leaching losses between all substances for the shallow-tilled plots varied between 40 and 92 %. The coefficient of variation in the relative leaching losses from all plots and for all substances combined (92–156 %) varied even more. In conclusion, the variation in relative leaching losses between plots within the same treatment was larger than that between different substances. This finding also demonstrates that the differences in transport pathways through the soil between plots have a larger effect on pesticide concentrations than the differences in pesticide properties.

Conclusions

Concentrations of the herbicides bentazone, clopyralid, fluroxypyr, MCPA and glyphosate were measured in subsurface drain discharge from a clay field during a four-year study. Despite hydrological conditions not representing a worst case scenario for leaching, the relative leaching losses of all herbicides studied were large compared to values reported in the literature. Measured concentrations of bentazone and MCPA exceeded Swedish guideline values based on predicted no effect on aquatic ecosystems for 50 and 33 % of the plots for MCPA and bentazone, respectively. All substances studied (except sulphonyl ureas which were not detected), irrespective of sorption strength, showed similar leaching patterns. These observations clearly demonstrate that preferential transport in macropores is the dominant transport process at this site. The variation in relative leaching losses between plots within the same treatment was greater than that between different substances. Crack stabilisation by gyttja, especially in the deeper subsoil, was suggested as an important explanatory factor for this large spatial

variation in pesticide leaching, although it was not possible to investigate differences in gyttja content between plots. Continuous macropores connecting the soil surface to the subsoil may be a factor contributing to the generally large pesticide losses observed after shallow tillage. However, careful studies of soil macropore systems, including topsoil and subsoil properties, are needed to explain the unpredictability in leaching at this site.

Assessment and conclusion by applicant:

The article describes a leaching experiment from a tile-drained Swedish marine clay soil with agricultural land use. Glyphosate among other herbicides was considered in analysis. Preferential transport in macropores was the dominant process for all investigated substances at the test site. Glyphosate losses in total were up to 0.23%. The study provides supportive information but not all parameters to derive endpoints are reported.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

RMS agrees with the applicant's analysis of the article. The use of a tile drained cracking clay focuses the analysis on preferential flow.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Ulen et al., 2012

Data point:	CA 7.1.4.3/002
Report author	Ulen, B.M. *et al.*
Report year	2012
Report title	Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains
Document No	DOI 10.1080/09064710.2012.697572 E-ISSN 1651-1913
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

Losses of commonly used chemical pesticides from agricultural land may cause serious problems in recipient waters in a similar way to phosphorus (P). Due to analytical challenges concerning determination of glyphosate (Gly), transport behaviour of this widely used herbicide is still not well known. The objective of the present study was to quantify and evaluate leaching of Gly in parallel with P. Leaching losses of autumn-applied Gly (1.06 kg/ha) via drainage water were examined by flow-proportional sampling of discharge from 20 drained plots in a field experiment in eastern Sweden. Samples were analysed for Gly in particulate-bound (PGly) and dissolved (DGly) form. The first 10 mm water discharge contained no detectable Gly, but the following 70 mm had total Gly (TotGly) concentrations of up to 6 µg/L, with 62 % occurring as PGly. On average, 0.7 g TotGly/ha was leached from conventionally ploughed plots, compared with 1.7 g TotGly/ha from shallow-tilled plots (cultivator to 12 cm working depth). Higher Gly losses occurred in snowmelt periods in spring, but then with the majority (60 %) as DGly. All autumn concentrations of PGly in drainage water were significantly correlated ($p < 0.001$) to the concentrations of particulate-bound phosphorus (PP) lost from the different

plots (Pearson correlation coefficient 0.84), while PP concentrations were in turn significantly correlated to water turbidity (Pearson correlation coefficient 0.81). Leaching losses of TotGly were significantly lower (by 1.3 g/ha; $p < 0.01$) from plots that had been structure-limed three years previously and ploughed thereafter than from shallow-tilled plots. Turbidity and PP concentration also tended to be lowest in discharge from structure-limed plots and highest from shallow-tilled plots. This difference in TotGly leaching between soil management regimes could not be explained by differences in measured pH in drainage water or amount of discharge. However, previously structure-limed plots had significantly better aggregate stability, measured as readily dispersed clay (RDC), than unlimited plots. The effects of building up good soil structure, with strong soil aggregates and an appropriate pore system in the topsoil, on mitigating Gly and P losses in particulate and dissolved form should be further investigated.

Materials and Methods

Experimental plots and soil characteristics

The experiment was done on 20 drained plots in an experimental field with a sub-surface drainage water collection system constructed on a flat plain close to the Lake Bornsjön reservoir. Drainage water flows to a sampling and measuring station and is recorded with tilting vessels and data logger. The data logger controls the flow proportional sampling by means of small tube pumps in the basement of the station. After a certain volume of water has passed, the suction tube is first cleaned by reverse pumping and thereafter a small volume is sampled. The flow-proportional (composite) sampling took place in dark glass vessels at relatively cold temperature and in darkness for a maximum of one week prior to freezing the water samples and transport to the laboratory before analysis.

Clay content (60 %), is high throughout the profile (Table 8.1.3.3-10), with small spatial variation in both topsoil and sub-soil (variance less than 0.5 %). pH and soil concentration of P are uniformly distributed in the experimental area (variance less than 15 %). In the soil profile, the pH (dry soil samples) varies between 5.2 and 6.9, with the lowest values occurring in the 70-100 cm layer, which includes the tile drains at approximately 90 cm depth. Under wet conditions the pH in the upper sub-soil is higher than that under dry conditions (6.9 compared with 6.6). Overall, the soil profile generally demonstrates a high ability to sorb P to the soil matrix.

The soil horizon has a strongly aggregated structure, especially in the deeper part, with approximately 10 cm wide and 10-20 cm prismatic aggregates in the layer 43-100 cm. Water retention is very high. In an adjoining field with an old drainage system, the deeper soil horizon is very wet, the aggregates similarly very prismatic and the structure is easily destroyed by digging.

Table 8.1.3.3-10: Selected physical and chemical properties of the soil at the study site.

	Soil depth (cm)					
Properties	0–10	10–30	30–50	50–70	70–100	Reference for the method
Particle size distribution						
<0.002 mm (clay) (%)	60	60	59	61	54	Eriksson et al. (1998)
0.002–0.02 mm (%)	31	30	30	31	34	Eriksson et al, (1998)
0.02–0.2 mm (%)	9	10	11	8	12	Eriksson et al. (1998)
Organic matter (%)	3.9	1.9	0.1	0.0	0.0	Eriksson et al. (1998)
pH_{H_2O}[a]	6.0	6.2	6.6	6.5	5.2	ISO (2005)
P_{Olsen} (mmol kg^{-1})[a]	0.59	0.53	0.13	0.17	0.68	Olsen and Sommers (1982)
P_{AL} (mmol kg^{-1})[a]	1.4	1.0	0.3	0.4	1.0	Egnér et al. (1960)
Al_{ox} (mmol kg^{-1})[a]	116	106	71	77	88	Schwertmann (1964)
Fe_{ox} (mmol kg^{-1})[a]	165	169	158	181	118	Schwertmann (1964)
Al_{AL} (mmol kg^{-1})[a]	10.3	9.9	9.8	9.6	16.1	Ulén (2006)
Fe_{AL} (mmol kg^{-1})[a]	9.4	10.1	8.8	9.4	12.5	Ulén (2006)
PSI_2 (mmol kg^{-1}).[a]	7.3	7.8	7.3	7.2	10.5	Börling et al. (2004)
P_{Olsen}/PSI_2[a] (%)	8.1	6.8	1.8	2.4	6.4	Börling et al. (2004)
DPS_{AL}[a] (%)	8.7	6.2	2.0	2.5	4.3	Ulén (2006)

[a]Data from Andersson et al. (2012).

Glyphosate application and cultivation practices.

No Gly had been applied to the actual experimental plots for the previous three years. Quicklime (CaO) had been applied in dry conditions on the stubble in four plots in 2007 Phosphorus fertilization was 11 kg/(ha year), always applied in mineral form in spring. This is a moderate load, since the area has special restrictions. When starting the experiment the aim was to avoid P limitation of the crop and therefore 20 kg/(ha year) were applied in 2007-2011 for all plots except four. Glyphosate was applied on 22 September 2010 as the commercial product Glypro Bio, at a rate equal to 1.06 kg/ha active substance. Twelve days later, the conventional and structure-limed plots were stubble-harrowed and eight plots were shallow-tilled (12 cm) twice and reconsolidated with a rib-roller. After a further 10 days, the conventionally ploughed plots (8) and the structure-limed plots (4) were mould-board-ploughed and the soil was inverted to a depth of 23 cm.

Table 8.1.3.3-11: Management regime in the different treatments (A-E) in 2010, where A+B (eight plots) represent regular conventional autumn ploughing, C (four plots) represents previous structure liming and D+E (eight plots) represent regular shallow tillage in autumn

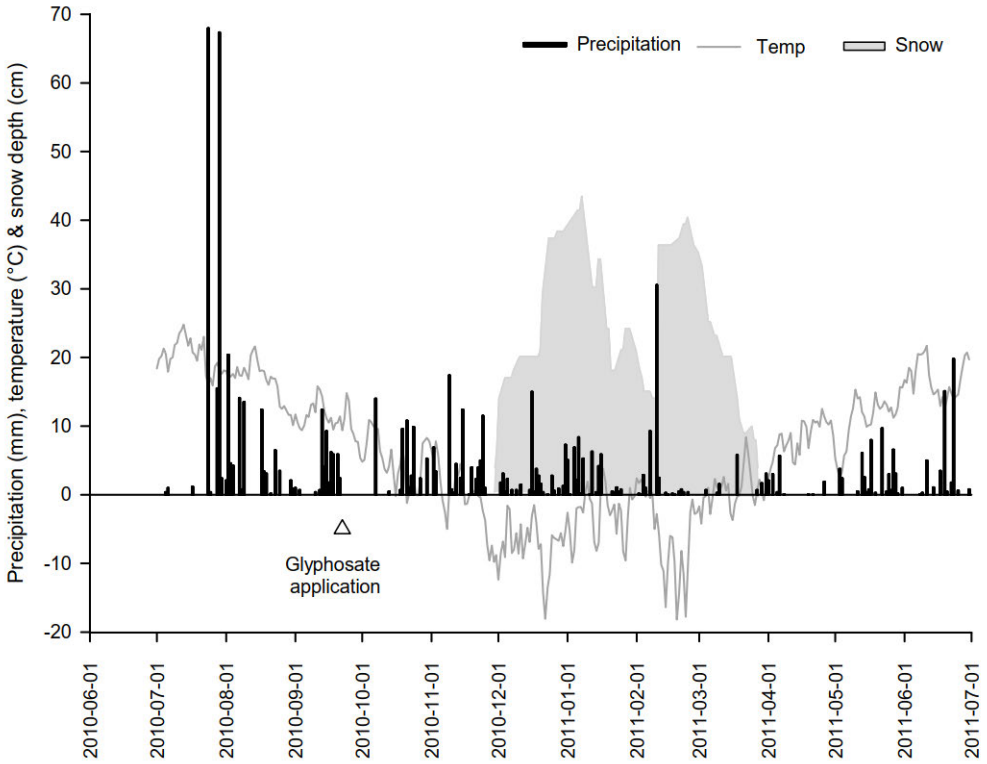
Treatment	Management	Date
A+B, C, D+E	Harrowing (0–5 cm)	16 May
A+B, C, D	Fertilization, seed drilling[a]	17 May
E	Fertilization (broadcasting)	17 May
A+B, C, D+E	Sowing (oats)	17 May
A+B, C, D+E	Harvesting	27 August
A+B, C, D+E	Glyphosate application (1.06 kg ha^{-1})	22 September
A+B, C	Stubble harrowing	4 October
D+E	Cultivation (8 cm) twice	4 October
A, B, C	Conventional ploughing (23 cm)	14 October

[a]No P fertilization to B plots.

Weather, discharge and water sampling procedure

Autumn 2010 was short, with permanent snow from the end of November (Figure 8.1.3.3-2). Owing to the thickness of the snow cover, soil freezing was limited despite low air temperatures. The main snowmelt took place in late March and the first two weeks in April. The glass vessels with flow-proportional samples in the station basement were observed regularly (at least weekly) and when at least 300 mL turbid water had been collected from most plots, sub-samples were taken from every plot for Gly analysis. When there was a moderate amount of water or less turbid water in the glass vessel, sampling was performed only for analysis of P and turbidity for reasons of economy. Such sampling occurred in total on five sampling occasions. On 28 March, 186 days after glyphosate application in autumn, turbidity was observed once again in the flow-proportionally sampled water and additional water was collected for Gly analysis, which was performed on the 14 most turbid samples.

Figure 8.1.3.3-2: Temperature (°C), precipitation (mm) and snow cover (mm) on the experimental field in 2010-2011



Water analysis

Total P was analysed as soluble molybdate-reactive P after acid oxidation with $K_2S_2O_8$ (ECS, 1996). DRP was analysed after pre-filtration using filters with pore diameter 0.45. Particulate P (PP) is the absolute dominant P fraction, while non-mineral forms of dissolved P are very small, and accordingly the difference between TotP and DRP was taken as PP. The concentration of particles was analyzed from thawed samples as turbidity on a HACH 2100 turbidometer. Before analysing Gly, each thawed sample was thoroughly shaken by hand, centrifuged and filtered. The filtered water was used for analysis of DGly, including AMPA, after pH adjustment (pH 7-8) with either diluted HCl or NaOH. After a few more rounds of extraction, centrifugation and filtration, the pH of the samples was adjusted to 2 in order to precipitate any humic acids and to harmonize with the method used for stream and lake sediment. After dilution, the pH was readjusted to 7-8.

The same analytical procedure was used for both PGly and DGly and involved ion-exchange and derivatization, using a modified version of Mogadati *et al.* (1996), followed by final identification and quantification by gas chromatography-mass spectrometry (GC-MS).

Soil aggregate stability

Soil samples from plots with structure liming, conventional ploughing and reduced tillage were analysed in the laboratory for aggregate stability, expressed as readily dispersed clay (RDC). Slightly moist samples were collected from the topsoil (0-20 cm) on 27 August 2010, before post-harvest stubble cultivation, and gently transported to the laboratory. Four sub-samples representing 12 aggregates (8-10 mm) were prepared for each plot and gently wet-sieved (0.6 mm mesh opening) with a slow oscillating movement. After 4 hours sedimentation (to allow all particles larger than clay to settle; Sheldrick & Wang, 1993), the content of dispersed clay still in solution was determined by turbidometer.

Data calculations and statistical analyses

The mean and standard deviation were calculated for the experimental parameters determined in all flow-proportional samples (four or eight parallel samples) from replicate plots for the different treatments. If no residue of Gly or AMPA was detected in a given sample, the value 0 was used for calculating the mean. Pearson correlation and regression linear relationships were determined between the parameters total glyphosate (TotGly_PGly_DGly), TotP, PGly, PP and turbidity for the autumn period (27 September – 15 November) and between TotP and turbidity for the spring period (21 March - 11April). Any differences in glyphosate concentrations between the different soil treatments were analysed using Bonferroni post test assuming equal variance and a significance level of $p < 0.05$. Leaching losses from the different plots in the autumn period were calculated by multiplying discharge by measured flow-proportional concentrations in the periods between sample collections. In the spring period, transport of TotGly was estimated from measured values from 14 plots on 28 March.

Results and Discussion

Glyphosate and phosphorus concentrations in water

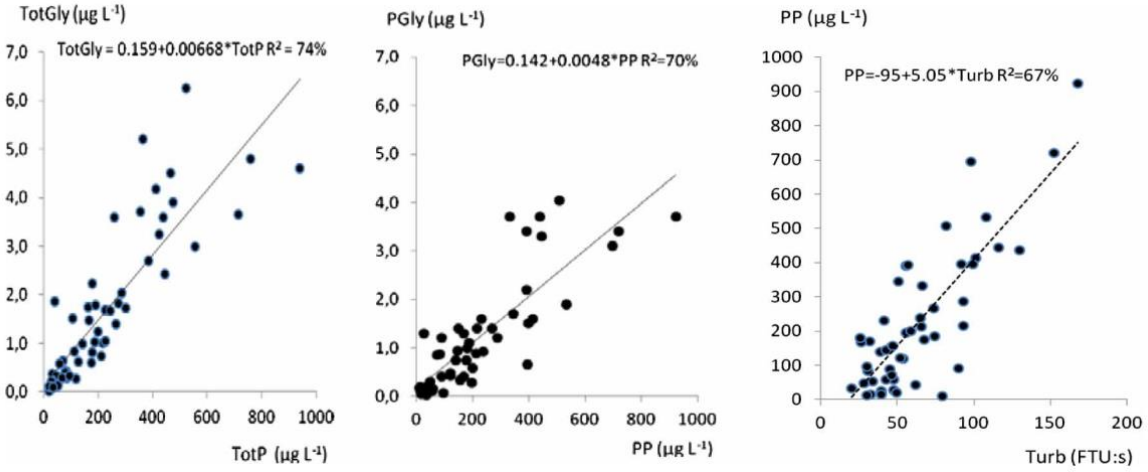
One week after Gly application in autumn, when 10 mm discharge had passed through the tile drainage system, no Gly or AMPA was present in detectable quantities in the discharge (Table 8.1.3.3-12). In the following 7-8 weeks, representing 70 mm water discharge, relatively high and quantifiable concentrations of both DGly and PGly were detected in practically all water samples and, in addition, dissolved AMPA was frequently observed. The concentrations varied greatly from plot to plot and TotGly concentrations of up to 5-6 µg/L were recorded for some plots. High PGly concentrations were generally associated with high DGly concentrations and the two forms of Gly were significantly correlated to each other (Pearson correlation 0.35; $p < 0.002$). Hence, more DGly seemed to leach with mobilized soil particles with high Gly content. Mean DGly concentration in discharge in the autumn (22/9-15/11) was 1.03 µg/L for plots with shallow tillage; 0.43 µg/L for plots with conventional ploughing and 0.36 µg/L for plots with structure liming (differences not statistically significant).

Similar to TotGly, the majority of TotP was lost in particulate form. The proportion of PP was higher (90 %) than the proportion of PGly (60 %). The present study site Gly was tilled down (10 or 23cm depth) in autumn after spraying which would facilitate the dispersion of Gly. A clear and positive correlation between TotGly and TotP concentrations and between PGly and PP concentrations was recorded (Figure 8.1.3.3-3). In turn, PP concentrations could be quite well predicted from turbidity (Figure 8.1.3.3-3). In contrast, DRP concentrations were generally low (0.018-0.027 mg/L) and DGly concentrations were more weakly correlated to DRP concentrations ($r = 0.65$; $p < 0.001$). Glyphosate is commonly suggested to compete with phosphate ions for adsorption sites, but at the present site, with high sorption capacity of the soil particles, this seemed not to be the case, since the correlation was positive. Mean PGly concentrations in the autumn were 1.73 µg/L in discharge from shallow-tilled plots; 0.62 µg/L for conventional ploughed plots; and 0.36 µg/L for structure-limed plots, all differences being statistically significantly different ($p < 0.001$). This implies that colloid P, colloid glyphosate and dissolved pesticides, although mobilized with different mechanisms (de Jonge *et al.*, 2009), may be transported via macropore flow.

Table 8.1.3.3-12: Discharge, pH (in stored composite samples) and flow-proportional concentrations of dissolved glyphosate (DGly), AMPA, particulate glyphosate (PGly), dissolved reactive phosphorus (DRP), particulate P (PP) and turbidity (Turb) in five periods 2010 - 2011 (n.d. = not detected)

Period	22/9–27/9	28/9–25/10	26/10–8/11	8/11–15/11	21/3–28/3
Conventional ploughing					
Discharge (mm)	8.2 ± 3.0	9.2 ± 4.3	25.5 ± 11.1	33.5 ± 13.1	72.9 ± 30.2
pH	6.5	7.2	7.0	6.7	6.6
DGly (μg L^{-1})	n.d.	0.43 ± 0.32	0.43 ± 0.34	0.39 ± 0.21	0.31 ± 0.34
AMPA (μg L^{-1})	n.d.	0.05	0.04	0.03	n.d.
PGly (μg L^{-1})	n.d.	0.67 ± 0.63	0.61 ± 0.67	0.60 ± 0.34	0.22 ± 0.43
DRP (mg L^{-1})	0.021 ± 0.011	0.021 ± 0.011	0.018 ± 0.007	0.020 ± 0.007	0.048 ± 0.019
PP (mg L^{-1})	0.132 ± 0.068	0.122 ± 0.010	0.161 ± 0.166	0.168 ± 0.144	0.124 ± 0.039
Turb (NTU)	64 ± 26	36 ± 7	62 ± 24	60 ± 20	30 ± 16
Structure liming					
Discharge (mm)	10.4 ± 4.1	13.5 ± 5.4	29.1 ± 11.4	30.1 ± 5.1	74.4 ± 25.5
pH	6.9	7.3	7.2	6.9	6.6
DGly (μg L^{-1})	n.d.	0.24 ± 0.20	0.30 ± 0.21	0.24 ± 0.27	0.23 ± 0.25
AMPA (μg L^{-1})	n.d.	0.03	e.d.	0.05	0.08
PGly (μg L^{-1})	n.d.	0.40 ± 0.48	0.41 ± 0.53	0.33 ± 0.58	0.16 ± 0.35
DRP (mg L^{-1})	0.018 ± 0.007	0.017 ± 0.008	0.015 ± 0.005	0.020 ± 0.006	0.047 ± 0.027
PP (mg L^{-1})	0.075 ± 0.066	0.066 ± 0.074	0.093 ± 0.131	0.100 ± 0.106	0.078 ± 0.032
Turb (NTU)	46 ± 30	34 ± 11	64 ± 26	46 ± 31	28 ± 6
Shallow tillage					
Discharge (mm)	10.8 ± 5.3	15.6 ± 6.6	25.9 ± 10.2	29.7 ± 6.4	76.4 ± 23.5
pH	6.8	7.2	7.1	6.8	6.6
DGly (μg L^{-1})	n.d.	1.15 ± 0.89	1.28 ± 1.42	0.99 ± 0.64	0.82 ± 0.93
AMPA (μg L^{-1})	n.d.	0.05	0.23	1.3	0.02
PGly (μg L^{-1})	n.d.	1.99 ± 1.64	1.42 ± 1.44	1.89 ± 1.48	0.57 ± 0.84
DRP (mg L^{-1})	0.024 ± 0.007	0.024 ± 0.007	0.023 ± 0.008	0.027 ± 0.007	0.047 ± 0.021
PP (mg L^{-1})	0.142 ± 0.078	0.236 ± 0.181	0.411 ± 0.355	0.275 ± 0.151	0.136 ± 0.029
Turb (NTU)	88 ± 44	50 ± 17	99 ± 45	81 ± 31	52 ± 43

Figure 8.1.3.3-3: Regression equation for the relationship between concentrations of: (a) total glyphosate (TotGly) and total phosphorus (TotP); (b) particulate glyphosate (PGly) and particulate P (PP); and (c) PP and turbidity (NTUs) in the period 27 September - 15 November 2010. Corresponding Pearson correlations (0.86, 0.84 and 0.82, respectively) were all significant (p < 0.001)



Glyphosate and phosphorus concentrations and losses in spring versus autumn period

As with Gly and P, pH was measured in the cumulative flow-proportionally sampled water and may have changed in the glass vessel. However, measured pH generally did not differ between the three treatments and pH in discharge from the previously structure-limed plots was similar to that in discharge from the unlimited plots (Table 8.1.3.3-12). The pH tended to be lower (6.6) in the snowmelt period (Table 8.1.3.3-12). The measured drop in (logarithmic-based) pH value is equal to 75 % less H^+ ions, which may have influenced both the electrical charge of Gly and the hydrogen bonds of the minerals, and which may explain the high concentrations of DGly in snowmelt. The snowmelt water had low electric conductivity and DRP concentrations that were twice as high as those in the autumn discharge water. The PGly concentrations found in snowmelt in the present study were generally lower than the

DGly concentrations and remained at nearly the same level as in autumn. Consequently, the relative proportions of DGly and PGly were reversed from autumn to spring (snowmelt) (Table 8.1.3.3-13). However, the latter case is based on a more limited number of analyses (n = 14).

Table 8.1.3.3-13: Number of samples analysed (n), relative proportions of dissolved glyphosate (DGly) and particulate glyphosate (PGly) in total glyphosate (TotGly) and relative proportions of dissolved reactive P (DRP) and particulate P (PP) in total phosphorus (TotP) in autumn (28/9 - 15/11 2010) and in a snowmelt period in spring (21 - 28/3 2011), based on flow-proportional concentrations

Glyphosate			Phosphorus		
Fraction	Autumn	Spring	Fraction	Autumn	Spring
n	80	14	No	80	20
DGly/TotGly (%)	40	60	DRP/TotP (%)	10	32
PGly/ TotGly (%)	60	40	PP/TotP (%)	90	68

In practice, half-life degradation rate may be several months. However, as indicated here; ratio PGly/turbidity was only 20-40 % lower in March than in November. Simultaneously, PGly/PP ratio decreased by 30 % on average (from 0.54 % in autumn to 0.15 % in spring). Correspondingly the topsoil colloids may be more depleted of P in spring than in autumn, since the ratio PP to turbidity was lower and had a lower slope in snowmelt than in autumn.

Therefore, there may be similarities between Gly and P transport behavior in spite of the fact that P exists in a large P pool in topsoil and that yearly net P load to the soil in recent years has been six-fold higher than the glyphosate load.

Since the major water discharge took place during the snowmelt period, glyphosate losses tended to be higher in spring than in autumn. In relation to applied amount, losses were approximately 0.1% in spring and 0.05% in autumn for the conventionally ploughed plots. The main reason for the high spring discharge was the intensive snowmelt taking place after a winter with much snow accumulation. These results indicate the importance of such a snowmelt period for Gly losses, confirming findings by Laitinen et al. (2009). Snow accumulation also had great consequences for P losses.

Table 8.1.3.3-14: Discharge and transport of dissolved glyphosate (DGly), particulate glyphosate (PGly), total glyphosate (TotGly), dissolved reactive phosphorus (DRP), particulate P (PP) and total P (TotP) from conventionally ploughed, structure-limed (and ploughed) and shallow-tilled plots in the period 28/9 - 15/11 2010

Period 28/9–15/11	Conventional	Structure-limed	Shallow-tilled
Discharge (mm)	69 ± 28	74 ± 23	72 ± 22
DGly (g ha^{-1})	0.25 ± 0.13	0.12 ± 0.10	0.65 ± 0.54
PGly (g ha^{-1})	0.45 ± 0.53	0.19 ± 0.19	1.01 ± 0.75
TotGly (g ha^{-1})	0.70 ± 0.60	0.31 ± 0.31**	1.65 ± 0.96
DRP (kg ha^{-1})	0.012 ± 0.004	0.012 ± 0.003	0.018 ± 0.007
PP (kg ha^{-1})	0.104 ± 0.082	0.048 ± 0.044	0.192 ± 0.111
TotP (kg ha^{-1})	0.117 ± 0.084	0.060 ± 0.044	0.209 ± 0.114

**Significantly lower ($p < 0.05$) than in shallow-tilled plots.

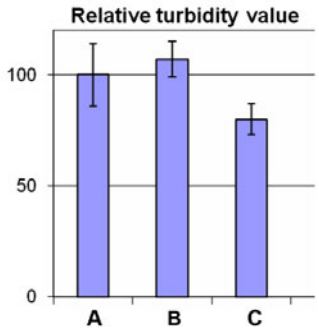
Table 8.1.3.3-15: Discharge (mm) and leaching losses of dissolved glyphosate (DGly), particulate glyphosate (PGly) and total glyphosate (TotGly) as a percentage of original amount applied from conventionally ploughed, structure-limed (and ploughed) and shallow-tilled plots based on measurements in autumn (28/9 - 15/11 2010) and more rough estimates in the most intensive spring snowmelt period (31/3 - 11/4)

	Conventional		Structure-limed		Shallow-tilled	
	Autumn	Snowmelt	Autumn	Snowmelt	Autumn	Snowmelt
Discharge (mm)	69	170	74	169	72	160
DGly (%)	0.024	–	0.011	–	0.061	–
PGly (%)	0.041	–	0.018	–	0.095	–
TotGly (%)	0.066	0.09	0.029	0.05	0.156	0.19

Glyphosate and phosphorus losses under different soil management regimes

In the autumn period, TotGly leaching losses were on average 0.70 g/ha from the conventionally ploughed plots (Table 8.1.3.3-15). TotGly losses from structure-limed plots were significantly lower ($p < 0.05$) than from shallow-tilled plots, expressed in absolute terms (Table 8.1.3.3-14), and also as a percentage of applied amount of Gly (Table 8.1.3.3-15). Fewer particles with attached Gly and P are expected to mobilize from soil aggregates that are less prone to dispersion. The structure-limed plots had significantly ($p < 0.05$) better aggregate stability (lower RDC values) in autumn than the conventionally ploughed and shallow-tilled plots (Figure 8.1.3.3-4), which may explain the clear tendency for lower losses of both PGly and PP from this treatment (Table 8.1.3.3-14).

Figure 8.1.3.3-4: Readily dispersed clay (RDC) in the topsoil from (A) conventionally ploughed, (C) structure-limed and (B) shallowtilled plots. The soil was sampled in September 2010, three years after structure liming



Leaching losses of both PGly and PP tended to be highest from the regular shallow-tilled plots (Table 8.1.3.3-14). Any enhanced amounts of stubble residues in the topsoil, combined with higher potential biological activity and organic matter content, did not seem to have improved aggregate stability from plots (Figure 8.1.3.3-4). However, significantly higher organic matter content was not expected, since such major changes may take at least 10 years. Sorption of Gly is generally not increased in the presence of more straw residues as a consequence of reduced tillage. Therefore, the straw may have facilitated water transport rather than providing new sorption sites after the mixing and reconsolidation of the soil surface. In addition, shallow and uneven accumulation of crop residues on the shallow-tilled plots possibly resulted in uneven infiltration and rapid lateral water movement compared with annually ploughed plots. This is a factor that should be further investigated.

There was no major difference in amount of discharge between the different treatments (Table 8.1.3.3-15). Topsoil structure should be further explored in connection with topsoil susceptibility to preferential flow and transport under different agricultural management regimes. In addition, there was a great variation in concentrations between different plots. Both ‘gyttja’ (cohesive matter of organic origin settled in marine or lake sediment) and oxidized iron (rust) have been frequently observed in soils at the present site. Such material might strengthen the crack walls and make them into permanent pathways, which could explain the general fast transport of particulate-bound glyphosate and P at the present site.

The source of the Gly leaching in this study was the tilled topsoil (0-12 or 0-23 cm), which was possibly the main source of P leaching too. Besides the total amount applied, risk assessment of leaching is often based on the sorption/desorption properties of the actual substance. However, according to the results of the present study, factors such as soil structure, macropore topology and macropore flow may be of great importance.

Conclusion

This study has demonstrated that a significant proportion of glyphosate (Gly) leaching losses may occur in particulate form from clay soils with high amounts of sorption sites available. Crack stabilization by gyttja, especially in the deeper sub-soil, might be an important explanatory factor for fast vertical transport of Gly and phosphorus (P) at the study site. The crack might also be an important explanatory factor for the great spatial variability in Gly and P, in both particulate and dissolved form, at the study

site. Structure liming of the topsoil was demonstrated to reduce total Gly leaching losses compared with unlimited soils, while shallow tillage may not be a suitable way to mitigate particle-facilitated transport of Gly and P via tile drains from this type of clay soil. Proper agricultural management and improved topsoil structure can counteract fast macropore flow in this type of clay soil.

Assessment and conclusion by applicant:

The article describes a field leaching experiment with glyphosate in Sweden on an agriculturally used soil. At this particular site with a clay soil, vertical transport of glyphosate through macropores (preferential flow) is the main transport process. The article provides no information to check the validity against current standards. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

In this study, preferential flow is the main transport process in the soil used, containing a a very high clay content (60%). As mentioned by the applicant, some of the relevant information needed for determining the validity of the study (especially in terms of recoveries and methods) are not available in the article.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Aronsson et al., 2010

Data point:	CA 7.1.4.3/003
Report author	Aronsson, H. *et al.*
Report year	2010
Report title	Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop
Document No	DOI 10.1111/j.1475-2743.2010.00311.x ISSN 0266-0032
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

During 2005–2007, studies were carried out in two field experiments in southwest Sweden with separately tile–drained plots on a sandy soil (three replicates) and on a clay soil (two replicates). The overall aim was to determine the effects of different cropping systems with catch crops on losses of N, P and glyphosate. Different times of glyphosate treatment of undersown ryegrass catch crops were examined in combination with soil tillage in November or spring. Drainage water was sampled continuously in proportion to water flow and analysed for N, P and glyphosate. Catch crops were sampled in late autumn and spring and soil was analysed for mineral N content. The yields of following cereal crops were determined. The importance of keeping the catch crop growing as long as possible in the autumn is demonstrated to decrease the risk of N leaching. During a year with high drainage on the sandy soil, annual N leaching was 26 kg/ha higher for plots with a catch crop killed with glyphosate in late September than for plots with a catch crop, while the difference was very small during 1 yr with less drainage. Having the catch crop in place during October was the most important factor, whereas the time of incorporation of a dead catch crop did not influence N leaching from either of the two soils.

However, incorporation of a growing catch crop in spring resulted in decreased crop yields, especially on the clay soil. Soil type affected glyphosate leaching to a larger extent than the experimental treatments. Glyphosate was not leached from the sand at all, while it was found at average concentrations of 0.25 µg/L in drainage water from the clay soil on all sampling occasions. Phosphorus leaching also varied (on average 0.2 and 0.5 kg/(ha x yr) from the sand and clay, respectively), but was not significantly affected by the different catch crop treatments.

Materials and methods

Experimental fields

The study was conducted over 2 years (2005–2007) in field experiments with a similar treatment design, but located at different sites, Lanna and Lilla Böslid in southwest Sweden. In both experiments, leaching was measured in separately tile–drained experimental plots where drainage flow was measured continuously and water was sampled in proportion to flow. Precipitation and air temperature were recorded at both sites.

Lanna site, clay soil.

Lanna research station (58°20′N, 13°07′E) is situated in a region which has a mean annual temperature of 6.1°C and mean annual precipitation of 558 mm (Lanna, 1961–1990). The experimental field, which was established in 2001, consists of 10 plots (790 m^2). Each plot was separately tile–drained at ca. 1 m depth and the drains were backfilled with 10–cm gravel at the bottom and then with the soil. The soil at Lanna consists of 47 % clay (<2 µm) in the topsoil (0–0.3 m depth) and 55–60 % clay in the subsoil (0.3–0.9 m depth). During the study, the topsoil had an organic matter content of 4.4 % and a mean pH of 6.6. The mean amount of ammonium lactate soluble P was 3.4 mg/100 g dry soil which is considered as low P status. The soil contains numerous cracks and macropores in the upper 1.0 m of the profile. More details on this soil are given by Bergström *et al.* (1994). At Lanna, the same plots were used during the two experimental years, with the same treatment being applied on each plot during the two consecutive years (with two replicates).

Lilla Böslid site, sandy soil.

Lilla Böslid experimental farm (56°35′N, 12°56′E) is located ca. 240 km south of Lanna. The mean annual temperature is 7.2°C and the mean annual precipitation is 803 mm (Halmstad, 1961–1990). The sandy soils in this region are commonly drained as the groundwater levels are often high because of a clay layer under the sand deposits. This experimental field was constructed in 2002, and consists of 36 separately tile–drained plots, each 320 m^2. The tile drains are at 0.9–m depth. The soil is an unstructured sand with 9 % clay in the topsoil (0–0.3 m depth) and 1–2 %in the subsoil (0.3–0.9 m depth). At the time of study, the topsoil had a mean organic matter content of 4.9 % and a pH value of 6.1. The mean amount of ammonium lactate soluble P was 12.8 mg/100 g dry soil. This value indicates that this soil is rich in P and that reduced P application rates are recommended for spring cereals. At Lilla Böslid, the experimental lay–out allowed two experimental years on different plots by dividing the field into two sections and using one section each year (with three replicates).

Experimental design and management practices

During the year before the experiment started, a spring cereal was grown at Lilla Böslid and winter wheat at Lanna. The experiments started in 2005 by undersowing a catch crop of ryegrass (*Lolium perenne L.*) in a cereal crop in three of five treatments at Lanna and in all treatments at Lilla Böslid (Table 8.1.3.3-16). At Lanna, glyphosate was applied in four treatments at the beginning of October, and in one in spring. Glyphosate treatment in October was combined with tillage in November (mouldboard ploughing, 25–cm depth) or in April (stubble cultivation, 6–cm depth). At Lilla Böslid, different times of glyphosate treatment in autumn were tested in combination with mouldboard ploughing (25–cm depth) in November or April. There was also one treatment without use of herbicide and spring ploughing, which was considered as a control treatment representing the best scenario for low N leaching. At Lilla Böslid, the soil was tine–cultivated to ca. 10 cm depth just before ploughing. Dates of tillage and glyphosate treatment are shown in Table 8.1.3.3-16. At Lanna, glyphosate was applied as Glyphomax Bio at a dose of 3.5 or 4.0 L/ha and at Lilla Böslid as Round–up Bio, 3.5 L/ha. The crop following incorporation of the catch crop was a spring cereal (oats or barley). It was fertilized

with 100–110 kg N/ha at Lanna and with 90 kg N/ha at Lilla Böslid. A dose of 10 kg/ha of mineral P was applied at Lilla Böslid in 2006 and the same amount at Lanna in 2007.

Sampling and analyses of water, soil and crops

Drainage water from the plots at both sites was led to an underground monitoring station with temperatures never >15°C and <10°C during the main drainage periods when discharge rates were recorded using tipping buckets connected to a data logger which stored accumulated daily drainage volumes from each plot. Flow–proportional water samples of 15 mL were taken using a peristaltic pump after every 0.2 mm discharge. The samples for each plot were collected in individual polyethylene bottles which were emptied every 2 weeks during drainage periods for analysis of total–N, NO_3–N, total–P and PO_4–P. During sampling, the bottles were prepared with sulphuric acid for conservation of glyphosate. Glyphosate and the degradation product of aminomethylphosphonic acid (AMPA) were analysed for the same samples on 5–6 occasions during each of the two drainage seasons. At Lanna, glyphosate was analysed in samples from treatments A–D and at Lilla Böslid, in treatments F–J (Table 8.1.3.3-16). These events were primarily chosen to represent periods when drainage started in autumn with high flow periods. The first samples were taken before glyphosate treatment to ensure that any leaching detected originated from the experimental treatments. During the first year, the samples from replicates were pooled for analyses of glyphosate because of the high cost of analyses, but during the second year, all samples were analysed individually. Prior to analysis, the water samples were pretreated with a C18 ion exchange column for removal of non–polar substances, which also caused some filtration of particles (unknown size). Then glyphosate was derived with trifluoroacetic acid/trifluorethanol before combined gas chromatograph/mass spectrometer (GC/MS) analyses. The partitioning between particle–bound and dissolved glyphosate was not examined and some particles were also filtered before analysis. Thus, the analysis mainly covered the amount of dissolved glyphosate, but it is also likely that some particle–bound glyphosate was included as water samples were acidified during storage, which may have resulted in some dissolution of particle–bound glyphosate.

Table 8.1.3.3-16 The different experimental treatments at the two sites during the 2 years, with planned and actual time of glyphosate treatment and catch crop incorporation

		Time of glyphosate treatment			Time of incorporation		
	Catch crop	Plan	Act yr 1	Act yr 2	Plan	Act yr 1	Act yr 2
Lanna, clay soil							
A	Per. ryegr.	1 Oct	4 Oct 2005	4 Oct 2006	10 Nov	11 Nov 2005	10 Nov 2006
B	Per. ryegr.	1 Oct	4 Oct 2005	4 Oct 2006	1 Apr	28 Apr 2006	12 Apr 2007
C	Per. ryegr.	1 Mar	14 Apr 2006	19 Mar 2007	1 Apr	28 Apr 2006	12 Apr 2007
D	–	1 Oct	4 Oct 2005	4 Oct 2006	10 Nov	11 Nov 2005	10 Nov 2006
E	–	1 Oct	4 Oct 2005	4 Oct 2006	1 Apr	28 Apr 2006	12 Apr 2007
Lilla Böslid, sandy soil							
F	Per. ryegr.	20 Sep	26 Sep 2005	26 Sep 2006	10 Nov	24 Nov 2005	24 Nov 2006
G	Per. ryegr.	20 Sep	26 Sep 2005	26 Sep 2006	1 Apr	12 Apr 2006	2 Apr 2007
H	Per. ryegr.	5 Oct	4 Oct 2005	10 Oct 2006	10 Nov	24 Nov 2005	24 Nov 2006
I	Per. ryegr.	5 Oct	4 Oct 2005	10 Oct 2006	1 Apr	12 Apr 2006	2 Apr 2007
J	Per. ryegr.	20 Oct	31 Oct 2005	22 Nov 2006	1 Apr	12 Apr 2006	2 Apr 2007
K	Per. ryegr.	–	–	–	1 Apr	12 Apr 2006	2 Apr 2007

Per. ryegr., perennial ryegrass.

Calculations and statistical analysis

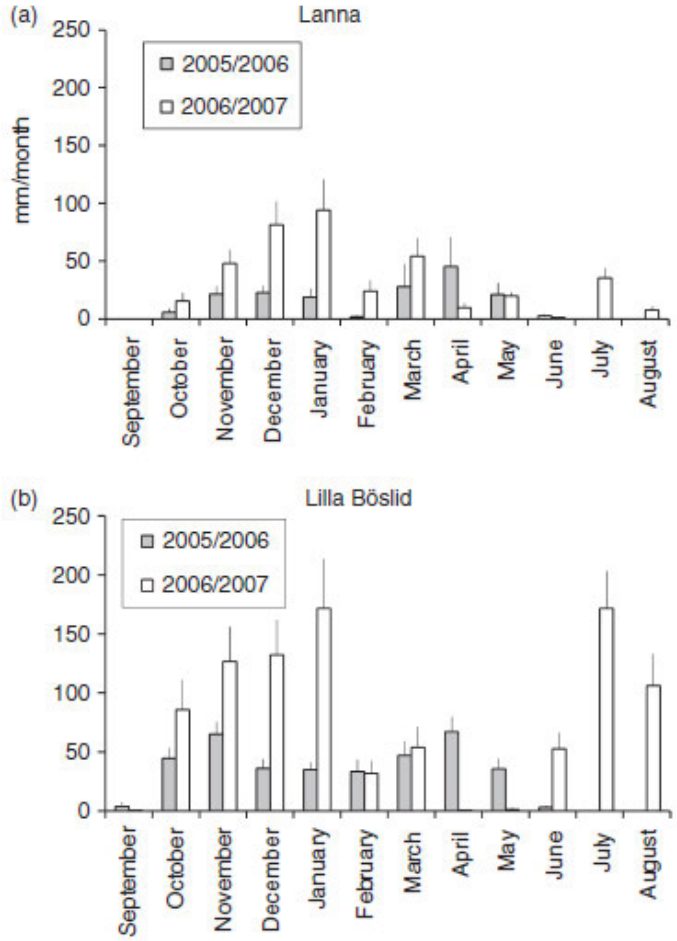
Analysis of variance was carried out by the Mixed procedure in SAS 9.1 (SAS Institute Inc. 2003: SAS/Stat 9.1 Users' Guide. Cary, NC, USA) for the statistical analysis of differences in yields, catch crop biomass and N and P contents, soil mineral N, leaching of N and P and concentrations of glyphosate between treatments. The t–test at P = 0.05 was used for pairwise comparisons by the PDIFF statement. Block was used as the random variable in analysis of a single year. For the Lanna site, the average for the 2 years was analysed by calculating an average per plot and by using block as random variable. For the Lilla Böslid site, where the experiment was carried out in separate plots during the 2 years, year was used as random variable when analysing the average for the 2 years.

Results

Drainage and climate conditions

The two experimental years represented varying climate and drainage conditions, 1 yr with a cold winter with relatively small drainage amounts, and one mild winter with high drainage. At Lanna, the mean temperature during December 2005–March 2006 was –3.5°C, while it was +2.1°C during the same period 2006–2007. For Lilla Böslid, corresponding values were –2.6°C and +4.3°C. At Lanna, the measured precipitation was 480 mm during 2005–2006 and 759 mm during 2006–2007 (1 September–31 August). At Lilla Böslid, the corresponding figures were 542 mm and 950 mm, respectively. During the first year, the precipitation was considerably lower than the long–term mean value for both sites, but higher during the second year. Different precipitation and temperature conditions during winter clearly affected drainage and the N and P leaching during the two experimental years. The high rainfall resulted in much drainage during the autumn and winter of 2006–2007. During summer 2007, southwest Sweden was exposed to several large low pressure cells, which resulted in extremely rainy conditions and major drainage events. There was some variation in drainage water totals between individual plots as shown in the figure below where standard deviations for all plots are included. These differences could not be attributed to different experimental treatments except for 2005–2006 at Lilla Böslid, when treatment K had higher drainage than most of the other treatments (P = 0.03).

Figure 8.1.3.3-5: Mean monthly drainage (mm) from all plots during the two experimental years at the two sites Lanna (a) and Lilla Böslid (b). Standard deviations are shown with narrow bars



Management practices, catch crop growth and crop yields

The planned time of glyphosate treatment in September and beginning of October corresponded quite well to the actual time at both sites (Table 8.1.3.3-16). From field observations, the catch crop was still intact 1 week after treatment, but after 3 weeks, it was totally killed in both years. However, glyphosate treatment in late October at Lilla Böslid was delayed by up to 4 weeks because of bad weather conditions, especially in 2006 (Table 8.1.3.3-16) when the catch crop was treated in late November. This resulted in a poor effect of the glyphosate, and only 50 % of the catch crop was killed 3 weeks after treatment. Glyphosate treatment in spring (treatment C at Lanna) resulted in problems with the timing.

Obtaining an effect of the herbicide, while simultaneously being able to cultivate this heavy clay soil, was a challenge. In spring 2007, when there was a very dense catch crop, it was particularly difficult to incorporate the catch crop material in this treatment, and about 20–40 % of the catch crop was estimated to be still growing at harvest of the following crop. Shallow cultivation in spring worked much better after glyphosate treatment in autumn (treatments B and E) with respect to incorporation of plant material, although this tillage practice is not common for this type of soil.

Leaching of glyphosate

At the sandy soil at Lilla Böslid, drainage water was analysed for glyphosate on eight occasions during the experimental period (November 2005, December 2005, April 2006, October 2006, November 2006, December 2006, January 2007 and March 2007). Glyphosate was only detected twice and occurred at trace levels, that is at concentrations above the detection limit (ca. 0.01 µg/L), but under the limit for determination of the concentration (ca. 0.05 µg/L). These occasions were in treatments F and I at sampling on 20 December 2006 and in treatment J on 8 January 2007.

AMPA was not found at all. As a result of bad weather conditions during October–November 2006, glyphosate application in treatment J was not possible until 22 November. If there had been a risk of glyphosate transport, it would probably have arisen during conditions like these, but the risk seemed to be very small for this soil. The adsorption of glyphosate in the sandy soil was probably very efficient, probably because of Al/Fe–oxides, the same as for P. At the Lanna clay soil, glyphosate was found at concentrations above the determination limit in all samples except two during the experimental period (Table 8.1.3.3-17). Thus, application of glyphosate both in autumn and in spring resulted in some transport to drainage water, but with this experimental design, it was possible that application of glyphosate during 2005–2006 also affected to some extent the results from 2006 to 2007. Even at sampling in spring 2005, before the start of the experiment at Lanna, traces of glyphosate were found in drainage water. This probably originated from autumn 2004 when glyphosate was applied to borders between the experimental plots. Concentrations were low, on average 0.25 µg/L, and only exceeded 1 µg/L on one occasion (January 2007 in treatment D). The concentrations of glyphosate measured at Lanna were similar to those found in monitoring of streams in agricultural catchments in southern Sweden (Adielsson *et al.*, 2007).

Table 8.1.3.3-17: Measured concentrations of glyphosate and its metabolite AMPA

	A		B		C		D	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
Glyphosate treatment	4 Oct 2005 4 Oct 2006		4 Oct 2005 4 Oct 2006		14 Apr 2005 20 Mar 2006		4 Oct 2005 4 Oct 2006	
24 Apr 2005	Trace*	nd*	Trace*		–	–	Trace*	nd*
15 Nov 2005	0.39	nd	0.86	Trace	–		0.85	0.40
27 Nov 2005	nd	nd	0.19	nd	–		0.01	ed
5 Apr 2006	0.23	nd	0.48	Trace	Trace*	Trace*	0.16	Trace
2 May 2006	–	–	–	–	0.29	Trace	–	–
1 Jun 2006	Trace	nd	0.08 (0.05)	nd	–	–	0.04 (0.01)	nd
1 Nov 2006	0.53[a] (0.09)	Trace	0.66[a] (0.48)	Trace	0.19[a] (0.04)	Trace	1.04[a] (0.37)	Trace
15 Nov 2006	0.21[a] (0.11)	Trace	0.44[b] (0.02)	0.10	0.10[a] (0.06)	Trace	0.51[a] (0.07)	0.10
8 Jan 2006	0.18[a] (0.01)	Trace	0.66[b] (0.12)	Trace	0.12[a] (0.06)	Trace	0.18[a] (0.06)	Trace
15 Jan 2006	0.13[a] (0.01)	Trace	0.50[b] (0.01)	Trace	0.10[a] (0.02)	Trace	0.20[a] (0.09)	Trace
13 Mar 2007	0.14[a] (0.04)	Trace	0.31[b] (0.02)	Trace	0.07[a] (0.02)	nd	0.13[a] (0.03)	Trace
21 May 2007	Trace	Trace	0.06[a] (0.04)	Trace	0.14[a] (0.07)	Trace	0.05[a] (0.03)	Trace

Standard deviations are shown in brackets. Different superscript letters indicate significant differences between treatments ($P = 0.01$). *Samples taken before treatment with glyphosate. nd, not detected. Trace, between detection and determination limit, ca. between 0.02 and 0.05 µg/L.

Discussion

Results from this study indicate that soil texture was the dominant factor in influencing both P and glyphosate losses, whereas different treatments had small or no effects. For glyphosate, this was not surprising, as soil structure and transport pathways have been shown to be of major importance for glyphosate leaching (Vereecken, 2005; Borggaard & Gimsing, 2008). The immediate detection of

glyphosate in drainage water from the clay soil at Lanna clearly shows that there are rapid pathways for water and solutes in this soil, as reported previously by Larsson & Jarvis (1999). The glyphosate analyses did not distinguish between dissolved and particle–bound glyphosate; however, as 70–80 % of the P losses were in particle–bound form, this might also be an important transport form for glyphosate. In studies on two soils in Denmark, the contribution of colloid–facilitated transport was up to 27 % and 52 % for a sandy loam and a sandy soil, respectively (de Jonge *et al.*, 2000). It is probable that total leaching of glyphosate, especially from the clay soil, was underestimated in this study as it is uncertain of the extent to which particle–bound glyphosate was included in the analyses. Soil tillage practices affect transport pathways through the soil. For example, conservation tillage has been shown to increase the amount of macropores and related preferential flow paths (Shipitalo *et al.*, 2000), but time of ploughing may also affect the partitioning between different types of losses. Spring ploughing instead of autumn ploughing protects the surface against destruction of soil aggregates over winter and is highly relevant in minimizing particle–bound P losses by erosion (Kronvang *et al.*, 2005), especially in combination with a catch crop (Ule´ n, 1997). In contrast, losses of dissolved compounds may increase when the soil is not cultivated in autumn. This was reported in studies of glyphosate losses in Norway (Stenrød *et al.*, 2007) and Denmark (Lærke Baun *et al.*, 2007) where tillage in autumn increased the leaching of particulate–bound glyphosate, while there was increased leaching of dissolved glyphosate when the soil was not tilled in autumn. These findings are supported by the results from Lanna, where there are indications of higher losses of total–P after ploughing in autumn, but differences in concentrations or yearly transport are ns. Spring tillage at Lanna (treatment B) gave significantly higher concentrations of glyphosate in drainage water than the other treatments on four occasions ($P = 0.01$) in 2006–2007, which may indicate that spring tillage conserved transport pathways through the topsoil during winter. However, it is not possible to draw conclusions about the partitioning between dissolved and particle–bound glyphosate. Another study on the Lanna soil in lysimeters shows that losses of particle–bound glyphosate were negligible and that almost all leached glyphosate was in dissolved form (Bergström *et al.*, 2010). There were no indications of increased transport of dissolved P in spring–ploughed plots, with or without a catch crop over winter. However, catch crop plant material may constitute a risk of dissolved P leaching if exposed to freezing, as shown by Bechmann *et al.* (2005).

In the sandy soil at Lilla Böslid, glyphosate was efficiently sorbed, which was also true for P. The high P status of this soil did not seem to increase the risk of P losses, although studies have shown a relationship between high P content of the soil and P leaching (Heckrath *et al.*, 1995). The larger proportion of dissolved P at Lilla Böslid, compared with Lanna, could be an indication of enhanced P desorption because of high soil P content, but this is probably not the case as P concentrations in drainage water were consistently low and stable. There is also considered to be an increased risk of glyphosate transport in soils with high P content, as PO_4–P and glyphosate may compete for the same surface binding sites on soil mineral particles (Gimsing & Borggaard, 2002). However, the P and glyphosate sorption capacity of the subsoil and the degree of saturation of sorption sites have a large impact on actual P losses, and there was no indication of saturated conditions in the sandy soil at Lilla Böslid.

The results from the sandy soil at Lilla Böslid show that the time available for catch crop growth and N uptake during autumn significantly affected the accumulation of N in the soil and the risk of N leaching during the following winter, although it is somewhat surprising that there is no clear correlation between soil mineral N in autumn and N leaching. The results also show that glyphosate treatment in September or early October resulted in fast release of N available for leaching. This confirms the findings by Snapp & Borden (2005) that N mineralization increases when the catch crop is treated with glyphosate 8 days before incorporation, compared with no treatment before incorporation. The time of catch crop incorporation after chemical kill–off in autumn seems to be of minor importance according to the results from both sites. This is somewhat surprising for the sandy soil, as several studies have shown that time of tillage in autumn clearly influences N mineralization and N leaching from this type of soil (e.g. Wallgren & Lindén, 1994; Djurhuus & Olsen, 1997; Stenberg *et al.*, 1999). In the present study, glyphosate treatment obviously had a similar effect to incorporation on N release in the soil, at least during the second year. For the clay soil at Lanna, the results are similar to those found in a study in an adjacent field (Aronsson & Stenberg, 2010), where time of tillage in autumn or spring did not affect N leaching to any large extent.

Growing a catch crop may affect the yield of the main crop because of inter–plant competition, although this effect is often small or negligible (Ohlander *et al.*, 1996). The catch crop may also affect the following crop after incorporation.

This effect can be positive as a result of the fast remineralization of catch crop N (Lyngstad & Borresen, 1996). It may also be negative as a result of the immobilization of catch crop N or depletion of soil mineral N content in spring as a result of N uptake by the catch crop. This pre–emptive effect (Thorup–Kristensen, 1993) probably contributed to decreased yields at Lanna together with regrowth of the catch crop and at Lilla Böslid. Incorporation of a living catch crop in November–December or in February–March instead of April would probably have been more suitable for remineralization of catch crop N, as suggested by Torstensson & Aronsson (2000). To improve synchronization with the N requirements of the following crop, the N mineralization dynamics must be considered rather than increasing N fertilization rates after incorporation of catch crops.

Conclusion

To develop recommendations to achieve decreased nutrient leaching and pesticide contamination of water, the leaching and contamination risks need to be considered in relation to each other, and crop production aspects also need to be considered. It is clear from this study that N leaching was considerably lower from the clay soil (2–22 kg N/ha/yr) than from the sandy soil (15–53 kg N/ha/yr). It was also clear that spring incorporation of a catch crop could not be recommended on the clay soil as it negatively affected crop yields. Glyphosate treatment causes some risk of contamination of percolating water on the clay soil, irrespective of time of application, while time of incorporation does not affect leaching of N and P on either clay soil or sandy soil. This suggests that clay soil should not be given special priority for the use of catch crops with chemical treatment or for the use of excluded tillage in autumn. The reasons are that the overall risk of N leaching is relatively low and that the beneficial effects on N leaching may be counteracted by some risk of glyphosate leaching. Moreover, there is no reduction in P losses.

For the sandy soil, N leaching was much higher than from the clay soil and keeping the soil covered with a catch crop until November or until spring considerably reduced N leaching during high–flow conditions compared with chemical kill–off in September or mid-October. It was difficult to achieve a good herbicide effect with delayed chemical treatment, indicating that there always has to be a compromise between N leaching and successful weed control. Despite the low risk of glyphosate and P leaching, the results suggest that for a sandy soil, glyphosate treatment should be excluded during autumn when growing catch crops to maximize the reduction in N leaching. Incorporation of the catch crop as late as possible in autumn or in very early spring probably reduces the risk of decreased crop yields of the following crop as a result of N uptake by the catch crop.

Assessment and conclusion by applicant:

The article describes a 2-years leaching experiment in Sweden on two agricultural soils (one soil and one sand) with glyphosate. The method is not sufficiently described to evaluate the validity of the results. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

RMS agrees with the applicant's conclusion.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Kjaer et al., 2011

Data point:	CA 7.1.4.3/004
Report author	Kjaer, J. *et al.*
Report year	2011

Report title	Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils
Document No	DOI 10.1016/j.chemosphere.2011.03.029 E-ISSN 1879-1298
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Geological Survey of Denmark and Greenland)
Acceptability/Reliability:	Reliable with restrictions

Full summary

Leaching of the strongly sorbing pesticides glyphosate and pendimethalin was evaluated in an 8–month field study focussing on preferential flow and particle–facilitated transport, both of which may enhance the leaching of such pesticides in structured soils. Glyphosate mainly sorbs to mineral sorption sites, while pendimethalin mainly sorbs to organic sorption sites. The two pesticides were applied in equal dosage to a structured, tile–drained soil, and the concentration of the pesticides was then measured in drainage water sampled flow–proportionally. The leaching pattern of glyphosate resembled that of pendimethalin, suggesting that the leaching potential of pesticides sorbed to either the inorganic or organic soil fractions is high in structured soils. Both glyphosate and pendimethalin leached from the root zone, with the average concentration in the drainage water being 3.5 and 2.7 µg/L, respectively. Particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13–16 % for glyphosate and 16–31 % for pendimethalin). Drain–connected macropores located above or in the vicinity of the drains facilitated very rapid transport of pesticide to the drains. That the concentration of glyphosate and pendimethalin in the drainage water remained high (>0.1 µg/L) for up to 7 d after a precipitation event indicates that macropores between the drains connected to underlying fractures were able to transport strongly sorbing pesticides in the dissolved phase. Lateral transport of dissolved pesticide via such discontinuities implies that strongly sorbing pesticides such as glyphosate and pendimethalin could potentially be present in high concentrations (>0.1 µg/L) in both water originating from the drainage system and the shallow groundwater located at the depth of the drainage system.

Materials and methods

Chemicals

Glyphosate [N–(phosphonomethyl)glycine] – the active ingredient in Roundup – is a broad–spectrum, post–emergence, non–selective herbicide that is one of the most used herbicides worldwide. In Denmark, glyphosate is the herbicide sold in the largest quantities; in 2003, glyphosate sales for agricultural purposes accounted for 44 % of all herbicide sales. By 2008, this had increased to 52 % (Danish Environmental Protection Agency, 2004, 2009). Pendimethalin [N–(1–ethylpropyl)–2,6–dinitro–3,4–xylidine] is a selective herbicide used to control most annual grasses and certain broadleaf weeds both pre–emergence (i.e. before weed seeds have sprouted) and early post–emergence. Pendimethalin ranks fourth among the herbicides used in Demark, accounting for 5 % of all herbicide sales for agricultural use in 2003 and 6 % in 2008 (Danish Environmental Protection Agency, 2004, 2009). Solubility of glyphosate is 10500 mg/L while that of pendimethalin is 0.33 mg/L pesticide properties database available at http://sitem.herts.ac.uk/aeru/footprint/index2.htm.

Site description

The study was conducted at the Estrup field research site in Denmark, a virtually flat systematically tile–drained loamy field located on glacial till with a cultivated area of 1.26 ha. The tile drains are located at an average depth of 1 m b.g.s., and the water table is relatively shallow, located 1–3 m b.g.s. The uppermost meter of the soil is heavily fractured and bioturbated, with plough layer containing 100–1000 biopores/m^2 (Lindhardt *et al.*, 2001). The geological structure is complex, comprising a clay till core with deposits of different age and composition. Of three pedological profiles available for the site, one is classified as Aquic Argiudoll, one as Abruptic Argiudoll and one as Fragiaquic Glossudalf. Details

on soil properties are reported in the tables and geological properties are further described in Lindhardt *et al.* (2001).

Agricultural management

After maize (Zea mays L.) had been harvested on 13 October 2005, glyphosate (1.44 kg/ha active ingredient; 4.0 L/ha Round–up Bio) and pendimethalin (1.44 kg/ha active ingredient; 3.6 L/ha Stomp) were applied simultaneously together with 30.0 kg/ha of potassium bromide as tracer on 9 November 2005. On 12 April 2006 the field was ploughed to a depth of 18 cm. Spring barley was sown on 27 April 2006. Whereas glyphosate had been applied previously (13 October 2000 and 2 September 2002) the field had been treated with pendimethalin 7 October 1997. The minor residues of glyphosate (0.01–0.03 µg/L) found in the drainage water before the current application of pesticides is thus likely to derive from these previous treatment.

Monitoring and sample preparation

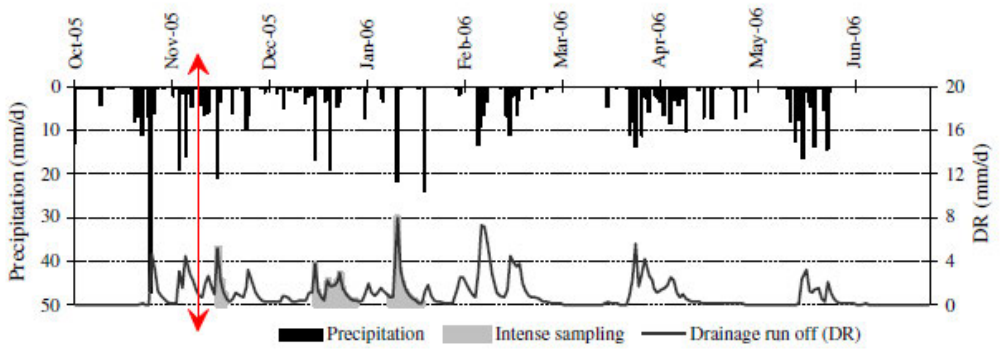
For a period of 8 months following application of the glyphosate and pendimethalin the concentration of the pesticides and bromide was measured on a weekly basis in drainage water sampled flow–proportionally. In addition, more intense sampling of drainage water was performed in connection with three flow events triggered by precipitation on 14 November 2005, 16 December 2005 and 11 January 2006 in order to enable detailed description of the transport of water and pesticides. Sampling lasted for 2, 13 and 9 d, respectively. Flow events are characterised by an initial rapid rise in the hydrograph followed by a less rapid drop (tailing). During these events, drainage water subsamples were collected for every 2 mm of drainage runoff using a refrigerated Isco sampler (Teledyne Isco, Inc., US) containing eight 2–L borosilicate bottles. Within 24 h of the onset of the flow events, each bottle from the Isco sampler was shaken thoroughly to resuspend the sediment. The particles in the individual samples were then separated by centrifugation at 3500 rpm using Teflon vials. The time required for separation of particles ≥0.24 µm was calculated according to Gimbert *et al.* (2005). The supernatant was removed using a pipette, cleaned with 20 % HCl. The supernatant of samples to be analysed for pendimethalin was placed in glass bottles and preserved by adjusting to pH 2.0 with sulphuric acid. The pellets were flushed into a glass bottle using demineralised water and preserved using sulphuric acid. The samples to be analysed for pendimethalin were stored at 2°C until analysis. The supernatant of samples to be analysed for glyphosate and AMPA was pipetted into polypropylene (PP) bottles and adjusted to pH 2.0 with sulphuric acid. The pellets were flushed into PP bottles and adjusted to pH 2.0 with sulphuric acid. The latter two types of sample were stored at –18°C until analysis. As the flow event on 16 December 2005 occurred at a weekend, it was not possible to conduct particle separation on the samples. With all the samples collected on a weekly basis and the intensive samples collected following the flow event on 16 December 2005, pesticide concentrations were measured on the entire water sample. Thus the reported concentrations refer to the total concentration of both dissolved and particle–bound pesticide. With samples collected intensively following the flow events on 14 November 2005 and 11 January 2006, pesticide concentrations are reported for both particle–bound pesticide (concentration in the pellets) and dissolved pesticide (concentration in the supernatant). Furthermore, measurements of turbidity, chloride concentration and conductivity were conducted on all water samples obtained from the Isco sampler.

Table 8.1.3.3-18: Physical and chemical properties of the soil

Profile[a]	Horizon	Depth	Clay[b] (%)	Silt[b] (%)	Sand[b] (%)	OM (%)	C/N	CEC (meq 100 g^{-1})	pH_{CaCl2}	Fe (mg kg^{-1})	Al (mg kg^{-1})
Estrup 2											
	Ap	0–26	13.8	12.7	70.8	2.7	13	12.1	6.5	2044	808
	Bt(g)	26–45	36.3	15.3	47.8	0.5	6	13.9	6.3	4144	1748
	Bt(g)2	45–121	33.0	15.9	50.9	0.2	4	16.8	6.6	2294	1034
	Cc[c]	121–150	31.1	24.9	7.5	0.5	6	19.7	7.5	2290	568
Estrup 3											
	Ap	0–28	9.9	7.1	77.5	5.5	17	15	7.0	1648	1024
	Bs/Bhs	28–58	8.8	4.7	85.7	0.8	12	10.2	6.6	1730	1340
	Bt(g)	58–115	12.2	4.3	83.1	0.4	8	9.2	4.2	1702	916
	2C	115–185	38.9	24.1	26.9	10.1	39	40.5	4.5	1576	2934

nd.: not determined; OM: organic matter determined as 1.72 total organic carbon; Fe and Al: oxalate extractable Fe and Al determined by the methods of McKeague and Day (1966).
a Profiles are classified as Abruptic Argiudoll (Estrup 2) and Fragiaquic lossudalf (Estrup 3).
b Clay: <2 µm; Silt: 2–20 µm; Sand: 20–2000 µm.
c Contains 36.1% $CaCO_3$. Contains 20.0% $CaCO_3$.

Figure 8.1.3.3-6: Precipitation (hanging bars on primary axis) and drainage runoff (solid line on secondary axis). The red vertical arrow indicates the date of application. The shaded grey area beneath the solid line indicates the flow events that were intensively monitored



Methods of analysis

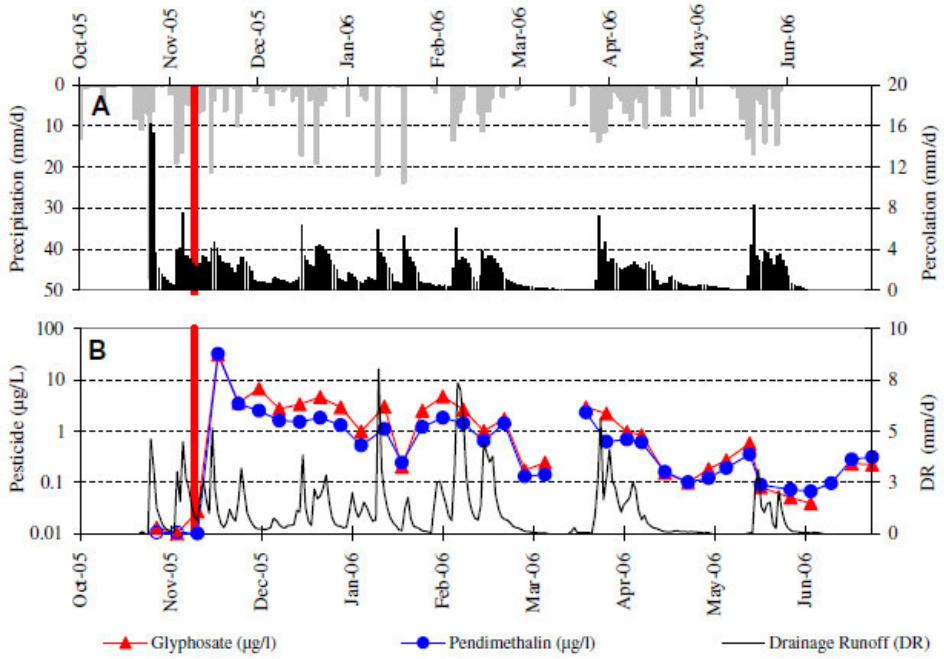
Glyphosate

The preserved water samples were first concentrated on a column of Chelex 100 resin, iron form 100–200 mesh from Bio–Rad. After washing with 0.1 M HCl, the analytes were eluted with 6 M HCl. The eluate was further cleaned on a column of AG 1–X8 resin, chloride form 200–400 mesh. The eluate was evaporated to dryness under nitrogen and redissolved in 200 µL of water–methanol–HCl (160:40:2.7). Derivatisation was carried out with 1 mL of trifluoroacetic anhydride–2,2,3,3,4,4,4–heptafluoro–1–butanol (2:1). The derivatives of glyphosate were measured by GC–MS using a 5 % phenyl methylsiloxane GC–column (HP–5) with the MS in electron impact (EI) mode. 2 µL sample was injected by splitless injection at 280°C with oven temperature at 65°C. After 2 min the oven temperature was raised to 310°C at 20°C min^{-1} and held at 310°C for 4 min. The glyphosate derivatives were identified by MS using m/z 612, 611 and 584. The calculations were made using the internal standard procedure with glyphosate–$^{13}C^{15}N$ as the internal standard. The LOD (limit of detection) was below 0.01 µg/L. The preserved pellet samples were treated with 1 M ammonia prior to analysis in order to extract the glyphosate from the solids. The extract was then diluted with water, adjusted to pH 2.0 with HCl, and analysed as described above for the water samples.

Inorganic analysis

The water samples were analysed for turbidity, conductivity and chloride concentration. Turbidity was measured with an infrared LED light source using a pHotoFlex Turb photometer (WTW Gmbh, Weilheim, Germany). Conductivity was measured using a Cond 340i conductivity pocket meter (WTW Gmbh, Weilheim, Germany). Chloride concentration was measured using a FIAstar™ 5000 flow injection analyser (Foss Analytical AB, Höganäs, Sweden).

Figure 8.1.3.3-7: Precipitation and simulated percolation (A) together with concentration of pendimethalin and glyphosate (B) in the drainage runoff (DR on secondary axis). The red vertical lines indicate the date of application. The open circles indicate concentrations below the LOD (0.01 µg/L)



Results and Discussion

Leaching of glyphosate and pendimethalin

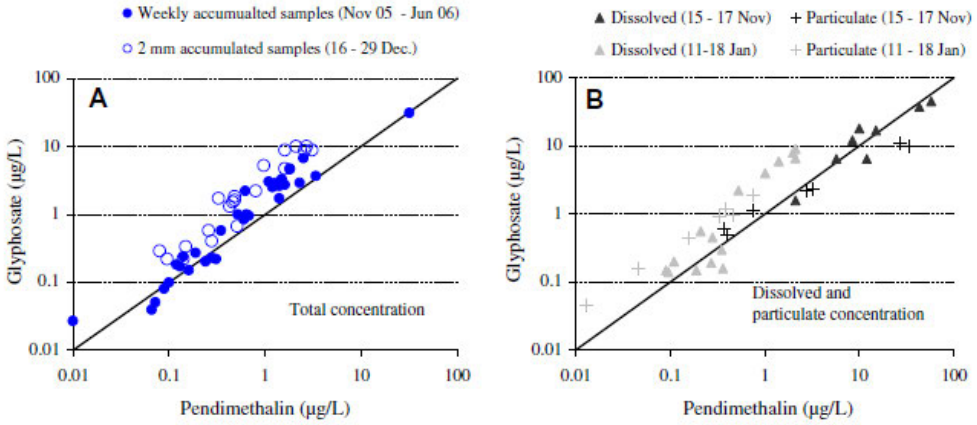
The leaching pattern of glyphosate resembled that of pendimethalin, thus suggesting (i) that the leaching potential of strongly bound pesticides from structured soil is high both with pesticides that bind to soil organic matter (e.g. pendimethalin) or to the inorganic fraction (e.g. glyphosate) and (ii) that the pathways governing the transport of these two pesticides are similar. Both glyphosate and pendimethalin leached from the root zone in average concentrations considerably exceeding the EU limit value for groundwater (0.1 µg/L) during the 8–month drainage flow period. The average concentration of glyphosate and pendimethalin in the drainage water was 3.5 and 2.7 µg/L, respectively. Both pesticides were found in all of the weekly drainage water samples. Among the 32 samples collected after pesticide application, the concentration exceeded 0.1 µg/L in 29. The similarity of the leaching patterns of the two pesticides was reflected in the close correlation between the measured concentration of pendimethalin and glyphosate. R^2 for measured total concentration (both dissolved and particle–bound) in samples collected (i) on a weekly basis during the entire monitoring period (32 samples) and (ii) for every 2 mm of drainage runoff occurring during a 13–d period in December (20 samples) was 0.962 and 0.963, respectively.

A). A similar tendency was found when comparing the particulate and dissolved concentrations of pendimethalin and glyphosate measured during two individual flow events, R^2 being 0.943 for dissolved pesticide (19 samples) and 0.928 for particle–bound pesticide (10 samples).

B). Pesticide leaching was governed by preferential transport, as evidenced by the soil hydraulic properties (Kjær *et al.*, 2005) and fast solute transport. Piston flow through the low–permeable soil matrix would entail a transport time to the drainage system of about 98 d (Kjær *et al.*, 2007). However, glyphosate and pendimethalin were detected in drainage water samples as early as 8 d after application. This finding is thus consistent with previous transport studies conducted at the Estrup site (Kjær *et al.*, 2005, 2007), as well as other field studies demonstrating rapid macropore–mediated transport of pesticides (for a review see Jarvis (2007)). As both glyphosate and pendimethalin leached in high concentrations following the same transport pathways, the difference in sorption characteristics of glyphosate, which sorbs strongly to the inorganic soil fraction, and pendimethalin, which sorbs strongly to the soil organic fraction, had little impact on leaching in this structured soil. Our finding is in line with previous studies showing that differences in the leaching of pesticides that differ widely in sorption properties are significantly reduced in the presence of macropore flow (Larsson and Jarvis, 1999). Likewise, Flury (1996) concluded from the transport studies of Kladivko *et al.* (1991), Traub–Eberhard

et al. (1995) and Flury *et al.* (1995) that part of the various pesticides applied simultaneously to the soil surface moved through structured soil in an identical manner irrespective of their chemical properties.

Figure 8.1.3.3-8: Measured concentration of glyphosate and pendimethalin in drainage water samples collected after pesticide application. A (left): Total concentration (both dissolved and particle–bound) in samples collected on either a weekly basis during the entire monitoring period (closed circles) or for every 2 mm of drainage runoff during a 13–d period in December 2005. B (right): Concentration of dissolved (triangles) and particle–bound (crosses) pesticide in samples collected for every 2 mm of drainage runoff during two selected flow events in November 2005 (black) and January 2006 (grey). Sampling periods are indicated in parentheses



Quantitative impact of particle–facilitated transport on total leaching

Measured concentration of particle–bound pesticides were marked lower than that of dissolved pesticides, ratio between measured concentration of dissolved pesticides and particle–bound ranging between 4–14 and 1.2–30 for glyphosate and pendimethalin respectively. Intensive monitoring of two individual flow events suggested that particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13–16 % of the leached mass of glyphosate and 16–31 % of the leached mass of pendimethalin). These values are in line with the few available field studies quantifying particle–facilitated transport of strongly sorbing pesticides. In Danish drainage water studies using a cut–off size of 0.7 µm, Petersen *et al.* (2003) found that 9 % of the leached pesticide was particle bound. Correspondingly, Vilholdt *et al.* (2000), using a cut–off size of 0.24 µm, found that 6 % of the leached pesticide was particle bound. In laboratory experiments with undisturbed 20–cm soil columns, de Jonge *et al.* (2000) found that particle–facilitated transport (particles >0.24 µm) accounted for <1–27 % of total glyphosate leaching. In a study by Gjettermann *et al.* (2009) using intact soil columns from ploughed and minimal tillage cultivation systems, colloid–facilitated glyphosate leaching (cut–off size >0.02 µm) accounted for 68 ± 10 % of total glyphosate leaching from the ploughed system as compared to only 17 ± 12 % from the minimal tillage system. That leaching of particle–bound glyphosate from the ploughed soil was markedly greater than that seen in our study and previous studies may be attributable to differences in experimental conditions, e.g. ploughing before or after pesticide application and precipitation intensity. In our field study the total amount of precipitation and maximum precipitation intensity were 12 mm within 11 h and 2.1 mm h^{-1} (11 January 2006) and 18 mm within 10 h and 4.6 mm h^{-1} (14 November 2005). In the study of Gjettermann *et al.* (2009) glyphosate was applied to the soil 1 d after the last of two rewettings and the soil then irrigated twice for 2 h using 15 mm h^{-1} on days 5, 8 and 12 following the last rewetting. In Denmark such high precipitation intensity is rare during the period relevant for autumn application of glyphosate (September–November). Analysis of precipitation data collected in a national grid of approximately 60 automatic climate stations run by the Danish Meteorological Institute revealed that there had only been 8 precipitation events exceeding 15 mm h^{-1} during the preceding 10 years (Birgit Sørensen, personal communication). The combination of wet, loose soil and very intensive precipitation shortly after the application of pesticide is likely to result in greater contact between pesticide and soil particles and enable greater mobilisation of soil particles. In soils having had time to consolidate, such as the minimal tillage soil studied by Gjettermann *et al.* (2009) and in the present study (ploughed 7 months before pesticide application) fewer particles will be available for contact with the pesticide.

Figure 8.1.3.3-9: Hourly precipitation (grey hanging bars in A and E) together with turbidity (B and F), particulate (crosses) and dissolved (circles) glyphosate (C and G), particulate (crosses) and dissolved (circles) pendimethalin (D and H) in the drainage runoff (DR on the secondary axis) following flow events on 14 November 2005 (right) and 11 January 2006 (left)

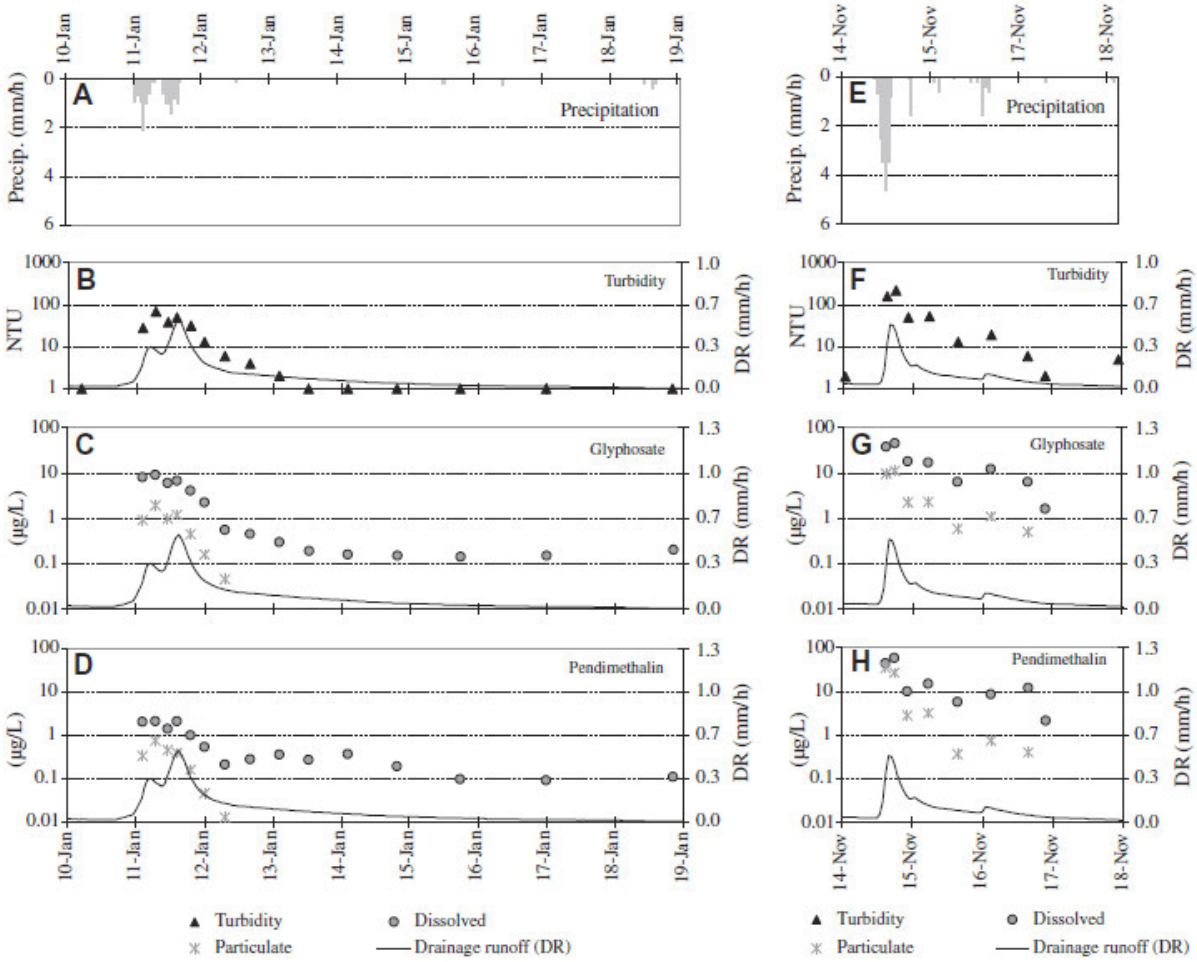
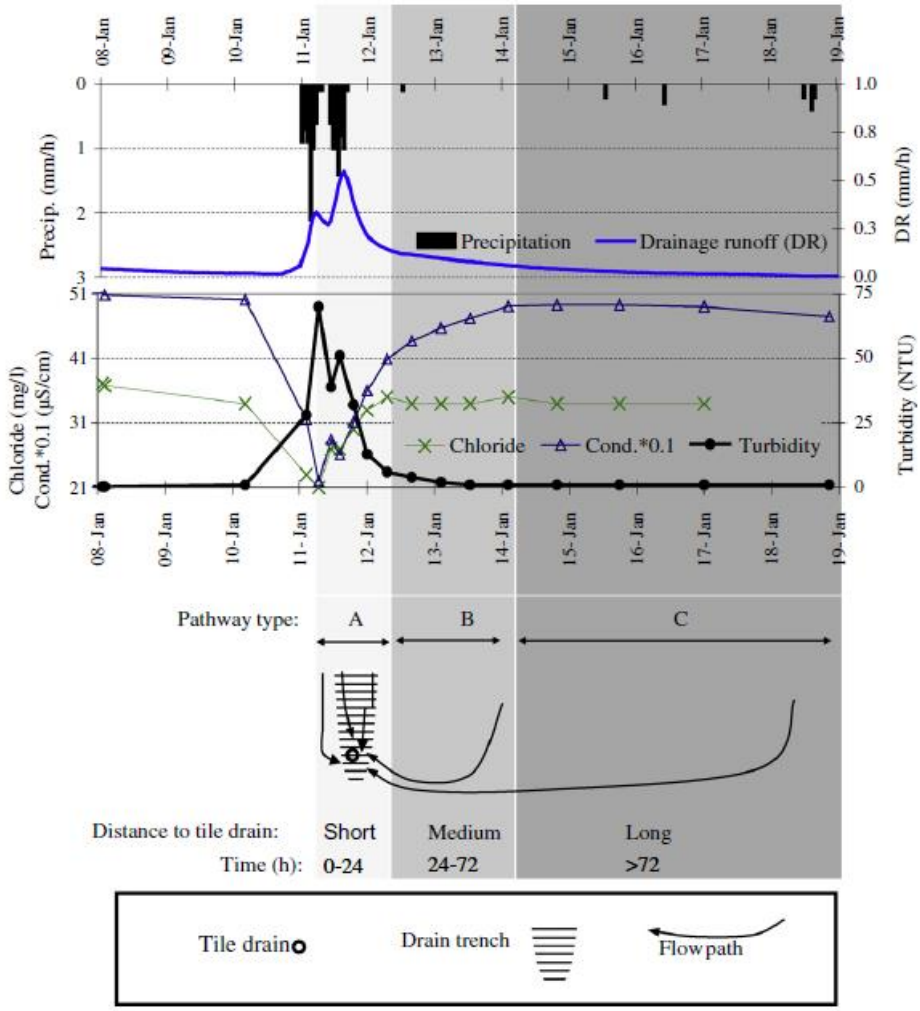


Figure 8.1.3.3-10: Hourly precipitation and drainage runoff together with measured chloride concentration, conductivity and turbidity (lower graph). The shaded areas indicate the dominant transport pathways (types A–C) feeding into the sampled drainage water during the flow event. While ''time'' and ''pathway types'' are classified directly from measured data, ''distance to tile drain'' and shown water flow pathways are indicative providing our interpretation of measured data. (see Section "*Through which pathways do strongly sorbing compounds enter the drainage system?*")



Water flow pathways

Drainage water consists of a mixture of water of different origins, with the dominant flow pathways varying over the course of time (Jacobsen and Kjær, 2007). Knowledge of the dominant transport pathways is thus important for the interpretation of measured pesticide concentrations. The transport pathways during the flow event of 11 January 2006 are indicated by the measured turbidity, chloride concentration and conductivity. Thus the chloride concentration and conductivity decreased markedly during the first precipitation event, while the turbidity increased. This indicates rapid transport of precipitation with low chloride concentration and conductivity, probably through drain–connected macropores. During the 24–h period from the end of the first precipitation event the turbidity decreased, while the chloride concentration and conductivity increased. This indicates that water entered the drainage system not only from macropores connected directly to the drains but also from the vicinity of the drain pipe, i.e. the trench dug when installing the tile drain system. Transport pathways is likely also to involve a lateral component in the shallow saturated zone through natural macro pores aided by gradients generated by inter–drain mounding of the water table during the high–flow condition following the rain event. Water from here would have a relative short travelling distance before entering the drainage system. These transport pathways characterised by having a short flow path to the drain and being active during the first 24–h period are designated type A. From 24 to 72 h the turbidity remained low and below the detection level of 1 NTU (nephelometric turbidity units), the chloride concentration plateaued out and the conductivity continued to increase. That the chloride concentration returned to the ‘‘background level’’ indicates cessation of the rapid entrance of precipitation low in chloride. Instead the length of the pathway increased with drainage water entering from in between the drain trench, designated pathway B. During these longer transport pathways, the particles are filtered by the soil causing the turbidity to decrease below the detection limit, while the longer retention time allows the

infiltrating water to interact with the soil matrix causing the conductivity to increase. By 72 h after the end of the first precipitation event the conductivity, chloride concentration and turbidity had returned to their background levels, and the drainage water is dominated by the longer transport pathways (designated type C). These transport pathways are likely to comprise precipitation that has infiltrated vertically some distance from the drain trench and subsequently been transported laterally to the drain via the saturated layer.

Pesticide transportation pathway

During the 24–h period following cessation of the first precipitation event on 11 January 2006 the leaching pattern was similar for both particles, particle–bound pesticide and dissolved pesticide, thus indicating that all three follow the same transport pathways, presumably involving drain–connected macropores located above or in the vicinity of the drains or rapid lateral transport near the drain line. Thereafter the leaching of particles and particle–bound pesticide ceased, whereas dissolved pesticide continued to leach in high concentrations (>0.1 µg/L) for up to 7 d after the precipitation had stopped. This ‘‘tailing’’ of dissolved pesticides indicates that the transport pathways involve transport through macropores between drains followed by lateral transport to the drains (types B and C). Moreover, it indicates that while particles (indicated by elevated turbidity) and particle–bound pesticide seems to be retained in the soil during the lateral transport in between the drain, dissolved pesticide can be transported laterally through the saturated zone to the drainage system. The leaching pattern following the flow event on 14 November 2005 was very similar to that observed after the flow event on 11 January 2006, although sampling conditions precluded the recording of transport occurring through pathways B and C. The flow event on 11 January 2006 was characterised by high precipitation (12 mm) followed by 7 d virtually free of precipitation (1 mm in total). Such conditions are ideal for describing variation in flow pathways over time and capturing the transport involving all three pathways (A–C). In contrast, the flow event on 14 November 2005 was characterised by one major (18 mm on 14 November) and several minor precipitation events (6 mm in total), and sampling was performed for just 2 d (E–H). The conditions were ideal for descryibing transport pathway A, but inadequate for describing pathways B and C. The fact that turbidity remained high for a much longer period (approx. 24 h) during the November 2005 event than during the January 2006 event (E–H) is attributable to the minor precipitation events on 16 and 17 November 2005 and resultant rapid preferential transport of leachate via pathway A. The direct transport from surface layers to drains via macropores (pathway A) reported here is in line with previous observations. Thus several studies report that the soil surface can be in direct contact with drains through macropores comprised of old root channels or earthworm burrows (Nielsen *et al.*, 2010; Nuutinen and Butt, 2003; Shipitalo and Gibbs, 2000). The same pathways were also responsible for the leaching of colloid–size particles (Nielsen *et al.*, 2011) and the strongly sorbing pesticides (both dissolved and particle–bound) pendimethalin (Petersen *et al.*, 2003) and prochloraz (Vilholdt *et al.*, 2000) on drained, loamy soils. The observed transport pathway involving transport through macropores located between drains followed by lateral transport to the drains (pathways B and C) is presumably attributable to connectivity between the vertical biopores and the three–dimensional fracture system in the soil, which enables rapid, lateral transport in the soil (Rosenbom *et al.*, 2008; Nilsson *et al.*, 2000, 2001; McKay *et al.*, 1999). Studies of the transport of two fluorescent tracers in clayey till (Rosenbom *et al.*, 2008) indicate that during periods of continuous drainage runoff the extent of rapid macropore transport in the soil between the drain lines is determined by the degree of connectivity between root zone biopores and high–permeability fractures. Evidence that such connectivity enables leaching of solutes from the surface of fractured till is also provided by forced gradient tracer experiments conducted at three different locations (Ringe, Avedøre and Lillebæk) in Denmark (Nilsson *et al.*, 2000, 2001; McKay *et al.*, 1999). These transport studies were all performed with conservative or slightly sorbing tracers (chloride, bromide, bacteriophage tracer PRD–1, colloidal tracer, sulforhodamine B, and acid yellow). Similar studies addressing the potential of pathways B and C to transport strongly sorbing pesticides are very limited, however. Transport of the strongly sorbing pesticides pendimethalin and prochloraz in drained structured soil has been studied by Vilholdt *et al.* (2000) and Petersen *et al.* (2003). However, the study design, while suitable for describing vertical transport from the top soil to the vicinity of the drain line (pathways A and B), was unsuitable for describing transport involving vertical infiltration between the tile drains followed by a subsequent lateral transport to the drain (pathway C). In Vilholdt *et al.* (2000), pesticide sampling was performed 2.5 m either side of the drain trajectory up to 7.5 h following a precipitation event. The study of Petersen *et al.* (2003) was conducted on a very

well drained soil with limited lateral water flow (drainage runoff accounting for only 2–10 % of total precipitation input during the sampling period) with most pesticide samples being collected within 36 h of the precipitation event. Under such conditions, lateral transport of pesticides (pathway C) is unlikely. The leaching pattern found in our study indicate that while particles (indicated by elevated turbidity) and particle–bound pesticides were retained in the soil during lateral transport, dissolved glyphosate and pendimethalin were transported through the saturated zone to the drainage system. Similar findings suggesting that dissolved, strongly sorbing pesticide can be transported over long distances via discontinuities are provided by Gooddy *et al.* (2007), who studied the concentration of dissolved and particle–bound diuron and its metabolites in chalk groundwater sampled 30 m b.g.s. Most of the pesticide–colloid complexes (particles >0.1 µm) formed in the soil were removed during migration of the water through the 30 m deep, unsaturated zone and/or the saturated zone, whereas pesticides in soluble form was detectable in the groundwater 30 m b.g.s. Moreover, in a study of the transport of brilliant blue, bromide and micropheres along macropores in sandy loam, Nielsen *et al.* (2011) found that while colloid–size particles were trapped in the bottom of the biopores, dissolved tracer (brilliant blue and bromide) migrated further into the soil.

Conclusion

Pesticides leaching from the unsaturated zone may eventually pose a risk to the aquatic environment. The present 8–month study of a loamy field demonstrates that: Strongly bound pesticides, whether bound to the organic or inorganic soil fraction, may leach from the root zone and enter the aquatic environment in average concentrations exceeding 0.1 µg/L. Particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of observed leaching (13–16 % for glyphosate and 16–31 % for pendimethalin). The pathway by which these strongly sorbing compounds entered the drainage system involved transport through drain–connected macropores (above or in the vicinity of the drains) as well as the macropores situated between the drains and connected to underlying fractures. Particle–bound pesticide (particles >0.24 µm) was transported solely by vertical transport in macropores and rapid lateral transport occurring nearby the drain line, whereas dissolved pesticide was also transported laterally over larger distances through the saturated zone via discontinuities in the soil. This newly identified transport pathway whereby dissolved pesticides are transported laterally via discontinuities in the soil needs to be taken into account when assessing the risk posed by pesticides to the aquatic environment. Our findings imply that strongly sorbed pesticides such as glyphosate and pendimethalin may be present in high concentrations (>0.1 µg/L) in both the water flowing from the drainage system and in the shallow groundwater located at the depth of the drainage system.

Assessment and conclusion by applicant:

The article describes a leaching experiment with glyphosate and pendimethalin in a Danish tile-drained agricultural soil over eight months. The substance properties are sufficiently reported. Pesticide leaching from the unsaturated soil zone may occur as particle-facilitated transport via drain-connected macropores as lateral flow with strongly bound pesticides. With regard to the data requirement, the study is too short for a comprehensive evaluation of the leaching behavior. In addition, no residues were determined in different soil layers after finalization of the study, and sample storage stability prior to analysis was not established. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

The article is well detailed. The soil used was, according to the authors, heavily fractured and bioturbated. This is consistent with the main transport processes identified in the study. As noted by the applicant, the study duration was limited to 8 months and no residues were determined in different soil layers at the end of the experiment, as recommended in Regulation 283/2013.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Candela et al., 2010

Data point:	CA 7.1.4.3/005
Report author	Candela, L. *et al.*
Report year	2010
Report title	Glyphosate transport through weathered granite soils under irrigated and non–irrigated conditions–Barcelona, Spain
Document No	DOI 10.1016/j.scitotenv.2010.03.006 E-ISSN 1879-1026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The transport of Glyphosate ([N–phosphonomethyl] glycine), AMPA (aminomethylphosphonic acid, CH6NO3P), and Bromide (Br–) has been studied, in the Mediterranean Maresme area of Spain, north of Barcelona, where groundwater is located at a depth of 5.5 m. The unsaturated zone of weathered granite soils was characterized in adjacent irrigated and non–irrigated experimental plots where 11 and 10 boreholes were drilled, respectively. At the non–irrigated plot, the first half of the period was affected by a persistent and intense rainfall. After 69 days of application, residues of Glyphosate up to 73.6 μg/g were detected till a depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot was detected till a depth of 0.5 m. According to the retardation coefficient of Glyphosate as compared to that of Br– for the topsoil and subsoil (80 and 83, respectively) and the maximum observed migration depth of Br– (2.9 m) Glyphosate and AMPA should have been detected till a depth of 0.05 m only. Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.

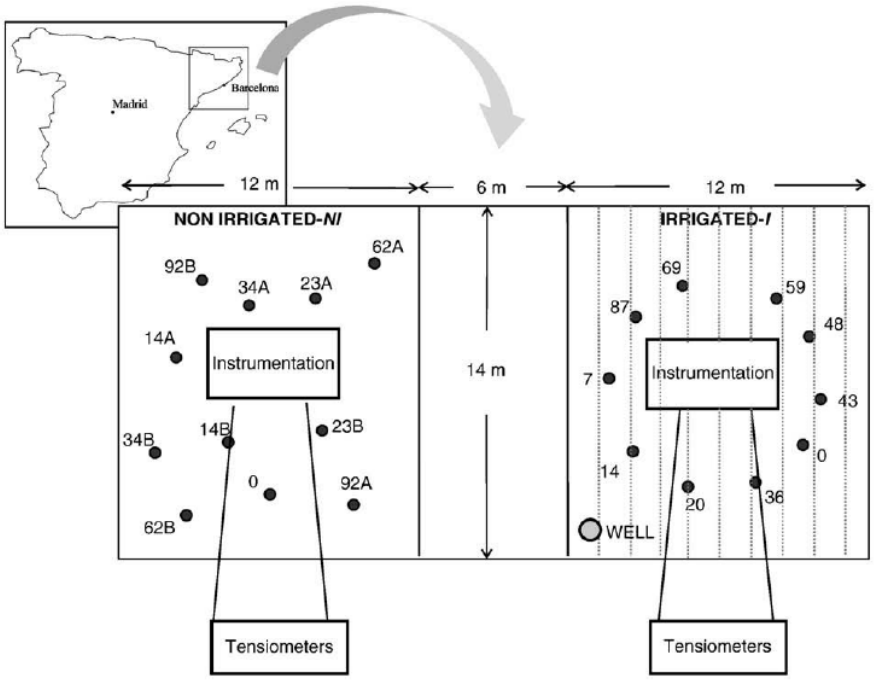
Materials and methods

Experimental site

The experimental site was located in a narrow coastal strip composed of weathered granite in the IRTA agricultural station of the Maresme region, located 30 km North of Barcelona– Spain. The area, under no–tillage farming, was only covered by a wheat crop to protect the soil from erosion for more than ten years. Groundwater was at a depth of 5.5 m; the hydrology of the study site has been described in detail by Guimerà *et al.* (1995).

Two individual plots of approximately 168 m^2 each, separated by a control area of 84 m^2, were selected. Initially, the weeds covering both plots were manually removed to allow installation of the irrigation and vadose zone monitoring equipment. Subsequently, the wheat cover was allowed to redevelop, prior to herbicide application. The upward–downward flux of water in the unsaturated zone was monitored by 7 tensiometers (Soilmoisture®). At the beginning of the experiment duplicate tensiometer sets were installed by manual drilling, in the middle of the plots, at a depth of 0.30, 0.60 and 0.90 m 106 and one tensiometer was installed at a depth of 1.20 m. Instrumentation remained in place until the end of the experimental activities.

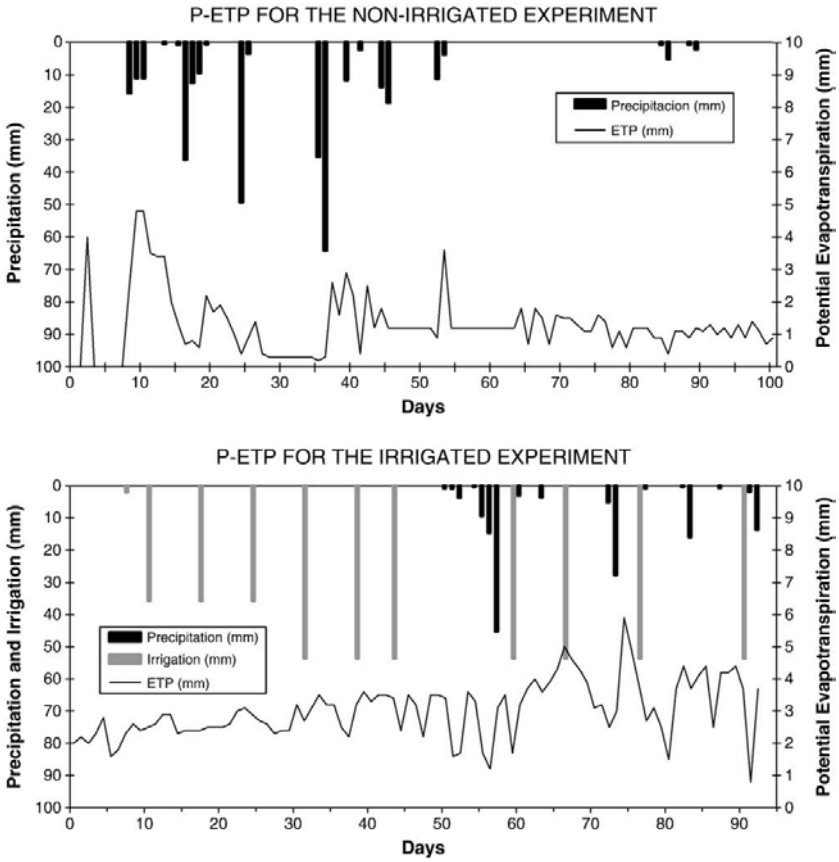
Figure 8.1.3.3-11: Study area location and non–irrigated (NI) and irrigated (I) experimental plots. The location of in situ field instrumentation and drillings is shown for the two sites; 0 denotes location of background drillings; duplicate drillings for *NI* are denoted as A and B (e.g., 1A and 1B). The vertical dashed lines (in *I*) denote the location of soak bands. The location of a groundwater well is also shown



For initial characterization of the unsaturated zone profile, before starting the experiments, two boreholes were drilled (location denoted as "0" in the non–irrigated–*NI* and irrigated–*I* plots) and undisturbed soil samples were taken down to 4.50 m. In both plots soil matrix characterization and the monitoring of pore water content was performed by destructive sampling. The amount of pesticide in the vadose zone at given times was determined in undisturbed soil sampling. Groundwater quality was monitored in an existing pumping well in plot *I*.

Precipitation amounts for both experimental periods were provided by the IRTA meteorological station. Irrigation was based on soak bands that were installed in the *I* plot with a separation of 0.30 m in order to obtain a uniform spatial distribution of water. Two irrigation doses of 36 mm per week, were applied during two hours for the first three weeks of the study period (March I-14 to I-27). Subsequently, the amount of irrigation increased to 53 mm per week.

Figure 8.1.3.3-12: Precipitation and evapotranspiration during the non-irrigated (September–December) and irrigated (February–June) experiments



Application of Glyphosate and bromide

Both Glyphosate (*Roundup*®, 36 % p/v, Montsanto Europe S.A.), and bromide (NaBr; conservative tracer) were applied under non–irrigated (*NI*) and irrigated (*I*) conditions. The first field experiment, non–irrigated, was conducted during the rainy season (September to December, 1994) and sampling and monitoring activities extended over 92 days. During the second field experiment, irrigated, that lasted 87 days, the area was irrigated from February to May (1995).

Glyphosate, along with a solution of NaBr, was applied on the soil surface on September 13 on the *NI* plot and on March 7 on the *I* plot using an automated spray system to ensure uniformity. The pesticide and bromide solutions were prepared at the study site before application. The concentrations of BrNa solutions for the *NI* and *I* plots were of 20 g/L and 17 g/L of BrNa respectively. Glyphosate solution was prepared by mixing 400 cm^3 and 420 cm^3 of a commercial 36 % (p/v) Glyphosate EC formulation with 20 and 21 L of groundwater for the *NI* and *I* plots, respectively. This procedure of pesticide application follows standard agricultural practice in the Maresme area.

Vadose zone soil and water sampling methodology

Soil samples were obtained with a hollow–stem auger after pesticide and bromide application in both field plots. A random sampling scheme with duplicate soil cores was applied in the non–irrigated area (*NI*–0(A,B) to 92 (A,B)) where undisturbed soil cores were taken at 0.20 m intervals till a depth of 1 m, and at 0.50 m intervals below it. Due to field and experimental constraints a circular sampling pattern and single cores, where undisturbed soil samples were obtained at 0.20 m intervals, was applied in the irrigated experimental plot (*I*–0 to 69). To prevent possible contamination from overlying layers, two samples of the soil to be analyzed were taken from the inner part of each core, after discarding the top and the bottom portions of it. One sample was carefully wrapped in aluminum foil, and frozen until pesticide and Br laboratory analyses. The other one was used for the determination of volumetric water content, saturated and unsaturated hydraulic conductivity, and bulk density (following ASTM 1993 standards) clay content and clay type (RX diffraction) and organic matter content. Also pH, CEC and Al and Fe oxides were determined in samples following standard techniques described in Melo (1996) and Candela *et al.* (2007).

During the field experiments (Table 8.1.3.1-18 and Table 8.1.3.1-19), groundwater samples, soil cores and soil–water potential measurements from the tensiometers were obtained after each rain or irrigation episode. Groundwater samples were obtained with a bailer from the existing open well where also the depth of the water table was monitored. Due to analytical constrains, the concentration of AMPA was monitored in the irrigated plot only.

Table 8.1.3.3-19: Sampling dates and precipitation amounts for the non–irrigated (NI) experiment conducted in 1994 (September–December)

Sampling survey	NI-0 Background	NI-14	NI-23	NI-34	NI-62	NI-92
Drilling date	Sep 6[a]	Sep 27	Oct 6	Oct 17	Nov 14	Dec14
Precipitation (mm)[b]	–	52.6	110.5	49.1	7.2	–
Days after glyphosate and NaBr application[c]	−7	14	23	34	62	92

[a] September 6 (*NI-0*), soil profile characterization.
[b] Cumulative values for the time interval between sampling. Total precipitation: 219.4 mm.
[c] Glyphosate and NaBr application on September 13.

Table 8.1.3.3-20: Sampling dates and precipitation amounts for the irrigated (I) experiment conducted in 1995 (February–June)

Sampling survey	*I-0* background	I-7	I-14	I-20	I-36	I-43	I-48	I-59	I-69	I-87
Drilling date	Feb 28[a]	Mar 14	Mar 21	Mar 27	Apr 12	Apr 19	Apr 24	May 5	May 15	Jun 2
Irrigation mm/week[b]	36	36	36	53	53	53	53	53	53	53
Precipitation (mm)[b]	–	–	–	–	–	0.7	4.7	75.3	32.8	32.9
Days after glyphosate and NaBr application[c]	−7	7	14	20	36	43	48	59	69	87

[a] February 28 (*I-0*), soil profile characterization.
[b] Total irrigation: 479 mm. Total precipitation: 146.4 mm. Precipitation (January–March 15) 8.6 mm.
[c] Glyphosate and NaBr application on March 7.

The total length of the sampled soil cores in each survey was determined according to: (a) the depth of penetration of water through the unsaturated zone as predicted from in situ tensiometer readings, (b) the hydraulic conductivity of soil samples as determined in the laboratory, (c) the predicted theoretical depth reached by the center of mass of Br^-, and (d) the retardation factor, R (Ghodrati and Jury, 1992) of glyphosate as determined in batch experiments for soils and sediments of the area. However, as a safety measure, soil drillings and sampling depths were always greater than the calculated theoretical depth of penetration of Glyphosate.

Chemical analyses

Chemical analysis of glyphosate and AMPA residues in soil and water samples was performed using an HPLC method (Hewtlett Packard, HPLC ChemStation G1034A) based on reversed–phase chromatography, with flourescent detection using pre-column derivatization with FMOC (9–fluorenylmethylchloroformate) to give the flourescent derivative. The liquid chromatography coupled column (LC–LC) methodology described by Sancho *et al.* (1996) was used to confirm the presence of glyphosate and AMPA residues in positive samples. The LC–LC technique presents several advantages, such as improved sensitivity, selectivity, and sample throughput. The detection limit of glyphosate and AMPA was 6 ng/g and 4 ng/g for soil, and 0.15 µg/L and 0.1 µg/L for water samples respectively, with extraction efficiency greater than 95 % for both analytes. Bromide content was determined by ionic chromatography (VYDAC column) and the detection limit was 0.1 ng/g.

Results

Soil properties

The soil profile, a Typic Xerorthent (Soil Survey Staff, 1999), is very homogeneous and consists of medium to coarse sand size with low clay content (clay, 5 %; silt, 20 %; sand, 75 %). The clay fraction is mainly composed of smectite, illite and kaolinite. The soil had no visual structure except for the presence of a coarse sand layer at 1.50–1.90 m and granite debris at a depth of about 4.50 m. However, according to physico–chemical properties a top soil layer and a subsoil horizon may be distinguished. The soil chemical properties determined from samples at a depth of 0–0.20 m (top soil) and 0.70–1 m (subsoil horizon) respectively, are: cation exchange capacity (CEC), 5.2 and 4.6 meq.100/g; pH (1:1 in H_2O), 7.9 and 7.3; organic matter 1.1 and 0.09 (%); P 0.2 mg.100/g (top soil), total Fe_2O_3, 1.92 and 5.43 g.100/g; and total Al_2O_3 1.75 and 7.22 g.100/g. Average values of soil bulk density from field

samples were 1.65 and 1.7 g/cm^3 for the top soil and subsoil, respectively. Residues of Glyphosate and Br^- were not detected along vadose zone profile before the experiments (*I*–0 and *NI*–0).

Figure 8.1.3.3-13: Volumetric content of water, bromide and glyphosate in the different soil profiles for the non–irrigated plot (September–December 1994). *NI*–0: soil profile prior to pesticide and bromide application. The high water content level (0.20–0.14 cm^3/cm^3) at a depth of 1.5 m reflects the presence of a coarse sand layer (LoD: 6 ng/g Glyphosate; 4 ng/g AMPA; 0.1 ng/g Br)

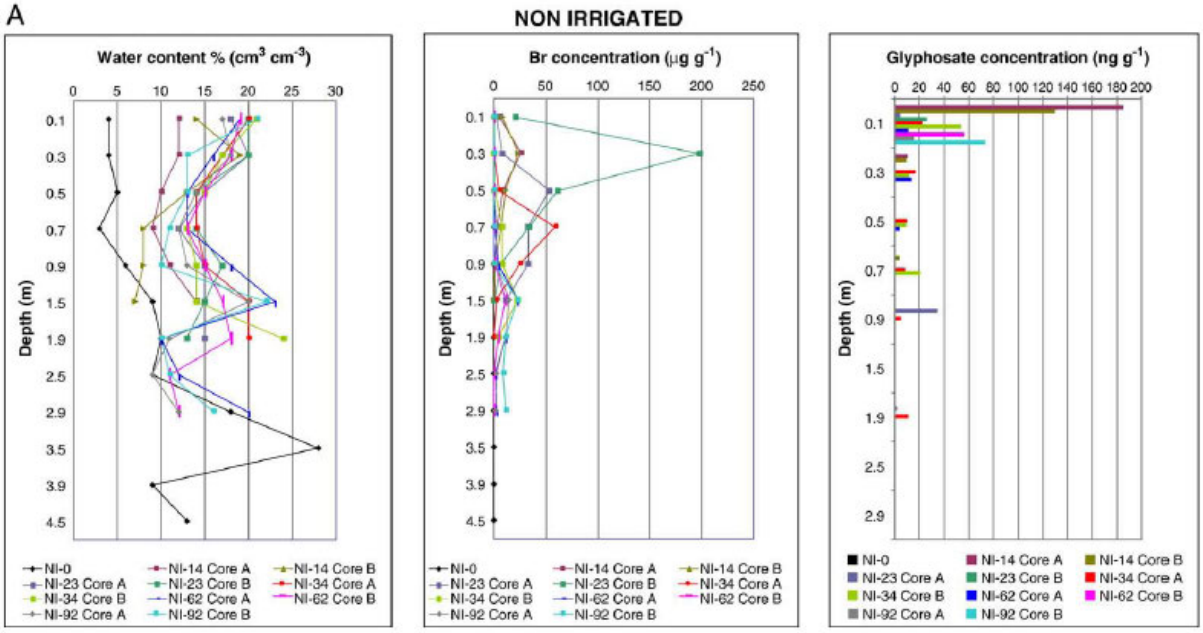
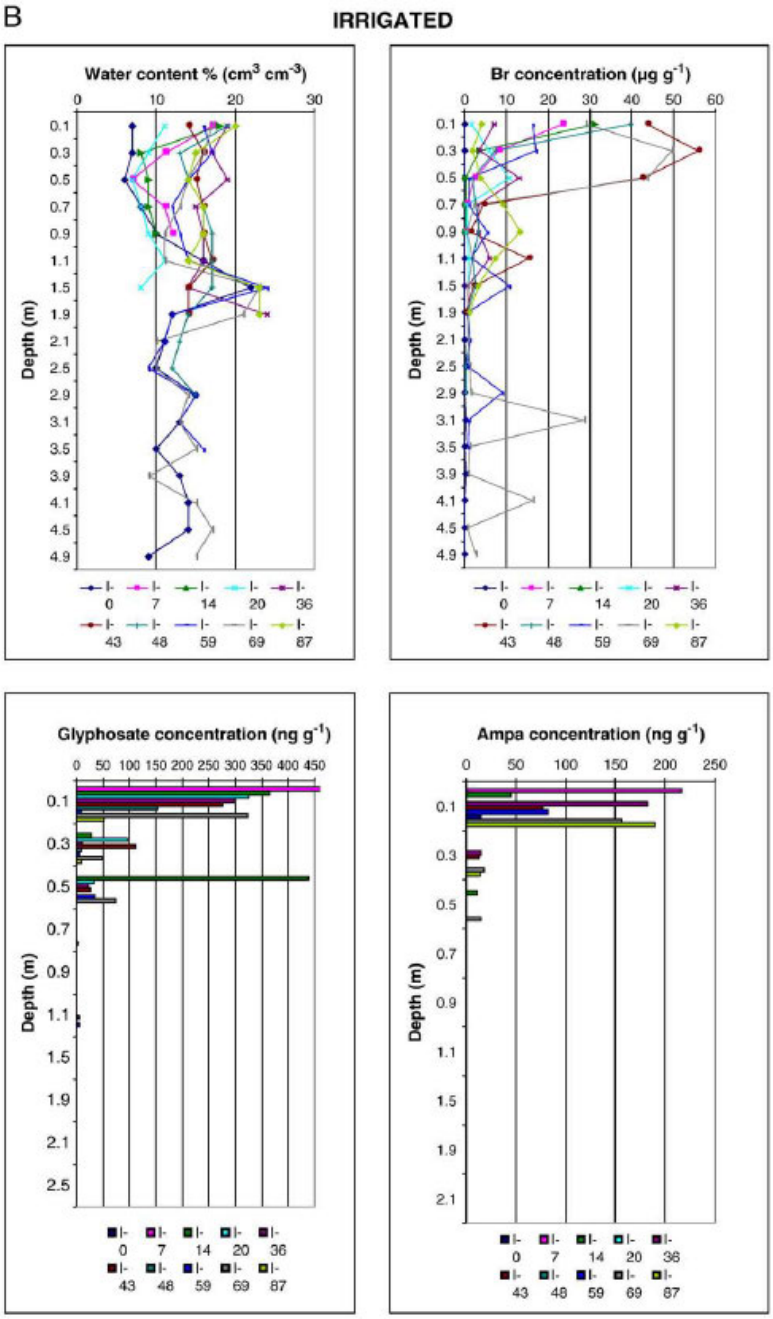


Figure 8.1.3.3-14: Volumetric content of water and bromide in the different soil profiles for the irrigated experiment (March–June 1995). *I*–0: soil profile prior to pesticide and bromide application. The high water content level (0.22–0.15 cm^3/cm^3) at a depth of 1.5 m reflects the presence of a coarse sand layer. (LoD: 6 ng/g Glyphosate; 4 ng/g AMPA; 0.1 ng/g Br)



Non-irrigated plot

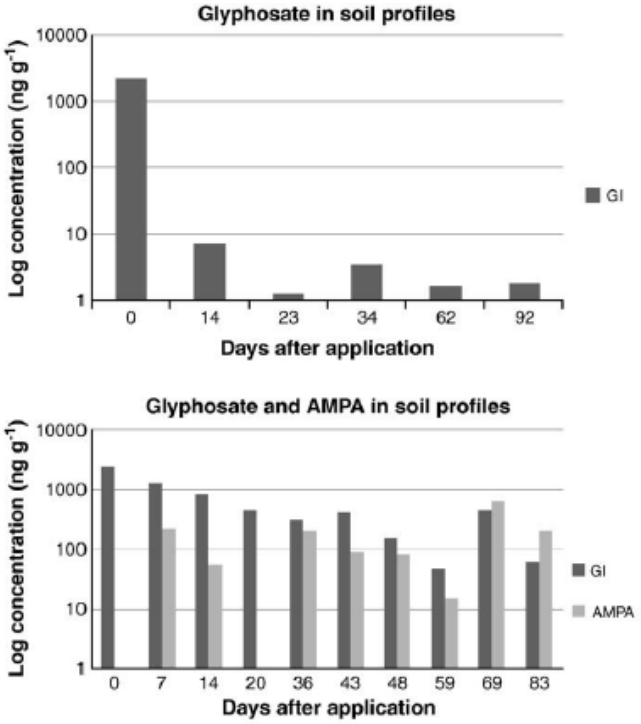
During the non–irrigated experiment the average temperature was 14.7°C, rainfall accounted for 219.4 mm, and more than 50 % of the total precipitation (163 mm) was due to three storm events in September and October. For this same time period, evapotranspiration was 118.9 mm. In this plot the water content till a depth of 1.5 m was extremely low (6 % background average) due to lack of precipitation and high temperature during the summer period (*NI*). After the first rain event, a week after Br and pesticide application, the movement of the wetting front is clearly observed (*NI*-14). In the upper 1.50 m the water content increases up to 20 % at the end of the experimental period and soil–water content seems to stabilize after 34 days (*NI*–34). The greatest water content along the profile was observed in the coarse sand layer at 1.5 m depth.

Maximum concentration of Glyphosate in the unsaturated zone were detected at a depth 0–0.30 m, except for *NI–23A* and *NI–34B* where residues were also detected at a depth of 0.9 and 0.7 m, respectively. The depth of penetration in individual cores varied widely. Glyphosate residues were also detected along the unsaturated zone, at concentration below the detection limit (LoD), up to a depth of

0.90 and 1.90 m after 23 and 34 days of application. After 14 days, the residual amount was 7 % of the total applied mass. After 23 days and till the end of the experiment, residual amounts account for 1 %. Glyphosate half–life (or half concentration time) calculated from in situ experimental values was 7 days, although it may be even lower considering that the first sampling campaign was undertaken after 14 days of pesticide application.

The following figure presents the amount of pesticide remaining in the soil profile till the end of the experiment for each core and sampled borehole. Mass estimation refers to the initial applied dose. A rapid initial dissipation phase, followed by a slower one is observed after 23 days. Degradation rate, estimated from logarithmic pesticide concentration vs. time (best fit equation) was 1.52 days. However, the small value of the correlation coefficient obtained (R^2 = 0.4) indicates the low accuracy of the calculations and the associated uncertainty.

Figure 8.1.3.3-15: Residual mass of glyphosate and AMPA remaining in soil profile as a function of time. Non–irrigated and irrigated experiment



Irrigated plot

In the irrigated plot experiment carried out during springtime, the total amount of water applied was three times higher than that of the NI plot as precipitation accounted for 146.4 mm and irrigation for 483 mm. The average temperature was of 12.3°C, and evapotranspiration (266.6 mm) was greater than in the non–irrigated experiment. The background average water content in the soil profile up to 1.5 m was 10.9 %. From *I–36* (when the irrigation dose is increased), until the end of the experiment the soil profile water content is quite constant. The increase in water content at 1.50–1.90 m is due to the presence of a coarse sand layer.

As shown above, maximum concentration of Glyphosate was always detected between the first 0–0.5 m of the soil profile and concentration values were greater than those found in the non–irrigated plot. Residues of Glyphosate (below LoD) were still found at 1.50 m after 69 days of application and continued to be detected after 87 days. Residual amount of Glyphosate in soil profile after 14 days was 34 % of the applied dose, being reduced to 2 % after 59 day, and up to the end of the experiment. Field half–life (or half concentration time) was around 7 days and estimated degradation rate was 0.04 days (R^2 = 0.6).

Glyphosate and bromide were not detected in groundwater samples obtained with a bailer along all the monitoring periods.

Discussion

For the non–irrigated experiment (NI) Br concentration along the soil profile was clearly affected by the rain episodes, and was detected up to a depth of 150 cm after 14 days of application, implying a flow velocity of 10 cm/day calculated according to Burns (1975). The observed deficit at *NI–14* profile (55 % recovery of applied dose) could be attributed to the uptake of bromide by plants (Kung, 1990a). After decomposition of plant residues, bromide may return to the soil and can be accounted for as an external input in the bulk mass balance. In the irrigated plot, tracer distribution over depth is fully controlled by irrigation dose, and Br^- concentration presents lower variability.

As shown in the results of both field experiments, concentrations of Glyphosate were detected, much deeper than expected according to the distribution coefficients calculated for the surface soil (K_f = 93), and subsoil (K_f = 154) in batch experiments (Melo, 1996; Candela *et al.*, 2007). The retardation factor (R, Ghodrati and Jury, 1992) of Glyphosate, as compared to that of Br for the topsoil and subsoil, is 80 and 83, respectively. Considering a worst–case scenario (R = 80) and the maximum migration depth of Br (2.90 m; and 4.90 m), then, the maximum transport depth of Glyphosate should have been 0.05 m only. We hypothesize that the deep transfer of both glyphosate and AMPA can be the result of: (a) preferential transport along the unsaturated zone (Kung, 1990b; Van den Bosch *et al.*, 1999; Scorza *et al.*, 2004; Coppola *et al.*, 2009), and/or (b) colloidal mediated transport of both components (Vereecken, 2005; Borggaard and Gimsing, 2008), a process that can be inferred from their relatively large K_f values.

The mobility of strongly adsorbing compounds as Glyphosate (Veiga *et al.*, 2001; Kjaer *et al.*, 2005; Vereecken, 2005, among others) has already been shown for pesticides such as propiconazole and fempropimorph (Krongvang *et al.*, 2004), regardless of how strongly they were found to be adsorbed under equilibrium conditions in the laboratory. For the two experiments reported here, the observed differences in soil profile distribution, and rate of degradation are probably conditioned by climatic factors prevailing during the experiments (autumn and springtime), agricultural practices (dryland–irrigated), inherent variability of soil spatial parameters, land cover and roughness of soil surface.

At the *NI* experiment the presence of glyphosate at greater depth than expected may be the consequence of rainfall events. In the *NI–34* profile, Glyphosate was detected along all the sampled profile showing a high concentration (20.3 ng/g) at 0.70 m although according to batch experiments (Candela *et al.*, 2007), after 34 days the pesticide should have been retained in the upper part of the soil. The high precipitation registered immediately after pesticide application could induce a rapid flux of water through the unsaturated zone, inhibiting adsorption onto soil particles. This process could be favored by the amount of Glyphosate available and the initial low water content in soil before rain which could promote the existence of preferential solute transport. In sandy soils with no visible structure in the top 1 m, preferential flow appears to be dependent on soil moisture and water flow tends to be channeled through low moisture zones. This effect has been observed by Kladivko *et al.* (1999) and Nolan *et al.* (2008). Previous laboratory soil column experiments carried out with the same soils and pesticide demonstrated the importance of non–equilibrium sorption under flow conditions. Mass loss is larger for longer residence times associated either to low pore–water velocity or long soil column lengths.

Mobility of AMPA is lower than Glyphosate and residues were only detected in the 0–0.30 m interval. Considering the molecular weight of both compounds, a 0.6 ratio glyphosate/AMPA concentration in soil and water samples could be expected. However, AMPA concentrations detected in soil samples only accounted for 15 % of glyphosate degradation. A slower glyphosate/AMPA transformation over time, or even AMPA degradation could explain the missing amount of herbicide. The analysis on dissipation of Glyphosate and AMPA formation was not the objective of this research and the available data are not sufficient to assess the importance of biological and chemical transformation of Glyphosate. Analysis of AMPA formation (0.08 days according to best fit equation) are highly uncertain due to the low correlation coefficient obtained (R^2 = 0.295).

Very little is known about the nature and kinetics of this process (Grunewald *et al.*, 2001), therefore, to gain insight into it, soil microbiological activity and the fast mineralization of both Glyphosate and AMPA should be the subject of future research.

Based on the non–reacting behavior of Br and the reduced mobility of pesticide induced by adsorption, estimation of glyphosate percentage found 3 times deeper than predicted, calculated following the Ghodrati and Jury (1992) approach, would account for 18 % and 28 % for the non–irrigated and irrigated areas, respectively (Table 8.1.3.1-20). Note that in the non–irrigated area the transport of the pesticide is clearly influenced by the two rain events (NI–34 and NI–62), a phenomenon not observed in the irrigated plot where water infiltration is mainly conditioned by continuous irrigation.

Table 8.1.3.3-21: Percentage of glyphosate found three times deeper than predicted (ZG) for the different soil profiles considering achievement of equilibrium adsorption

Non irrigated (NI)			Irrigated (I)		
Profile	$3Z_G$ (cm)	PF (%)	Profile	$3Z_G$ (cm)	PF (%)
NI-14A	0.6	5	I-7	0.2	0
N1-14B	0.6	9	I-14	0.1	56
NI-23A	1.0	0	I-20	0.6	28
NI-23B	1.0	0	I-36	0.2	10
NI-34A	1.4	64	I-43	0.6	33
NI-34B	2.4	43	I-48	0.2	5
NI-62A	3.0	62	I-59	0.6	82
NI-62B	3.0	0	I-69	0.6	28
NI-92A	3.4	0	I-87	1.3	15
NI-92B	3.8	0			
Average (%)		18	Average (%)		28

Z_G : theoretical depth to the center of mass of the pesticide (Ghodrati and Jury, 1992). PF: preferential flow; $Z_G = Z_{Br}/R$, where R is the retardation factor and Z_{Br} the depth reached by the center of mass of a pulse of the conservative tracer (Br). Center of mass depth of tracer (Z_{Br}) was estimated at each sampling profile (see Fig 3).

Although in both plots detectable amounts of tracer and pesticide have been found in the soil profile, direct comparison of results is not possible as they are conditioned by climatic parameters and water application regime. The experiment under non–irrigated conditions was undertaken in autumn; the most important aspect of the precipitation pattern is its concentration in a few unevenly distributed events of heavy storms, characteristics of the Mediterranean environment. Evapotranspiration presents a decreasing trend and water content in soil profile was low at the beginning of the exercise. For the irrigated experiment, spring climatic conditions prevail and evapotranspiration is much higher than in autumn. The weekly applied irrigation dose controls water content in soil presenting a more uniform distribution along the soil profile and experimental period.

As far as the authors are aware, such deep penetration of Glyphosate has not been reported from field studies for granite soils, such as the studied ones in the Maresme area of Spain.

Conclusion

Glyhosate is commonly considered a pesticide strongly sorbed on soils, presenting a low risk for groundwater pollution due to the phosphonate functional group strong adsorption to clay minerals, Fe and Al–oxides and OM according to laboratory experiments. A problem is whether pesticide parameters measured in the laboratory are representative for predicting pesticide behaviour under field conditions. Field investigation and monitoring of pesticide leaching present the complexity of profiling pesticide concentration in soil and the difficulty of sampling pesticide migration through preferential flow paths.

As shown in the field experiments described above, Glyphosate deep leaching in a weathered granite soil profile was observed under natural field conditions regardless of the irrigated or non–irrigated conditions and climatic season. Laboratory miscible displacement experiments performed with the same soils showed that Glyhosate adsorption in soils is essentially a kinetic process and depends on the pore water velocity and residence time of soil solutions. If flow velocities are slow and enough time is given to react with the soil matrix, surface complexation and precipitation takes place. Complexation with iron and aluminum oxides, transition metals or alkaline–earth metals has been reported in literature (Sprankle *et al.*, 1975; Vereecken, 2005). Since Glyphosate adsorption is not an instantaneous process, needing time to attain equilibrium conditions, under heavy rain or irrigation just after its application on soil surface, it could leach more than predicted.

Given the typical conditions of the Maresme region vadose zone, highly permeable medium–coarse sand with low organic matter and clay content and containing Al, Fe, oxides and hydroxides, the principal mechanism affecting Glyphosate transport through the vadose zone may not be chemical equilibrium with the solid matrix alone. At field scale two possible explanations accounting for physical non–equilibrium will be of decisive importance on the transport of pesticide through the vadose zone. Since the phosphate compound of the molecule can be strongly adsorbed by Al, Fe, oxides and hydroxides, organic matter and humic acids of colloidal size, transport of colloid–bound Glyphosate and AMPA and preferential flow pathways driven by rainfall events or water application dose is likely. Presence of Glyphosate residues below detection level at depth up to 1.10 and 1.9 m in the irrigated and non–irrigated plot suggests that the pesticide may migrate into deep soil layers. This observation emphasizes the potential risk of Glyphosate transport to groundwater.

At field scale, the half–life was found to be shorter than 7 days in both experiments, much shorter than values reported in the literature (47 days average). This can be attributed to the fact that under field conditions a multitude of factors and processes contribute to herbicide disappearance, while laboratory studies are generally designed to study one of these processes. It is important to note here that these results are conditioned by the low correlation coefficient obtained (best fit equation). From the limited information obtained during the experimental study, AMPA accumulation in soil from pesticide degradation accounts for 15 % of the initial herbicide application. The parent compound transformation rate is always superior to the above–mentioned rate, leading to the conclusion that Glyphosate/AMPA transformation is a slow process or rapid AMPA degradation has occurred. Whether the transformation of the herbicide during the course of the experiment is basically controlled by chemical or biochemical processes is unknown and more research is needed regarding microbiological transformation.

Assessment and conclusion by applicant:

The article describes a leaching experiment with glyphosate in an agricultural area in Spain. Leaching over a period of several months in spring and in autumn was observed under irrigated and un–irrigated conditions. Glyphosate and AMPA were found in deeper soil layers than expected from the calculations based on a tracer experiment. Two possible explanation given were colloid-facilitated transport of glyphosate adsorbed by Al, Fe, oxides, hydroxides, organic matter and humic acids on one hand and preferential flow pathways driven by rainfall events or water application dose on the other hand. Without direct comparison of the timing and magnitude of the tracer in the actual field experiment the relevance of both processes cannot be assessed. Duration of the study was not long enough to evaluate the leaching behavior for a long–time perspective. Details regarding field sampling and sample handling practices and analysis are not sufficient to classify the study as fully reliable.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

The hypothesis given in the study for transport of glyphosate were colloid-facilitated transport of glyphosate adsorbed by Al, Fe, oxides, hydroxides, organic matter and humic acids or preferential flow pathways.

Duration of the study is limited, and the analysis of leachates in this article was performed over two periods, the first one in September to December over 92 days and the second one from February to May for 87 days. The mean annual concentration of glyphosate and AMPA in leachates can therefore not be calculated.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

Rasmussen et al, 2015

Data point:	CP 9.2.4/003
Report author	Rasmussen, S., et al.
Report year	2015
Report title	Effects of Single Rainfall Events on Leaching of Glyphosate and Bentazone on Two Different Soil Types, using the DAISY Model
Document No	Vadose Zone Journal; Advancing Critical Zone Science; Published November 13, 2015
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Full summary

The purpose of the present modeling study was to contribute to an improved understanding of the mechanisms involved in pesticide leaching during a single rainfall event with temporal variability. Rainfall intensity of the first event after pesticide application has great effect on the amount of pesticide transported to groundwater and subsurface drains, especially in soils containing preferential flow pathways. One way to improve the understanding of single event properties on pesticide leaching is to use a transport model. The soil–plant–atmosphere model Daisy was used to simulate pesticide leaching during and after single rainfall events of different durations and intensities. Designed temporally variable single rainfall events based on the Chicago Design Rain were inserted in the original weather file. A combination of different intensities (13, 20, 24, 28, 34, and 39 mm/h) at different event durations (1, 3, 5, and 9 h) where the intensity peak was placed in the middle of the event ware applied, resulting in 24 different design events. The model setup included two different soil types: a coarse sandy soil and a sandy loam containing macropores and subsurface drains. The fates of the herbicides bentazone [3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] and glyphosate [*N*-(phosphonomethyl) glycine] were simulated. The leaching dynamics of both pesticides showed high variability at the hourly level, illustrating the importance of high model resolution when estimating pesticide leaching. For the coarse sandy soil different intensities did not appear to have an effect, as pesticide leaching was controlled by event volume. In contrast, results for the sandy loam showed an effect of intensity, especially for glyphosate, at initially wet soil conditions. Short intense events (1 h) resulted in high leaching to drains (1.7 % of matrix infiltration) compared to events of longer duration (up to 0.4 % of matrix infiltration). This indicates that it might be more prudent to view leaching as a risk that occurs under certain conditions, rather than something that can be averaged.

Materials and Methods

Soils

Two different agricultural soils from Denmark were chosen as model soils—a coarse sandy soil and a sandy loam. The DAISY (deterministic and dynamical two-dimensional soil–plant–atmosphere model developed for simulating agrohydrological systems) parameterization of this location (Jutland, Denmark) originates from Jacobsen (1989), and selected soil properties are illustrated Table 8.1.3.3-22. Because the coarse sandy soil is considered completely homogeneous in the Ap and C horizons and contains no biopores or subsurface drains, it is modelled in 1D. The sandy loam is a heterogeneous soil developed in a glacial till in the eastern part of Zealand, Denmark. It shows signs of long-term agricultural use with the development of a plow pan that has a lower hydraulic conductivity than the surrounding soil layers (Petersen et al., 2001).

Table 8.1.3.3-22: Selected soil characteristics of the two locations, coarse sand and sandy loam, used in the DAISY simulations. Data originate from Jacobsen (1989) and Hansen et al. (2012b).

Soil type	Horizon	Soil depth	Clay	Silt	Sand	Humus	K_{sat}
		cm			%		cm h^{-1}
Coarse sand	Ap	0–30	3.8	7.2	86.7	2.3	21.7
	C	30–200	2.8	2.3	94.5	0.4	92.5
Sandy loam	Ap	0–25	10.4	21.6	65.1	2.9	0.174
	Bplow	25–33	14.6	21.1	62.8	1.6	0.046
	Bt	33–120	21.9	19.2	57.4	1.6	0.269
	C	120–200	20.5	23.3	55.2	1.0	1.500

The sandy loam contains biopores and subsurface tile drains. The drains are placed in a depth of 1.1 m, and 16 m apart, and the biopores are divided into classes according to the depth at which they begin and end in the soil profile. Selected soil properties for this location, which originates from Hansen et al. (2012b) are illustrated in Table 8.1.3.3-22.

Pesticide Management

Bentazone was applied on 17 June with a rate of 960 g/ha, on grass for cutting. Hence, bentazone is located in the crop canopy and will there be mixed with the first rainfall and washed off the plants when the interception capacity is exceeded. Glyphosate was applied on 30 October at a rate of 1440 g/ha on bare soil (stub after harvesting of maize, *Zea mays* L.) (Table 8.1.3.3-23). Glyphosate will therefore be located at the soil surface, from where it will enter the soil system together with the first rainfall. The fate of both pesticides was simulated for 4 yr, and their leaching at 2 m depth was logged. For the sandy loam, transport of pesticides into drains was included in the leaching assessment.

Table 8.1.3.3-23 Pesticide management plan used in the DAISY simulations.

Rotation year	Crop	Pesticide	Application date	Dosage
Warm-up	Spring barley			
Warm-up	Spring barley			
Warm-up	Spring barley			
Warm-up	Spring barley w. grass			
1	Grass	Bentazone	17 June	960 g ha^{-1}
2	Grass			
3	Grass			
4	Maize	Glyphosate	30 October	1440 g ha^{-1}
5	Spring barley			
6	Spring barley			
7	Spring barley			
8	Spring barley			

Weather Data

The weather file used in the DAISY simulations was provided by University of Copenhagen and originated from Taastrup, Denmark. It contains hourly values of precipitation, temperature, relative humidity, wind speed, and global radiation and covers the period 1999 to 2008 (8 yr). The weather data covered the 8 yr of pesticide tracking in the model simulations (Table 8.1.3.3-23). To initiate the soil water content in the model, 4 yr of weather data (1999–2003) and a simple spring barley (*Hordeum vulgare* L.) crop rotation were used as a warm-up period before the first pesticide application. The weather record was then reset as the 8 yr of pesticide simulations began (Table 8.1.3.3-23). The weather of the warm-up period was kept constant, while the weather of the 8 yr of pesticide simulations were permutated.

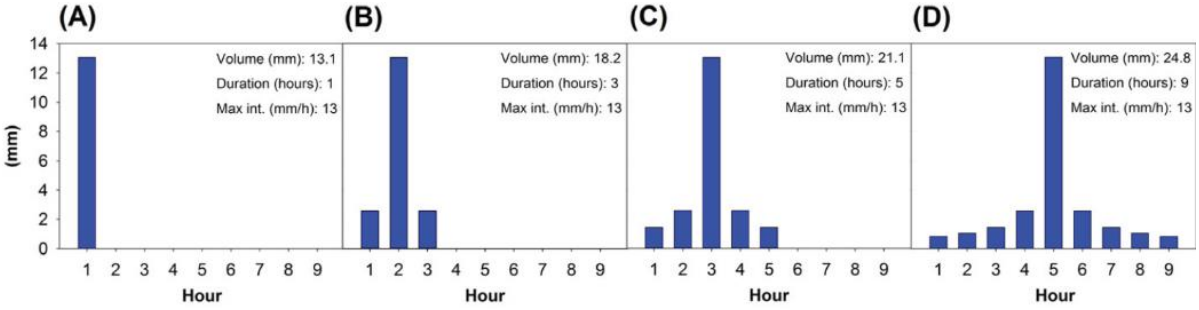
Artificial Rainfall Events

A single artificial rainfall event was inserted in the weather file 4 d after application of the pesticide. Intermittent rain that occurred between pesticide application and the artificial rainfall event were removed. The artificial event originates from the CDS rain, developed in the 1950s, for the use in city sewage planning. The durations of the events studied were 1, 3, 5, and 9 h combined with six different levels of maximum intensities of 13, 20, 24, 28, 34, and 39 mm/h (Table 8.1.3.3-24). When the duration increases, the maximum intensity of the event will be displaced to the middle of the event, and small pre- and post-tails of rain will be added to the event compared to a 1-h event (Figure 8.1.1.2-1). Hence, the event volume is connected to the event duration, as increased duration results in increased volume.

Table 8.1.3.3-24 Characteristics of the inserted Chicago Design Storm rain (CDS rain. All combinations of maximum intensities and durations were investigated at eight different initial conditions, and with different post-event weather.

Max. intensity	Duration	Volume	Repeat interval
mm h^{-1}	h	mm	yr
13	1	13	1
13	3	18	1
13	5	21	1
13	9	25	1
20	1	20	5
20	3	28	5
20	5	33	5
20	9	39	5
24	1	24	10
24	3	33	10
24	5	39	10
24	9	46	10
28	1	28	20
28	3	39	20
28	5	45	20
28	9	53	20
34	1	34	50
34	3	48	50
34	5	55	50
34	9	65	50
39	1	39	100
39	3	55	100
39	5	64	100

Figure 8.1.3.3-16 Examples of Chicago Design Storm rain (CDS-rain) (Kiefer and Chu, 1957), modified by Madsen et al. (2002) and Arnbjerg-Nielsen et al. (2006), inserted in the weather files used in the DAISY simulations. (A) to (D) illustrate the range of event durations investigated and the differences in rain distribution pattern and consequently the differences in total volumes at different durations, with the same maximum intensity.

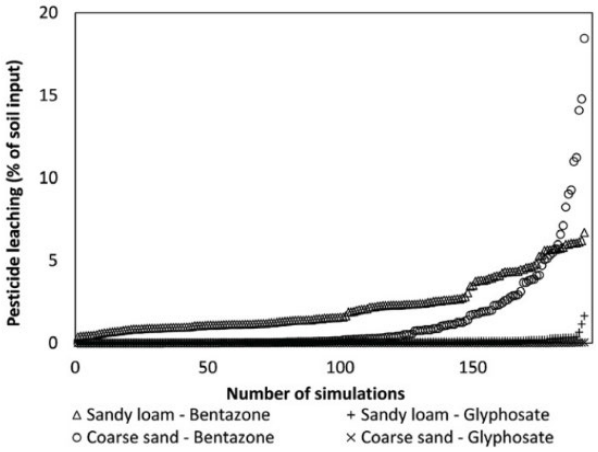


Results and Discussion

The complete set of total leaching (leaching after 4 yr as percentage of soil input) from the 192 DAISY simulations is illustrated in Figure 8.1.3.3-17. A quick overview of the dataset is supplied, as the

leaching percentages are shown in a sorted sequence from lowest to highest leaching. It is seen that there is an effect of soil type on leaching of both pesticides and that bentazone leaching is substantially larger than glyphosate leaching. In the following responsible processes and mechanisms will be described, and where feasible, related to field or laboratory findings. For each of the two soil types, a description of pesticide leaching during the 4-yr simulations (leaching dynamics) will be followed by a clarification of the effects of CDS event structure (event characteristics) and completed with a description of the importance of initial soil water conditions and post-event weather (rotated weather).

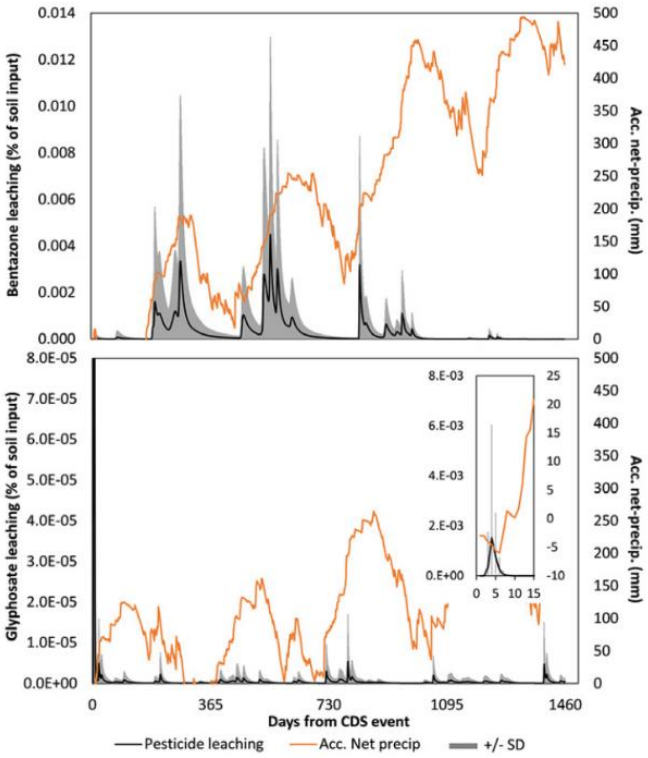
Figure 8.1.3.3-17 Overview of all pesticide fate simulations made by the DAISY model. Total bentazone and glyphosate leaching as percentage of soil input (matrix and biopore infiltration), at the coarse sandy and sandy loam soils. The 192 simulations for each pesticide, at each soil type, are sorted from smallest to largest.



Pesticide Leaching in the Coarse Sand—Leaching Dynamics

The pesticides entered the soil with the first rainfall after application and were thereafter transported vertically through the soil profile with the water. Figure 8.1.3.3-18 illustrates bentazone and glyphosate leaching (daily values) during 4 yr after pesticide application. The amount of pesticide leaching is calculated at the soil depth of 2 m and is given as the percentage of input to the soil. The average ± SD of all 24 investigated CDS events are shown together with accumulated net precipitation (precipitation minus evapotranspiration) (Figure 8.1.3.3-18). The inserted figure illustrates the negative values of accumulated net precipitation during the first 10 d and the importance of glyphosate leaching the first 5 to 10 d. A seasonality in rainfall is seen, as increased accumulated net-precipitation occurs during autumn and winter.

Figure 8.1.3.3-18. Four-year pesticide leaching as percentage of soil input (matrix and biopore infiltration) in the coarse sandy soil (daily values). Pesticide leaching is given as an average ± SD of the 24 different events investigated within Weather Rotation 8. The second *y* axis shows the accumulated net precipitation (precipitation minus evapotranspiration), with negative values only shown in the inserted figure. The inserted figure also illustrates the full magnitude of glyphosate leaching.



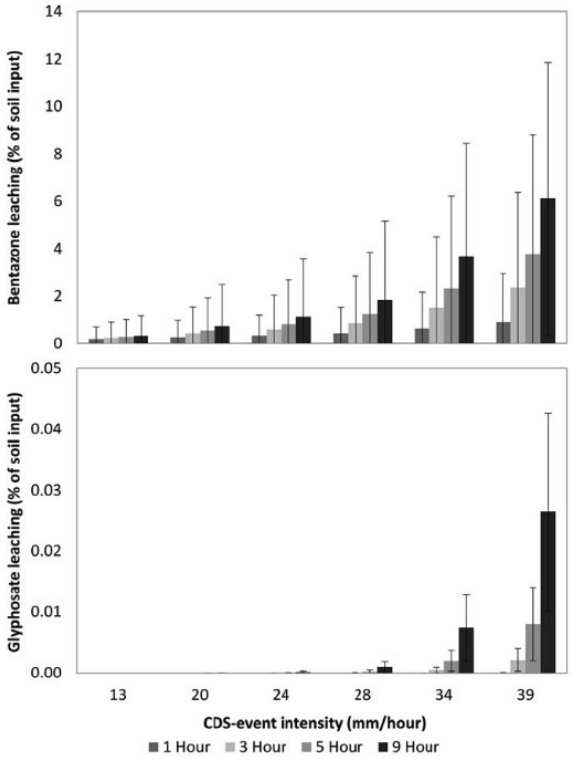
It can be seen that bentazone leaching took place over a 3-yr period, with the highest leaching occurring during winter. Within the first 10 d after the CDS event only 2.7 x 10^{-5} % of total leached bentazone had reached the 2-m depth, which indicate a limited direct effect of the 24 CDS events. Bentazone was applied 17 June (Table 8.1.3.3-23), where evapotranspiration most often exceeds the rainfall volume, resulting in dry soil water conditions. The CDS event transports bentazone into the soil profile, but not below the depth of 1 m, where biodegradation is zero in the model. Hence, under these conditions the first rainfall after bentazone application appears of minor importance at the two layered homogeneous coarse sandy soil. In contrast, the direct response of the CDS event on glyphosate leaching (Figure 8.1.3.3-18, illustrating variation of the 24 events and effect of WR 8), was found to be substantially larger. Within 10 d after the CDS event 86 % of total leached glyphosate had been transported to the 2-m depth. However, total glyphosate leaching (3.5 x 10^{-3} % of soil input) is two magnitudes smaller than total bentazone leaching (4.5 x 10^{-1} % of soil input), which is a result of the higher sorption properties of glyphosate. Glyphosate was applied 30 October, which is a time of year where the net rainfall is substantially larger than at the time of bentazone application (17 June), resulting in wetter soil water conditions, especially at the top 0.5 m. This means that smaller amounts of rainfall is needed to facilitate the transport of glyphosate, and a direct and visible effect of the CDS events is possible (Figure 8.1.3.3-18).

Pesticide Leaching in the Coarse Sand—Effect of Single Event Characteristics

Even though the immediate effect of the CDS events were small (Figure 8.1.3.3-18), total bentazone leaching at the coarse sandy soil, showed a systematic response to the 24 investigated CDS events. Bentazone leaching increased with increased duration (1, 3, 5 and 9 h) and maximum intensity (13, 20, 24, 28, 34, and 39 mm/h) of the CDS event. This is illustrated in Figure 8.1.3.3-19, where the average ± SD of total bentazone leaching of all 8 WRs are shown, but divided into event durations and maximum

intensities. This was to be expected, as a higher rainfall volume has a higher potential of transporting pesticides. With increased CDS event volume, the pesticide was transported faster through the degradation zone (upper 1 m), which left less time for degradation. These results also indicate that higher intensities will result in increased leaching percentages. Glyphosate leaching at the coarse sandy soil showed the same pattern as the one found for bentazone, although only small amounts of glyphosate were leached (<0.025 % of soil input).

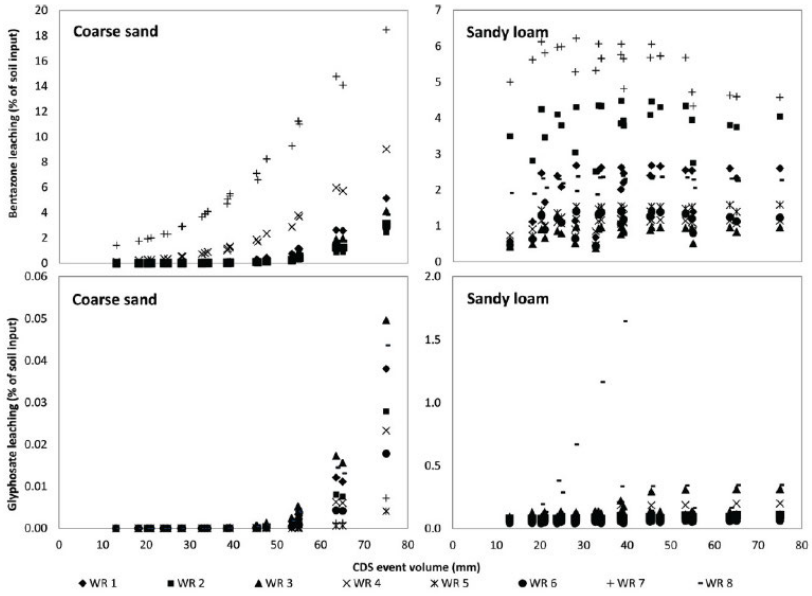
Figure 8.1.3.3-19 Average ± SD of 4 yr accumulated pesticide leaching, based on results from all eight weather rotations, shown as percentage of soil input (matrix and biopore infiltration), in the coarse sandy soil, and as a function of Chicago Design Storm (CDS) event intensity and duration.



Pesticide Leaching in the Coarse Sand—Effect of Rotated Weather

The rotated weather produced different soil water conditions at the time of pesticide application, and different post-CDS event weather, which all may influence the pesticide fate. Figure 8.1.3.3-20 illustrates these effects on pesticide leaching, where daily values of leaching are given as a function of CDS event volume, for all 8 WRs. These results indicate that the leaching pattern can be explained by the volume of the CDS event, as the accumulated leaching of especially bentazone produced smooth curves at the coarse sandy soil and showed almost no effect of increased intensity. In the case where intensity had an effect, the curves would be irregular, showing a spread of leaching results. This indicates that the effect of intensity shown in Figure 8.1.3.3-19 is solely explained by the increase in volume, as intensity increases. The leached amounts of glyphosate were smaller than those of bentazone and showed a steeper curve. This is due to the high number of low leaching percentages even at relatively high CDS event volumes (up to 50 mm). When event volume exceeds 50 mm, a steep increase in leaching percentages was observed (Figure 8.1.3.3-20). The difference between the leaching caused by the different WRs in Figure 8.1.3.3-20 is either due to the initial soil water conditions (water conditions at the time of pesticide spraying), the post-event rainfall, or a combination thereof. Bentazone leaching at the coarse sandy soil appeared to be mostly affected by CDS event volume. In the case of glyphosate leaching at the coarse sandy soil, no connection to initial soil water conditions or P_{10} was found, except at WR 3 which had the highest P_{10} and the highest leaching.

Figure 8.1.3.3-20 Four-year cumulative pesticide leaching shown as percentage of soil input (matrix and biopore infiltration), and as a function of Chicago Design Storm (CDS) event volume. Each of the eight weather rotations (WRs) represents a new set of initial soil water conditions and post-CDS event weather conditions. Please note the different scales on the *y* axis.

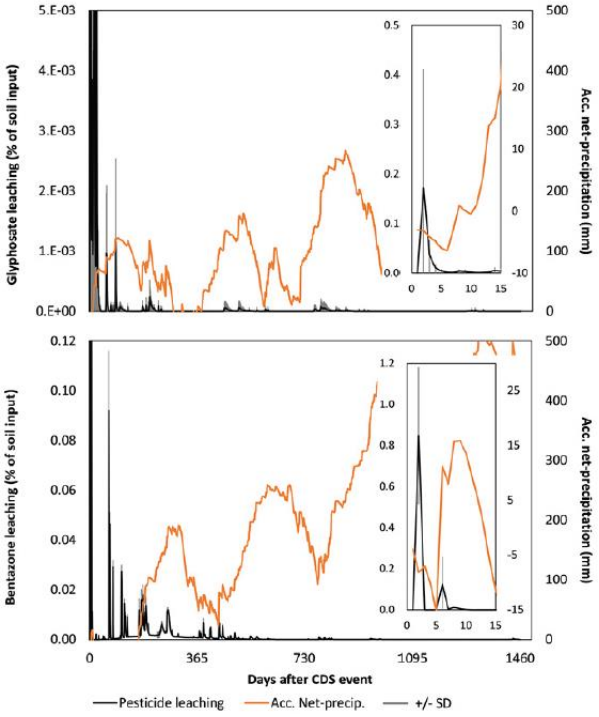


Pesticide Leaching in the Sandy Loam—Leaching Dynamics

Figure 8.1.3.3-21 constitutes an example of how daily values of pesticide leaching (to drain plus leaching at 2 m depth) evolved over the 4 yr period of which the pesticides were tracked. Average ± SD of the 24 CDS events from WR 8 is shown. Compared to similar graphs for the coarse sandy soil (Figure 8.1.3.3-17), it can be seen that the largest proportion of both pesticides were leached during or shortly after the CDS event, as was the case for glyphosate at the coarse sandy soil. On average, 87 % of total bentazone leaching and 75 % of total glyphosate leaching occurred within 24 h from the beginning of the CDS event. Hence, the first single rainfall event after pesticide leaching is of considerable importance on the sandy loam, compared to the coarse sandy soil. Even though both pesticides were affected by the CDS event (Figure 8.1.3.3-21), it was found that different processes control the leaching of the two pesticides. It was found that a substantial part of drain leached bentazone was transported via biopores leading directly from the surface to the tile drain. A total of 51 % of drain leached bentazone (average of the 24 CDS event in WR 8) completely bypassed the soil matrix where sorption would have occurred. The second way of entering biopores via the soil matrix is how glyphosate predominantly migrated. Only 23 % of drain leached glyphosate, was transported directly from the soil surface into the biopores, completely bypassing the soil matrix. Further investigation into the transport mechanisms revealed that the agricultural practice was important, in particular the presence of a plant cover in the model setup. Bentazone was applied on grass for cutting, and thus had to be washed off the crop canopy before getting into contact with the soil surface. The CDS event is the first rainfall after pesticide application and is heavy enough to wash bentazone off the canopy and *at the same time* activate the preferential flow pathways where some led directly to the drain. Glyphosate was applied directly to the soil surface and the main fraction entered the soil matrix together with the very first water that hit the surface. When the pesticide had entered the soil matrix it either sorbed to soil particles or followed the water route in dissolved form through the soil matrix into the biopores and from here to the drain pipe. Despite high sorption of glyphosate a small fraction was present as dissolved glyphosate, which was subject to leaching through transport route B. This explains the dependence of amount of water that comes after the maximum intensity in the CDS event: the hour of maximum intensity does not result in

relatively higher transport of glyphosate, since the majority of this water is led directly into macropores and thereby bypassing the soil matrix where glyphosate is located.

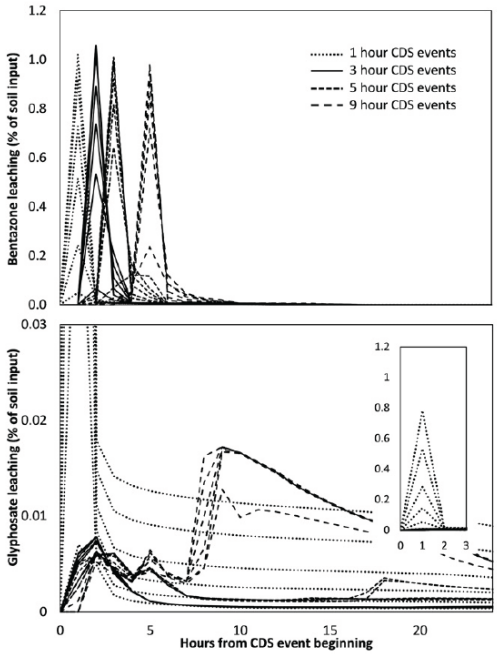
Figure 8.1.3.3-21 Four-year pesticide leaching shown as percentage of soil input (matrix and biopore infiltration) in the sandy loam soil (daily values). Pesticide leaching is given as an average ± SD of the 24 different events investigated within Weather Rotation 8. The second *y* axis shows the accumulated net precipitation (precipitation minus evapotranspiration), with negative values only shown in the inserted figures.



Pesticide Leaching in the Sandy Loam—Effect of Single Event Characteristics

Bentazone leaching was found to increase as CDS event volume increased. The CDS events of 3, 5, and 9 h caused leaching that tended to level out as CDS event volumes increased. This trend represents the prevailing leaching patterns, but some WRs showed limited effect of CDS event volume. The differences in leaching dynamics between bentazone and glyphosate in the sandy loam (Figure 8.1.3.3-22) were also observed at the 4-yr cumulated leaching (Figure 8.1.3.3-23). The applied glyphosate was protected against soil infiltration by small rainfall events when located in a litter layer, and a substantial amount of glyphosate was transported via the biopores to the drains during a heavy event that generated preferential flow. Long CDS event durations resulted in high leaching percentages of glyphosate.

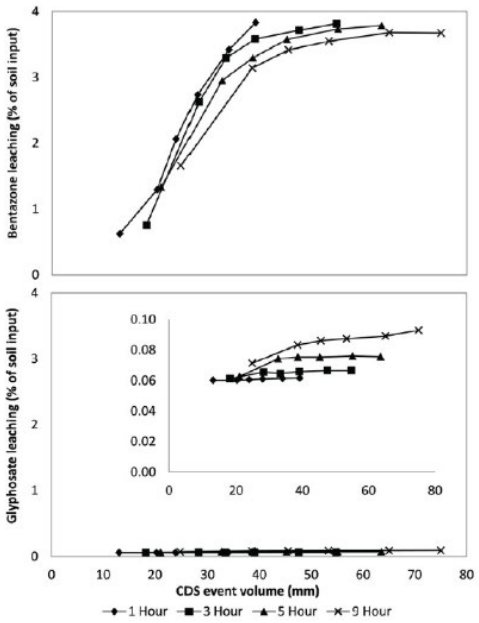
Figure 8.1.3.3-22 Four-year pesticide leaching shown as percentage of soil input (matrix and biopore infiltration) in the sandy loam soil (hourly values). Pesticide leaching is shown for each of the 24 different events investigated within Weather Rotation 8, but divided into groups according to the respective Chicago Design Storm (CDS) event duration.



Pesticide Leaching in the Sandy Loam—Effect of Rotated Weather

The smooth curves produced at the coarse sandy soil (Figure 8.1.3.3-20) were not reproduced, indicating that CDS event volume alone did not explain the leaching in the sandy loam. The increased bentazone leaching caused by increased CDS event volume (Figure 8.1.3.3-23) is not repeated by all WRs, and the effect of CDS event characteristics appear of less importance compared to the effect of initial soil water conditions and post-event weather. Glyphosate leaching appeared to be affected by CDS event intensity to a higher degree than bentazone.

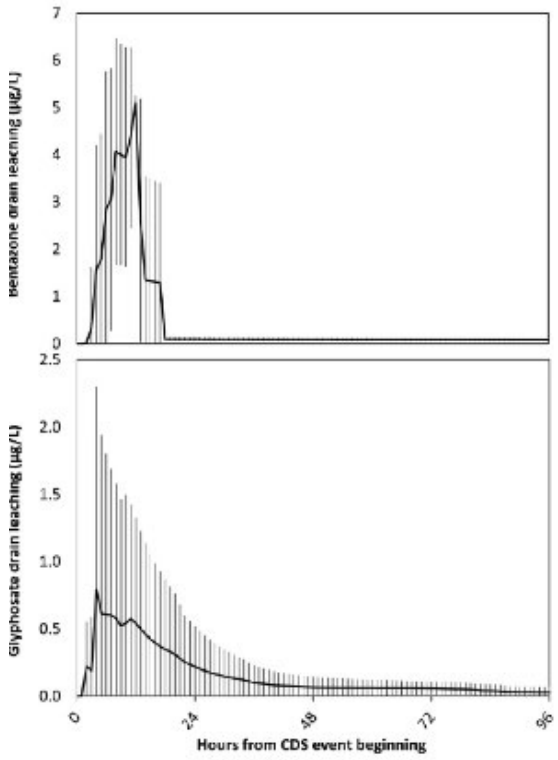
Figure 8.1.3.3-23. Four-year accumulated pesticide leaching shown as the percentage of soil input (matrix and biopore infiltration) in the sandy loam and as a function of the Chicago Design Storm (CDS) event volume, Weather Rotation 1.



Simulated Pesticide Concentrations in the Sandy Loam

The simulated concentrations of bentazone and glyphosate in drainage water at WR 8 are shown in Figure 8.1.3.3-24. Both pesticide concentrations are seen to peak shortly after the CDS event beginning. Previous studies strongly indicate that glyphosate leaching is highly event driven, where especially rainfall intensity affects the leaching dynamics. High intensity rainfall events occurred as the first rainfall after application at initially wet soil conditions, which supports the findings of this work.

Figure 8.1.3.3-24 Pesticide leaching (µg/L), during the first 4 d after the Chicago Design Storm (CDS) event in the sandy loam soil. Pesticide leaching is given as an average ± SD of the 24 different events investigated within Weather Rotation 8. Negative SD values are not shown.



Conclusions

Based on the 192 model simulations, testing the effect on pesticide leaching of duration and intensity of the first rainfall event after pesticide application at an hourly temporal resolution, the following trends were observed: The importance of the first single rainfall event after pesticide application depended highly on soil types. On average, 87 % of total bentazone leaching and 75 % of total glyphosate leaching occurred within 24 h from the CDS event beginning in the sandy loam. Hence, the first single rainfall event after pesticide leaching is of considerable importance in the sandy loam. Preferential flow transport in the biopores was responsible for this immediate transport of both pesticides. In the coarse sandy soil, the first rainfall event was of minor importance, and the effect was only visible if the soil was relatively wet at the time of application. The influence of rainfall characteristics on pesticide leaching depends on the hydrological conditions of the investigated soil types. In the coarse-textured soil, where non-threshold matrix flow dominates, solute leaching was found to increase with increased rainfall volume, whereas in the sandy loam, varying rainfall intensity also affected pesticide leaching, especially for the strongly sorbing pesticide glyphosate. For strongly sorbing pesticides like glyphosate it might be more prudent to view leaching as a risk that occurs under certain conditions, rather than something that can be averaged. Under most initial conditions glyphosate leaching did not vary much (up to 0.4 % of soil input), but at specific initial wet surface conditions glyphosate leaching greatly increased (1.7 % of soil input). It may therefore be equally important to have knowledge of the weather preceding the pesticide application, as knowledge of the weather following the pesticide application.

Assessment and conclusion by applicant:

The article describes a modelling assessment for the leaching of glyphosate through two different soil types. The effect of single rainfall events is analysed. The modelling approach is not in line with current FOCUS guidelines.

Therefore, the article is classified as reliable with restrictions.

Assessment and conclusion by RMS:

This article, as mentioned by the applicant, describes the modelling assessment for leaching of glyphosate and the influence of rainfall events on two type of soils. The approach is indeed not in line with current FOCUS guidelines. The experiment still seems to be well performed and may be considered as supportive. Glyphosate leaching was predicted to be low, in the two soils, at less than 2% of the soil input.

The article provides supportive information on the mobility of glyphosate, but no reliable endpoints can be derived for use in risk assessment.

B.8.1.3.4. *Summary on mobility of glyphosate in soil*

Since reliable adsorption coefficients for glyphosate and AMPA were obtained in adsorption/desorption studies, additional mobility studies are not strictly required. However several column and aged column leaching studies were provided.

Only one aged column leaching study provided reliable results. Under the conditions of the study peformed on a sandy soil, glyphosate and AMPA were found to be immobile, with no residues found deeper than 12 cm. The radioactivity in the leachates did not exceed 0.1% AR. These results support the results from the batch adsorption studies.

The mobility of glyphosate was investigated in several literature studies (column leaching, lysimeter and field leaching). Although results from these studies cannot be used to derive reliable endpoints for regulatory risk assessment, the studies bring supportive information regarding the mobility of glyphosate. In particular, preferential flow was demonstrated in some of the studies, mainly in clay soils, as expected for many herbicides applied on bare soil.

B.8.2. FATE AND BEHAVIOUR IN WATER AND SEDIMENT

B.8.2.1. Chemical and photochemical degradation

B.8.2.1.1. Hydrolytic degradation

The hydrolysis of glyphosate was investigated in 10 existing studies, see table below. No new study was provided in this renewal dossier.

RMS notes that an additional study ([redacted] 1993), not mentioned by the applicant in this renewal dossier, was available in DAR 2001. However DAR (2001) indicated: "There are only summary reports which can't be evaluated because quality of data can't be checked". Therefore this study was not deemed necessary and was not requested to the applicant.

In the scientific literature review for glyphosate (2010-2020), no article was identified to provide further information relevant to the data point.

Table 8.2.1.1-1: List of existing hydrolytic degradation studies

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)	Remark
CA 7.2.1.1/004	[redacted], 1993	Not mentioned in RAR (2015) but accepted in DAR (2001)	Acceptable	
CA 7.2.1.1/005	[redacted], 1992	Not mentioned in RAR (2015) but accepted in DAR (2001)	Acceptable	

CA 7.2.1.1/007	[redacted], 1990	Accepted in RAR 2015	Acceptable	
CA 7.2.1.1/009	[redacted]., 1983	Accepted in RAR 2015	Supportive	
CA 7.2.1.1/001	Anonymous, 1995	Not mentioned in RAR (2015) but not accepted in DAR (2001)	Not acceptable	Report not available
CA 7.2.1.1/002	[redacted], 1995	Accepted in Phys-Chem section of RAR (2015)	Not acceptable	
CA 7.2.1.1/003	[redacted], 1993	Not mentioned in RAR (2015) but accepted in DAR (2001)	Not acceptable	
CA 7.2.1.1/006	[redacted], 1991	Accepted in Phys-Chem section of RAR (2015)	Not acceptable	
CA 7.2.1.1/008	[redacted], 1990	Not mentioned in RAR (2015) but accepted in DAR (2001)	Not acceptable	
CA 7.2.1.1/010	[redacted], 1978	Not mentioned in RAR (2015) and not accepted in DAR (2001)	Not acceptable	

[redacted], *1993*

Data point:	CA 7.2.1.1/004
Report author	[redacted]
Report year	1993
Report title	Glyphosate isopropylamine salt. Hydrolysis in water at 3 different pH-values
Report No	PR93/009
Guidelines followed in study	BBA-Merkblatt No. 55, part I and II (October 1980)
Deviations from current test guideline	From OECD 111: - Only single vessels prepared for each combination of pH and temperature - The test was conducted at pH 5 instead of a pH of 4 - No information on dark conditions
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentioned in RAR (2015) but accepted in DAR (2001)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate isopropylammonium salt (non-labelled)

Lot No.: 10819

Chemical purity: 98 %

2. Buffers:

The following buffer solutions were prepared for the test:

- pH 5: 2.25 g KH_2PO_4 were dissolved in 250 mL water, 0.01 M Na_2PO_4-solution (about 100 mL) was added until pH 5 was reached
- pH 7: 0.97 gKH_2PO_4 were dissolved in 100 mL water, 150 mL 0.01 M Na_2PO_4-solution were added. pH 7 was adjusted with 0.1 M NaOH
- pH 9: 1.05 g $NaHCO_3$ were dissolved in 250 mL water, pH 9 was adjusted with 0.1 M NaOH

Buffer solutions were filtered through a sterile filter and collected into 20 mL volumetric flasks containing about 40 mg (accurately weighed) of the test substance. The flasks were topped up with buffer solution and the flasks were directly closed with glass stoppers.

All glassware used was heated at 180 °C for 2 hours.

B. STUDY DESIGN

1. Experimental conditions

The initial concentration of the test substance was 2 g/L.

The test solutions each prepared at pH 5, 7 and 9 were incubated at 23 °C and 50.0 °C, respectively.

2. Sampling

Samples from single test vessels were taken at day 0, 4, 7, 14 and 29 under sterile conditions.

3. Analytical procedures

Samples were analysed with HPLC-UV. With the method used, only the glyphosate-anion was determined.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC method were not reported.

II. RESULTS AND DISCUSSION

A. DATA

Glyphosate concentrations are summarised below for the respective pH values.

Table 8.2.1.1-2: Degradation of glyphosate in sterile buffer solutions at pH 5, 7 and 9 in g/L

		Glyphosate (g/L)					
Days	Sample [1)]	pH 5		pH 7		pH 9	
		23 °C	50 °C	23 °C	50 °C	23 °C	50 °C
0	1	2.02	2.02	1.97	1.97	2.02	2.02
	2	2.04	2.04	1.89	1.89	1.96	1.96
	mean	2.03	2.03	1.93	1.93	1.99	1.99
4	1	2.00	2.00	1.98	1.95	1.94	2.00
	2	1.99	1.99	1.96	1.94	1.93	1.94
	mean	2.00	2.00	1.97	1.95	1.94	1.97
7	1	1.98	2.04	1.96	1.94	1.96	1.94
	2	1.95	2.04	1.90	1.89	1.90	1.97
	mean	1.97	2.04	1.93	1.92	1.93	1.96
14	1	1.96	2.02	1.98	n.i.	1.95	1.98
	2	1.96	2.02	1.88	n.i.	1.93	1.97
	mean	1.96	2.02	1.93	n.i.	1.94	1.98
29	1	1.88	2.08	1.93	2.00	1.91	1.97
	2	1.93	2.06	1.85	2.01	1.90	1.95
	mean	1.91	2.07	1.89	2.01	1.91	1.96

n.i. = not indicated

[1)] Analytical replicate, true replicates are not available as per combination of pH and temperature only one vessel was prepared

B. TRANSFORMATION OF THE TEST ITEM

Glyphosate mean concentrations at pH 5 changed from 2.03 to 1.91 g/L and from 2.03 to 2.07 g/L at 23 °C and 50 °C, respectively, each from 0 DAT to 29 DAT. At pH 7, mean concentrations decreased from 1.93 (0 DAT) to 1.89 g/L (29 DAT) at 23 °C and were 1.93 at 0 DAT and 2.01 g/L at 29 DAT at 50 °C. At pH 9 mean concentrations marginally decreased from 1.99 to 1.91 g/L and from 1.99 to 1.96 g/L at 23 °C and 50 °C, respectively. As hydrolysis of < 10 % of applied amount was observed at study end after 29 days at pH 5, 7 and 9, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

C. KINETICS

No assessment of degradation kinetics was performed.

III. CONCLUSIONS

Glyphosate isopropylammonium salt was stable under the conditions of the test.

Assessment and conclusion by applicant:

The hydrolysis of glyphosate was examined in sterile buffer solutions at pH 5, 7 and 9 at 23 and 50.0 °C. As hydrolysis of < 10 % of applied amount was observed at all pH values at study end after 29 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. The study has some minor deviations from current guideline requirements, e.g. only single vessels were prepared per combination pH – temperature, which however do not have an impact on the results.

Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

The test was conducted at pH 5 instead of pH 4, but this is considered as a minor deviation. Only single vessels were used and it is not specified whether the study was conducted in the dark. However, since no degradation was observed, RMS considers that these deviations do not invalidate the study which confirms that glyphosate is hydrolytically stable at pH 5, 7 and 9.

The study is acceptable.

[redacted], *1992*

Data point:	CA 7.2.1.1/005
Report author	[redacted]
Report year	1992
Report title	MON-8722: Determination of hydrolysis as a function of pH
Report No	91/MON024/1207
Guidelines followed in study	OECD 111 (1981)
Deviations from current test guideline	From OECD 111: - Not reported whether study was conducted under sterile conditions
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentioned in RAR (2015) but accepted in DAR (2001)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate monosodium salt (MON-8722)

Lot No.: LLNHB210491

Chemical purity: 97.5 %

2. Buffers:

The following buffer solutions were prepared:

- pH 4.0: Disodium hydrogen phosphate (10.9 g) and citric acid monohydrate (12.9 g) were dissolved in distilled water (1900 mL)
- pH 7.0: Potassium dihydrogen phosphate (13.6 g) was dissolved in distilled water (1900 mL), 1 M sodium hydroxide (60 mL) was added

- pH 9.0: Disodium tetraborate decahydrate (33.1 g) and potassium dihydrogen phosphate (3.59 g) were dissolved in distilled water (1900 mL)

The volume of all solutions was adjusted to 2000 mL with distilled water. The pH was adjusted to the target value with 1 M hydrochloric acid or 1 M sodium hydroxide.

B. STUDY DESIGN

1. Experimental conditions

Portions of about 250 mL of each buffer solution were placed in Pyrex bottles and incubated at 50 °C in the dark. Following equilibration, a sample (100 mL) of each buffer solution was added to weighed amounts (about 180 mg) of glyphosate in separate Pyrex bottles resulting in test concentrations of about 1.8 g/L. The samples were purged with nitrogen. Storage areas were monitored for temperature.

Test solutions were incubated at 50 °C for 5 days. The pH of each test solution was measured at the beginning and the end of the test period.

2. Sampling

Samples were taken after 0, 2.4 and 120 hours. On each sampling, duplicate aliquots (1 mL) were removed from each sample.

3. Analytical procedures

Sampled aliquots were diluted to volume (10 mL) with HPLC mobile phase (0.005 M potassium dihydrogen phosphate in water/methanol (96:4; v/v) adjusted to pH 2.0 with orthophosphoric acid) and analysed for glyphosate by HPLC using a UV detector.

The limit of detection was approximately 5 mg/L.

4. Calculations

The concentration of glyphosate in the injection solution (C_A) was calculated as:

C_A [mg/L] = sample peak area x standard concentration [mg/L] / mean peak area of bracketing standards

The concentration of glyphosate in the sample solution (C_B) was calculated as:

C_B [mg/L] = C_A [mg/L] x dilution factor (V_A / V_B)

II. RESULTS AND DISCUSSION

A. DATA

The results of glyphosate concentration determinations are summarised below for the respective pH values.

Table 8.2.1.1-3: Degradation of glyphosate in sterile buffer solutions at pH 4, 7 and 9 (in g/L)

	Glyphosate (g/L)					
pH	0 hours		2.4 hours		120 hours	
	replicates	mean	replicates	mean	replicates	mean
4	1.80 / 1.76	1.78	1.88 / 1.85	1.87	1.91 / 1.91	1.91
7	1.52 / 1.71	1.62	1.59 / 1.53	1.56	1.67 / 1.65	1.66
9	1.82 / 1.68	1.75	1.71 / 1.70	1.71	1.68 / 2.06	1.87

B. TRANSFORMATION OF THE TEST ITEM

Measurements of pH values showed that there was no significant change in the pH of the buffer solutions with time.

Glyphosate concentrations did not decrease until study end for pH 4, 7 and 9. Thus, glyphosate was considered as hydrolytically stable under the conditions of the test.

C. KINETICS

Kinetic assessments of the data were not conducted.

III. CONCLUSIONS

Glyphosate was considered as hydrolytically stable in aqueous buffer solutions of pH 4, 7 and 9 under the conditions of the test.

Assessment and conclusion by applicant:

The hydrolysis of glyphosate was examined in buffer solutions at pH 4, 7 and 9 at 50 °C in the dark. As hydrolysis of < 10 % of applied amount was observed at all pH values at study end after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. It was not reported whether sterile conditions were applied and the study duration was only five days.

Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

OECD 111 indicates that if the preliminary test performed at 50°C shows less than 10% degradation after 5 days, the substance is considered hydrolytically stable and no additional testing is required. The study duration is therefore considered appropriate.

It is not reported whether the study was conducted under sterile conditions. However, since no degradation was observed, RMS considers that this deviation does not invalidate the study which confirms that glyphosate is hydrolytically stable at pH 4, 7 and 9.

The study is acceptable.

[redacted], *1990*

Data point:	CA 7.2.1.1/007
Report author	[redacted]
Report year	1990
Report title	Hydrolysis determination of 14C-Glyphosate (PMG) at different pH values
Report No	238500
Guidelines followed in study	US EPA 540/9-85-013: section 161-1
Deviations from current test guideline	From OECD 111: - Test performed in aqueous buffer of pH 5 instead of pH 4 - Test performed solely at temperature of 25 °C
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Radiolabelled Test Material

Identification:	[^{14}C]glyphosate (PMG), labelled in the methyl position
Lot No.:	CFA.745 C5
Specific activity:	11.2 MBq/mg (304 µCi/mg)
Radiochemical purity:	97.4 %

Unlabelled Test Material

Identification:	Glyphosate
Lot No.:	185-ff-131
Chemical purity:	99.5 %

2. Buffers

The following aqueous buffer solutions were prepared for the test:

- 0.71 g potassium hydrogen phthalate were combined with 350 mL water, pH 5 was adjusted with 0.01 M NaOH (170 ml); pH of 4.99 was determined
- 75 mL buffer pH 7 (Merck No. 9439, phosphate) were combined with 425 mL water, pH 7 was adjusted with monopotassium hydrogen phosphate; pH of 7.03 was determined
- 100 mL buffer pH 9 (Merck No. 9461, boric acid/potassium chloride – sodium hydroxide) were combined with 400 mL water; pH of 8.96 was determined

The buffer solutions were sterilised at 120 °C for 30 minutes.

B. STUDY DESIGN

1. Experimental conditions

A stock solution was prepared from 200 µL of the acetonic solution supplied (radioactive concentration: 200 µCi/mL; specific activity of the test article: 304 µCi/mg) were combined with 800 µL of water to 1.0 mL (stock solution). By liquid scintillation counting (LSC) the total content of [^{14}C]glyphosate in the aqueous stock solution was found to be 0.128 mg. For the preparation of the test solutions aliquots of 100 mL of the respective sterile buffer solution were combined each with 250 µL of the stock solution (128 mg/L of test article in water) in three neck round-bottomed flasks. Therefore, the concentration in each test solution was 0.32 mg/L. For each pH, one test solution was prepared.

The study was performed in a glass-apparatus with an open gas (nitrogen)-flow system. For the incubation procedure the flasks containing the test solutions were connected with the absorption bottles and the nitrogen flow was adjusted to about 1-2 bubbles per second. Finally, the flasks were incubated at 25 + 0.1 °C in the dark. The incubation flasks were controlled by weighing at each sampling interval to detect possible evaporation of water, these water losses were negligible. The incubation apparatus was sterilised at 120°C during 30 minutes before use.

Sterility of the test solutions was checked by adding 1 ml of each test solution on the top of agar plates, which were exposed for 24 to 48 h at 37 °C, afterwards the number of colonies was counted.

A high germ formation was determined after 30 days of hydrolysis at pH 7 and 9. One germ was counted in sample 9/0 (pH 9, 0 days). But, as the results of the study demonstrate, no influence on the hydrolytical behaviour of the test substance could be observed. The other samples tested proved to be sterile.

2. Sampling

Approximately 4 mL of test samples were taken each for analyses at day 0 and after 5, 9, 15, 20, 26 and 30 days. The CO_2 absorption bottles (Sodium hydroxide solutions) and volatile absorption bottles (2-methoxy-ethanol solutions) were exchanged at the same intervals.

3. Analytical procedures

The radioactivity in the test solution, as well as the solutions in the CO_2 and volatile absorption bottles, was determined on a Packard Instrument (section 2.2) equipped with OPM and luminescence options. For this purpose, 100 µL test solution were measured in 10 mL scintillation mixture. 0.5 mL of the sodium hydroxide solutions from CO_2-absorption bottles were mixed with 4.0 mL of water and 10 mL of scintillation mixture. 0.5 mL of 2-methoxy-ethanol from volatiles absorption bottles were mixed with 10 mL of scintillation mixture. The radioactivity was determined by LSC.

Samples were analysed by TLC performed on pre-coated plates (20 cm x 20 cm) of cellulose with a layer thickness of 0.50 mm. The plates were developed with chamber saturation (at least 30 min.). Two different solvent system were used, SS 2: methanol / water (50:10, v/v), and SS 4: methanol / water / trichloroacetic acid / acetic acid / 15N ammonia hydroxide (55:35:3.5:2:2.5, v/v/w/v/v). The characterisation of the radioactivity in the test solutions was performed at each sampling date. The unlabelled parent compound was used for co-chromatography and visualised by spraying with ninhydrin reagent and drying for 10 to 20 minutes at 100 to 120 °C. The radioactive zones on TLC-plates were detected by using a scanner equipped with a data processing system.

II. RESULTS AND DISCUSSION

A. DATA

The radioactivity and the balance of radioactivity is presented in the table below.

Table 8.2.1.1-4: Overall mass balance in % of applied radioactivity

Test solution		Radioactivity assigned to the test item							
		Incubation time (days)							Balance of radioactivity (%)
Temp.(°C)	pH	0 *	5	9	16	20	26	30	
25	5	100.0	99.9	97.7	101.9	99.1	98.3	101.3	101.2
25	7	100.0	101.3	100.3	103.1	99.4	98.6	99.2	99.6
25	9	100.0	99.0	100.0	100.4	96.1	99.3	100.6	99.8
Mean ± S									100.2 ± 0.9

S = Standard deviation

* = initial value set at 100 %

B. MASS BALANCE

The balance of radioactivity was calculated by relating the radioactivity determined in the test solutions at the end of the incubation period to the difference of the radioactivity at the start and the total radioactivity sampled from the test solutions.

No significant amount of volatile radioactivity was determined in the absorption traps and therefore these results are not included in the calculation of the balance. With 101.2, 99.6 and 99.8 % of applied radioactivity at pH 5, 7 and 9, respectively, a mean balance for the three test solutions of 100.2 + 0.9 % was obtained and further showed that the entire amount of radioactivity was kept back in the test solutions.

C. VOLATILE RADIOACTIVITY

The results reveal that during the test period of 30 days and at a temperature of 25 °C, no significant amounts of radioactivity disappeared from the test solutions. The amount of volatile radioactivity liberated from the test solutions was < 0.1 % at each sampling interval, except for one sample (sodium hydroxide trap, pH 7, 9 days) which contained an amount of volatile radioactivity of 0.17 % (presumable due to an inaccuracy of the scintillation counter). This result demonstrates that finally no significant part of the test article was hydrolytically degraded to volatile molecules.

D. CHARACTERISATION OF RADIOACTIVITY

The results of the TLC-analysis showed that besides the parent compound no further components were detected.

III. CONCLUSIONS

In conclusion, with respect to the study design, it can be stated that [^{14}C]glyphosate was stable to abiotic hydrolysis under the conditions of the test. After 30 days of incubation at 25 °C and pH 5, 7 and 9, respectively, no hydrolysis products were observed. Furthermore, no significant amount of the test article (< 0.1 %) was degraded to volatile products.

Assessment and conclusion by applicant:

The hydrolytic degradation of glyphosate was assessed according to pertinent guideline requirements at the time of conduct, i.e. pH range for 5 to 9 was tested at a temperature of 25 °C. While conditions required by the current guidelines slightly differ, the study adequately demonstrates that glyphosate is stable at the conditions tested. Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

The study is well performed and considered acceptable. Deviations from OECD 111 (study conducted at 25°C only, pH buffer of 5 instead of 4) are minor.

[redacted], *1983*

Data point:	CA 7.2.1.1/009
Report author	[redacted]
Report year	1983
Report title	Hydrolysis and photolysis degradation studies of SC-0224
Report No	WRC-83-85
Guidelines followed in study	U.S. Environmental Protection Agency Report EPA 540/9-82-021, October 18, 1982, Pesticide Assessment Guidelines, Subdivision N; Chemistry: Environmental Fate, Series 161
Deviations from current test guideline	From OECD 111: - Glassware was not sterilised - pH 5 instead of pH 4 and a test temperature of 25 °C - mass balance not reported but expected to be outside 70-110% at some sampling dates
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate as glyphosate-trimesium salt (SC-0224)
Lot No.: WRC-7746-9-1
Composition: 19.3 % glyphosate-trimesium, 75.6 % water, 0.6 % isopropanol, 1.9 % sodium, 3.0 % chloride
Measured molar ratio: Glyphosate (CMP) : trimesium (TMS) = 1.00 : 1.03

2. Buffers

Aqueous phosphate buffer solutions were prepared at pH 5, 7 and 9 as published.

B. STUDY DESIGN

1. Experimental conditions

Sterilized test solutions of 10 mg/L (ppm) and 100 mg/L (ppm) glyphosate-trimesium (SC-0224) were prepared in phosphate buffer at pH 5, 7 and 9. An adequate number of aliquots of each solution were placed in individual non-sterile, Teflon®-sealed, screw top test tubes. The tubes were placed in a 25°C thermostat-controlled water bath (±0.5°C) in the dark for 32 days in maximum.

2. Sampling

Samples were removed for analysis each for the carboxymethylaminomethylphosponate anion on days 0, 1, 4, 8, 12, 24 and 32.

3. Analytical procedures

For glyphosate and AMPA (CMP and aminomethylphosphonic acid anions) single samples were analysed, if not stated otherwise. Determinations of glyphosate and AMPA were carried out by derivatisation with 9-fluorenylmethyl chloroformate followed by HPLC analysis. The typical recovery via the method was 93 ± 10 % for anions.

II. RESULTS AND DISCUSSION

A. DATA

The results of hydrolysis of glyphosate at the test temperature of 25 °C are summarised below.

Table 8.2.1.1-5: Glyphosate-trimesium concentrations (mg/L) at 25 °C, 10 mg/L test concentration, based on analysis for glyphosate anion

pH	5.0	7.0	9.0
Time (days)	Observed concentration (mg/L)		
0	8.9	9.2	10.2 / 10.3
1	10.0	9.7	10.7
4	8.2	8.6	9.8
8	8.6	9.2	11.2
12	10.8	14.8	11.2
18	9.4	9.3	9.3
24	10.2	9.7	9.3
32	9.7	8.8	9.0

Table 8.2.1.1-6: Glyphosate-trimesium concentrations (mg/L) at 25 °C, 100 mg/L test concentration, based on analysis for glyphosate anion

pH	5.0	7.0	9.0
Time (days)	Observed concentration (mg/L)		
0	78.4	105.6	94.3
1	86.8	105.6	107.5
4	82.3	70.6	97.6
8	91.7	96.4	83.5
12	133.3	147.8	130.0
18	100.0	103.0	95.0
24	91.3	102.6	94.8
32	100.0	99.0	100.0

B. Transformation of the test substance

For glyphosate anion (CMP), no detectable loss was observed for any pH at either glyphosate-trimesium concentration (10 or 100 mg/L) during the 32-day test period.

III. CONCLUSIONS

Glyphosate was stable under the conditions of abiotic hydrolysis.

Assessment and conclusion by applicant:

The hydrolytic degradation of glyphosate was tested in line with pertinent guideline requirements at the time of conduct. While conditions required by the current guidelines slightly differ, the study provides information on the hydrolytic stability of glyphosate at the conditions tested. In view of the lacking sterilisation of glassware used, the study is considered as supportive information.

Assessment and conclusion by RMS:

The study was performed at 25°C only and at pH buffer of 5 instead of 4 but these are considered as minor deviations. Sterile conditions were not used but no degradation is observed, confirming that glyphosate is hydrolytically stable. Mass balance is not reported but from results presented in the

above table, it is expected that it is outside the acceptable range of 70-110% at some sampling dates. RMS also notes that a high variability of glyphosate concentrations is observed at the 100 ppm test.

The study is considered as supportive.

Anonymous, 1995

Data point:	CA 7.2.1.1/001
Report author	Anonymous
Report year	1995
Report title	Stability in water
Report No	R 500, WAS95-00282
Guidelines followed in study	Cannot be checked
Deviations from current test guideline	Cannot be checked
GLP/Officially recognised testing facilities	Cannot be checked
Previous evaluation	Yes, not mentioned in RAR (2015) but not accepted in DAR (2001)
Acceptability/Reliability:	No

Assessment and conclusion by applicant:

The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Information in the monograph is limited to the results presented here. The study was not accepted in the monograph.

Assessment and conclusion by RMS:

This study report is not available to the RMS. However DAR (2001) indicated: "There are only summary reports which can't be evaluated because quality of data can't be checked". Therefore this study was not deemed necessary and was not requested to the applicant.

[redacted], *1995*

Data point:	CA 7.2.1.1/002
Report author	[redacted]
Report year	1995
Report title	Determination of the hydrolysis of Glyfosaat
Report No	141784
Guidelines followed in study	EEC A 7
Deviations from current test guideline	From OECD 111: - Basic data is missing (see details in RMS' conclusion)
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in Phys-Chem section of RAR (2015) and previously accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate (dutch term: Glyfosaat, non-labelled)
Lot No.: 22022
Chemical purity: 99 %

2. Buffers:

The following buffer solutions were prepared:

- sterile 0.05 M acetate buffer with pH 4: sodium acetate was combined with acetic acid in Milli-Q water
- sterile 0.05 M phosphate buffer with pH 7: potassium dihydrogenphospate was combined with sodium hydroxide in Milli-Q water
- sterile 0.05 M borate buffer with pH 9: boric acid was combined with potassium chloride and sodium hydroxide in Milli-Q water

B. STUDY DESIGN

1. Experimental conditions

Two standard solutions of glyphosate were prepared in aqueous solution at a concentration of 130 - 248 mg/L (n=2). After sonication, solutions were analysed without further pre-treatment.

An amount of approximately 20.0 mg glyphosate was added to 50.0 mL buffer solutions at pH 4, 7 and 9. After sonication, solutions were filter-sterilised through a 0.2 µm membrane filter and transferred into sterile glass vessels. To exclude oxygen, nitrogen gas was bubbled through each solution for approximately 5 minutes. Each test vessel was tightly sealed with a septum-crimcap.

In addition to test solution with glyphosate, blank buffer solutions were prepared.

Prepared test solution at pH 4, 7 and 9 were placed in a thermostatically controlled waterbath at 50.0 ± 0.5 °C in the dark.

2. Sampling

The concentration of glyphosate was determined immediately after preparation of test solutions as well as after 2.4 hours and 5 days.

pH values of test solutions were determined at study beginning and study end.

3. Analytical procedures

Immediately after samples of ≤ 5 mL were taken, they were cooled down to room temperature. Then, each test solution was analysed by HPLC without any further pre-treatment.

4. Calculations

The decrease in concentration was calculated as:

$[(C_0 - C_t) / C_0] \times 100$ %

Where
C_0 = concentration at time 0
C_t = concentration at time t

The relative concentration C_r was calculated as:

$Cr = [C_t / C_0] \times 100$ %

II. RESULTS AND DISCUSSION

A. DATA

Glyphosate concentrations are summarised below for the respective pH values.

Table 8.2.1.1-7: Degradation of glyphosate in sterile buffer solutions at pH 4, 7 and 9

pH	Measured pH value (study start / study end)	Glyphosate concentration (mg/L) after 0 hours	2.4 hours	5 days
4	4.0 / 4.0	203.04	204.66 (101 % [2])	203.06 (100 % [2])
7	7.0 / 7.0	222.60	224.07 (101 % [2])	216.46 (97 % [2])
9	8.9 / 8.9	212.02	212.02 (100 % [2])	205.03 (97 % [2])

[1] mean value of duplicate analysis
[2] relative concentration

B. HYDROLYSIS

Glyphosate concentrations at study end (5 DAT) were 100 %, 97 % and 97 % of applied amount in buffers at pH 4, 7 and 9. The difference in test concentration of Glyphosate was determined to less than < 10 % of applied amount at study start and after 5 days of incubation in maximum.

C. KINETICS

No degradation kinetics was calculated due to stability under the test conditions.

III. CONCLUSIONS

Results indicated that glyphosate is stable to abiotic hydrolysis in aqueous buffer at pH 4, 7 and 9 under the conditions of the test.

Assessment and conclusion by applicant:

The hydrolysis of glyphosate was examined in sterile buffer solutions at pH 4, 7 and 9 at 50.0 ± 0.5 °C in the dark. As hydrolysis of < 10 % of applied amount was observed at all pH values at study end after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. However, basic data is missing, e.g. on application procedure or preparation of buffers, or unclear, i.e. number of test solutions and the study duration was only five days.

The study is considered as supportive information.

Assessment and conclusion by RMS:

OECD 111 indicates that if the preliminary test performed at 50°C shows less than 10% degradation after 5 days, the substance is considered hydrolytically stable and no additional testing is required. The study duration is therefore considered appropriate.

However the study report is very brief. RMS agrees with the applicant that some basic data are missing (*e.g.* the form of glyphosate applied is not reported, no information on application procedure, no information to check the validity of the analytical method). As a consequence, the reliability of the study cannot be fully checked.

RMS considers that the study is not acceptable.

[redacted], *1993*

Data point:	CA 7.2.1.1/003
Report author	[redacted]
Report year	1993
Report title	Glyphosate, ammonium salt: Determination of hydrolysis as a function of pH
Report No	93/MON033/0344
Guidelines followed in study	OECD 111 (1981) EEC Directive 84/449/EEC (Annex V)
Deviations from current test guideline	From OECD 111: - Basic data is missing (see details in RMS' conclusion)

GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentioned in RAR (2015) but accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate ammonium salt (non-labelled)
Lot No.:	PSGA 1128
Chemical purity:	97.9 % glyphosate ammonium salt
Measured concentration:	88.9 % w/w glyphosate acid

2. Test Buffers:

The following aqueous buffer solutions were prepared:

pH 4.0: Disodium hydrogen phosphate dodecahydrate (27.6 g) and citric acid (12.9 g) were dissolved in distilled water (1900 mL).

pH 7.0: Potassium dihydrogen phosphate trihydrate (6.8 g) was dissolved in distilled water (1900 mL) and 1 M sodium hydroxide (30 mL) was added.

pH 9.0: Disodium tetraborate decahydrate (33.1 g) and potassium dihydrogen phosphate trihydrate (3.59 g) were dissolved in distilled water (1900 mL)

The volume of buffer solutions was adjusted to 2000 mL with distilled water. The pH was adjusted to the target value with 1 M hydrochloric acid or 1 M sodium hydroxide.

B. STUDY DESIGN

1. Experimental conditions

Aliquots (250 mL) of each buffer solution were measured into reagent bottles containing approximately 465 mg of glyphosate in the form of its ammonium salt to give nominal concentrations of about 1860 mg/L. The pH of the solutions was readjusted to the required values with 1 M sodium hydroxide and sealed bottles placed in a thermostatically controlled water bath at 50 °C. Following equilibration, initial samples (about 20 mL) were removed and the samples again stored in the water bath until further sampling.

Test solutions were incubated at 50 °C for 5 days in maximum.

2. Sampling

The concentration of glyphosate in test solutions was determined immediately after preparation as well as after 2.4, 72, 91.5, 96, 115.5 and 120 hours. pH values of test solutions were determined at all samplings.

3. Analytical procedures

Duplicate aliquots (1 mL) were diluted to 10 mL with the HPLC mobile phase (0.005 M aqueous potassium dihydrogen orthophosphate: methanol (97:3, v:v) adjusted to pH 2.0 with orthophosphoric acid) and then followed by analysis via HPLC.

The limit of detection was approximately 1 mg/L.

4. Calculations

The concentration of glyphosate, ammonium salt in the injection solution (C_A) was calculated from the mean response of bracketing standards:

C_A [mg/L] = sample peak area x standard concentration [mg/L] / mean peak area of bracketing standards

The concentration of glyphosate, ammonium salt in the sample solution (C_B) was then calculated as follows:

C_B [mg/L] = C_A [mg/L] x dilution factor (V_A/V_B)

Where:

V_A = volume of injection solution (10 mL)

V_B = volume of aqueous samples (1 mL)

II. RESULTS AND DISCUSSION

A. DATA

The concentration of glyphosate, ammonium salt is presented below for buffer solutions at pH 4, 7 and 9.

Table 8.2.1.1-8: Degradation of glyphosate, ammonium salt in buffer solutions at pH 4, 7 and 9 (in mg/L)

Time (hours)	Replicate	Glyphosate, ammonium salt (mg/L)		
		pH 4	pH 7	pH 9
0	1	1794	1906	1857
	2	1793	1873	1879
	mean	1794	1890	1868
2.4	1	1752	1905	1895
	2	1736	1900	1876
	mean	1744	1903	1886
72	1	1646	1871	1846
	2	1639	1891	1862
	mean	1643	1881	1854
91.5	1	1708	1883	1894
	2	1726	1941	1876
	mean	1717	1912	1885
96	1	1717	1811	1808
	2	1729	1852	1820
	mean	1723	1832	1814
115.5	1	1682	1860	1817
	2	1642	1910	1790
	mean	1662	1885	1804
120	1	1744	1860	1866
	2	1735	1855	1850
	mean	1740	1858	1858

B. TRANSFORMATION OF THE TEST ITEM

The mean concentration of glyphosate decreased from 1794 to 1740 mg/L at pH 4 and from 1890 to 1858 mg/L at pH 7, each time after 0 and 120 hours, respectively. At pH 9, mean concentrations were 1868 and 1858 mg/L after 0 and 120 hours, respectively. At the end of the test, concentration of glyphosate, ammonium salt represented 96%, 98% and 98% of the initial concentration at t0, at pH 4, 7 and 9, respectively. As less than 10 % of glyphosate was degraded after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

pH values in test solutions did not change significantly with time.

C. KINETICS

No degradation kinetics was calculated due to stability under the test conditions.

III. CONCLUSIONS

Results indicated that glyphosate is stable to abiotic hydrolysis in aqueous buffer at pH 4, 7 and 9 under the conditions of the test.

Assessment and conclusion by applicant:

The hydrolysis of glyphosate (as ammonium salt) was examined in buffer solutions at pH 4, 7 and 9 at 50.0 °C. As hydrolysis of < 0 % of applied amount was observed at all pH values at study end after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. Basic data is unclear (application rate, number of test solutions, sterile conditions, dark conditions) and the study duration was only five days. The study is considered as supportive information.

Assessment and conclusion by RMS:

OECD 111 indicates that if the preliminary test performed at 50°C shows less than 10% degradation after 5 days, the substance is considered hydrolytically stable and no additional testing is required. The study duration is therefore considered appropriate.

However RMS agrees with the applicant that some basic data are missing (*e.g.* it is not reported whether the study was performed under sterile and dark conditions, the number of test solutions is not reported, examples of HPLC are not available).

The study is not considered acceptable.

[redacted], *1991*

Data point:	CA 7.2.1.1/006
Report author	[redacted]
Report year	1991
Report title	Behaviour of Glyphosate in water and soil. Part 1: Hydrolysis as a function of pH
Report No	PR90/002
Guidelines followed in study	BBA-Guideline "Prüfung des Verhaltens von Pflanzenschutzmitteln in Wasser" (Merkblatt 55, part I and II)
Deviations from current test guideline	From OECD 111: - The analytical procedure is not described - The test was conducted at pH 5 instead of a pH of 4 - The test period was longer than 30 days - Not reported whether study was conducted under sterile test conditions
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in Phys-Chem section of RAR (2015) and previously accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate, free acid (non-labelled)

Lot No.: 00516

Chemical purity: 99 %

2. Buffers:

The following buffer solutions were prepared for the test:

pH 5: 9 g/L KH2PO4; about 4 mL of Na2HPO4-solution (23.8 g/L) were added to reach pH 5

pH 7: 9.65 g/L (0.071 M) KH2PO4 plus 0.01 M Disodiumhydrogenphosphate (1.42 g/L)

pH 9: 0.05 M NaHCO3 (4.2 g/L); pH 9 was adjusted with 1 N NaOH

B. STUDY DESIGN

1. Experimental conditions

A solution containing 20.6 mg glyphosate in 100 mL water was prepared (1.03 mg/ 5 mL). 5 mL of this solution was diluted to 1 L of each buffer solution resulting in a test concentration of 1.03 mg test item/L. Eight brown glass bottles each filled with 125 mL treated buffer solution were prepared per buffer solution.

The test was performed at 22 °C.

2. Sampling

Samples were taken at days 0, 4, 7, 28 and 56. At each sampling day, two samples were investigated.

3. Analytical procedures

Procedures for determination of glyphosate residues are not detailed in the study report.

II. RESULTS AND DISCUSSION

A. DATA

Degradation of glyphosate in buffer solutions at pH 5, 7 and 9 is summarised below.

Table 8.2.1.1-9: Recovery of glyphosate at pH 5, 7 and 9 (mg/L)

Day	Replicate	Glyphosate (mg/L)		
		pH 5	pH 7	pH 9
	1	988	1080	1064
0	2	862	1193	1034
	mean	925	1137	1049
	1	779	909	819
4	2	758	996	1171
	mean	769	953	995
	1	938	978	1098
7	2	889	904	1068
	mean	914	941	1083
	1	999	1077	1014
15	2	966	1145	1102
	mean	983	1111	1058
	1	785	1222	1099
28	2	970	983	1103
	mean	878	1103	1101
	1	951	1066	1042
56	2	937	990	1039
	mean	944	1028	1041

B. HYDROLYSIS

Glyphosate concentrations varied slightly between start and at the end of the study: at pH 5, mean concentrations of 925 and 944 µg/L were measured, whereas at pH 7 mean concentrations of 1137 and 1028 µg/L were detected at 0 DAT and 56 DAT, respectively. At pH 9, mean values of 1049 and 1041 µg/L were determined at 0 DAT and 56 DAT, respectively.

As hydrolysis of less than 10 % of applied amount was observed at study end at all pH values investigated, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

C. KINETICS

Glyphosate was stable to abiotic hydrolysis in aqueous buffer at pH 5, 7 and 9.

III. CONCLUSIONS

For none of the three pH values there was hydrolysis to be seen within the time of investigation.

Assessment and conclusion by applicant:

The hydrolysis of glyphosate in buffer solutions at pH 5, 7 and 9 was examined at an application rate of 1.03 mg/L. The analytical procedure is not described. It is not reported whether study was conducted with sterile buffers and under sterile conditions.

Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

The study was conducted at pH 5 instead of pH 4 and study duration was longer than 30 days. These deviations are considered as minor.

However, the study report is very brief. It is not reported whether the study was performed under dark and sterile conditions. In addition, the analytical procedure is not described. As a consequence, the study is not considered acceptable.

[redacted], *1990*

Data point:	CA 7.2.1.1/008
Report author	[redacted]
Report year	1990
Report title	Stability of Glyphosate to hydrolysis
Report No	WAS95-00278
Guidelines followed in study	OECD 111 (1981)
Deviations from current test guideline	From OECD 111: - Basic data is missing (see details in RMS' conclusion)
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentioned in RAR (2015) but accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate (sample identification No 21/00)

Chemical purity: 99 %

2. Buffers

The following aqueous buffer solutions were prepared:

pH 4.0: 0.4 mL 0.1 N NaOH + 50 mL 0.1 M potassium biphtalate and then diluted to 100 mL with distilled water at 20°C.

pH 7.0: 29.63 mL 0.1 N NaOH + 50 mL 0.1 N KH2PO4 and then diluted to 100 mL with distilled water at 20°C.

pH 9.0: 21.3 mL 0.1 N NaOH + 50 mL boric acid and then diluted to 100 mL with distilled water at 20°C.

B. STUDY DESIGN

1. Experimental conditions

Approximately 39.5 mg of glyphosate were dissolved into 100 mL of each buffer solution. Concentration was determined to be approximately 0.0231 mole/L.

Incubation was performed at 50°C for 5 days.

2. Sampling

Samples were removed for analysis on days 0 and 5.

3. Analytical procedures

Glyphosate was analysed by HPLC.

II. RESULTS AND DISCUSSION

Less than 10% degradation was observed after 5 days at 50°C.

III. CONCLUSIONS

Based on results from preliminary test, glyphosate is considered hydrolytically stable and no additional testing is required

Assessment and conclusion by applicant:

The hydrolysis of glyphosate was examined in buffer solutions at pH 4, 7 and 9 at 50.0 °C for 5 days. Glyphosate was hydrolytically stable under the conditions of the test. As some basic data is unclear (sterile conditions, temperature records, pH values measured at study end), samples were only analysed at start and end of the study and the study duration was only five days and the limit of detection of the analytical method is not reported,the study is considered as supportive.

Assessment and conclusion by RMS:

The report is very brief. Basic data such as application of the test substance, conditions of incubation (dark / sterile), analytical method, *etc.* are not reported. As a consequence the quality of the study cannot be checked. The study is not considered acceptable.

[redacted], *1978*

Data point:	CA 7.2.1.1/010
Report author	[redacted]
Report year	1978
Report title	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 0101
Report No	MSL-0207
Guidelines followed in study	None
Deviations from current test guideline	Deviations from OECD Guideline 111 (April 2004): Radiochemical purity < 95% Basic data is missing (see details in RMS' conclusion)

	Recovery >110 % in two cases
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, study not mentioned in RAR (2015) but not accepted in DAR (2001)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test item: [^{14}C] glyphosate, phosphonomethyl-label (94 % radiochemical purity)

2. Buffers:

The following buffer solutions were prepared:

- 0.05 M potassium biphthalate-hydrochloric acid (pH 3.0);
- 0.05 M potassium phosphate monobasic-sodium hydroxide buffer (pH 6.0);
- 0.1 M boric acid-potassium chloride-sodium hydroxide buffer (pH 9.0)

2. Natural waters:

Natural water samples were taken from:

- Cattail Swamp (Wisconsin) (pH 6.2),
- Sphagnum bog (Wisconsin) (pH 4.2),
- Ballard pond (Missouri) (pH 7.3)

B. STUDY DESIGN

1. Experimental conditions

Hydrolysis was investigated in two buffers and three natural waters.

Buffer tests: The buffers and all glasswares were autoclaved at 120°C for 20 minutes. Test labelled item was diluted with the unlabelled test item, was sterilised by Millipore filtration 0.45 µm and was applied to the buffers at concentrations of 25 and 250 ppm. Buffer test vials were incubated in the dark at 5 and 35°C for 32 days.

Natural water tests: Test labelled item was applied at concentrations of 0.1 ppm to the waters, and they were sterilised by Millipore filtration 0.45 µm. Natural water solutions were incubated at 30°C for 5 weeks.

2. Sampling

For the buffer tests, samples were taken in duplicate 0, 7, 14, 21 and 32 days after treatment. For natural water tests, samples were taken after 0, 1, 3 and 5 weeks after treatment.

3. Analytical procedures

Buffer samples were analysed by LSC and TLC (all samples) and by HPLC (32 DAT). Natural water samples were analysed by LSC, TLC and HPLC.

II. RESULTS AND DISCUSSION

A. DATA

Buffer tests: The data from the final sampling date are summarised below.

Table 8.2.1.1-10: Distribution of radioactivity in sterile buffer solutions after 32 days incubation

Temperature (°C)	pH	Concentration (ppm)	Total recovery (% AR)	Glyphosate (% AR, TLC)	Glyphosate (% AR, HPLC)	AMPA (% AR, HPLC)
35	3	25	102.4	91.6	94.6	5.4
		250	100.3	92.9	na	na

	6	25	113.4	91.9	93.7	6.3
		250	98.4	93.3	na	na
	9	25	99.2	92.7	94.1	5.9
		250	107.4	94.4	na	na
	3	25	101.4	91.8	94.2	5.8
		250	106.6	92.0	na	na
5	6	25	103.7	92.9	94.1	5.9
		250	98.1	92.7	na	na
	9	25	104.8	93.3	93.7	6.3
		250	106.0	93.9	na	na

na: not analysed

Natural water tests: The data from the final sampling date are summarised below.

Table 8.2.1.1-11: Distribution of radioactivity in natural water

Natural water	pH	Sampling time (days)	Total recovery (% AR)	Glyphosate (% AR, HPLC)	AMPA* (% AR, HPLC)
Cattail Swamp	6.25	21	102.0	90.4	3.9
		35	104.7	74.7	19.4
		49	101.9	80.7	13.5
Sphagnum bog	4.23	21	104.0	94.4	0.0
		35	104.3	94.0	0.1
Ballard pond	7.30	21	104.3	83.8	10.3
		35	119.0	82.8	11.3

* Data corrected for 5.9% AMPA present in stock solution

B. TRANSFORMATION OF THE TEST ITEM

Buffer tests: There is no indication that the test item is hydrolysed under these conditions, as all the results are within error of the analysis of the starting materials which contained 5.4% AMPA when analysed by HPLC.

Natural water tests: The recovery of radioactivity is within error of the analytical method, with the exception of 119% recovery in Ballard pond water after 35 days. This is probably due to loss of water from the sample by evaporation. Biodegradation occurs in Cattail Swamp and Ballard pond waters. Degradation to AMPA is 13 to 19% (corrected for 5.9 % present in stock solution) in these two samples after 35 days but appears to remain constant indicating that degradation is dependent upon a fixed amount of activator in the system. Sterilization by filtration would rule out bacterial mechanisms but enzymatic reactions would not be inactivated and may contribute to the degradation of glyphosate. Because glyphosate and AMPA do not undergo chemical hydrolysis by virtue of their chemical structures the degradation in natural water is probably by soluble enzymes.

III. CONCLUSIONS

There is no evidence of hydrolysis of glyphosate in sterile buffers. Slow biodegradation occurs in natural waters.

Assessment and conclusion by applicant:

The study was considered invalid due to the following deficiencies from OECD Guideline 111 (April 2004):

- pH 3.0 buffer solution used instead of pH 4
- Usage of natural water for hydrolysis test
- Measures to avoid oxygen were not taken
- Recovery >110 % in two cases

Hydrolysis and biodegradation cannot be separated within the experiment.

Assessment and conclusion by RMS:

The study report is brief. The radiochemical purity of the test substance is below the minimal purity recommended in OECD 111 (95%). Some basic information is missing (*e.g.* no information to check the validity of the analytical method). For buffer tests, results are available only for the last sampling point. OECD 111 indicates that test should be performed with buffer solutions and not natural water.

The study is not considered acceptable.

B.8.2.1.2. Direct photochemical degradation

The aqueous photolysis of glyphosate was investigated in 8 existing studies. A review of direct and indirect photolysis is also provided. No new study was provided in this renewal dossier. In the scientific literature review for glyphosate (2010-2020), no article was identified to provide further information relevant to the data point.

Table 8.2.1.2-1: List of existing and news direct photochemical degradation studies

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)	Remark
CA 7.2.1.2/002	[redacted], 2005	Not evaluated	Acceptable	Direct and indirect photolysis
CA 7.2.1.2/006	[redacted], 1992	Accepted in RAR 2015	Acceptable	Direct photolysis
CA 7.2.1.2/005	[redacted]., 1990	Not evaluated	Acceptable	Direct photolysis
CA 7.2.1.2/003	[redacted], 2001	Not evaluated	Supportive	Indirect photolysis
CA 7.2.1.2/001	[redacted], 2012	Not evaluated	Supportive	Review on direct and indirect photolysis
CA 7.2.1.2/004	[redacted], 1992	Accepted in RAR (2015)	Supportive	Direct photolysis
CA 7.2.1.2/007	[redacted]., 1983	Not evaluated (only the hydrolysis part was evaluated in RAR (2015))	Not acceptable	Direct photolysis
CA 7.2.1.2/008	[redacted], 1978	Not accepted in RAR (2015)	Not acceptable	Indirect photolysis

[redacted], 2005

Data point:	CA 7.2.1.2/002
Report author	[redacted]
Report year	2005
Report title	Degradation Study: Photodegradation of [^{14}C]glyphosate in Sterilized Pure Water and Natural Water by Artificial Light
Report No	1318W-2
Guidelines followed in study	Japan MAFF 12-Nousan-No. 8147, Part 2-6-2 Photodegradation in Water
Deviations from current test guideline	From OECD 316: - For direct photolysis test, distilled water was used instead of buffer solution.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not previously evaluated
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [C1-^{14}C]glyphosate

Lot No.: 040806

Specific activity: 55.0 mCi/mmol)

Radiochemical purity: 96.9 %

Identification: [C3-^{14}C]glyphosate

Lot No.: C-2278

Specific activity: 39.0 mCi/mmol)

Radiochemical purity: 98.6 %

2. Test systems

Glass distilled HPLC grade water with a conductivity of 7µS/cm at experimental start and natural water were used.

Water from Lake Herman, Benicia, CA (38°5'47.4" N latitude, 122°9'3.9" W longitude) was collected on October 28, 2004 and characterised as follows:

Table 8.2.1.2-2: Physico-chemical characteristics of the lake water Herman

pH	8.0
Calcium	29 ppm
Magnesium	19 ppm
Sodium	43 ppm
Hardness	150 mg equiv. $CaCO_3$/L
Conductivity	61 µS/cm
Sodium absorption ratio (SAR)	1.52
Total dissolved solids	386 ppm
Turbidity	10.6 NTU
Chloride	32.3 ppm

The pH of the test systems was measured at each sampling and averaged 8.08, 7.24 and 8.29 for distilled water and natural water containing [C1-^{14}C]glyphosate and natural water containing [C3-^{14}C]glyphosate, respectively.

B. STUDY DESIGN

1. Experimental conditions

Photolysis set-up was conducted in Quartz sample tubes (10 mm i.d., 80 nm length), equipped with Teflon-lined silicon septum screw for the irradiated samples. For the dark control samples, amber borosilicate glass vials with Teflon-lined caps were used. The following tests were conducted

- Sterile distilled water treated with [C1-^{14}C]glyphosate
- Sterile natural water treated with [C1-^{14}C]glyphosate
- Sterile natural water treated with [C3-^{14}C]glyphosate

The test systems were sterilised by filtering through a 0.22 micron Falcon Bottle top filter into sterile Erlenmeyer flasks immediately prior to use. The sterility of the test systems was confirmed throughout the experiment. The pH of the sterile solutions was checked with a pH meter prior to dosing. The test substances arrived at PTRL as aqueous solutions prepared in sterile water. Concentration of these stock solutions were 0.1 mCi/mL and 39.6 µCi/mL for [C1-^{14}C]glyphosate and [C3-^{14}C]glyphosate, respectively. The dose solutions were prepared by transferring aliquots of corresponding test substance stock solution in water to sterile amber glass bottles and combining with an aliquot of the respective sterile test system.

Samples were prepared by transferring aliquots (5 mL) of the respective dose solution to sterile quartz or Pyrex sample holders using a 10 mL sterile glass pipette. Aliquots (3 x 100 µL) of the dosing solutions were taken at least before and after application of each set to determine the dose concentration and the homogeneity of the solutions during the dosing process. Stability of the dosing solutions under conditions of administration was demonstrated by HPLC analysis of the time zero samples. The nominal concentration of glyphosate in the samples was 1.0 µg/mL.

The light exposed samples for the natural water set dosed with [C3-^{14}C]glyphosate test substance were set up with continuous trapping of volatiles. Additionally, a set of tubes containing natural water dosed with [C1-^{14}C]glyphosate was equipped with a trapping system and volatiles were collected at the end of the test period. The aeration set up for continuous trapping of headspace was performed during the whole photolysis period. An air pump was used to circulate air through the sample holders. The samples were connected to the air source via Teflon tubing threaded through the septum caps and connected to manifolds. The circulating air was first pumped through a vessel containing sterile deionized water before connecting to the samples to minimize evaporation losses. Each sample was connected at the outlet, to an individual set of traps consisting of one ethylene glycol trap (20 mL) to collect organic volatiles, and two 10 % aqueous sodium hydroxide traps (20 mL each) for CO_2 collection. Trap solutions were housed in glass vials (40 mL capacity) fitted with open top caps with Teflon-lined silicon septa through which the Teflon tubing was threaded in the same fashion as the samples, except that the inlet tubing was placed under the surface of the liquid to bubble the headspace through the trap solutions.

After dosing, light exposed sample tubes were placed in a deionized water bath maintained at an average temperature of 25 ± 1°C by continuous circulation using a circulation bath. Dark control samples were placed in a Hotpack constant temperature chamber maintained at 25 ± 1 °C during the incubation period. Aliquots (2 x 0.1 mL) of the dose solutions were plated on trypticase soy agar for sterility assay at the time of application. The [C3-^{14}C]glyphosate light exposed sample set and the Day 12 samples (duplicate light exposed samples and dark control samples) with natural water, containing [C1-^{14}C]glyphosate were connected to the traps for volatiles and connected to an aeration set up for continuous trapping of headspace during the photolysis period.

The apparatus utilized for exposure of [^{14}C]glyphosate in aqueous solutions to artificial light was a Heraeus Suntest CPS+ unit, equipped with a xenon arc lamp with a filter blocking the radiation from the wavelengths below approximately 290 nm. The Suntest CPS+ was set at a light intensity of 600 W/m^2, which gave an average intensity of 457 W/ m^2 for the 300-800 nm range at the level of the photolysis sample tubes. Continuous irradiation was used for the irradiated samples.

2. Sampling

Duplicate light exposed and dark control samples (when applicable) were removed from the water bath and Hotpack chamber and analysed on days 0, 1, 3, 5, 7, 9, and 12 after treatment (DAT). Samples were analysed by LSC and HPLC on the same day they were collected.

At each sampling, trapping solutions for the collection of volatiles were measured (volumes) and aliquots (3 x 1 mL) were radioassayed by LSC.

3. Analytical procedures

All radioassays utilised 5 mL or 15 mL of scintillation cocktail in 7 mL or 20 mL standard polyethylene counting vials and Beckman LS 6500 or LS 6000IC liquid scintillation spectrometers.

[^{14}C]glyphosate and degradates were analysed and quantitated based on HPLC analyses. For all samples the structural assignments for [^{14}C]glyphosate and degradates were based on co-chromatography with reference standards upon HPLC analysis. The limit of detection for individual degradates in the HPLC radiochromatograms were determined by the dpm injected, and the liquid scintillation counting detection limit. As a typical example a limit of 0.002 µg/mL is given for a background of 50 dpm and a sample size of 50,000 dpm injected of a matrix containing 1.0 µg/mL. Representative samples were co-spotted with reference standards and analysed by one-dimensional TLC. After elution, the reference standards were visualized by spraying with ninhydrin reagent and warming the plates to 135 °C to develop the spots. The plates were then scanned with an optical scanner and the radioactive spots matched against the UV trace of the standards.

Initial HPLC analyses of the irradiated samples were conducted by fraction collection followed by LSC counting. The reconstructed radiochromatograms for these analyses showed glyphosate as the only radioactive component. However, the HPLC column recoveries for light exposed natural water samples treated with [C1-^{14}C]glyphosate declined steadily throughout the study period, reaching an average of 20.9 % of the dose by the end of the irradiation period. This suggested the possibility of the presence of a volatile component that was not trapped in the scintillation cocktail during fraction collection and subsequent radioassay by LSC assumed to be $^{14}CO_2$. Since the pH of the aqueous samples averaged 7.24 and the samples were sealed during the exposure period, the $^{14}CO_2$ would be expected to stay in the solution predominantly as non-volatile bicarbonate ($^{-}H^{14}CO_3$). HPLC analysis required the use of acidic mobile phase (pH = 2), which would be expected to shift the solution equilibrium toward the volatile $^{14}CO_2$ during HPLC analyses, resulting in low HPLC column recovery due to loss of $^{14}CO_2$ during fraction collection and LSC counting. To confirm this hypothesis, the [C1-^{14}C]glyphosate-irradiated samples were reanalyzed by HPLC using a Beta-Ram flow through detector for ^{14}C detection. HPLC analyses showed the presence of a distinct peak eluting at approximately 6 minutes in addition to the glyphosate peak. The identity of the 6-minute eluting peak was confirmed as $^{14}CO_2$ by comparing its retention time to HPLC analysis of an authentic standard of $NaH^{14}CO_3$ solution. Further confirmation of the formation of $^{14}CO_2$ in the [C1-^{14}C]glyphosate light exposed natural water samples was obtained from the contingency samples that were set up with continuous trapping of volatiles over the entire study period, resulting in an average of 12.1 % of the applied dose recovered in the NaOH traps for the light exposed samples. The radiocarbon in these caustic traps was precipitated with $BaCl_2$ as $Ba^{14}CO_3$, confirming that the trapped radiocarbon was $^{14}CO_2$. As described above, due to the basic pH of the natural water used in the study, some carbon dioxide remained dissolved in the natural water samples. Partial precipitation of the radiocarbon in solution by treatment with $BaCl_2$ also confirmed the presence of dissolved carbonate in the natural water samples.

Confirmation of the identity of methanediol as the major degradate in the [C3-^{14}C]glyphosate irradiated samples was accomplished by derivatisation and co-chromatography with the 2,4-DNPH hydrazone derivative of an authentic standard of methanediol. The presence of methanediol in EG traps was confirmed qualitatively by bubbling air through an aliquot of a selected aqueous sample containing large amount of methanediol [C3-^{14}C]glyphosate treated Natural Water Light Day 9 and passing the headspace gases through three EG traps connected in series. Small amount of radiocarbon was detected in the three EG traps connected in series confirming that the radioactivity from the aqueous sample (e.g. methanediol) was relatively volatile.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate in the separate test systems are summarised below.

Table 8.2.1.2-3: Mass balance of [C1^{14}C]glyphosate in sterile distilled water (expressed as percent of applied radioactivity)

DAT		Light exposed	Dark control
0	Replicate A	102.9	-
	Replicate B	102.8	-
1	Replicate A	102.5	103.1
	Replicate B	102.1	105.3
3	Replicate A	101.1	104.0
	Replicate B	101.2	104.3
5	Replicate A	105.6	107.2
	Replicate B	105.4	107.2
7	Replicate A	99.4	103.7
	Replicate B	98.6	103.4
9	Replicate A	97.3	103.6
	Replicate B	94.9	103.5
12	Replicate A	100.8	107.1
	Replicate B	101.2	106.8
Mean recovery	-	101.1 ± 2.9	104.9 ± 1.7

Table 8.2.1.2-4:Mass balance of [C1^{14}C]glyphosate in sterile natural water (expressed as percent of applied radioactivity)

DAT		Light exposed	Dark control
0	Replicate A	99.5	-
	Replicate B	99.9	-
1	Replicate A	99.8	99.9
	Replicate B	99.9	100.9
3	Replicate A	96.3	100.6
	Replicate B	95.3	101.1
5	Replicate A	98.3	101.8
	Replicate B	97.4	102.5
7	Replicate A	99.2	100.6
	Replicate B	98.1	102.0
9	Replicate A	97.5	99.8
	Replicate B	95.8	100.2
12	Replicate A	96.2	102.9
	Replicate B	96.6	101.6
Mean recovery	-	97.8 ± 1.6	101.2 ± 1.0

Table 8.2.1.2-5: Mass balance of [C3^{14}C]glyphosate in sterile natural water (expressed as percent of applied radioactivity)

DAT		Solution	NaOH traps	Ethylene glycol traps	Total recovery
Light exposed					
0	Replicate A	100.5	NA	NA	100.5
	Replicate B	100.5	NA	NA	100.5
1	Replicate A	98.5	0.0	0.0	98.5
	Replicate B	100.4	0.0	0.0	100.4
3	Replicate A	99.6	0.0	0.1	99.7
	Replicate B	101.6	0.0	0.0	101.6
5	Replicate A	97.7	0.0	0.1	97.8
	Replicate B	97.7	0.1	0.2	98.0
7	Replicate A	99.0	0.1	0.4	99.5
	Replicate B	98.1	0.4	0.3	98.8
9	Replicate A	93.9	0.7	1.2	95.8
	Replicate B	98.9	0.2	0.6	99.7
12	Replicate A	97.4	0.6	0.8	98.8
	Replicate B	100.0	0.2	0.7	100.9
Mean recovery					99.3 ± 1.5
Dark control					
1	Replicate A	100.6	NA	NA	100.6
	Replicate B	100.2	NA	NA	100.2
3	Replicate A	100.3	NA	NA	100.3
	Replicate B	98.9	NA	NA	98.9
5	Replicate A	97.6	NA	NA	97.6
	Replicate B	95.8	NA	NA	95.8
Mean recovery					99.3 ± 1.8

Table 8.2.1.2-6: Degradation of [C1^{14}C]glyphosate in sterile distilled water (expressed as percent of applied radioactivity)

DAT		Total	Glyphosate	Others
Light exposed				
0	Replicate A	102.9	101.5	1.4
	Replicate B	102.8	102.7	0.1
Average		102.9	102.1	0.8
1	Replicate A	102.5	102.4	0.1

Table 8.2.1.2-6: Degradation of [C1^{14}C]glyphosate in sterile distilled water (expressed as percent of applied radioactivity)

	Replicate B	102.1	100.1	2.0
Average		102.3	101.3	1.1
3	Replicate A	101.1	101.0	0.1
	Replicate B	101.2	101.2	0.0
Average		101.2	101.1	0.1
5	Replicate A	105.6	105.3	0.3
	Replicate B	105.4	105.3	0.1
Average		105.5	105.3	0.2
7	Replicate A	99.4	99.0	0.4
	Replicate B	98.6	98.6	0.0
Average		99.0	98.8	0.2
9	Replicate A	97.3	95.5	1.8
	Replicate B	94.9	92.9	2.0
Average		96.1	94.2	1.9
12	Replicate A	100.8	99.8	1.0
	Replicate B	101.2	100.8	0.4
Average		101.0	100.3	0.7
Dark control				
1	Replicate A	103.1	103.0	0.1
	Replicate B	105.3	105.2	0.1
Average		104.2	104.1	0.1
3	Replicate A	104.0	103.9	0.1
	Replicate B	104.3	103.8	0.5
Average		104.2	103.9	0.3
5	Replicate A	107.2	107.2	0.0
	Replicate B	107.2	107.2	0.0
Average		107.2	107.2	0.0
7	Replicate A	103.7	103.5	0.2
	Replicate B	103.4	103.4	0.0
Average		103.6	103.5	0.1
9	Replicate A	103.6	103.6	0.0
	Replicate B	103.5	103.1	0.4
Average		103.6	103.4	0.2
12	Replicate A	107.1	106.8	0.3
	Replicate B	106.8	104.9	1.9
Average		107.0	105.9	1.1

Table 8.2.1.2-7: Degradation of [C1-^{14}C]glyphosate in sterile natural water (expressed as percent of applied radioactivity)

DAT		Glyphosate	others	CO_2	Escaped CO_2[1)]	Total recovery CO_2[2)]
Light exposed						
0	Replicate A	99.9	0.1	0.0	0.0	0.0
	Replicate B	99.8	0.2	0.0	0.0	0.0
Average		99.9	0.2	0.0	0.0	0.0
1	Replicate A	76.7	0.0	11.0	12.2	23.2
	Replicate B	77.5	0.0	10.5	11.9	22.4
Average		77.1	0.0	10.8	12.1	22.8
3	Replicate A	66.8	0.0	17.2	12.3	29.5
	Replicate B	52.6	0.0	19.3	23.4	42.7
Average		59.7	0.0	18.3	17.9	36.1
5	Replicate A	37.8	0.0	38.9	21.6	60.5
	Replicate B	34.1	0.0	36.7	26.6	63.3
Average		36.0	0.0	37.8	24.1	61.9
7	Replicate A	56.9	0.0	26.6	15.7	42.3

	Replicate B	35.2	0.0	25.3	37.6	62.9
Average		46.1	0.0	26.0	26.7	52.6
9	Replicate A	37.7	0.0	37.2	22.6	59.8
	Replicate B	14.9	0.0	53.7	27.3	81.0
Average		26.3	0.0	45.5	25.0	70.4
12	Replicate A	21.4	0.0	16.2	58.6	74.8
	Replicate B	18.2	2.4	0.0	76.0	76.0
Average		19.8	1.2	8.1	67.3	75.4
Dark control						
1	Replicate A	99.9	0.1	0.0	NA	0.0
	Replicate B	99.9	0.1	0.0	NA	0.0
Average		99.9	0.1	0.0	NA	0.0
3	Replicate A	100.0	0.0	0.0	NA	0.0
	Replicate B	99.8	0.2	0.0	NA	0.0
Average		99.9	0.1	0.0	NA	0.0
5	Replicate A	99.7	0.3	0.0	NA	0.0
	Replicate B	99.6	0.4	0.0	NA	0.0
Average		99.7	0.4	0.0	NA	0.0
7	Replicate A	95.3	4.7	0.0	NA	0.0
	Replicate B	94.6	5.4	0.0	NA	0.0
Average		95.0	5.1	0.0	NA	0.0
9	Replicate A	98.9	1.1	0.0	NA	0.0
	Replicate B	97.3	2.7	0.0	NA	0.0
Average		98.1	1.9	0.0	NA	0.0
12	Replicate A	98.3	1.7	0.0	NA	0.0
	Replicate B	97.1	2.9	0.0	NA	0.0
Average		97.7	2.3	0.0	NA	0.0

[1)] Loss of radioactivity from solution during storage of light exposed samples assumed to be due to loss of CO_2; calculated as (% of dose in aqueous immediately after sampling)-(% of dose remaining in aqueous solution after storage and prior to HPLC analysis).
[2)] Calculated from % CO_2 from HPLC + % CO_2 from loss of activity attributed to CO_2 escape
NA = not applicable

Table 8.2.1.2-8: Degradation of [C3^{14}C]glyphosate in sterile natural water and the dark control (expressed as percent of applied radioactivity)

DAT		Glypho-sate (%)	AMPA (%)	D-2 2.3 min (%)	D-3 4 min (%)	Methane-diol (%)	Others	Ethylene glycol	NaOH	Total
Light exposed										
0	Replicate A	99.3	0.0	0.0	0.0	0.0	0.7	NA	NA	0.0
	Replicate B	99.3	0.0	0.0	0.0	0.0	0.7	NA	NA	0.0
Average		99.3	0.0	0.0	0.0	0.0	0.7	NA	NA	0.0
1	Replicate A	83.7	2.7	0.3	0.2	11.6	0.0	0.0	0.0	0.0
	Replicate B	85.0	3.5	0.6	0.3	10.9	0.0	0.0	0.0	0.0
Average		84.4	3.1	0.5	0.3	11.3	0.0	0.0	0.0	0.0
3	Replicate A	50.0	11.5	1.0	1.4	35.8	0.0	0.1	0.0	0.1
	Replicate B	55.5	10.6	1.1	1.3	32.9	0.2	0.0	0.0	0.0
Average		52.8	11.1	1.1	1.4	34.4	0.1	0.1	0.0	0.1
5	Replicate A	56.4	10.1	2.8	1.5	26.8	0.0	0.1	0.0	0.1
	Replicate B	31.9	14.9	3.6	2.3	44.9	0.0	02	0.1	0.3
Average		44.2	12.5	3.2	1.9	35.9	0.0	0.2	0.1	0.2
7	Replicate A	25.7	17.3	1.4	3.5	51.1	0.0	0.4	0.1	0.5
	Replicate B	25.3	18.8	1.5	3.3	49.2	0.0	0.3	0.4	0.7
Average		25.5	18.1	1.5	3.4	50.2	0.0	0.4	0.3	0.6
9	Replicate A	27.5	16.9	2.2	2.8	46.3	0.0	1.2	0.7	1.9
	Replicate B	25.5	17.2	3.4	2.5	50.3	0.0	0.6	0.2	0.8
Average		26.5	17.1	2.8	2.7	48.3	0.0	0.9	0.5	1.4
12	Replicate A	21.5	19.6	1.5	2.9	52.0	0.0	0.8	0.6	1.4

	Replicate B[1]	45.6	13.6	2.6	3.8	34.4	0.0	0.7	0.2	0.9
Average		21.5	19.6	1.5	2.9	52.0	0.0	0.8	0.6	1.4
Dark control										
3	Replicate A	98.2	0.0	0.0	0.0	0.0	1.8	NA	NA	0.0
	Replicate B	97.8	0.0	0.0	0.0	0.0	2.2	NA	NA	0.0
Average		98.0	0.0	0.0	0.0	0.0	2.0	NA	NA	0.0
7	Replicate A	92.3	5.2	0.6	0.0	1.7	0.2	NA	NA	0.0
	Replicate B	99.4	0.1	0.0	0.5	0.0	0.0	NA	NA	0.0
Average		95.9	2.7	0.3	0.3	0.9	0.1	NA	NA	0.0
12	Replicate A	95.7	0.6	0.3	0.0	0.9	0.1	NA	NA	0.0
	Replicate B	94.7	0.1	0.2	0.0	0.5	0.3	NA	NA	0.0
Average		95.2	0.4	0.3	0.0	0.7	0.2	NA	NA	0.0

NA = not applicable

[1] Outlier Not used for product distribution / half-life calculations.

B. MASS BALANCE

The material balance for the study was determined as the radiocarbon recovered in the aqueous samples, and as the sum of radiocarbon in the aqueous samples and volatile traps for those samples with continuous trapping of volatiles and is expressed as percent of applied radiocarbon based on aliquots of the dosing solution. Mass balance for irradiated samples averaged from 101.1 ± 2.9 % and 97.8 ± 1.6 % of the dose in distilled water and natural water containing [C1-^{14}C]glyphosate samples, respectively. In the [C3-^{14}C]glyphosate photolysis experiment, the average radiocarbon recovered in the light exposed samples following 12 days of continuous irradiation was 99.3 ± 1.5 % of the dose.

C. VOLATILE RADIOACTIVITY

The radioactivity trapped in the ethylene glycol (EG) trap (maximum of 1.2 % of the dose) was characterised as methanediol. Additionally, from the irradiation of [C3-^{14}C]glyphosate treated natural water, a maximum of 0.6 % of the applied dose was recovered in the NaOH traps. Treatment of the caustic traps with $BaCl_2$ resulted in very little precipitation of radioactivity as $Ba^{14}CO_3$, demonstrating that majority of the radioactivity collected in the caustic traps was not due to $^{14}CO_2$ but may have originated as a result of methanediol volatilization.

D. TRANSFORMATION OF THE TEST SUBSTANCE

Glyphosate is relatively stable to photodegradation in distilled water as expected based on its UV spectrum and represented >92% of the applied dose throughout the irradiation period. Significant degradation is observed in natural water when exposed to artificial light with mean values of 19.8 % AR and 21.5 % AR for C1- and C3 labelled glyphosate, respectively, and the end of the irradiation period. $^{14}CO_2$ was the major degradate observed in the [C1-^{14}C]glyphosate treated light exposed natural water samples and represented an average of 75.4 % of the dose following 12 days of continuous irradiation.

In the [C3-^{14}C]glyphosate experiments, AMPA and methanediol were observed at levels above 10%AR with maximum amounts of 19.6 % AR and 52.0 % AR, respectively, after 12 days of irradiation in test water treated with [C3-^{14}C]glyphosate.

The proposed pathway in the natural water experiments is indirect photodegradation of glyphosate, induced by active oxidising species (such as peroxides or hydroxyl radicals), which are known to form from the photolysis of natural humic acids present in natural waters. The photoinduced oxidation of glyphosate in natural water may proceed via N-hydroxylation, followed by dehydration of the hydroxylamine, hydrolysis and decarboxylation to obtain methanediol, AMPA and CO_2. AMPA is expected to undergo similar oxidative transformation because of its structural similarity to glyphosate. However, since the AMPA concentration gradually increased during the irradiation period, it can be concluded that its oxidation rate was slower than its rate of formation from glyphosate. Two minor degrades were also observed represented an average of 1.5 % and 2.9 % of dose, respectively, by the end of the photolysis period.

E. HALF-LIFE OF [^{14}C]GLYPHOSATE

The half-life of glyphosate was calculated using pseudo-first order kinetics based on hours of continuous irradiation (light exposed) or days of incubation (dark controls). Very little degradation was observed during photolysis of glyphosate in distilled water and in all dark control samples. Therefore, no meaningful degradation half-life could be calculated when small changes in the concentration of glyphosate were fitted to SFO kinetics.

The artificial photolysis half-life of glyphosate in natural water was determined as 128 and 126 hours (5.33 and 5.25 days) for [C1-^{14}C] and [C3-^{14}C]glyphosate treated samples, respectively, please refer to the table below. This is equivalent to 34.4 and 33.9 solar days in [C1-^{14}C] and [C3-^{14}C]glyphosate treated samples, respectively, based on Tokyo spring solar day irradiation.

Table 8.2.1.2-9: Determined DT_{50} and DT_{90} of glyphosate

Sample Set	Artificial Light (days)		Solar Days (Tokyo)		R^2
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	
Natural Water [C1-^{14}C]glyphosate Light Exposed	5.33	17.8	34.4	115	0.9247
Natural Water [C3-^{14}C]glyphosate Light Exposed	5.25	17.5	33.9	113	0.9208

III. CONCLUSIONS

Aqueous photolysis of glyphosate was studied using sterile distilled water (pure water) and natural water from Lake Herman, Benicia, CA and exposing a 1.0 µg/mL solution of [^{14}C]glyphosate to an artificial light source for up to 12 days of continuous irradiation. Glyphosate was relatively stable to photolysis in distilled water and represented > 92 % of the applied dose throughout the irradiation period. In contrast, glyphosate degraded rapidly in natural water when exposed to artificial light and represented 19.8 % and 21.5 % of the dose in [C1-^{14}C] and [C3-^{14}C]glyphosate samples, respectively, at the end of the exposure period. The major degradates detected during photolysis in natural water were CO_2 from the [C1-^{14}C]glyphosate (up to 75.4 % of dose), and AMPA and methanediol (up to 19.6 % and 52.0 %, respectively) from the [C3-^{14}C]glyphosate. The photo induced degradation half-life of glyphosate in natural water ranged from 33.9 to 34.4 solar days (Tokyo, spring) based on pseudo-first order kinetics.

Assessment and conclusion by applicant:

The use of distilled water instead of buffer solution for the direct photolysis test, does not have a significant impact on the outcome of the study as pH was measured.

Therefore, the experiment on direct and indirect photolysis is considered valid.

Assessment and conclusion by RMS:

Agrees with notifier conclusion. The use of distilled water has no impact on the reliability of the direct photolysis test. Results clearly indicate that no direct photolytic degradation occurred.

RMS notes that the indirect photolysis test was performed with 2 different labels, [C3-^{14}C] being the most relevant since the other label does not allow following the metabolites formation.

DT_{50} were determined based on pseudo first-order kinetics. A data gap is set for the applicant to update the kinetic analysis according to FOCUS guidance for light exposed systems with natural water (no degradation is observed in the dark controls and in the system with distilled water, then no update is deemed necessary in these cases). The study is considered acceptable.

[redacted], *1992*

Data point: CA 7.2.1.2/006

Report author	[redacted]
Report year	1992
Report title	Photodegradation study of [^{14}C]glyphosate in water at pH 5, 7 and 9
Report No	250751
Guidelines followed in study	U.S. EPA 540/9-82-021 Section 161-2 Photodegradation Studies in Water
Deviations from current test guideline	From OECD 316: - Impurities of up to 7 % were present at day 0.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate (N-(Phosphonomethyl)-glycine)

Lot No.: 185-ff-131

Specific activity: 0.9 mCi

Radiochemical purity: 95.8 %.

2. Test systems:

Since the test article may reversibly ionize within the pH range 5-9, photolysis was performed at three different pH's:

- 67.8 mL sodium acetate (0.1 mol/1) was combined with 32.2 mL acetic acid (0.1 mol/1) for a pH 5.1 buffer solution.
- 29.6 mL 0.1 N NaOH (0.1 mol/1) was combined with 50.0 mL monopotassium phosphate (0.1 mol/1) for a pH 7.3 buffer solution.
- 21.3 mL 0.1 N NaOH (0.1 mol/1) was combined with 50.0 mL boric acid (0.1 mol/1) for a pH 9.2 buffer solution.

To minimize buffer effects during incubation, the buffer solution was used at a final concentration of 0.01 mol/L. In a pre-test, based on the total amount needed, 130 mg glyphosate were dissolved in buffer solutions pH 5.1 and pH 9.2 at a concentration of 2.6 mg/mL. No significant pH-changes were observed, indicating that the addition of the test article did not affect the pH of the buffer solutions. Total plate counts (bacteria) were determined after 48 hours of exposure for 0, 7 and 15 days at every pH. The results indicated that microbial degradation did not play a significant role in the present study.

B. STUDY DESIGN

1. Experimental conditions

The incubation vessel with an aliquot of 200 mL buffer solution containing the test article (diameter: 9.5 cm; height: 6 cm) consisted of pyrex glass covered with a quartz glass-plate and was equipped with a septum to take samples by means of a Hamilton syringe. The system was continuously ventilated through a sterile filter with air (about 30 mL per minute) and pre-moistened by bubbling through a flask with sterile bidistilled water. The outcoming air was passed through a CO_2-trapping system (2N NaOH) and through 2-methoxy-ethanol at room temperature for absorption of volatiles. Since the occurrence of methylamine was assumed, the methoxy-ethanol trap was acidified with glacial acetic acid (2 %, v/v) from day l on. At the beginning of the incubation period, the depth of the buffer solution in the reaction vessel was about 2.82 cm.

For each buffer solution, in addition to the illuminated reaction vessel, a reaction vessel (diameter: 8.5 cm; height: 7 cm) with an aliquot of 150 mL sterile buffer solution containing the test article was incubated under identical conditions in the dark. The outcoming air was trapped through NaOH and 2-methoxy-ethanol/acetic acid at room temperature. At the beginning of the incubation period, the depth of each buffer solution in the corresponding reaction vessel was about 2.64 cm. Sterility of the test systems throughout the experiments was confirmed.

The study was performed in the Hanau Suntest apparatus which is equipped with a xenon burner (l.l kW) and an UV-filter (290 to 800 nm) with controllable irradiance between 400 W/m^2 and 765 W/m^2 to a preset value. Specimen Area was about 50 cm^2 per reaction vessel. Light Intensity was measured by means of a Lux-meter and ranged from 80-94 KLux which was comparable to the light intensity of natural daylight in the summer with vertical incidence of the sun on a clear, cloudless day (about 90-100 KLux). Irradiated samples were exposed to continuous irradiation.

The photolytis apparatus was set at a target temperature of 25 °C and cooled by means of a waterjacket connected to a waterbath. The actual temperature in the main test was monitored at regular time intervals. In the illuminated incubation vessels, the temperature during the first 7 days ranged from 24.5 - 24.8 °C. Due to disfunctioning of the cooling system at day 11 and an increase to at least 40 °C, the last illumination sampling interval (day 16) had to be repeated. Except for about 2 hours at day 11 (33.3 °C), the temperature for the repeated illumination during 15 days ranged from 24.5 - 24.7 °C. The temperature of the controls during 16 days of incubation ranged from 24.3 - 25.1 °C.

Based on a target specific radioactivity of 6 µCi/mg and an amount of 38.5 mg (including an excess of 10 %) three stock solutions were prepared for each pH. An amount of 1.2 mL (0.8 mg [^{14}C]glyphosate) was diluted with 39.6 mg (for pH 5.1), 39.4 mg (for pH 7.3) and 39.6 mg (for pH 9.2) unlabelled glyphosate, respectively. Each aliquot was made up to 20.0 mL with the respective buffer solution and determined by liquid scintillation counting (LSC).

2. Sampling

Aliquotes of 10 mL for irradiated samples and 5 mL for dark controls were taken at 0, 1, 2, 4, 7 and 16 DAT. The repeated illumination was incubated for 15 days. Appropriate aliquots (50 µl) were used to determine the amount of radioactivity. Remaining samples were stored at -20 °C until further analyses, performed within 11 weeks or 17 weeks for irradiated and dark control, respectively.

Except for day 0, at each time interval samples for ^{14}C-CO_2 and volatiles were taken for both test and control solution.

At the end of the incubation period, the incubation vessel was washed with acetone to dissolve possible precipitates and to exclude possible glass adsorption during incubation. Further, the remaining volume was noted. The difference as compared to the theoretical volume represented the amount of evaporation during incubation.

3. Analytical procedures

The radioactivity was determined on a Packard liquid scintillation. All values were corrected for instrumental background. Measurements were performed at least in duplicate. Additional characterisation of the radioactivity in the NaOH-absorption solutions of day 15 was obtained by precipitation of ^{14}C-CO_2, to barium carbonate in a subsample (5.0 mL) after addition of 15 mL bidistilled water and 20 mL saturated barium hydroxide solution. After centrifugation, the supernatant was counted and the amount of precipitated radioactivity was obtained by means of subtraction.

The aqueous samples of illuminated solutions were directly analysed by one-dimensional TLC. Aqueous samples of control solutions of days 0, 7 and 16 were analysed accordingly. TLC was performed on precoated plates of silica gel with a layer thickness of 0.25 mm or on precoated cellulose plates with a layer thickness of 0.50 mm. All non-labelled reference compounds were visualized on TLC-plates after moistening with ninhydrin-spray solution followed by heating at about 50 – 100 °C for about 10 minutes. The radioactive zones on TLC-plates were detected by using a Berthold Automatic TLC-Linear Analyser equipped with an Epson PC AX Processing System. Additionally, selected aqueous samples

were submitted to high performance liquid chromatography (HPLC) after appropriate dilution in 0.05 M potassium dihydrogen phosphate, pH 3.4.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in illuminated and dark control samples are summarised in the tables below for the test systems at pH 7.3, 5.1 and 9.2. In the illuminated solutions, the time interval at day 16 represented the results after transient elevated temperature and were therefore not further discussed.

Table 8.2.1.2-10: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 5.1 after exposure to artificial sunlight at various time intervals (values in % AR)

	Sampling Interval (Days)						
	0	**1**	**2**	**4**	**7**	**15 ***	**16**
Aqueous solution (pH 5.1)	100.0	100.0	102.9	100.4	99.3	94.0	97.6
^{14}C-CO_2-(NaOH-trapped)	n.d.	0.1	0.1	0.1	0.1	0.3	0.3
2-methoxy-ethanol-trapped**	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	100.1	103.0	100.5	99.4	94.3	97.9
TOTAL MEAN (except day 16)		99.5 ± 3.2					

n.d. : Not determined.
*: Repeated incubation, clear solution; somewhat less optimal total recovery assumed to be due to a CO_2-saturated NaOH trap.
**: With additionally 2 % acetic acid from day 1 on

Table 8.2.1.2-11: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 5.1 in the dark at various time intervals (values in percentage % AR)

	Sampling Interval (Days)					
	0	**1**	**2**	**4**	**7**	**16**
Aqueous solution (pH 5.1)	100.0	99.8	101.4	99.3	98.7	97.9
Cumulative volatiles						
^{14}C-CO_2 (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
2-methoxy-ethanol-trapped*	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	99.8	101.4	99.3	98.7	97.9
TOTAL MEAN		99.4 ± 1.3				

n.d.: Not determined.
*: With additionally 2 % acetic acid from day 1 on

Table 8.2.1.2-12: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 7.3 after exposure to artificial sunlight at various time intervals (values in % AR)

	Sampling Interval (Days)						
	0	1	2	4	7	15 *	16
Aqueous solution (pH 7.3)	100.0	101.2	102.2	99.3	97.1	95.8	97.2
^{14}C-CO_2-(NaOH-trapped)	n.d.	0.1	0.1	0.1	0.2	0.1	1.0
2-methoxy-ethanol-trapped**	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	101.3	102.3	99.4	97.3	95.9	98.2
TOTAL MEAN (except day 16)		99.2 ± 2.7					

n.d.: determined.
* Repeated incubation
**: With additionally 2 % acetic acid from day 1 on

Table 8.2.1.2-13: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 7.3 in the dark at various time intervals (values in % AR)

	Sampling Interval (Days)					
	0	1	2	4	7	16
Aqueous solution (pH 7.3)	100.0	101.2	102.8	100.8	100.4	97.9
Cumulative volatiles						
^{14}C-CO_2 (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	<0.05	0.5
2-methoxy-ethanol-trapped*	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	101.2	102.8	100.8	100.4	98.4
TOTAL MEAN		100.7 ± 1.6				

n.d.: Not determined
*: With additionally 2 % acetic acid from day 1 on

Table 8.2.1.2-14: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 9.2 after exposure to artificial sunlight at various time intervals (values in %AR)

	Sampling Interval (Days)						
	0	1	2	4	7	15 *	16
Aqueous solution (pH 9.2)	100.0	99.4	102.6	99.8	99.5	96.1	98.7
^{14}C-CO_2 (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	<0.05	0.1	0.2
2-methoxy-ethanol-trapped**	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	99.4	102.6	99.8	99.5	96.2	98.9
TOTAL MEAN (except day 16)		99.5 ± 2.3					

n.d.: Not determined.
*: Repeated incubation
**: With additionally 2 % acetic acid from day 1 on

Table 8.2.1.2-15: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 9.2 in the dark at various time intervals (values in % AR)

	Sampling Interval (Days)					
	0	1	2	4	7	16
Aqueous solution (pH 9.2)	100.0	100.8	104.3	103.6	102.7	96.7
Cumulative volatiles						
^{14}C-CO_2 (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	0.2	0.4
- 2-methoxy-ethanol-trapped: *	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	100.8	104.3	103.6	102.9	97.1
TOTAL MEAN		101.7± 2.9				

n.d.: Not determined.
*: With additionally 2 % acetic acid from day 1 on

Table 8.2.1.2-16: Degradation patterns of [^{14}C]glyphosate in aqueous samples at pH 5.1 after exposure to artificial sunlight and in dark control samples at various time intervals (values in % AR)

DAA/Identity	Irradiated/ dark	0	1	2	4	7	15
Parent	irradiated	95.5	93.0	94.3	86.9	80.8	70.7
	dark	93.0	-	-	-	92.6	90.7
AMPA	irradiated	1.8	3.2	4.1	5.8	10.1	16.0
	dark	2.0	-	-	-	3.3	1.9
Unknown M3	irradiated	2.7	3.8	4.5	7.7	8.4	7.3
	dark	5.0	-	-	-	2.8	5.3
Total	irradiated	100.0	100.0	102.9	100.4	99.3	94.0
	dark	100.0	-	-	-	98.7	97.9

n.d.: not detected; -: not determined

Table 8.2.1.2-17: Degradation patterns of [^{14}C]glyphosate in aqueous samples at pH 7.3 after exposure to artificial sunlight and in dark control samples at various time intervals (values in % AR)

DAA/Identity	Irradiated/ dark	0	1	2	4	7	15
Parent	irradiated	94.0	94.2	93.5	90.2	83.9	82.3
	dark	92.9	-	-	-	93.7	92.4
AMPA	irradiated	2.2	3.1	3.9	5.0	6.7	11.6
	dark	3.0	-	-	-	3.5	1.6
Unknown M3	irradiated	3.8	3.9	4.8	4.1	0.4	n.d.
	dark	4.1	-	-	-	n.d.	4.4
Unknown M4	irradiated	n.d.	n.d.	n.d.	n.d.	6.1	1.9
	dark	n.d.	-	-	-	n.d.	n.d.
Total	irradiated	100.0	101.2	102.2	99.3	97.1	95.8
	dark	100.0	-	-	-	100.4	98.4

n.d.: not detected; -: not determined

Table 8.2.1.2-18: Degradation patterns of [^{14}C]glyphosate in aqueous samples at pH 9.2 after exposure to artificial sunlight and in dark control samples at various time intervals (values in % AR)

DAA/Identity	Irradiated/ dark	0	1	2	4	7	15
Parent	irradiated	95.0	93.9	--	93.8	89.0	83.1
	dark	94.5	-	-	-	96.7	91.0
AMPA	irradiated	2.2	2.3	--	1.8	4.0	6.5
	dark	2.7	-	-	-	3.5	2.2
Unknown M3	irradiated	2.8	3.2	--	4.2	6.5	6.5
	dark	2.8	-	-	-	2.7	3.9
Total	irradiated	100.0	99.4	--	99.8	99.5	96.1
	dark	100.0	-	-	-	102.9	97.1

n.d.: not detected; -: not determined; --: not analysed due to sample loss

B. MASS BALANCE

During illumination, radioactivity was almost completely recovered at all time intervals and amounted, on average, to 99.2 + 2.7 % for pH 7.3. At pH 5.1, during illumination, recovery of radioactivity was virtually complete and amounted, on average, to 99.5 ± 3.2 %. During illumination at pH 9.2, radioactivity was almost completely recovered at all time intervals and amounted, on average, to 99.5 + 2.3 %. In dark controls, the mean total recovery ranged between 99.4±1.3 % AR and 101.7±2.9 % AR for all pH values.

C. VOLATILE RADIOACTIVITY

At pH 5.1, volatiles (0.3 %) were only trapped by NaOH from the illuminated solution. At pH 7.3 and 9.2, low amounts of radioactivity (0.1 - 0.5 %) were trapped by NaOH from both illuminated and control solutions.

No volatiles (<0.05 %) were trapped by 2-methoxy-ethanol/acetic acid in irradiated and dark control test system.

D. TRANSFORMATION OF THE TEST SUBSTANCE

At pH 7.3 and pH 9.2, the parent compound was degraded to a similar extend. At pH 5.1, degradation was somewhat more pronounced. At all time intervals, the major radioactive fraction was the parent compound.

The parent compound accounted for 93.0 % after 1 day at pH 5.1, thereafter, the amount of parent compound steadily decreased to 86.9 %, 80.8 % and 70.7 % after 4, 7 and 15 days of illumination, respectively. Accordingly, radioactive fraction AMPA steadily increased from 3.2 % at day 1 to 5.8 % at day 4, 10.1 % at day 7 and 16.0 % at day 15. Radioactive fraction M3 increased from 3.8 % at day 1 to 7.7 % at day 4. After 0, 7 and 16 days of incubation in the dark, besides the parent compound (90.7 - 93.0 % of the radioactivity applied), minor amounts of radioactive fractions AMPA and M3 were found, ranging from 1.9 - 5.0 %.

The amount of parent compound amounted to 94.2% after 1 day of illumination at pH 7.3. Radioactive fraction AMPA increased from 3.1 % (day 1) to 5.0 % (day 4). Radioactive fraction M3 remained constant and ranged from 3.9 - 4.8 %. In dark control, after 0, 7 and 16 days of incubation in the dark, besides the parent compound (92.4 - 93.7 % of the radioactivity applied), minor amounts of radioactive fractions AMPA and M3 were found at all time intervals, ranging from 1.6 - 4.4 %.

The parent compound remained constant up to 4 days (93.8 %) in the pH 9.2 irradiated buffer solution. Thereafter, it decreased to 89.0 % and 83.1 % at days 7 and 15, respectively. Accordingly, radioactive fraction AMPA increased from 1.8 % at day 4 to 4.0 % at day 7 and 6.5 % at day 15. Radioactive fraction M3 increased from 3.2 % at day 1 to 6.5 % at days 7 and 15. After 0, 7 and 16 days of incubation in the dark, besides the parent compound (91.0 - 96.7 as), minor amounts of radioactive fractions AMPA and M3 were found at all time intervals, ranging from 2.2 - 3.9 %.

At pH 7.3 and 5.1, the amount of AMPA accounted for more than 10 % of the radioactivity applied at the end of the illumination period (day 15). Radioactive fraction M3 occurred at minor amounts (below 9 %) at each time interval and every pH. Furthermore, only at pH 7.3 radioactive fraction M4 occurred in minor amounts (below 7 %) after 7 and 15 days of illumination.

After incubation in the dark at every pH, the parent compound was not degraded, i.e. the amount of parent compound from day 0 to day 15 did not decrease more than 3.5 %. Additionally, radioactive fractions AMPA and M3 were found in minor amounts (below 6 %) at every pH and at all time intervals.

E. KINETICS

After continuous illumination half-lives of 77, 69 and 33 days were obtained (regression analysis assuming first order kinetics) for the photolysis rate of [^{14}C]glyphosate at pH 9.2, 7.3 and 5.1, respectively.

III. CONCLUSIONS

The data demonstrated that after 15 days of continuous illumination (the equivalent of 30 days natural sunlight, 12 hours of light per day), the photolytic degradation of [^{14}C]glyphosate in aqueous solutions at pH 9.2, 7.3 and 5.1 proceeded with decreasing half-lives of 77, 69 and 33 days, respectively.

In the dark, at every pH the parent compound was not significantly degraded.

Low but significant amounts of radioactivity (0.1 - 0.5 %) were trapped by NaOH. At pH 5.1, volatiles were only detected in the illuminated solution, indicating that significant amounts of parent compound could be completely degraded at acidic pH 5.1 due to the process of photolysis. At pH 7.3 and pH 9.2, volatiles were also found in the corresponding dark controls, assuming an additional breakdown process at more alkalic pH-values (7.3 and 9.2). Volatile radioactivity mainly represented ^{14}C-$C0_2$, although at more acidic pH 5.1, additional volatile compounds may occur.

At all three pH-values, radioactivity was almost completely recovered (on average above 98 %) during illumination and in the dark controls.

After analyses of the illuminated aqueous solutions, at every pH, mainly parent compound was found at all time intervals. Radioactive fractions AMPA and M3 were common at every pH. Radioactive fraction M4 was exclusively found at pH 7.3.

The major radioactive fraction M2, characterised as AMPA, accounted at pH 7.3 and pH 5.1 for more than 10 % of the radioactivity applied at the end of the illumination period. All other degradation products (M3 and M4) occurred in minor amounts (below 9 %) at any time interval during illumination.

During incubation in the dark, radioactive fractions AMPA and M3 were detected in minor amounts (below 6 %) at every pH at all time intervals. Taking into account the occurrence of radioactive fractions AMPA and M3 in similar minor amounts already in the stock solution, no significant amounts of hydrolytic products of [^{14}C]glyphosate occurred in the aqueous solutions at various pH-values.

Finally, taking into account the sterility of the aqueous solutions and the elimination of the process of hydrolysis by means of the control values in the dark, the present data reflect merely the process of photolysis of [^{14}C]glyphosate in aqueous solution, mainly resulting in aminomethylphosphonic acid.

Assessment and conclusion by applicant:

The study was conducted mainly in line with the current guideline. At day 0 impurities of up to 7 % were present in the test systems but this does not have a serious impact on the results.

Therefore, the study it is considered valid.

Assessment and conclusion by RMS:

According to the review of [redacted] 2012 (CA 7.2.1.2/001), fractions identified as M3 and M4 in this study may not be distinct metabolites of glyphosate but rather chromatographic artefacts of the silica gel TLC method used in the study. No impact on the outcome of the study is expected.

DT75 was not reached during the study duration, however this is not considered as a deviation since the duration of the study (15 days continuous artificial sunlight, equivalent to 30 days natural sunlight) seems to be compliant with the maximum duration recommended in OECD 316 (30 days of sunlight exposure during an appropriate season and at an appropriate latitude).

SFO model was used, however the visual fits and statistical parameters are not available. A data gap is set for the applicant to update the kinetic analysis according to FOCUS guidance for the light exposed systems (no degradation is observed in the dark controls, then no update is deemed necessary in these cases). Moreover, the applicant should provide data on the equivalence between continuous artificial sunlight used in the study and natural sunlight conditions .

The study is acceptable.

[redacted], *1990*

Data point:	CA 7.2.1.2/005
Report author	[redacted]
Report year	1990
Report title	Degradation Study: Photodegradation of [^{14}C]glyphosate in Buffered Aqueous Solution at pH 5, 7 and 9 by Natural Sunlight
Report No	233W-1
Guidelines followed in study	U.S. EPA 161-2
Deviations from current test guideline	From OECD 316: - duration of the experiment exceeding slightly 30 days.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not previously evaluated
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate

Lot No.: C927-51B

Specific activity: 8.08 mCi/mmole

Radiochemical purity: 100 %

2. Test systems:

All water used in the preparation of buffer solutions was filtered using a Barnstead NANO-Pure II system which produces Type I Reagent Grade water per ASTM-D1193 (conductivity: 70fiS, dissolved solids: 30 ppm as NaCl, 27 ppm as $CaC0_3$).

- pH 5: Acetic Acid-Sodium Acetate: 146 mL of 0.1 M acetic acid added to 100 mL of 0.1 M NaOH and then deionized water added to a final volume of one liter.
- pH 7: Potassium Dihydrogen Phosphate-Disodium Hydrogen Phosphate: 22.4 mL of 0.1 M KH_2PO_4 was added to 25.8 mL of 0.1 M Na_2HP0_4 and then deionized water added to a final volume of one liter.
- pH 9: Sodium Borate-Hydrochloric Acid: 11.5 mL of 0.04 M HCl added to 125 mL of 0.01 M $Na_2B_4O_7$ and deionized water then added to a final volume of 250 mL.

All solutions were adjusted to the precise pH by addition of NaOH or HCl as indicated. The nominal ionic strength of each buffer solution was 0.01 M. The solutions were sterilised by filtering through a 0.2 micron Falcon filter and the pH was rechecked. The pH was also measured in the test samples and found to be stable throughout each of the study periods. Sterility of the test systems throughout the experiments was confirmed.

B. STUDY DESIGN

1. Experimental conditions

Sample tubes used for exposure of [^{14}C]glyphosate in pH 7 buffer to natural sunlight were made of quartz for the irradiated samples, those used for the dark control samples were made of pyrex. Individual pyrex tubes (8 mL) with teflon lined caps sealed with Parafilm were used for the pH 5 and 9 buffer solutions. All dark control samples were covered with aluminum foil to prevent irradiation. The sample tubes were placed in a distilled water bath at a 60 degree vertical angle to maximize irradiation during periods of strong sunlight intensity. The temperature in the water bath was maintained at approximately 25° C by continuous circulation. The temperature was continuously monitored and recorded at 20 minute intervals throughout the study.

Ethylene glycol and 10% NaOH were used to trap volatile organic compounds and CO_2, respectively in the pH 7 test. Air was drawn through sterilised bacterial filters into both the light and dark sample tubes and then into separate sets (light and dark) of three traps (1 EG, 2 10 % NaOH). Gas dispersion tubes were used to maximize the trapping efficiency. Trapping efficiency for $^{14}CO_2$ (100.9 %) was determined, using the identical system, by introducing a measured amount of ^{14}C-sodium bicarbonate (Sigma) as an aqueous solution into a sample holder and adding an excess (3 mL) of glacial acetic acid while air was being drawn through the system. $^{14}CO_2$ was trapped by two sodium hydroxide traps in series over a 2 day period. Volatiles were not trapped in the pH 5 and 9 samples.

Mean light intensity and daily total light energy ranged from 9953 µW/cm² to 16789 µW/cm² and 8.19 to 11.08 W min/cm² for the pH 7 test period. For pH 5 and pH 9 ranges of 7684 µW/cm² to 13897 µW/cm² and 6.5 to 11.08 W min/cm² were determined.

Application solutions for each pH were prepared by adding aliquots of [^{14}C]glyphosate to the sterilised buffer solutions. For pH 7, 144 µL was added to 300 mL buffer. For pH 5 and 9, 24 µl was added to 50 mL of each buffer solution. The resulting solutions were stirred. Aliquots (10 mL) of the pH 7 test solution were transferred into each sample holder using aseptic technique. Aliquots taken from the time 0 samples were averaged to determine the applied radiocarbon. Similarly, aliquots (5 mL) of the pH 5 and 9 test solutions were transferred to pyrex tubes. Aliquots from the stock solutions were used to determine the applied radiocarbon. The measured concentration of glyphosate in the pH 5, 7 and 9 solutions was 0.9, 0.9 and 0.8 ppm respectively.

2. Sampling

For pH 7, duplicate light exposed and dark control samples were removed from the water bath at 0, 5, 11, 17, 26 and 31 DAT. Volumes and pH were measured and the samples were analysed promptly. Aliquots of the samples were subjected to LSC in triplicate (3 x 50 µl). A separate rinse of the sample holders with approximately 2 mL of ammonium bicarbonate was analysed by LSC to determine if any radiocarbon had deposited on the walls. Total volumes in each gas dispersion trap were measured and aliquoted (3 x 0.5 mL) for radioassay (LSC) at each sampling time. Recovered radiocarbon from each trap was divided equally among the contributing samples.

Duplicate light exposed and dark control samples for the pH 5 and 9 samples were taken from the water bath at 0 DAT and 29 DAT. The volumes and pH were measured and the samples were analysed first by LSC then by HPLC.

3. Analytical procedures

Samples were analysed by LSC immediately following their removal from the water bath. All radioassays utilised 5 mL of scintillation cocktail in 7 mL standard polyethylene counting vials and Beckman LS 5000 CE liquid scintillation spectrometers.

Test and control samples were analysed by HPLC by direct injection of 100 µl of the aqueous samples typically within 48 hours of sampling. Chromatographic methods (HPLC) were validated with authentic standards achieving the necessary resolution and sensitivity. Both the UV and radiocarbon peak of glyphosate using the initial HPLC method were characteristically broad peaks. Selected samples were re-analysed with a second HPLC method that provided better resolution and peak shape. LOQ and LOD are described as 0.6 % AR 0.1 % AR, respectively.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate in pH 7 buffer under irradiation and respective dark controls and mass balance for pH 5 and pH 9 buffers are summarised below for the respective pH values.

Table 8.2.1.2-19: Material balance of [^{14}C]glyphosate in pH 7 buffer under irradiation with natural sunlight (expressed as percent of applied radioactivity)

Sample/Replicate	Buffer solution pH 7	Volatiles		Total Recovery
		CO_2	Ethylene glycol	
Hour 0				
Irradiated 1	100.8			100.8
Irradiated 2	100.6			100.6
Day 5				
Irradiated 1	92.2	0.2	0	92.4
Irradiated 2	92.3	0.2	0	92.5
Day 11				
Irradiated 1	100.2	0.2	0	100.5
Irradiated 2	100	0.2	0	100.2
Day 17				
Irradiated 1	102	0.3	0.1	102.4
Irradiated 2	97.6	0.3	0.1	98
Day 26				
Irradiated 1	96.5	0.3	0.3	97.2
Irradiated 2	92.6	0.3	0.3	93.3
Day 31				
Irradiated 1	86.9	0.4	0.5	87.8
Irradiated 2	98	0.4	0.5	98.9

Table 8.2.1.2-20: Material balance of [^{14}C]glyphosate in pH 7 buffer dark controls (expressed as percent of applied radioactivity)

Sample/Replicate	Buffer solution pH 7	Volatiles		Total Recovery
		CO_2	Ethylene glycol	
Hour 0				
Dark Control (I)	100.2	-	-	100.2
Dark Control (2)	98.4	-	-	98.4
Day 5				
Dark Control (1)	91.5	0.2	0	91.7
Dark Control (2)	90.1	0.2	0	90.3
Day 11				
Dark Control (1)	100.7	0.2	0	101
Dark Control (2)	93.8	0.2	0	94
Day 17				
Dark Control (1)	104.2	0.3	0.1	104.5
Dark Control (2)	98.1	0.3	0.1	98.5
Day 26				
Dark Control (1)	91.1	0.3	0.1	91.6
Dark Control (2)	90.7	0.3	0.1	91.1
Day 31				
Dark Control (1)	94.1	0.4	0.1	94.5
Dark Control (2)	93.1	0.4	0.1	93.6

Table 8.2.1.2-21: Degradation of [-^{14}C]glyphosate in pH 7 buffer solution irradiated with natural sunlight irradiation and dark controls (expressed as percent of applied radioactivity)

Compound	Replicate	DAT					
		0	5	11	17	26	31
Glyphosate	Irradiated 1	100.8	92.2	100.2	102.0	96.5	86.9
	Irradiated 2	100.6	92.3	100.0	97.6	92.6	98.0
	Dark Control (1)	100.2	91.5	100.7	104.2	91.1	94.1
	Dark Control (2)	98.4	90.1	93.8	98.1	90.7	93.1
CO2	Irradiated 1	-	0.2	0.2	0.3	0.3	0.4
	Irradiated 2	-	0.2	0.2	0.3	0.3	0.4
	Dark Control (1)	-	0.2	0.2	0.3	0.3	0.4
	Dark Control (2)	-	0.2	0.2	0.3	0.3	0.4
Unknowns	Irradiated 1	0.0	0.0	0.0	0.1	0.3	0.5
	Irradiated 2	0.0	0.0	0.0	0.1	0.3	0.5
	Dark Control (1)	0.0	0.0	0.0	0.0	0.1	0.1
	Dark Control (2)	0.0	0.0	0.0	0.0	0.1	0.1
Total recovery	Irradiated 1	100.8	92.4	100.4	102.4	97.1	87.8
	Irradiated 2	100.6	92.5	100.2	98.0	93.2	98.9
	Dark Control (1)	100.2	91.7	100.9	104.5	91.5	94.6
	Dark Control (2)	98.4	90.3	94.0	98.4	91.1	93.6

DAT: days after treatment

Table 8.2.1.2-22: Degradation of [-14C]glyphosate in pH 5 buffer solution under natural sunlight irradiation and dark controls (expressed as percent of applied radioactivity)

Compound	Replicate	DAT	
		0	29
Glyphosate	Irradiated 1	100.1	103.2
	Irradiated 2	99.8	100.9
	Dark Control (1)	100.3	100.9
	Dark Control (2)	99.8	100.9
Total recovery	Irradiated 1	100.1	103.2
	Irradiated 2	99.8	100.9
	Dark Control (1)	100.3	100.9
	Dark Control (2)	99.8	100.9

DAT: days after treatment

Table 8.2.1.2-23: Degradation of [-14C]glyphosate in pH 9 buffer solution under natural sunlight irradiation and dark controls (expressed as percent of applied radioactivity)

Compound	Replicate	DAT	
		0	29
Glyphosate	Irradiated 1	100.4	100.7
	Irradiated 2	101.1	100.9
	Dark Control (1)	99.2	99.7
	Dark Control (2)	99.3	98.3
Total recovery	Irradiated 1	100.4	100.7
	Irradiated 2	101.1	100.9
	Dark Control (1)	99.2	99.7
	Dark Control (2)	99.3	98.3

B. MASS BALANCE

Mass balance for pH 7 averaged 97.1±4.5 % AR and 95.7±4.7 % AR in light and dark samples, respectively. Radiocarbon recoveries based on solute measurements only for pH 5 averaged 101.0±1.5 % and 100.5±0.5 % for the light and dark samples, respectively. For pH 9, light and dark samples averaged 100.8±0.3 % and 98.8±0.6 % of the applied radiocarbon, respectively.

D. VOLATILE RADIOACTIVITY

Carbon dioxide at study end amounted to 0.4 % AR in buffer pH 7 for both, irradiated and dark control samples. Organic volatiles determined were ≤ 0.5 % AR for pH 7 buffer at the end of the study (31 DAT).

E. TRANSFORMATION OF THE TEST ITEM

Glyphosate degraded only minimally over the study period in any of the pH buffer test solutions. No difference in degradation was observed for the separate buffer solutions tested over the study period.

F. KINETICS

The extrapolated half-life (regression analysis assuming first-order kinetics) of light exposed and dark control samples in pH 7 buffer was determined in the report as 413 days (R^2=0.15) and 555 days (R^2=0.09), respectively.

III. CONCLUSIONS

[^{14}C]glyphosate degrades very slowly in pH 5, 7 or 9 buffer solutions when exposed to natural sunlight for up to 31 days. The extrapolated half-life of light exposed and dark control samples in pH 7 buffer is 413 days (R^2=0.15) and 555 days (R^2=0.09) respectively. The poor correlation coefficients reflect the minimal amount of degradation observed during the study period with respect to the long half-life. No significant difference in material balance or degradation is detected among pH 5, 7 or 9 samples. These results indicate that photodecomposition is a minor process for degradation of glyphosate in the environment.

Assessment and conclusion by applicant:

The test systems were exposed to natural sunlight instead of artificial light. Nevertheless, the study indicates that photodegradation is a minor process in aquatic degradation process.

Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

The duration of the experiment exceeded slightly 30 days but it is considered as a minor deviation.

Test items were exposed to natural sunlight. As recommended in the OECD guideline 316, information relative to temperature (minimum, maximum and mean), light intensity, total light energy and cloud cover are available in the study report. In addition, events including sunrise and sunset

times for each day throughout the study periods are also available. The temperature of the water bath was measured continuously and recorded at 20 minute intervals throughout the study.

The study is acceptable.

[redacted], *2001*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation (TMS) are not reported below for easiness of reading.

Data point:	CA 7.2.1.2/003
Report author	[redacted]
Report year	2001
Report title	Glyphosate trimesium Determination of the rate of photolytic degradation in natural water under laboratory conditions
Report No	ZCA/069
Guidelines followed in study	Requirements of Japanese Ministry of Agriculture, Forestry and Fisheries Guideline: Photolysis of a Pesticide in Water
Deviations from current test guideline	From OECD 316: - No traps for volatiles. - Only the test substance was quantified in test solutions, transformation products were not assessed. - Radiochemical purity of [^{14}C-PMG]glyphosate is below 95%.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not previously evaluated
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C-PMG]glyphosate trimesium

Batch.: 00-J12

Specific activity: 2.04 GBq/mmol

Radiochemical purity: 94.5 %

2. Test system:

The natural water used to prepare the samples was from the Great River Ouse, Huntingdon, and Cambridgeshire, UK and was stored at +4 °C until required. The temperature, pH and oxygen saturation of the water was measured at the time collection. The pH was in a range of 7.8 and 8.3 through the study.

Table 8.2.1.2-24: Characterisation of the natural river water

pH[a]	7.75
pH[b]	7.84
Oxygen saturation (%)[a]	70.1
Oxygen saturation (%)[c]	87.8
Oxygen saturation (%)[d]	85.4
Temperature (°C)[a]	11.7
Electrical conductivity (µS/cm)[b]	60 ± 117
Suspended solids (g/L)[b,e]	0.0036 ± 0.0021
Total residue on evaporation (g/L)[b,d]	0.50 ± 0.032

[a] Measured at the time of collection.
[b] Measured in the laboratory following filtering and sterilisation.
[c] Measured prior to addition of [^{14}C]trimesium.
[d] Measured prior to addition of [^{14}C]glyphosate.
[e] Mean of three replicates determinations.

B. STUDY DESIGN

1. Experimental conditions

- The test was conducted in sterile natural water treated with [^{14}C-PMG]glyphosate.

First the natural water was filtered through a 212 µm filter to remove large particulate matter prior to filtration through Whatman Grade 5 filter paper to remove further particulate matter. The filtered water was stored at +4 °C in the dark when not in use. Water was stored for no longer than two months. Sterile water was aseptically dispensed into a sterile plastic bottle. An aliquot of the prepared stock solution of [^{14}C-PMG]glyphosate was added and the solution mixed by inversion to obtain a nominal concentration of 2 mg/L.

Aliquots of the test solutions (20 mL) were transferred into each of the 22 pre-weighed, sterile photolysis and dark control vessels. The photolysis and control vessels were then capped and re-weighed to determine the exact weight of test solution dispensed. Aliquots (1 mL) of the test solution were taken and radioassayed. The actual application rate was determined as 2.00 µg/mL for [^{14}C-PMG]glyphosate.

The samples to be irradiated were placed in the Suntest apparatus and irradiation started. The study was conducted using a Suntest Accelerated Exposure Unit (Heraeus Equipment Ltd, Brentwood, Essex, UK) fitted with a xenon arc light source. A system of mirrors and filters prevented ultra-violet radiation with a wavelength of less than 290 nm from reaching the test solutions. Light intensity (irradiance) measurements were made at five representative positions in the Suntest apparatus at the beginning and end of the irradiation period over the wavelength range 250 – 800 nm. The measurements were integrated to provide the total light intensity over the wavelength range 300 – 400 nm. A mean value was determined and then used to calculate the equivalent time of irradiation of natural Tokyo spring sunlight (latitude 35 °N) received by each test solution. Continuous irradiation was used for the irradiated samples.

Irradiated test solutions were maintained within the range 25 ± 2 °C and were stirred continuously. Control vessels were maintained in darkness in a temperature controlled growth room within the range 25 ± 2 °C, and were oscillated continuously.

2. Sampling

Duplicate test solutions were taken for analysis immediately after test substance application and provided a zero-time analysis for both irradiated and dark control experiments. Duplicate irradiated and single non-irradiated treated solutions were taken for analysis at approximately 0.5, 1, 1.5, 2, 2.5 and 3 days after application for [^{14}C-PMG]glyphosate. Three further treated samples were taken, one at zero-time and one irradiated and one dark control at the final sample time for sterility testing.

3. Analytical procedures

Measurement of Radioactivity was conducted by liquid scintillation counting, using liquid scintillation counters with automatic quench correction. Twice the background was considered as the limit of accurate determination.

High Performance Liquid Chromatography with radiodetection was carried out for analysis of [^{14}C-PMG]glyphosate samples. For quantitative analysis, following sample injection, 1 minute fractions of column eluate were collected and radioassayed. The proportion of the total net eluted radioactivity in each fraction was calculated, as well as the recovery of radioactivity from the column. The proportion of [^{14}C-PMG]glyphosate in test solutions was derived from this data using only those fractions greater than or equal to twice the background value. Normal phase TLC was carried out to provide confirmatory quantitative data for representative [^{14}C]glyphosate samples.

II. RESULTS AND DISCUSSION

A. DATA

Recoveries of radioactivity of PMG glyphosate, respectively in irradiated and dark control samples are summarised below.

Table 8.2.1.2-25: Total radioactivity and concentration of [^{14}C]glyphosate (PMG) in irradiated and dark control test solutions (expressed as % AR)

DAT	Equivalent duration[1] (days)	Irradiated		Dark control	
		Total radioactivity	PMG	Total radioactivity	PMG
0	0	98.1	93.3	-	-
		97.6	94.1		
0.5	2.6	99.7	68.3	100	92.9
		99.8	67.2		
1	5.5	100	59.2	101	93.8
		100	54.7		
1.5	8.1	99.4	42.5	99.8	90.9
		99.3	42.5		
2	10.9	100	42.0	101	93.4
		100	35.3		
2.5	13.8	100	30.9	101	93.0
		99.9	28.4		
3	16.1	99.3	26.0	99.3	92.6
		98.6	24.2		

[1] for Tokyo at latitude 35 °C spring sunlight

Table 8.2.1.2-26: Photodegradation Half-Life of [^{14}C-PMG]glyphosate in Natural Water

Sample Set	Artificial Light (days)	Solar Days (Tokyo)	R^2
	DT_{50}	DT_{50}	
Natural Water	1.6 (35.5 hours)	8.8	0.98

[a] No significant degradation was observed after irradiation to > 30 days

B. MASS BALANCE

[^{14}C-PMG]glyphosate material balances ranged from 97.6 to 100 % of applied radioactivity.

C. TRANSFORMATION OF THE TEST SUBSTANCE

At zero-time, [^{14}C-PMG]glyphosate accounted for a mean of 93.7 % AR. After 5.5 days equivalent of Tokyo spring sunlight at latitude 35 °N [^{14}C-PMG]glyphosate accounted for a mean of 57.0 % AR. This declined further to a mean of 25.1 % AR after 16.1 days sunlight equivalents. This compared to a 92.6 % AR in the terminal dark control sample treated with this radiolabel.

D. KINETICS

Assuming first order kinetics, the estimated DT_{50} value for [^{14}C-PMG]glyphosate in natural river water was reported as 38.5 hours equivalent to 8.8 days of natural spring sunlight in Tokyo (latitude 35 °C). The correlation coefficient (r^2) of the data was 0.98. There was no significant degradation of [^{14}C-PMG]glyphosate in dark control solutions.

III. CONCLUSIONS

The phosphonomethyl anion of glyphosate trimesium is photolytically labile and degraded in natural river water under sterile conditions with a DT_{50} of approximately 8.8 days of natural spring sunlight in Tokyo (latitude 35°N).

Assessment and conclusion by applicant:

The study describes the indirect photodegradation rate of glyphosate trimesium in sterilised natural water. Samples were analysed for glyphosate only. The study is considered as supportive information.

Assessment and conclusion by RMS:

Radiochemical purity is slightly below 95%.

No complete mass balance can be established since there was no traps for volatiles. However, the radioactivity recovered in the aqueous solutions is good (≥97.6% AR), indicating that no significant formation of volatiles occurred.

Only glyphosate was analyzed; there was not attempt to analyze the degradation products.

Therefore the study cannot be used to describe the route of degradation under indirect photolytic degradation. However it gives supportive information regarding the rate of indirect photolytic degradation.

The study is considered as supportive.

[redacted], *2012*

Data point:	CA 7.2.1.2/001
Report author	[redacted]
Report year	2012
Report title	Review of Direct and Indirect Photolysis of Glyphosate
Report No	MSL0024051
Guidelines followed in study	None
Deviations from current test guideline	Not applicable (review report). Only aqueous photolysis aspect of study is summarised
GLP/Officially recognised testing facilities	Not applicable
Previous evaluation	Not previously evaluated (submitted in AIR2 but not evaluated)
Acceptability/Reliability:	Supportive

I. INTRODUCTION

In an expert statement, results of several aqueous photolysis studies are summarised and the impact on direct and indirect photolytic process on the degradation of glyphosate are discussed.

Photodegradation of pesticides in the environment can occur either by direct or indirect absorption of light. A prerequisite for direct photolysis of pesticides is its ability to absorb light at wavelengths equal or greater than 290 nm to reach excited electronic states. The excited species then may undergo chemical transformations. In contrast, during indirect photolysis, light energy is absorbed by other substances in soil or water and the excited species can then transfer the energy to a pesticide, undergo electron transfer with the pesticide, or form highly reactive species which may enter into a series of reactions with pesticides. Glyphosate does not absorb light significantly at wavelengths longer than 230 nm. Thus, in highly purified sterile water, in which direct photolysis is the only mechanism for photo-transformation, glyphosate is expected to be photo-stable. Indeed, aqueous photolysis studies have shown that glyphosate is relatively stable to photodegradation in distilled water; confirming that it does not absorb incident radiation directly as expected based on its UV spectrum. However, photo-induced degradation of glyphosate can occur in water under certain conditions. Studies using artificial light and solutions containing calcium ions show that glyphosate is susceptible to a slow indirect photodegradation. Similarly, under intense artificial light, glyphosate in natural river water degrades via oxidative transformation induced by photochemical excitation of humic acids as reported for other pesticides. Naturally-occurring organic and inorganic solutes such as humic acid, tryptophan, tyrosine, organic peroxides, and various metal ions are known to absorb strongly in the ultraviolet and visible region to form reactive species such as singlet molecular oxygen, hydrogen peroxide, hydroxyl radical, organic peroxyradicals, and other free radicals. These photooxidants then could react with oxidizable compounds like glyphosate in the aqueous environment.

Although indirect photodegradation of glyphosate in water can occur, under normal environmental conditions photolysis is expected to be a slow process and compared to microbial degradation is, at most, a very minor pathway for the degradation of glyphosate in the environment. Several glyphosate

aqueous photolysis studies have been conducted. Two studies were conducted in distilled water utilising artificial light and two more recent studies were conducted in natural water using artificial light in one and natural sunlight in the other study. The results of these studies are discussed below.

II. DISCUSSION OF PHOTOLYSIS STUDIES

1. Direct photolysis

[redacted] ***(1990), please refer to CA 7.2.1.2/005 (supportive in this submission)***

The rate of photodegradation and the nature and extent of formation of degradation products of glyphosate in pure sterile pH 5, 7, and 9 aqueous buffers were investigated in this study.

No ^{14}C-activity was detected in the ethylene glycol traps at levels greater than 0.5 % of the applied. The amount of ^{14}C-activity evolved as $^{14}CO_2$ from the irradiated solutions was minimal and was approximately the same as that evolved from the non-irradiated solutions. In addition to minor amounts of CO_2, no other degradation product of glyphosate was detectable in the pH 5, 7, and 9 buffer solutions after 31 days of irradiation. The fluctuation in glyphosate concentrations in both light exposed and dark control samples during various sampling intervals is not indicative of glyphosate degradation but rather is attributed to variations in mass balance data due to normal errors in radioactivity measurement by liquid scintillation counting. This study suggests that in the purified sterile water glyphosate is not susceptible to photodegradation.

[redacted] ***(1992), please refer to CA 7.2.1.2/006 (valid in this submission)***

In this study, the rate of photolysis of [^{14}C]glyphosate in aqueous buffers at pH 5.1, 7.3, and 9.2 under the influence of simulated, artificial sunlight was investigated. TLC on cellulose and HPLC analyses of illuminated aqueous buffers at all pHs showed glyphosate as a major constituent and AMPA as a minor product. No other significant unknown degrades were detected by analyses at any sampling point.

The TLC immobile radioactive fraction was detected at all sampling points including day zero and in the [^{14}C]glyphosate dosing solution as well. The immobile radioactive fraction detected near the origin of the TLC plate (TLC Rf value in the range of 0.00-0.02) was assigned by the authors of the report as M4 unidentified fraction and those which were slightly more mobile relative to M4 (TLC Rf values in the range of 0.02-0.19), was assigned as M3 unidentified fraction we now believe that fractions identified as M3 and M4 in this study are not distinct metabolites of glyphosate but rather are chromatographic artefacts of the silica gel TLC method used in the study. It is stated that the relatively immobile radioactive fractions observed in this study are glyphosate and AMPA which are strongly and reversibly binding to the polar surface of silica gel causing the smear of the radioactivity in the TLC plates. The binding of glyphosate and AMPA to silica gel and other minerals and organic matter has been widely reported and is consistent with the highly polar nature of these molecules.

Taking into account the lack of significant degradation of glyphosate during the 15-day photolysis period and the fact that AMPA was also detected in the non-irradiated control samples, coupled with the results from the aqueous photolysis study conducted by [redacted], it is concluded that glyphosate is stable to direct photodegradation in purified sterile water. This conclusion is consistent with the fact that glyphosate does not absorb incident radiation based on its UV spectrum.

2. Indirect photolysis

[redacted] ***(1978), please refer to CA 7.2.1.2/008 (invalid in this submission)***

The rate of aqueous photodegradation of [^{14}C]glyphosate was determined in natural water, purified natural water, and deionized water fortified with $CaCl_2$.

Extensive photodegradation of [^{14}C]glyphosate to AMPA was found in this study. In natural water, irradiation for 14 and 21 days resulted, respectively, in 58.4-68.8 % and 78.6-86.7 % degradation of glyphosate to AMPA, compared to 5.8-9.8 % and 7.2-13.5 % degradation in the non-irradiated controls. Carbon dioxide evolution accounted for 0.5 % of the applied activity after 21 days of irradiation. Degradation of glyphosate to AMPA was 67.1 and 78.1 % after 14 days of irradiation in deionized water containing 3 and 30 ppm $CaCl_2$, respectively. In contrast, only 38.3 % degradation of glyphosate to AMPA occurred in purified natural water after irradiation for 14 days. The purified natural water

contained 0.4 ppm $CaCl_2$ compared to 26.0 ppm for unpurified natural water. These results indicate that calcium sensitises the photodegradation of glyphosate to AMPA in water.

[redacted] (2005), please refer to CA 7.2.1.2/002 (valid in this submission)

In [redacted] (2005), the aqueous photolysis of glyphosate was studied using test substances labelled with 14C in either the glycine portion of the molecule [C1-^{14}C]glyphosate, or the phosphonomethylene carbon of the molecule, [C3-^{14}C]glyphosate.

Consistent with the previous studies, glyphosate was stable to photolysis in distilled water and showed low degradation throughout the 12 days of continuous irradiation. However, in natural water, glyphosate degraded rapidly when exposed to artificial light and represented an average of 19.8 % and 21.5 % of the dose in [C1-^{14}C] and [C3-^{14}C]glyphosate labelled photolysis experiments, respectively, following 12 days of continuous irradiation. In [C1-^{14}C]glyphosate photolysis experiments, the main degradate detected was $^{14}CO_2$, which represented an average of 75.4 % of dose at the end of the exposure period. In the [C3-^{14}C]glyphosate experiments, aminomethylphosphonic acid (AMPA) and methanediol were the main degradates detected and represented 19.6 % and 52.0 % of the dose, respectively, at the end of the exposure period. Glyphosate was relatively stable in dark control samples in both test systems and represented > 92 % of the dose throughout the incubation period for all sample sets. The photo-induced degradation half-life of glyphosate in natural water ranged from 33.9 to 34.4 solar days based on pseudo-first order kinetics.

Degradation of glyphosate in natural water when exposed to artificial light is proposed to proceed via oxidative transformation induced by photochemical excitation of humic acids in natural water. A mechanistic pathway as described below is based on detailed mechanistic work conducted on the oxidation reaction of glyphosate and glycine with sodium hypochlorite, conducted by the author of this report. It is proposed that photooxidation of glyphosate is induced by active oxidising species (such as peroxides or hydroxyl radicals), which are known to form from the photolysis of natural humic acids present in natural waters. Oxidative breakdown of glyphosate with hydrogen peroxide and sodium hypochlorite to form methanediol, glycine, and AMPA are described as having been previously reported. Further, reference to the degradation of glyphosate in the presence of Mn(II) and molecular oxygen is made, suspected to occur in the dark via intramolecular electron transfer mechanism.

The photoinduced oxidation of glyphosate in natural water may proceed via N-hydroxylation, followed by dehydration of the hydroxylamine, to form Imines I and II depending on which hydrogen is eliminated from glyphosate. Imines are known to hydrolyse rapidly under the reaction conditions, and hydrolysis of Imines I and II would lead to formation of glycine and AMPA, methanediol, orthophosphoric acid, and CO_2. The detection of significant amounts of AMPA in the photolysis experiment coupled with the lack of glycine detection suggests that Imine I was formed preferentially under the reaction conditions.

AMPA and glycine are expected to undergo similar oxidative transformation because of their structural similarities to glyphosate. However, since AMPA concentration gradually increased during the irradiation period, it can be concluded that its oxidation rate was slower than its rate of formation from glyphosate.

Methanediol is the hydrated form of formaldehyde in dilute aqueous solutions. Methanediol is present in the environment from both natural and non-natural sources and is derived from natural metabolic processes as well as combustion processes (automobile exhaust or burning of wood) and building materials. Large quantities of methanediol are formed in the troposphere by the oxidation of hydrocarbons. Methanediol is stated to be a metabolic intermediate involved in one-carbon metabolic processes and several publications on the presence of methanediol in plants and drinking are referenced. Reference is further made to the fact that production of methanediol under certain laboratory conditions is not unique to glyphosate and would also be expected from the oxidative fragmentation of many carbon containing small molecules, amino acids, and other natural organic compounds such as humic and fulvic acids. In the environment, formaldehyde/methanediol is rapidly metabolized in soil or water by bacteria and in the air by oxidative photolytic processes. Therefore, it is not expected that photoinduced oxidation of glyphosate will result in any additional accumulation of methanediol in the environment.

III. CONCLUSIONS

The outcome of several aqueous photolysis studies is summarised in this review report. Further, the impact on direct and indirect photolytic process on the degradation of glyphosate are discussed. Direct photolysis as assessed in sterile buffer solutions under standard conditions are considered to be negligible, whereas an impact of indirect photolysis in natural waters to the degradation of glyphosate in the environment was found.

Assessment and conclusion by applicant:

The review plus expert statement provides a concise overview of the impact of direct and indirect photolysis on the transformation processes of glyphosate observed in the studies considered. As such the review is regarded as supportive information.

Assessment and conclusion by RMS:

Agrees that this is a supportive study. The studies mentioned in this review were submitted in the renewal dossier and are summarized and assessed by RMS. Please note that only the studies [redacted] 2005, [redacted] 1992 and [redacted] 1990 are considered as acceptable by RMS.

The conclusion that direct photolysis cannot be important for glyphosate since the substance does not absorb light in the right wavelength spectrum, but that there are indications for indirect photochemical degradation is consistent with results from acceptable studies.

[redacted], *1992*

Glyphosate – Trimesium was applied in this study. As previously explained, the results for the trimesium cation (TMS) are not reported below for easiness of reading.

Data point:	CA 7.2.1.2/004
Report author	[redacted]
Report year	1992
Report title	Glyphosate-Trimesium – aqueous photolysis
Report No	RR91-065B
Guidelines followed in study	U.S. EPA 161-2
Deviations from current test guideline	From OECD 316: - No traps for volatiles. - LOQ not specified. - Test systems were sterilised but sterility was not confirmed
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate trimesium, glyphosate (phosphono[^{14}C]methyl)labeled, [^{14}C]PMG

Lot No.: WRC 13269-04

Specific activity: 56 Ci/mol

Radiochemical purity: 95 %

2. Buffers:

Phosphate buffer solutions (25 mM, pH 7) were prepared from potassium dihydrogen phosphate and sodium hydroxide using distilled water. The pH was adjusted to 7.00 ± 0.05 with sodium hydroxide. Buffer solutions were then autoclaved at 121 °C for 1 hour.

B. STUDY DESIGN

1. Experimental conditions

Photolysis tubes (1 cm x 10 cm) consisted of cylindrical quartz tubes. Each tube had a tapered ground-glass joint fitted with a polytetrafluoroethylene (PFTE) stopper. Irradiated and dark control samples were placed in a separate tank covered with aluminium foil to exclude light. Distilled water was then poured into the photolysis chamber covering all test samples. The photoreactor was a stainless steel chamber closed with a quartz window at the top and equipped with a cooling system keeping test solutions at approximately 25 ± 1 °C during irradiation. The temperature of samples was monitored with a thermocouple inserted into a photolysis tube filled with distilled water and a recirculating water bath was used to control the temperature of test solutions. Sterility of the test systems throughout the experiments was not confirmed.

For each test, the glyphosate-trimesium stock solution was prepared by adding radiolabelled glyphosate-trimesium in water to a sterilized volumetric flask (100 or 200 mL) and filled up with pH 7 buffer. The resulting solution was filtered through a sterile 0.2 µm filter in a laminar flow hood. A 3 to 7 mL aliquot of the filtered glyphosate-trimesium stock solution was added to each photolysis and dark control photolysis tube. The concentration of glyphosate-labelled test substance was 186 mg/L (as glyphosate-trimesium), which consisted of 182.3 mg/L of non-labelled and 3.3 mg/L of labelled glyphosate-trimesium. The radioactivity concentration was 1690 and 1677 dpm/µL in irradiated and dark control samples, respectively.

The photolysis chamber was irradiated continuously under a Heraeus Suntest xenon arc lamp. The lamp output was collimated with aluminum parabolic reflectors. UV filters were used to remove wavelengths below 290 nm. A spectroradiometer (spectral range 300 – 850 nm) was used to measure the light intensity and emission spectrum of the xenon arc lamp inside the chamber. The integrated xenon light intensity over the wavelength range 300 – 800 nm was measured at least at beginning and end of the study. The averaged intensity was used to calculate the sunlight equivalent received by samples. The local solar spectrum at Richmond, CA (latitude 37° 56´N) was similarly measured for comparison of solar and xenon lamp emission spectra. Three and two measurements of light intensity were averaged for anion and cation labelled glyphosate trimesium. The integrated light intensities for the samples treated with glyphosate labelled test substance over 13.6 days of continuous artificial light were equivalent to 29.3 days of natural sunlight.

2. Sampling

Duplicate tubes of photolysed and dark control samples were withdrawn on days 0, 1.7, 4.0 6.7, 8.7, 11.8 and 13.6 for all [^{14}C]glyphosate irradiated samples. Dark control samples for [^{14}C]glyphosate were sampled on days 0, 2.0, 5.9, 8.1, 10.0, 11.9 and 13.9.

3. Analytical procedures

Photolysis and dark control samples were analysed by directly injecting the solutions into HPLC. HPLC fractions were collected in scintillation vials after the eluent was mixed with scintillation cocktail. Later the radioactivity in each fraction was measured by LSC. The radioactivity in the solutions was measured by counting 20 or 25 mL aliquots of each sample solution. All components were analysed relative to the radioactivity in solution at the start of the test, which served as a check on losses by volatilisation, sorption, or precipitation of test substance or products. Analysis by TLC was performed as confirmatory method.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/radiodetection method were not reported.

4. Calculations

The pseudo first-order rate constant was determined from the slope of a line generated by a linear least-squares fit of the natural logarithm of the glyphosate-trimesium concentration versus time.

The net pseudo first-order photolytic DT_{50} was calculated as:
$t_{1/2} = \ln 2 / (k_i - k_d)$

Where
$t_{1/2}$ = net photolysis DT_{50}
k_i = pseudo first-order rate constant for the irradiated samples
k_d = pseudo first-order rate constant for the dark control samples

As glyphosate was stable in the dark controls, the above equation can be simplified to
$t_{1/2} = \ln 2 / k_i$

The integrated light intensities (300 – 800 nm) were calculated as follows:

$I_{ave} = (I_0 + \ldots I_f) / n$

Where
I_0 = integrated intensity (watt/m^2) measured at the beginning of irradiation period
I_f = integrated intensity (watt/m^2) measured at the end of irradiation period
n = number of intensity measurements
I_{ave} = average intensity during the test

The amount of radiation received from the continuous exposure for time t (days) can be converted to natural summer equivalent days (SED) with the following equation:

$SED = (I_{ave} \times 24 \times t) / 5030$

Where
5030 = the averaged daily sunlight irradiation measured for three consecutive days (June 21-23, 1988) at Richmond, CA
I_{ave} = average intensity during the test

II. RESULTS AND DISCUSSION

A. DATA

Radioactivity measurements for glyphosate labelled test substance in irradiated and dark control samples are summarised below.

Table 8.2.1.2-27: Degradation of glyphosate labelled glyphosate trimesium (^{14}C-PMG) and metabolites in irradiated test solutions (expressed as % of applied radioactivity)

Sample point	Irradiation time (days)	Solar sunlight equivalent (days)	% AR			
			^{14}C-PMG	AMPA	Unassigned radioactivity	Mass balance[1]
0	0	0	94.0	4.0	1.6	100
			97.3	4.1	2.1	104
1	1.7	3.8	95.5	6.9	2.4	105
			97.3	6.6	1.5	105
2	4.0	8.6	93.5	7.2	2.5	103
			90.3	9.4	3.2	103
3	6.7	14.4	80.8	12.0	3.7	96
			87.7	12.7	4.5	105
4	8.7	18.6	83.2	11.6	3.6	98
			86.3	9.0	4.1	99
5	11.8	25.3	82.9	12.6	4.7	100
			80.0	10.7	4.7	95
6	13.6	29.3	75.8	18.1	6.1	100
			71.2	18.9	6.9	97
Average						**100.8**
Standard deviation						**3.4**

[1] Total radioactivity collected after each HPLC injection expressed as a percent of the initial radioactivity per unit volume multiplied by the sample loop volume.

Table 8.2.1.2-28: Degradation of glyphosate labelled glyphosate trimesium (^{14}C-PMG) and metabolites in dark control test solutions (expressed as % of applied radioactivity)

Sample point	Sampling dates (days)	% AR			
		^{14}C-PMG	AMPA	Unassigned radioactivity	Mass balance[1]
0	0	93.0	3.9	2.1	99
		93.7	4.0	2.0	100
1	2.0	93.0	3.2	1.6	98
		94.7	3.4	1.5	100
2	5.9	94.5	3.6	1.2	99
		97.7	4.3	3.6	106
3	8.1	89.5	3.4	1.3	94
		94.5	3.8	1.7	100
4	10.0	92.4	3.3	0.9	97
		94.7	3.6	1.6	100
5	11.9	95.9	3.6	1.6	101
		95.5	4.2	2.3	102
6	13.9	94.9	3.4	1.2	99
		95.0	4.0	2.3	101
Avarage					99.7
Standard deviation					2.6

[1] Total radioactivity collected after each HPLC injection expressed as a percent of the initial radioactivity per unit volume multiplied by the sample loop volume.

B. MASS BALANCE

Mass balances were determined by relating the ratio of radioactivity collected during an HPLC run to the initial total radioactivity. [^{14}C]glyphosate material balances ranged from 95 to 100 % AR.

C. TRANSFORMATION OF THE TEST SUBSTANCE

At 0 DAT, [^{14}C]glyphosate accounted for 95.7 % AR (mean of two duplicates). After 33.5 days equivalent of natural sunlight at latitude 38 °N [^{14}C]glyphosate was decreased to 73.5 % AR(mean of two duplicates). In the dark control samples treated with this radiolabel 95 % AR were encountered at the end of the test period. At the beginning of the irradiation period, AMPA accounted for 4.05 % AR (mean of two duplicates). After 33.5 days equivalent of natural sunlight at latitude 38 °N AMPA had increased to 18.5 % AR (mean of two duplicates). No other degradation products were identified in the study. In the dark control, AMPA remained at a level of ca. 4 % AR throughout the study period.

D. KINETICS

The photolysis DT_{50} reported was 81 sunlight equivalent days for glyphosate at pH 7 and 25 °C.

III. CONCLUSIONS

After irradiation by light from a xenon arc lamp, [^{14}C]glyphosate photolysed in solution at pH 7 and 25 °C, to yield aminomethylphosphonic acid. The pseudo first-order DT_{50} was 81 days of clear weather summer sunlight at 38° N.

Assessment and conclusion by applicant:

The study was conducted mainly in agreement with the current guideline. The test items were sterilized but sterility was not checked throughout the experiment. Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

No complete mass balance can be established since there was no traps for volatiles. However, the radioactivity recovered in the aqueous solutions is good (≥95% AR), indicating that no significant formation of volatiles occurred.

LOQ is not reported.

Since the sterility of the test items was not checked during the experiment, it cannot be ensured that the degradation observed for the active substance was only photo-degradation.

DT75 was not reached during the study duration, however this is not considered as a deviation since the duration of the study (29.3 days equivalent natural sunlight) is compliant with the maximum duration recommended in OECD 316 (30 days of sunlight exposure during an appropriate season and at an appropriate latitude).

The study is considered as supportive.

[redacted], *1983*

Data point:	CA 7.2.1.2/007
Report author	[redacted]
Report year	1983
Report title	Hydrolysis and photolysis degradation studies of SC-0224
Report No	WRC-83-85
Guidelines followed in study	U.S. EPA 161
Deviations from current test guideline	From OECD 316: - Test was conducted at 40 °C. - No duplicate samples were used. - UV light was used. - Test systems were sterilized but sterility was not confirmed. - No material balance provided. - Exact application rate was not reported.
GLP/Officially recognised testing facilities	No
Previous evaluation	No, only the hydrolysis part was evaluated in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate as glyphosate-trimesium (SC-0224)

Lot No.: WRC-8146-27-1

Composition: 90.9 % glyphosate-trimesium, 4.2 % water

Measured molar ratio: Glyphosate (CMP) : trimesium (TMS) = 1.00 : 1.09

2. Buffers:

Buffer solutions were prepared in buffer systems of pH 5.0 (biphthalate), 7.0 (phosphate) and 9.0 (borate).

B. STUDY DESIGN

1. Experimental conditions

Test solutions between 50 mg/L and 60 mg/L were prepared in buffer systems of pH 5.0 (biphthalate), 7.0 (phosphate) and 9.0 (borate). The water used was free of bacteria, having passed through a 0.2 µm filter. Flasks and photoreactor tubes were sterilised prior to use. Sterility of the test systems throughout the experiments was not confirmed.

Each of the three reactor tubes was filled to the 1300 mL level with one of the test solutions. Reactor tubes were placed into a 40 °C thermostated bath and UV lamps were turned on. Baths of dark control samples were covered. Irradiated and dark control samples were incubated for 29 and 30 days, respectively.

UV black-light lamps (GE Lamp No. F40 BL) were used as artificial light source. Each lamp was mounted vertically inside a double-walled, cylindrical Pyrex glass photoreactor. Comparative measurements with natural sunlight have shown that distribution for the GE F40 BL lamp is similar to that of the sunlight at the high energy (low wavelength) end of the spectrum. Light intensity emitted by UV lamps was measured chemically at the beginning and the end of the study period. The light intensity was calculated to be 2.097 x 10^4 erg/sec/cm^2, or approximately 2100 µWatt/cm^2.

The temperature of each test solution remained constant at 40 ±0.5 °C throughout the study period.

2. Sampling

Reactor tubes of irradiated samples were removed for sampling on days 0, 4, 7, 11, 15, 19, 22 and 29. Dark controls were sampled after 30 days. Single samples were analysed.

3. Analytical procedures

At each sampling, 2 to 3 mL aliquots were removed from the bottom sampling port of the reactor and submitted for analysis.

Determinations of glyphosate and AMPA (CMP and aminomethylphosphonic acid anions) were carried out by derivatisation with 9-fluorenylmethyl chloroformate followed by HPLC analysis. The trimesium cation (TMS) was dealkylated to dimethylsulfide prior to analysis by gas chromatography. Typical recoveries via these methods are 93 ± 10% for anions and 94 ± 7% for TMS.

In the pH 5 solution an unknown response was observed, as well as discoloration. To identify the unknown compound, the extract was directly analysed by GC/MS but no response was detected. In a separate study, about 1000 mL of the final (aqueous) pH 5 solution was evaporated to dryness using a rotary evaporator at 38 °C. About 2.5 mL of D_2O and 0.5 mL of 50 % NaOH were added to the residue. The resulting deuterium oxide solution was analysed by phosphorous NMR.

II. RESULTS AND DISCUSSION

A. DATA

Glyphosate, trimesium and AMPA concentrations are summarised in below.

Table 8.2.1.2-29: Concentrations (mg/L) of glyphosate and AMPA at 40 °C

pH	5.0		7.0		9.0	
Time (days)	Observed concentration (mg/L)					
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
0	45.1	0.3	45.0	0.3	49.2	0.3
4	34.5	4.4	45.0	1.5	44.1	1.8
7	29.4	8.5	43.4	2.1	43.0	2.9
11	22.9	10.0	40.0	2.9	40.0	3.2
15	21.6	8.5	40.7	2.7	37.0	3.8
19	16.6	8.1	n.a.	4.2	35.8	6.0
22	16.1	8.5	38.1	3.7	34.1	5.5
29	10.5	7.0	34.9	3.9	29.4	6.7
Dark Control (30 days)	46.3	n.a.	49.7	n.a.	45.7	n.a.

n.a. = not available

Table 8.2.1.2-30: Trimesium concentrations (mg/L) at 40 °C

pH	**5.0**	**7.0**	**9.0**
Time (days)	**Observed concentration (mg/L)**		
0	18.7	21.1	20.0
4	23.6	21.3	20.4
7	20.7	23.5	18.2
11	18.3	22.3	20.1

15	23.5	22.1	14.8
19	18.8	21.6	11.9
22	18.8	23.5	11.3
29	20.8	20.9	13.1
Dark Control (30 days)	20.8	20.6	19.9

B. TRANSFORMATION OF THE TEST ITEM

Glyphosate concentrations decreased from 45.1 at study start to 10.5 mg/L after 29 days at pH 5.0 and from 45.0 to 34.9 mg/L at pH 7.0. At pH 9.0, glyphosate concentrations decreased from 49.2 to 29.4 mg/L from study start to study end.

The only photolytic decomposition products identified for the glyphosate anion (CMP) were AMPA and phosphoric acid. Maximum concentrations of AMPA were 10.0 %, 4.2 % and 6.7 % at pH 5.0, pH 7.0 and pH 9.0, respectively, with concentrations qualifying AMPA as major metabolite at pH 5 and pH 9.

No other responses were observed in the analytical chromatographic scans except for pH 5 solutions, where an unknown compound represented <4 % of the original compound. Since this response was present and the pH 5 solution was discolored, the solution was further analysed by phosphorous NMR. The NMR spectrum indicated that the solution contained CMP, AMPA and phosphoric acid in a molar ratio of 2.5 : 1.0 : 1.9.

No decomposition of trimesium occurred at pH 5 or pH 7.

C. KINETICS

For glyphosate, DT_{50} values of 14.6, 77.9 and 41.6 days were calculated based on pseudo first-order.

Assessment and conclusion by applicant:

In view of the test conditions using UV light and of lacking information e.g. on mass balance or the exact application rate the study is considered invalid.

Assessment and conclusion by RMS:

Several deviations from OECD 316 are identified: test conducted at 40°C, no duplicate samples, use of UV light, sterility not confirmed, no material balance, exact application rate not reported.

The study is not acceptable.

[redacted], *1978*

Data point:	CA 7.2.1.2/008
Report author	[redacted]
Report year	1978
Report title	Photodegradation and anaerobic aquatic metabolism of Glyphosate, N-Phosphono-Methylglycine
Report No	MSL-0598
Guidelines followed in study	None
Deviations from current test guideline	From OECD 316: The study was conducted with artificial light with wavelengths of 350-450 nm, no details of light source are reported. No details on characteristics of test systems are reported. Test temperature is not reported. Test systems were sterilised by microfiltration but sterility was not confirmed. Only four sampling dates within experimental period. AMPA was present as impurity of test solution with 7.4 %. No analysis results for glyphosate are reported.

GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate (N-(phosphono-methyl-^{14}C-glycine), PMG)
Lot No.:	not indicated
Specific activity:	10.12 mC/mM
Radiochemical purity:	98 – 99 % (TLC)

2. Test system:

The natural lake water used was sampled at lake number 34 at the Busch Wildlife Area, Weldon Springs, Missouri, USA. A pH of 6.6 was determined. For test no. 1, the test water was purified by AG 50-X8 resin. Further, for test no. 2, a second natural water sample was obtained to assess the photosensitising impact of $CaCl_2$ on degradation of glyphosate natural water. The test water for the second test was deionised and $CaCl_2$.was added at a level of 3 or 30 mg/L, respectively. Further, the test water was analysed for metal ions before and after clean up.

B. STUDY DESIGN

1. Experimental conditions

The photolysis reactors were sterilised at 20 psi and 120 °C for 20 minutes. After dosing, the test waters were sterilised by Millipore filtration (0.20 µm). The test solutions were fortified with 1.0 mg/L glyphosate (0.1 mg/L [^{14}C]glyphosate mixed with unlabelled glyphosate at a ratio 1:10). Prior to use, the radiolabeled test material applied in the second test was purified by D-50 column chromatography to remove AMPA present in the stock solution. Ascarite towers were placed on the reactors to monitor the formation of $^{14}CO_2$ over the study duration. Sterility of the test systems throughout the experiments was not confirmed.

The test solutions were exposed to artificial light emitting wave lengths between 350-450 nm for two to three weeks. An exposure period of 14 days to this source of artificial light corresponds to 112 eight-hour days of exposure to sunlight at Davis, California, USA. Simultaneously, dark control test solutions were maintained.

2. Sampling

In the first test using purified natural water, aliquots were removed at 0, 1, 7, 14 and 21 days. Test no. 2 solutions were sampled after 0, 1, 7 and 14 days.

3. Analytical procedures

The samples were analysed via HPLC for glyphosate and AMPA (aminonethylphospohnic acid) by collecting eluant at 0.5 min intervals for LSC. The respective retention times were determined using radiolabelled standards. TLC was further used as confirmatory method for aliquots taken at the last sampling event. The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/TLC//LSC were not reported.

II. RESULTS AND DISCUSSION

A. DATA

Mass balances or recoveries of glyphosate are not given in the study report. The degradation of glyphosate in irradiated and dark control test solutions is reflected by results for AMPA as indicated in

the table below.

Table 8.2.1.2-31: Recovery of AMPA in irradiated and dark control samples after treatment with [^{14}C]glyphosate (expressed as percent of applied radioactivity)

		DAT				
Variant	Incubation	0	1	7	14	21
Water no. 1	Irradiated	7.4	25.9	39.5	58.4	78.6
	Dark control	7.4	11.3	8.8.	9.8	14.6
Water no. 2	Irradiated	-	18.4	68.8	86.7	-
	Dark control	-	2.0	5.8	13.5	-
Water no. 2 deionised	Irradiated	-	6.0	23.7	38.3	-
	Dark control	-	2.1	1.0	3.2	-
Water no. 2 deionised with 3 mg/L $CaCl_2$	Irradiated	-	7.5	57.5	67.1	-
	Dark control	-	1.0	1.0	1.2	-
Water no. 2 deionised with 30 mg/L $CaCl_2$	Irradiated	-	5.3	38.4	78.1	-
	Dark control	-	0	0	2.5	-

- not determined

B. MASS BALANCE

The total recovery of the irradiated and dark control samples was reported to be 96.4 % and 106.3 % AR, respectively.

C. VOLATILISATION

CO_2 was reported to amount to 0.5 % after 21 days of irradiation.

D. TRANSFORMATION OF THE TEST SUBSTANCE

In the first test, 78.6 % AR formation of AMPA was encountered after 21 days of continuous irradiaton. There was no evidence of any photodegration product other than AMPA. Initially, the test substance contained 7.4 % AMPA and corrected for this initial value, 7.2 % photodegradation in the dark control sample was encountered after 21 days. This degradation was suspected to be due to enzymes present in the water samples after Millipore filtration rather than microbial contamination.

In the second test, formation of AMPA amounted to 86.7 %, 38.3 %, 67.1 % and 78.1 % of applied radioactivity after 14 days of irradiation in natural water, deionised natural water, deinonised natural water with 3 mg/L $CaCl_2$ and deinonised natural water with 30 mg/L $CaCl_2$, respectively. In the dark controls 13.5 % AMPA was found in natural water, but only 3.2 % in deionised natural water. Degradation seems markedly reduced in the natural water after deionisation, while $CaCl_2$ enhances photodegradation of glyphosate. However, sodium, silica, and calcium ions were still present in the water after deionisation. All in all, the results are considered to indicate that while $CaCl_2$ has a sensitising effect photodegradation may also be influenced by other unknown factors.

E. KINETICS

The half-life of glyphosate designated in the abstract of the report was 19 days, however it's not further elaborated how the half-life was derived from the given data.

Assessment and conclusion by applicant:

In view of the limited information on test conditions, test systems and analytical results and due to the wavelength spectrum, the study is considered invalid.

Assessment and conclusion by RMS:

The report is very brief, but several deviations from OECD 316 are identified: no details on light sources, wavelength spectrum limited, limited data relative to the test conditions and the test items, sterility not confirmed, only 4 sampling dates, no analysis for glyphosate.

The study is not acceptable.

B.8.2.1.3. Indirect photochemical degradation

Experimental studies on indirect photolysis are formally not required. For completeness, the available studies are submitted as supportive information. The results are presented above together with the studies on direct photodegradation.

B.8.2.1.4. Summary on route and rate in aquatic systems

Glyphosate was found to be hydrolytically stable in sterile buffers of pH 4, 5, 7 and 9.

Direct photolysis of glyphosate is not expected to be an important process since the substance does not absorb light in the right wavelength spectrum. Glyphosate was stable in experiments on direct photolysis in sterile distilled water under artificial sunlight and in buffer solutions at pH 5, 7 and 9 under natural sunlight. In another study, degradation of glyphosate was slightly enhanced under artificial irradiated conditions compared to dark conditions. AMPA was found at levels above 10 % at pH 7.3 and 5.1 with maximum amounts of 11.6 and 16.0 %, respectively. A data gap is identified to update the kinetic adjustments provided to determine a photolysis DT_{50} for glyphosate.

Glyphosate was significantly degraded by indirect photolysis. Besides the natural compound methanediol (up to 52 %AR after 12 days) and the known metabolite AMPA (up to 19.6% AR after 12 days), no degradation products were observed above 10 %.

B.8.2.2. Route and rate of biological degradation in aquatic systems

B.8.2.2.1. 'Ready biodegradability'

Ready biodegradability of glyphosate was investigated in 3 existing studies. No new study was provided in this renewal dossier. In the scientific literature review for glyphosate (2010-2020), no article was identified to provide further information relevant to the data point.

Table 8.2.2.1-1: List of existing studies on ready biodegradability with glyphosate

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)	Remark
CA 7.2.2.1/001	[redacted], 2009	Accepted in RAR (2015)	Acceptable	
CA 7.2.2.1/002	[redacted], 1991	Accepted in RAR (2015)	Acceptable	
CA 7.2.2.1/003	[redacted], 1990	Accepted in RAR (2015)	Acceptable	

[redacted], 2009

Data point:	CA 7.2.2.1/001
Report author	[redacted]
Report year	2009
Report title	Ready biodegradability of Glyphosate in a manometric respirometry test
Report No	53981163
Guidelines followed in study	OECD 301 F Commission Regulation 440/2008/EC, Method C.4-D
Deviations from current test guideline	From OECD 301 F - The concentration of activated sludge slightly exceeded the concentration of suspended solids given in OECD 301 F
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate
Lot No.:	07-b-151
Chemical purity:	97.7 % (w/w)
Molecular formula:	$C_3H_8NO_5P$
Molecular weigth:	169.01 g/mol (calculated)

Reference substance:

Identification:	Sodium benzoate
Lot No.:	098K0700
Chemical purity:	100 % (w/w)
Molecular formula:	$C_7H_5O_2Na$
Molecular weigth:	144.1 g/mol

2. Inoculum and test medium:

Inoculum

A sample of activated sludge was supplied from a domestic waste water treatment plant by the sewage plant Darmstadt, Germany. Activated sludge was used as inoculum with a concentration corresponding to 31 mg dry solids per litre. Dry solid of the activated sludge was 1.5 g/L by weight measurements. The activated sludge was washed three times by centrifugation of the sludge, decanting the supernatant and re-suspending the sludge in tap water. After the last washing step, the pellet was re-suspended in test water and aerated overnight.

Test medium

Analytical grade salts were added to deionised water to prepare the following stock solutions:

8.5 g KH2PO4, 21.75 g K2HP04, 33.4 g Na2HP04 x 2 H20, 0.5 g NH4Cl filled up with deionised water to 1000 mL volume

22.5 g MgS04 x 7H20 filled up with deionised water to 1000 mL volume

36.4 g CaCl2 x 2H20 filled up with deionised water to 1000 mL volume

0.25 g FeC13 x 6H20 filled up with deionised water to 1000 mL volume

In order to avoid precipitation of iron hydroxide in the stock solution (D) after storage and before use, one drop of concentrated HCl per litre was added.

10 mL of stock solution (A) and 1 mL of the stock solutions (B) to (D) were combined and filled up to a final volume of 1000 mL with deionised water. The pH-value was 7.5, thus no adjustment had to be done. 5 mL activated sludge was filled up to 244 mL with 239 mL mineral medium corresponding to 31 mg/L dry solids.

B. STUDY DESIGN AND METHODS

1. Experimental conditions

Five treatment groups were established:

- Inoculum Control: inoculated mineral salts medium
- Procedure Control: inoculated mineral salts medium plus sodium benzoate at 104 mg/L organic carbon

Glyphosate: inoculated mineral salts medium plus test substance at 103 mg/L, corresponding to an oxygen demand of about 59 mg/L (ThODNH4) and 97 mg/L (ThODNO3)

Toxicity Control: inoculated mineral salts medium plus the test substance at 103 mg/L and the reference substance at 104 mg/L

Abiotic Control: not inoculated mineral salts medium plus test substance at 103 mg/L, poisoned with HgCL2 (5 mL of stock solution with 48.72 mg/mL was made up to a final volume of 244 mL)

The purpose of the toxicity control was to assess the biodegradation of the reference substance in the presence of the test substance. Duplicate vessels were established for the glyphosate treatment and the inoculum control. Single vessels were established for the procedure, the abiotic and the toxicity control.

The amounts of test item and reference item were directly weighed into the test flasks of approximately 500 mL volume. No emulsifiers or solvents were used, but the solutions were dispersed by stirring to stirring to achieve a homogeneous solution of the test item.

2. Analytical procedures

The closed test flasks were incubated in a climatised room under continuous stirring in the dark. The consumption of oxygen was determined daily by measuring the change of pressure in the flasks by means of a manometric method (BSB/BOD-Sensor-System). The temperature was measured each working day in the climatised room and was 22 ± 1 °C throughout the whole study.

The pH-values were measured in control, procedure control and a separately prepared test flask with test item at test start (to prevent loss of test item in the test flasks) and in all flasks at the end of the test using a pH-electrode WTW pH 340i.

Evolved carbon dioxide was absorbed in an aqueous absorbed in an aqueous solution (45%) of potassium hydroxide.

The pH value was 7.5 and 6.8 – 7.6 measured at start and at the end of the test, respectively.

3. Calculations

Biodegradation related to oxygen demand

The biodegradability (% BOD = mg O_2 per mg test item) exerted after each period was calculated as:

BOD = (mg O_2 uptake of test item – mg O_2 uptake of inoculum control) / mg test item in flask

The percentage biodegradation of the test item and of the reference item sodium benzoate was calculated as:

% degradation = (BOD (mg O_2 / mg test item or reference item)) / ($ThDO_{NH4}$ (mg O_2 / mg test item or reference item)) x 100

or in case of nitrification of the test item:

% degradation = (BOD (mg O_2 / mg test item or reference item)) / ($ThOD_{NO3}$ (mg O_2 / mg test item or reference item)) x 100

II. RESULTS AND DISCUSSION

A. DATA

Biodegradation of glyphosate, sodium benzoate and the toxicity control based on $ThODN_{H4}$ and $ThOD_{NO3}$ are summarised in the table below.

Table 8.2.2.1-2: Percentage biodegradation of glyphosate, sodium benzoate and the toxicity control based on $ThOD_{NH4}$ and $ThOD_{NO3}$

Time (days)	Glyphosate				Sodium benzoate	Toxicity control	
	$ThOD_{NH4}$ [1]		$ThOD_{NO3}$ [3]		$ThOD_{NH4}$ [2]	$ThOD_{NH4}$ [1,2]	$ThOD_{NO3}$ [3,4]
	Flask 1	Flask 2	Flask 1	Flask 2	Flask 5	Flask 7	Flask 7
1	0	0	0	0	29	22	18
2	0	0	0	0	43	28	24
3	-4	-4	-3	-3	65	46	40
4	-9	-9	-5	-5	67	52	44
5	-9	-9	-5	-5	72	56	48
6	0	0	0	0	75	58	50
7	0	0	0	0	78	60	52
8	-4	-4	-3	-3	80	61	53
9	-9	-9	-5	-5	81	62	53
10	-9	0	-5	0	81	62	53
11	0	0	0	0	84	65	55
12	0	0	0	0	87	65	55
13	0	0	0	0	87	65	55
14	0	0	0	0	90	65	55
15	0	9	0	5	90	65	55
16	9	9	5	5	93	67	57
17	4	4	3	3	94	66	56
18	0	0	0	0	94	65	55
19	9	9	5	5	96	65	55
20	9	9	5	5	96	65	55
21	9	9	5	5	96	65	55
22	9	9	5	5	96	65	55
23	9	9	5	5	96	65	57
24	9	17	5	10	98	67	57
25	17	17	10	10	98	67	57
26	17	17	10	10	98	67	57
27	26	26	15	16	98	69	59
28	26	26	15	16	98	69	59

[1] $ThOD_{NH4}$ of glyphosate: 0.568 mg/mg
[2] $ThOD_{NH4}$ of sodium benzoate: 1.666 mg/mg
[3] $ThOD_{NO3}$ of glyphosate: 0.946 mg/mg

B. BIODEGRADATION

The relevant pass levels for ready biodegradability of glyphosate are 60 % of ThOD for respirometric methods. The mean percentage biodegradation at the end of the 28-day exposure period was 26 % ($ThOD_{NH4}$). The occurrence of nitrification was considered but not experimentally confirmed. Based on $ThOD_{NH3}$, the mean percentage biodegradation at the end of the exposure period at 28 DAT was 16 %. The degradation rate of glyphosate did not reach 60% within 28 days of incubation. Therefore, glyphosate is considered not to be readily biodegradable.

The reference item sodium benzoate was sufficiently degraded to 90 % after 14 days and to 98 % after 28 days of incubation. The percentage biodegradation of the reference item confirms the suitability of the used aerobic activated sludge inoculum.

In the toxicity control containing both the test item and reference 65 % or 55 % biodegradation was noted within 14 days based on $ThOD_{NH4}$ and $ThOD_{N03}$, respectively. After 28 days of incubation

biodegradation of the toxicity control was 69 % or 59 %, respectively. According to the test guidelines the test item can be assumed to be not inhibitory on the aerobic activated sludge micro-organisms because degradation was >25 % within 14 days.

The oxygen demand in the abiotic control was zero. No correction of the test item degradation rates had to be done.

Assessment and conclusion by applicant:

The study was conducted in line with the relevant guideline OECD 301 F. It is therefore considered valid to describe the ready biodegradability of glyphosate. Glyphosate is considered not to be readily biodegradable.

Assessment and conclusion by RMS:

Except that the concentration of activated sludge slightly exceeded the concentration of suspended solids given in OECD 301 F, no other deviation was identified.

The study is acceptable.

[redacted], *1991*

Data point:	CA 7.2.2.1/002
Report author	[redacted]
Report year	1991
Report title	A study to evaluate Ready Biodegradability of Glyphosate Technical
Report No	RB-09
Guidelines followed in study	OECD 302 B (1981) EEC Directive 87/302 Biodegradation, Zahn Wellens Test
Deviations from current test guideline	From OECD 302 B: - Information on inoculum is limited.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate Technical

Lot No.: 0206-JAK-25-1

Chemical purity: 97.7 %

2. Inoculum and test solutions:

Inoculum

Activated sludge from Kendal sewage treatment plant was used as inoculum in an amount corresponding to approximately 0.2 g dry material/L.

Test medium

The test medium (2 L per flask) was prepared according to OECD 302 B:

38.5 g NH_4Cl, 33.4 g $NaH_2PO_4 \times 2H_2O$, 8.5 g KH_2PO_4 and 21.75 g K_2HPO_4 were dissolved in 1000 mL bidistilled water.

2.5 mL of this stock solution were added to 1000 mL test water (1:1 drinking water/bidistilled water).

On the basis of the suspended solids determination, the medium of all treatment groups was inoculated with activated sludge in an amount corresponding to approximately 0.2 g dry material/L.

The total volume used per flask was 2 litres. Per flask, 2.5 mL nutrient solution/L was added.

B. STUDY DESIGN AND METHODS

1. Experimental conditions

Prior to the biodegradation test a microbial toxicity test was carried out to check that microbial inhibition was not greater than 50 % at the test substance DOC concentration of 50 mg/L as required in the biodegradation test. In this test, a range of glyphosate concentrations (100, 50, 10 and 1 mg/L) with a fixed concentration of biodegradable standard (glucose/glutamic acid solution) were dissolved in BOD dilution water. The mixtures were saturated with air, seeded and then measured volumes stirred in partially filled bottles connected to closed-end mercury manometers. Oxygen consumption was measured by observing the change in level of the mercury columns, with any carbon dioxide evolved into the bottles absorbed by alkali held in small cups within the bottle caps. The test as carried out at 20 ± 1 °C for 5 days, and the amount of oxygen taken up was determined and compared to that from the standard solution.

For the biodegradation test four treatment groups were established:

- Blank solution: inoculated mineral salt medium
- Control solution: inoculated mineral salt medium plus sodium acetate at 50 mg/L DOC

Glyphosate solution: inoculated mineral salt medium plus test substance at 50 mg/L DOC

Adsorption check: inoculated mineral salt medium plus the test substance at 50 mg/L DOC

Three vessels were established for the glyphosate treatment. Single vessels were established for the blank, the standard control and the adsorption control.

The test was conducted over a period of 28 days. Flasks were placed in a tank through which water at 22 ± 3°C was circulated from a temperature controlled unit. The test flasks were stirred with continuous aeration to ensure that the sludge did not settle or the oxygen concentration fall below 2 mg/L. Sides and top of the tank was covered but 15 cm holes under each of the tests flasks at the bottom of the tank allowed a small amount of diffuse daylight into the system.

Evaporation losses from the flasks were made up with deionised water just prior to sampling by marking the liquid levels in the flasks before starting the test, and after each sampling. Samples were taken 3 hours after the start of the test in order to allow for any adsorption of glyphosate by the activated sludge.

2. Analytical procedures

Daily samples (weekdays) were removed for DOC analysis. A 20 mL sample was removed from each flask and filtered through a washed filter paper with the first 5 mL filtrate returned to the test flask.

The pH of the glyphosate and blank test solutions was checked at regular intervals and adjusted to pH 7-8 by addition of M NaOH.

3. Calculations

Inhibition of microbial activity was calculated as:

$$((BOD_{std} - BOD_{test}) / BOD_{std}) \times 100$$

The degradation rate was calculated as:

$$D\ (\%) = [(1 - (C_T - C_B)) / (C_A - C_{BA})] \times 100$$

Where

D_T = biodegradation (%) at time T

C_T = DOC value at time of sampling (mg/L)

C_B = DOC value of the blank (mg/L)

C_A = initial DOC value in the test solutions (mg/L) measured three hours after the beginning of the test

C_{BA} = DOC value of the blank (mg/L) measured three hours after the beginning of the test

II. RESULTS AND DISCUSSION

A. DATA

Results of the microbial inhibition test are summarised below, whereas biodegradation of glyphosate, sodium benzoate and results on adsorption are summarised below.

Table 8.2.2.1-3: Pre-test: microbial inhibition as BOD value and percent inhibition

Concentration of test substance in BOD solution (mg TOC/L)	3 day BOD value (mg O_2/L)		Inhibition (%)	
	Flask 1	Flask 2	Flask 1	Flask 2
100	138	142	8.6	8.4
50	162	153	-7.3	1.3
10	142	148	6.0	4.5
1	133	141	11.9	9.0
Control Standard	151	155	-	-
Blank	0	0	-	-

Table 8.2.2.1-4: Percentage biodegradation of glyphosate and sodium acetate as well as DOC values for adsorption check

Day	% Biodegradation				Arithmetic mean	Adsorption check DOC
	Control (Sodium acetate)	Glyphosate				
		Flask 1	Flask 2	Flask 3		
2	100	0	-3	-1	-1	57.8 [1]
7	-	-1	0	-4	-2	60.0
14	-	3	5	1	3	56.7
21	-	1	2	4	3	59.3
28	-	2	2	1	2	55.6

[1] initial adsorption check value

B. BIODEGRADATION

In flasks with glyphosate, 2 % (mean of three replicates) biodegradation was calculated at 28 DAT.

Therefore, glyphosate is considered not to be readily biodegradable.

The test is considered valid if the procedural control shows the removal of the reference compound by at least 70 % within 14 days. The validity of this study was ratified by the 100 % biodegradation of the sodium acetate control within 2 days confirming the viability of the inoculum.

The temperature of the flasks was maintained between 22.15 - 22.55 °C (hourly logged values) and the lowest measured oxygen concentration in the flasks was 8.3 mg O_2.

Assessment and conclusion by applicant:

The study was conducted in line with the relevant guideline OECD 302 B. It is therefore considered valid to describe the ready biodegradability of glyphosate. Glyphosate is considered not to be inherently biodegradable.

Assessment and conclusion by RMS:

The study was performed before the publication of OECD 301 guideline (recommended in Regulation 283/2013). However it follows OECD 302B and RMS agrees that it can be used to describe the inherently biodegradability of glyphosate.

Although information on inoculum is limited, the study is acceptable.

The test shows that glyphosate is not inherently biodegradable under the conditions of the test, and can therefore be considered as not readily biodegradable.

[redacted], *1990*

Data point:	CA 7.2.2.1/003
Report author	[redacted]
Report year	1990
Report title	Glyphosate technical: inherent biodegradability: "modified Zahn-Wellens Test"
Report No	271653
Guidelines followed in study	OECD 302 B (1981)
Deviations from current test guideline	From OECD 302 B : - Ratio between inoculum and test compound as TOC was lower than recommended (1.5-1.6:1). - The pH was slightly higher in some tests than recommended - 28 DAT samples were stored at 4 °C for more than 48 hours due to defect of TOC analyser.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate technical (N-(Phosphonomethyl)-glycine)

Lot No.: 229-Jak-5-1

Chemical purity: 98.9 %

2. Inoculum and test solutions:

Inoculum

Two sets of microorganisms were used, both originating from the secondary effluent of a domestic waste water sewage plant, with not adapted and adapted microorganisms in sets 1 and 2, respectively. Microorganisms from set 1 were provided by ARA Sissach/Switzerland, whereas those from set 2 were supplied by the sponsor.

Test medium

The test medium (2 L per flask) was prepared according to OECD 302 B:

38.5 g NH_4Cl, 33.4 g NaH_2PO_4 x $2H_2O$, 8.5 g KH_2PO_4 and 21.75 g K_2HPO_4 were dissolved in 1000 mL bidistilled water.

2.5 mL of this stock solution were added to 1000 mL test water (1:1 drinking water/bidistilled water).

An amount of sludge from a domestic waste-water sewage plant corresponding to 0.2 g dry material was added per litre final test medium.

B. STUDY DESIGN

1. Experimental conditions

Three treatment groups were established:

- Inoculum Control: inoculated mineral salt medium
- Functional solution: inoculated mineral salt medium plus aniline at 100 mg/L corresponding to a theoretical amount of 77.4 mg TOC/L

Glyphosate solution: inoculated mineral salt medium plus test substance at 1240 mg/2 L (620 mg/L) corresponding to 121.5 mg TOC/L in Test set 1 and 131 mg TOC/L in Test set 2

Three vessels were established for the glyphosate treatment. Single vessels were established for the inoculum control and the functional control.

A pre-test was conducted investigating potential inhibitory effects of different glyphosate concentrations on sludge.

The study was run at 20 – 23 °C protected from light. The flasks were aerated with a flow rate of about 0.5 – 0.7 L/minute, resulting in an oxygen concentration of 7.7 – 9.0 mg O_2 per litre. The pH was adjusted to 7.0 and 8.2.

2. Analytical procedures

Per sampling interval, two subsamples of 30 mL were taken per flask and analysed for TOC in duplicate. Samples were taken at day 0 (0 and 3 hours after treatment), 7, 14, 21 and 28 of the incubation period. Water evaporation losses were compensated by adding bidistilled water.

Samples were filtered through a washed fluted filter paper. The first 5 mL of the filtrate were replaced into the reactor. The remaining 25 mL were used for TOC analysis. Samples were analysed on day of sampling, except on 28 DAT where the samples were stored at 4 °C for four days due to a defect of the TOC-Analyser.

TOC analyses were performed with the various filtrates using a total carbon analyser.

3. Calculations

The degradation rate was calculated as:

Dt (%) = (1 – (Ct - Cbl) / (C0 - Cbl)) x100

Where

Dt = degradation in percent TOC-removal at time t
C0 = starting TOC-concentration of the culture medium (mg TOC/L)
Ct = TOC-concentration of the culture medium at time t (mg TOC/L)
Cbl(0) = starting TOC-concentration of the blank (mg TOC/L)
Cbl(t) = TOC-concentration of the blank at time t (mg TOC/L)

Degradation is stated as the percentage TOC-removal within 28 days with respect to the test article (% TOC-removal).

II. RESULTS AND DISCUSSION

A. DATA

Biodegradation of glyphosate technical and reference compound aniline expressed as percent TOC removal is summarised below for.

Table 8.2.2.1-5: Degradation of glyphosate technical by activated sludge (microorganisms set 1 and set 2, respectively) expressed as percent TOC-removal

Replicate	% TOC-removal after				
	3 hours	7 d	14 d	21 d	28 d
Microorganisms test set 1 (supplied by ARA Sissach, Switzerland)					
1	-12	-17	-2	-25	-2
2	-10	-14	-3	-21	-6

Microorganisms test set 2 (supplied by Sponsor)					
1	-15	0	-17	-19	-11
2	-8	-13	-15	-23	-3

Table 8.2.2.1-6: Degradation of aniline by activated sludge (microorganisms set 1 and set 2, respectively) expressed as percent TOC-removal

Replicate	% TOC-removal after 3 hours	7 d	14 d	21 d	28 d
Microorganisms test set 1 (supplied by ARA Sissach, Switzerland)					
1	-27	88	91	94	100
Microorganisms test set 2 (supplied by Sponsor)					
1	-12	89	92	96	99

B. BIODEGRADATION

No removal of glyphosate was detected in flasks treated with sludge sets 1 and 2 demonstrated by unchanged TOC. Glyphosate was neither eliminated nor degraded within 28 days of incubation. Therefore, glyphosate is considered not to be readily biodegradable.

The test is considered valid as the reference compound aniline was biodegraded within 14 days by 91 % and 92 %, respectively, with microorganisms sets 1 and 2, respectively.

III. CONCLUSIONS

The test article glyphosate technical appeared to be non-degradable (unchanged TOC). The reference compound aniline was degraded within 14 days by 91 and 92 % by microorganisms of two different sources, demonstrating the viability of the microorganisms.

Assessment and conclusion by applicant:

The study was conducted in line with the relevant guideline OECD 302 B with minor deviations that did not have an impact on the outcome. It is therefore considered valid to describe the ready biodegradability of glyphosate. Glyphosate is considered not to be ready biodegradable.

Assessment and conclusion by RMS:

The study was performed before the publication of OECD 301 guideline (recommended in Regulation 283/2013). However it follows OECD 302B and RMS agrees that it can be used to describe the inherently biodegradability of glyphosate.

The deviations identified (ratio between inoculum and test compound as TOC was lower than recommended, in some tests pH was slightly higher than recommended, 28 DAT samples were stored at 4 °C for more than 48 hours due to defect of TOC analyser) do not invalidate the results of the study.

The study is acceptable.

The test shows that glyphosate is not inherently biodegradable under the conditions of the test, and can therefore be considered as not readily biodegradable.

B.8.2.2.2. Aerobic mineralisation in surface water

The aerobic mineralisation of glyphosate in surface water was investigated in a new study ([redacted], 2020, CA 7.2.2.2/001).

In the scientific literature review for glyphosate (2010-2020), no article was identified to provide further information relevant to the data point.

[redacted], *2020*

Data point:	CA 7.2.2.2/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate – Aerobic Mineralisation of [^{14}C]Glyphosate in Surface Water
Report No	815731
Guidelines followed in study	OECD Guideline 309
Deviations from current test guideline	From OECD 309: - Material balance below 90% for some samples. - Procedural recovery for HPLC analysis was <90 % for some samples. - Single replicates for sterile samples.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	No, not previously submitted
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Identification: [phosphonomethyl-^{14}C]glyphosate
Batch ID: 6848SXD008-2
Specific activity: 12.18 MBq/mg
Radiochemical purity: 98.3 % (HPLC-radiodetection, from certificate of analysis)

2. Test Surface Water and Sediment

Freshly collected natural sediment and water from a lake (Calwich Abbey Lake, Staffordshire, UK) was used. Upon collection, sediment was passed through a 2-mm sieve and water through a 0.2-mm sieve. Sediment and water were stored under aerobic conditions at *ca* 4°C until use for 7 days until acclimation of test systems. Characteristics of test water and sediment are summarised in the table below.

Table 8.2.2.2-1: Characteristics of test surface water and sedimentacteristics of test surface

Parameter	Results
Test system	Calwich Abbey
Country	UK
Sediment:	
Textural Class (USDA)	Silt Loam
Sand [50 µm – 2 mm] (%)	10
Silt [2 µm – 50 µm] (%)	73
Clay [< 2 µm] (%)	17
pH (in water)	7.6
pH (in 0.01 M $CaCl_2$)	7.5
Organic matter (%)	7.94
Organic carbon (%)	4.60
Maximum water holding capacity – pF0 disturbed sediment (% w/w)	100.5
Cation exchange capacity [meq/100 g]	18.4
Nitrogen, Total (% w/w)	0.35
Phosphorus, Total (mg/kg)	1312
Carbonate Content as CO_3 (%)(w:w)	30.3
Water:	
Organic Carbon (mg/L)	5.50
Dissolved Organic (mg/L)	5.64
Nitate (mg/L) (-N)	12.51 (2.83)
Nitrite (mg/L) (-N)	<0.66 (<0.20)
Total Nitrogen (mg/L)	3.55
Ammonium (mg/L) (-N)	<0.26 (<0.20)
Phosphate (mg/L) (-P)	<0.06 (<0.20)

Total phosphorous (mg/L)	<0.02
Total Suspended Solids (mg/L)	53.6
Electrical Conductivity (µS/cm)	544
CBOD (mg/L)	<1.0
pH	8.2
Total Hardness (EDTA, mg/L as calcium carbonate)	261
Alkalinity (mg $CaCO_3$/L)	149
Carbonate (mg/L)	2.4
Bicarbonate (mg/L)	177

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Preliminary tests were performed to establish the sampling regime and chromatographic methods for use in the definitive experiment. It was also checked that there was no adsorption to the incubation vessels, centrifuge tubes or storage jars. The solubility test showed the test item to be soluble at the highest test concentration.

For the definitive test, first, Calwich Abbey sediment and Calwich Abbey surface water were added to a 10-L glass duran bottle and thoroughly shaken. After settling for 130 minutes, the supernatant was removed and the sediment concentration was determined. Afterwards, sediment was added to the supernatant and left to settle again. This process was repeated until a sediment concentration of 0.535 g/L was reached. The test system was then stored aerobically for four days at +4 ºC prior to being weighed into test vessels.

The study was performed in 250 mL Erlenmeyer flasks filled with approximately 100 g of the test water containing sediment (0.535 g/L). Test vessels were contained on an orbital shaker and the water was gently agitated through the study. Each flask was connected to a series of four liquid traps, the first being a safety trap, the second containing ethanediol to trap organic volatiles and the final two containing 2 M NaOH to trap CO_2. Moist air was drawn through the test apparatus (via a dip tube just below the bulk inlet water surface) and the air leaving each test vessel was drawn through the series of traps. The air had at a rate of flow such that only one air bubble was observed in the trapping solutions at any time.

Flasks containing the test water were each treated with the corresponding treatment solution, prepared in ultrapure water to receive final nominal concentrations of 10 µg/L (low concentration) and 95 µg/L (high concentration). For both test concentrations sterile samples were prepared. Volumes and dosing technique were the same as for non-sterile flasks. Measured concentration were 9.8 and 96.2 µg/L.

Additionally, two further vessels were treated with sodium [ring-U-^{14}C]benzoate at a concentration of 10 µg/L as a reference control to prove biological viability of the test systems.

Samples were maintained under aerobic conditions for 62 days at 20 ± 2°C in the laboratory in the dark.

2. Sampling

Seven sampling intervals were distributed over the entire incubation period of 62 days. For each of the two test concentrations (10 and 95 µg/L) duplicate flasks removed 0, 3, 7, 14, 30, 44 and 62 days after treatment (DAT). The sterile controls were sampled after 0 and 62 days. Reference controls were sampled with other terminal samples at day 62. Traps were collected and replenished at 7, 14, 30, 44 and 62 days. The sodium benzoate reference controls had an additional trap change on Day 3.

3. Analytical Procedures

At each sampling interval, the dissolved oxygen content (mg/L) and pH of the water was measured in control vessels.

Sediment and water were separated by filtration using 0.45-µm filter membrane and Büchner apparatus. The test vessels were rinsed with ultrapure water which was passed through the filter membrane and combined with the sample filtrate. Duplicate aliquots of the water were taken for analysis by liquid scintillation counting (LSC).

The amount of $^{14}CO_2$ trapped in the water samples was determined by LSC after acidification of subsamples of the filtered surface water to *ca* pH 2-3 and shaking on an orbital shaker overnight. An additional test for entrained $^{14}CO_2$ was performed analogously for two contingency samples.

The loss on filtration was tested with one contingency sample of the 95 µg/L group. Recovery before and after filtration through a 0.45 µm filter membrane was compared to determine if the filtering process led to a loss.

The filter membrane containing the sediment after separation of the surface water and sediment was extracted in three steps. First, the membrane was extracted in a centrifuge tube using 30 mL 0.5 M aqueous NH_4OH solution by shaking for 1 hour. Afterwards, the filter membrane was placed on the Büchner apparatus and the extractant passed through it. Final volume was made to 30 mL with extractant. The second extract was created as above, with the exception that day 3, 7 and 14 were not passed through their respective filter membrane. Final volume was made to 30 mL with extractant. The third extraction was performed as above on samples from the Day 14 timepoint onwards. For the day 30 samples onwards, the centrifuge tube was rinsed with 10 mL extractant, passed through the membrane and combined with extract 3. Final volume was made to 40 mL with extractant. Duplicate aliquots of all three extracts were analysed by LSC.

Non-extractable residues (NER) were determined by combustion of the filter membrane followed by LSC measurement of the evolved $^{14}CO_2$. As the extract 2 samples from the Day 3, 7 and 14 timepoints were not passed through their respective filter membranes after shaking, the samples were re-filtered using a fresh filter membrane and these membrane were also combusted.

Trap solutions were removed for analysis at each sampling time and duplicate aliquots were analysed by LSC.

Surface water samples of the first sampling (0 DAT) were analysed directly by HPLC. All other time points were admixed (50:50, v:v) with mobile phase A of the respective HPLC method and analysed by HPLC without further processing.

Extract 1 was analysed by HPLC for all samples while extract 2 was only analysed by HPLC when containing >5 % AR and extract 3 was not analysed by HPLC. An aliquot (5 mL) of the sediment extract was concentrated to dryness under a stream of nitrogen. The samples was reconstituted with mobile phase A, sonicated for 10 min and analysed by HPLC.

HPLC involving a porous graphitic carbon (PGC) 'Hypercarb' column was used as primary analytical method for radiochemical purity determination of the test item, stock and application solutions of the test item and for determining the initial patterns of degradation in surface water and sediment extract samples. The limit of quantification (LOQ) for HPLC was deemed to be 200 dpm in a single peak for online radiodetector analysis. The limit of quantification for low dose samples (10 µg/L) was 1.5 % AR for surface water and 0.22 % AR for sediment extracts. The limit of quantification for high dose samples (95 µg/L) was 0.15 % AR for surface water and 0.02 % AR for sediment extracts. All values reported are in excess of the limit of detection, unless stated otherwise.

A secondary method based on HPLC involving a strong cation exchange column to differ from the primary method was used in addition to confirm the initial profiles for selected water and sediment extracts.

Following the observation of an unknown component from use of the confirmatory secondary method, additional attempts were made for characterisation. Tests to characterise the unknown as AMPA and/or glyphosate associated to metal ions failed. The unknown was thus isolated by fraction collection using the secondary analytical method for a representative sample of 62 DAT (high dose). The isolated unknown was subject to investigation by a tertiary chromatographic method, i.e. HPLC involving a strong anion exchange (SAX) column to result in separation of the unknown peak into three components. The result has to be confirmed for the whole range of samples of the low and high dose being subject of ongoing work and to an amendment to report.

LC-MS experiments were performed on selected surface waters, sediment extracts and the isolated unidentified peak observed using the secondary method, to confirm assignments made by HPLC through co-chromatography with reference standards.

Control samples treated with sodium benzoate were analysed by reversed phase HPLC.

The identity of carbon dioxide was confirmed by precipitation with barium chloride.

4. Determination of transformation kinetics

The transformation kinetics of glyphosate in both test systems were evaluated using CAKE version 3.3. The replicate data were directly fitted un-weighted with the complete data set and unconstrained initial concentration (M_0). IRLS was used as solver. Single first-order (SFO), double first-order in parallel (DFOP), and first-order multicompartment (FOMC) kinetics were tested.

As indicated in the expert statement [redacted] 2020, the material balance values at 0 DAT were corrected for purity of test item of 96.3%, resulting in corrected mass balance values of 89.7 %AR and 92.3 %AR at 0 DAT for the 10 µg/L test concentration. Similarly, the material balance values for the 95 µg/L test concentration were corrected for the purity of 96.5%. These radiochemical purities were measured on the day of the test item application. No other corrections to the data were performed.

II. RESULTS AND DISCUSSION

The pH value of the water remained relatively constant during the study between 7.32 and 8.61. The dissolved oxygen decreased from 0 DAT to 62 DAT from ≥8.69 to ≤6.34 mg/L. At each sampling interval of sterile samples, sterility was proven.

Recovery from the sodium [^{14}C]benzoate reference controls had a mean value of 97 % AR at Day 62. Radioactivity accounted for a mean of 0.7 % AR in surface water only and 0.8 % AR in the sediment/filter membrane extracts. The sediment/filter membrane combustions accounted for a mean of 8.0 % AR. The majority of the recovery was in the NaOH traps, which accounted for a mean of 79.0 % AR after 14 days and 87.3 % AR by Day 62, showing that the test system was viable.

Radioactive mass balance and distribution of glyphosate and metabolites in surface water and suspended sediment are summarised in the following tables. Comparison of analyses by primary and secondary method are also presented.

A. DATA

Table 8.2.2.2-2: Material balance of radioactivity from [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT						
		0	3	7	14	30	44	62
Surface Water	1	77.0	59.8	45.9	49.0	48.7	44.2	45.4
	2	78.7	56.1	48.1	52.3	50.3	47.8	44.8
	Mean	77.9	58.0	47.0	50.7	49.5	46.0	45.1
Sediment Extract 1	1	15.0	15.4	23.2	14.2	6.1	3.6	3.0
	2	15.8	15.9	24.9	16.1	6.0	3.9	3.9
	Mean	15.4	15.7	24.1	15.2	6.1	3.8	3.5
Sediment Extract 2	1	1.0	2.0	2.7	2.8	1.5	1.4	0.6
	2	1.1	2.0	1.3	2.9	1.3	0.9	0.5
	Mean	1.1	2.0	2.0	2.9	1.4	1.2	0.6
Sediment Extract 3	1	NA	NA	NA	2.7	0.6	0.8	0.3
	2	NA	NA	NA	2.7	0.7	0.8	0.3
	Mean	NA	NA	NA	2.7	0.7	0.8	0.3
NER [1]	1	0.1	10.6	14.8	7.0	12.1	11.5	14.7
	2	0.2	13.8	9.3	8.9	11.9	13.8	13.2
	Mean	0.2	12.2	12.1	8.0	12.0	12.7	14.0
Volatiles	1	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Mean	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
$^{14}CO_2$ [2]	1	NA	0.5	<LOQ	10.3	19.0	23.8	27.9
	2	NA	0.6	3.8	11.0	20.0	23.2	25.1
	Mean	NA	0.6	1.9	10.7	19.5	23.5	26.5
Apparatus Wash	1	<LOQ	<LOQ	<LOQ	0.8	<LOQ	0.6	<LOQ
	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.2	0.8

	Mean	<LOQ	<LOQ	<LOQ	0.4	<LOQ	0.9	0.4
Centrifuge Tube Wash	1	NA	0.4	1.4	0.6	0.9	0.7	NA
	2	NA	1.4	NA	NA	NA	NA	0.5
	Mean	NA	0.9	1.4	0.6	0.9	0.7	0.5
Total	1	93.1	88.7	88.0	87.4	88.9	86.6	91.9
	2	95.8	89.8	87.4	93.9	90.2	91.6	89.1
	Mean	94.5	89.3	87.7	90.7	89.6	89.1	90.5

DAT: days after treatment
<LOQ = below the limit of quantification
NA = Not Applicable
[1] Combined sediment and filter membrane
[2] Combined total recoveries from both NaOH traps

Table 8.2.2.2-3: Material balance of radioactivity from [^{14}C]glyphosate at an application rate of 95 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT						
		0	3	7	14	30	44	62
Surface Water	1	63.7	46.8	47.2	41.0	48.1	43.7	48.6
	2	57.0	46.2	24.3	49.6	41.0	48.2	45.0
	Mean	60.4	46.5	35.8	45.3	44.6	46.0	46.8
Sediment Extract 1	1	28.4	33.9	27.4	24.8	15.9	11.8	7.8
	2	35.1	31.5	42.9	23.8	17.8	11.6	10.0
	Mean	31.8	32.7	35.2	24.3	16.9	11.7	8.9
Sediment Extract 2	1	2.1	3.3	3.2	3.3	3.6	3.4	1.5
	2	2.5	4.0	7.9	3.1	3.4	4.0	2.3
	Mean	2.3	3.7	5.6	3.2	3.5	3.7	1.9
Sediment Extract 3	1	NA	NA	NA	1.6	1.3	1.8	0.5
	2	NA	NA	NA	2.8	1.4	1.6	1.0
	Mean	NA	NA	NA	2.2	1.4	1.7	0.8
NER [1]	1	0.3	5.7	6.9	2.4	7.4	8.7	7.5
	2	0.2	6.8	5.5	4.7	8.7	9.4	10.1
	Mean	0.3	6.3	6.2	3.6	8.1	9.1	8.8
Volatiles	1	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Mean	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
$^{14}CO_2$ [2]	1	NA	0.4	2.4	11.0	15.2	20.6	20.9
	2	NA	0.4	6.2	8.3	17.1	19.1	25.3
	Mean	NA	0.4	4.3	9.7	16.2	19.9	23.1
Apparatus Wash	1	<LOQ	0.1	0.3	0.4	0.4	0.1	0.5
	2	<LOQ	0.1	0.1	0.2	0.3	0.1	0.2
	Mean	<LOQ	0.1	0.2	0.3	0.4	0.1	0.4
Centrifuge Tube Wash	1	NA	0.4	1.3	0.4 [3]	NA	NA	0.3
	2	NA	0.5	0.7	NA	0.3	NA	NA
	Mean	NA	0.5	1.0	0.4	0.3	NA	0.3
Total	1	94.5	90.6	88.7	84.9	91.9	90.1	87.6
	2	94.8	89.5	87.6	92.5	90.0	94.0	93.9
	Mean	94.7	90.1	88.2	88.7	91.0	92.1	90.8

DAT: days after treatment
<LOQ = below the limit of quantification
NA = Not Applicable
[1] Combined sediment and filter membrane
[2] Combined total recoveries from both NaOH traps
[3] Combined total recovery from centrifuge tube wash containing filter membranes for extractions 1 and 2

Table 8.2.2.2-4: Material balance of radioactivity from [^{14}C]glyphosate at two test concentrations in sterilised surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound		DAT

	Test concentration [µg/L]	0	62
Surface Water	10	97.5	92.8
	95	96.0	93.1
Sediment Extract 1	10	0.4	3.6
	95	2.0	4.0
Sediment Extract 2	10	<LOQ	0.4
	95	0.1	0.3
Sediment Extract 3	10	NA	0.3
	95	NA	0.1
NER [1]	10	<LOQ	2.0
	95	<LOQ	0.5
Volatiles	10	NA	<LOQ
	95	NA	<LOQ
$^{14}CO_2$ [2]	10	NA	1.2
	95	NA	1.2
Apparatus Wash	10	<LOQ	<LOQ
	95	0.1	0.1
Total	10	97.9	100.3
	95	98.2	99.3

DAT: days after treatment
<LOQ = below the limit of quantification
NA = Not Applicable
[1] Combined sediment and filter membrane
[2] Combined total recoveries from both NaOH traps

Table 8.2.2.2-5: Degradation of [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Phase	Replicate	DAT						
			0	3	7	14	30	44	62
Glyphosate	Surface Water	1	71.5	55.2	39.6	31.8	9.7	2.6	2.6
		2	73.7	52.3	40.9	35.9	9.4	4.0	1.4
		Mean	72.6	53.8	40.3	33.9	9.6	3.3	2.0
	Sediment extracts	1	14.2	15.1	22.2	12.5	3.2	NS	NS
		2	15.5	15.6	23.7	14.5	2.7	NS	NS
		Mean	14.9	15.4	23.0	13.5	3.0	NS	NS
	Total	1	85.7	70.3	61.8	44.3	12.9	2.6	2.6
		2	89.2	67.9	64.6	50.4	12.1	4.0	1.4
		Mean	87.5	69.1	63.2	47.4	12.5	3.3	2.0
AMPA	Surface Water	1	3.9	4.6	6.3	17.2	39.0	41.6	41.7
		2	3.1	3.8	7.2	16.4	40.9	43.8	42.3
		Mean	3.5	4.2	6.8	16.8	40.0	42.7	42.0
	Sediment extracts	1	0.8	0.3	1.0	1.7	2.9	NS	NS
		2	0.3	0.3	1.2	1.6	3.3	NS	NS
		Mean	0.6	0.3	1.1	1.7	3.1	NS	NS
	Total	1	4.7	4.9	7.3	18.9	41.9	41.6	41.7
		2	3.4	4.1	8.4	18.0	44.2	43.8	42.3
		Mean	4.1	4.5	7.9	18.5	43.1	42.7	42.0
Total Minor Unidentified Degradation Products [1]	Surface Water	1	1.6	ND	ND	ND	ND	ND	1.1
		2	1.9	ND	ND	ND	ND	ND	1.0
		Mean	1.8	ND	ND	ND	ND	ND	1.1
	Sediment extracts	1	ND	ND	ND	ND	ND	NS	NS
		2	ND	ND	ND	ND	ND	NS	NS
		Mean	ND	ND	ND	ND	ND	NS	NS
	Total	1	1.6	ND	ND	ND	ND	ND	1.1
		2	1.9	ND	ND	ND	ND	ND	1.0
		Mean	1.8	ND	ND	ND	ND	ND	1.1

DAT: days after treatment

NS = sample not analysed as insufficient radioactivity in sample
ND = not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 8.2.2.2-6: Degradation of [^{14}C]glyphosate at an application rate of 95 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Phase	Replicate	DAT						
			0	3	7	14	30	44	62
Glyphosate	Surface Water	1	61.1	42.8	42.6	31.5	25.8	12.5	8.5
		2	54.1	41.6	18.8	39.4	18.3	14.5	3.6
		Mean	57.6	42.2	30.7	35.5	22.1	13.4	6.1
	Sediment extracts	1	27.6	31.9	26.0	22.9	13.4	7.8	3.9
		2	33.5	30.2	46.4	22.2	14.1	7.5	3.6
		Mean	30.6	31.1	36.2	22.6	13.8	7.7	3.8
	Total	1	88.7	74.7	68.6	54.4	39.2	20.3	12.4
		2	87.6	71.9	65.2	61.6	32.4	22.0	7.2
		Mean	88.2	73.3	66.9	58.0	35.8	21.2	9.8
AMPA	Surface Water	1	2.6	4.0	4.6	9.5	21.3	30.1	39.1
		2	2.9	4.5	5.5	9.3	21.6	33.2	40.5
		Mean	2.8	4.3	5.1	9.4	21.5	31.7	39.8
	Sediment extracts	1	0.8	2.0	1.4	1.9	2.5	4.0	3.9
		2	1.6	1.2	4.4	1.6	3.7	4.1	6.4
		Mean	1.2	1.6	2.9	1.8	3.1	4.1	5.2
	Total	1	3.4	6.0	6.0	11.4	23.8	34.1	43.0
		2	4.5	5.7	9.9	10.9	25.3	37.3	46.9
		Mean	4.0	5.9	8.0	11.2	24.6	35.7	45.0
Total Minor Unidentified Degradation Products [1]	Surface Water	1	ND	ND	ND	ND	1.0	1.2	1.0
		2	ND	0.2	ND	0.9	1.1	0.6	0.9
		Mean	ND	0.1	ND	0.5	1.1	0.9	1.0
	Sediment extracts	1	ND	ND	ND	ND	ND	ND	ND
		2	ND	ND	ND	ND	ND	ND	ND
		Mean	ND	ND	ND	ND	ND	ND	ND
	Total	1	ND	ND	ND	ND	1.0	1.2	1.0
		2	ND	0.2	ND	0.9	1.1	0.6	0.9
		Mean	ND	0.1	ND	0.5	1.1	0.9	1.0

DAT: days after treatment
NS = sample not analysed as insufficient radioactivity in sample
ND = not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 8.2.2.2-7: Degradation of [^{14}C]glyphosate at two test concentrations in sterilised surface water containing suspended sediment (0.54 g/L) under aerobic conditions (total system; expressed as percent of applied radioactivity)

Compound	Test concentration [µg/L]	DAT	
		0	62
Glyphosate	10	93.7	89.6
	95	92.7	89.9
AMPA	10	3.8	3.2
	95	3.1	3.2
Total Minor Unidentified Degradation Products [1]	10	ND	ND
	95	0.2	ND

DAT: days after treatment
ND = not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 8.2.2.2-8: Comparison of degradation of [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions through analysis by the primary and secondary chromatographic methods (expressed as percent of applied radioactivity)

Compound	Chromatographic method	DAT										
		0	3	7		14	30		44		62	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Surface water												
Glyphosate	Primary	71.5	52.3	39.6	40.9	35.9	9.7	9.4	2.6	4.0	2.6	1.4
	Secondary	75.7	51.3	20.1	23	34.5	6.9	3.9	ND	ND	ND	ND
	Difference	4.2	1.0	19.5	17.9	1.4	2.8	5.5	2.6	4.0	2.6	1.4
AMPA	Primary	3.9	3.8	6.3	7.2	16.4	39.0	40.9	41.6	43.8	41.7	42.3
	Secondary	1.3	3.1	2.7	2.7	14.8	35.5	39.3	38	40.3	38.0	37.0
	Difference	2.6	0.7	3.6	4.5	1.6	3.5	1.6	3.6	3.5	3.7	5.3
Unidentified Peak	Primary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Secondary	ND	1.7	23.2	22.5	3.0	6.3	7.1	6.2	7.5	7.4	7.8
	Difference	ND	1.7	23.2	22.5	3.0	6.3	7.1	6.2	7.5	7.4	7.8
Total Minor Unidentified Degradation Products [1]	Primary	1.6	ND	ND	ND	ND	ND	ND	ND	ND	1.1	1.0
	Secondary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Difference	1.6	ND	ND	ND	ND	ND	ND	ND	ND	1.1	1.0
Sediment extracts												
Glyphosate	Primary	14.2	NS	NS	NS	14.5	NS	NS	NS	NS	NS	NS
	Secondary	15.0	NS	NS	NS	14.9	NS	NS	NS	NS	NS	NS
	Difference	0.8	NS	NS	NS	0.4	NS	NS	NS	NS	NS	NS
AMPA	Primary	0.8	NS	NS	NS	1.6	NS	NS	NS	NS	NS	NS
	Secondary	ND	NS	NS	NS	0.8	NS	NS	NS	NS	NS	NS
	Difference	0.8	NS	NS	NS	0.8	NS	NS	NS	NS	NS	NS
Unidentified Peak	Primary	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
	Secondary	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
	Difference	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
Total Minor Unidentified Degradation Products [1]	Primary	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
	Secondary	ND	NS	NS	NS	0.4	NS	NS	NS	NS	NS	NS
	Difference	ND	NS	NS	NS	0.4	NS	NS	NS	NS	NS	NS

DAT: days after treatment
NS = sample not analysed as insufficient radioactivity in sample
ND = not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 8.2.2.2-9: Comparison of degradation of [^{14}C]glyphosate at an application rate of 95 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions through analysis by the primary and secondary chromatographic methods (expressed as percent of applied radioactivity)

Compound	Chromatographic method	DAT										
		0	3	7		14	30		44		62	
		Rep 2	Rep 2	Rep 1	Rep 2	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Surface water												
Glyphosate	Primary	54.1	42.8	42.6	18.8	39.4	25.8	18.3	12.5	14.5	8.5	3.6
	Secondary	53.8	42	33.7	9.0	39.7	25.2	17.3	10.6	13.9	6.3	2.6
	Difference	0.3	0.8	8.9	9.8	0.3	0.6	1.0	1.9	0.6	2.2	1.0
AMPA	Primary	2.9	4.0	4.6	5.5	9.3	21.3	21.6	30.1	33.2	39.1	40.5
	Secondary	2.4	3.4	3.4	2.7	8.5	19.4	19.8	27.2	29.3	36.9	35.6
	Difference	0.5	0.6	1.2	2.8	0.8	1.9	1.8	2.9	3.9	2.2	4.9
Unidentified Peak	Primary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Secondary	0.7	1.4	10.0	12.6	1.3	3.5	4.0	5.0	5.0	5.4	6.8
	Difference	0.7	1.4	10.0	12.6	1.3	3.5	4.0	5.0	5.0	5.4	6.8

Total Minor Unidentified Degradation Products [1]	Primary	ND	ND	ND	ND	0.9	1.0	1.1	1.2	0.6	1.0	0.9
	Secondary	ND	ND	ND	ND	ND	ND	ND	0.9	ND	ND	ND
	Difference	ND	ND	ND	ND	0.9	1.0	1.1	0.3	0.6	1.0	0.9
Sediment extracts												
Glyphosate	Primary	33.5	NS	NS	NS	22.2	NS	NS	NS	7.5	3.9	NS
	Secondary	34.0	NS	NS	NS	22.0	NS	NS	NS	7.3	3.4	NS
	Difference	0.5	NS	NS	NS	0.2	NS	NS	NS	0.2	0.5	NS
AMPA	Primary	1.6	NS	NS	NS	1.6	NS	NS	NS	4.1	3.9	NS
	Secondary	1.1	NS	NS	NS	1.5	NS	NS	NS	3.5	3.7	NS
	Difference	0.5	NS	NS	NS	0.1	NS	NS	NS	0.6	0.2	NS
Unidentified Peak	Primary	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS
	Secondary	ND	NS	NS	NS	0.2	NS	NS	NS	0.9	0.7	NS
	Difference	ND	NS	NS	NS	0.2	NS	NS	NS	0.9	0.7	NS
Total Minor Unidentified Degradation Products [1]	Primary	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS
	Secondary	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS
	Difference	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS

DAT: days after treatment
NS = sample not analysed as insufficient radioactivity in sample
ND = not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

B. MATERIAL BALANCE

Mean material balances ranged from 87.7 to 94.5 % AR for the low dose and from 88.2 to 94.7 % AR for the high dose. Material balances for the sterile test system were between 97.9 and 100.3 % AR.

The decreased material balances obtained from 3 DAT onward were likely due to one of two factors, or a combination of both. One factor involved the challenge in accounting for relatively low levels of radioactivity across multiple compartments. The other factor is that $^{14}CO_2$ generated during the course of the study may not have been fully accounted for because of low amounts entrained in surface waters, which were lost during sample processing. Results from testing for entrained $^{14}CO_2$ support this possibility. The relatively low amounts that were lost ultimately have no impact on the data for the calculation of biotransformation and kinetic data.

C. VOLATILES

Total mineralisation of the samples accounted for 26.5 and 23.1 % AR, for the low and high dose, respectively. Formation of other volatiles was not significant as demonstrated by values <LOQ in all samples. The amount of carbon dioxide determined in sterile samples after 62 days was 1.2 % AR for the low and high dose, respectively. Formation of other volatiles was not significant as demonstrated by values <LOQ in all samples.

The results from the acidified surface water sub-samples showed some entrained $^{14}CO_2$ to be present in the surface water. Samples in the 10 µg/L and 95 µg/L groups lost between 0.9 % AR and 5.2 % AR upon acidification. In the sterile samples, a mean of 5.8 % AR $^{14}CO_2$ was evolved in the 0 DAT samples. However, these results are considered anomalous as the rest of the sampling data does not support such a rapid degradation to $^{14}CO_2$ as the cumulative total in the $^{14}CO_2$ traps at 62 DAT for the sterile samples was only 1.2 % AR.

D. NON-EXTRACTABLE RESIDUES

The amount of non-extractable residues (NER) increased from 0 DAT to 62 DAT from 0.2 to 14.0 % AR for the low dose and from 0.3 to 8.8 % AR for the high dose.

E. DEGRADATION OF PARENT COMPOUND (based on primary chromatographic method)

At the low (10 µg/L) dose level, glyphosate in the water phase declined from 72.6 % AR on 0 DAT to 2.0 % AR on 62 DAT. In the sediment extracts, glyphosate increased from 14.9 % AR on 0 DAT to 23.0 % AR on 7 DAT and declined to 3.0 % AR on 30 DAT and was not detectable afterwards. In the

total system, glyphosate decreased from 87.5 % AR at 0 DAT to 2.0 % AR at 62 DAT. The only degradation product observed was aminomethylphosphonic acid (AMPA). AMPA was mainly detected in the water phase, increasing from 3.5 % AR on 0 DAT to 42.7 % AR on 44 DAT, slightly decreasing to 42.0 % AR at 62 DAT. In the sediment extracts, AMPA increased from 0.6 % AR on 0 DAT to 3.1 % AR on 62 DAT. In the total system, AMPA increased from 4.1 % AR on 0 DAT to 42.7 % AR on 44 DAT and then slightly decreased to 42.0 % AR at 62 DAT. Minor metabolites accounted for a maximum of 1.8 % AR.

At the high (95 µg/L) dose level, glyphosate in the water phase declined from 57.6 % AR on 0 DAT to 6.1 % AR on 62 DAT. In the sediment extracts, glyphosate increased from 30.6 % AR on day zero to 36.2 % AR on 7 DAT and declined to 3.8 % AR at the end of the study (62 DAT). In the total system, glyphosate decreased from 88.2 % AR on 0 DAT to 9.8 % AR on 62 DAT. The only degradation product observed was AMPA. It was mainly detected in the water phase, increasing from 2.8 % AR on 0 DAT to 39.8 % AR on 62 DAT. In the sediment extracts, AMPA increased from 1.2 % AR on 0 DAT to 5.2 % AR on 62 DAT. In the total system, AMPA increased from 4.0 % AR on 0 DAT to 45.0 % AR on 62 DAT. Minor metabolites accounted for a maximum of 1.1 % AR.

In sterile samples, degradation of glyphosate was negligible. Glyphosate decreased from 0 DAT to 62 DAT from 93.7 to 89.6 % AR for the low dose and from 92.7 to 89.9 % AR for the high dose. AMPA was determined in low dose samples with 3.8 % AR at 0 DAT and 3.2 % AR at 62 DAT and in high dose samples with 3.1 % AR at 0 DAT and 3.2 % AR at 62 DAT.

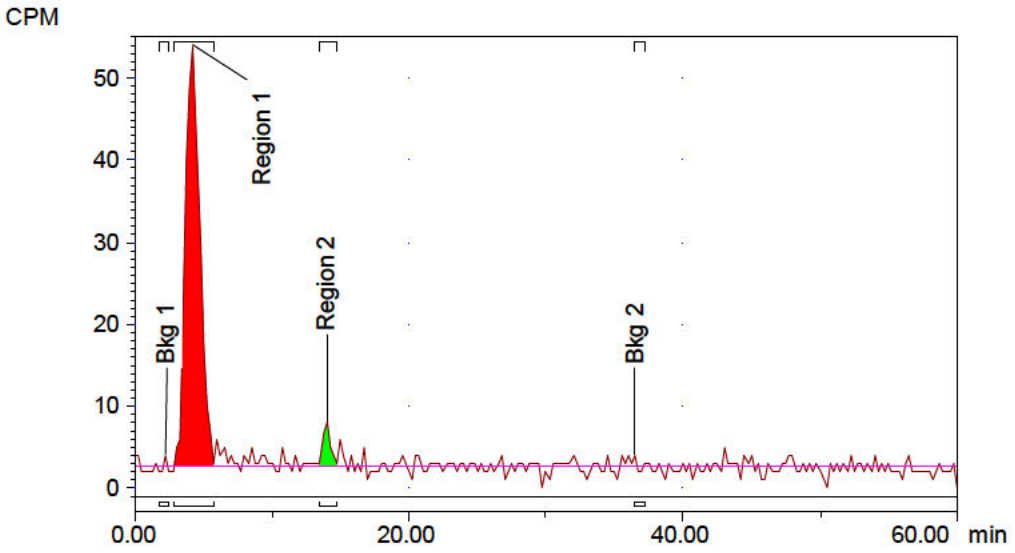
The results of the secondary HPLC analysis for glyphosate were mostly comparable to those obtained from the primary analysis. Absolute differences between primary and secondary analysis (excluding values of 7 DAT) were <5 % (mean values, if applicable) for surface water and sediment extracts of both concentrations. On 7 DAT, absolute differences for glyphosate determined by both methods were 18.7 and 9.4 % AR for the low and high dose samples, respectively. In the secondary analysis an unidentified peak with a retention time between 3 and 4 minutes was observed in surface water accounting for a maximum of 22.9 and 11.3 % AR at 7 DAT for the low and high dose samples, respectively. The maximum of the unknown peak in the secondary analysis coincides with the drop of the glyphosate associated radioactivity compared to the primary method. At the following sampling days the amount of the unidentified peak was <8 % AR and the amounts of glyphosate determined by both methods differed by maximum 5.5 % AR for individual samples.

An LC-MS/MS analysis was performed using reference items to confirm the identity of glyphosate and AMPA and to investigate whether the isolated unknown peak could be assigned to the known water/sediment metabolite hydroxymethylphosphonic acid (HMPA). Analyses confirmed the identity of glyphosate and AMPA but did not support confirmation of the unknown peak as HMPA.

Further attempts were made to characterise the unidentified peak using the secondary method after addition of EDTA solution to surface water to test whether the peak was comprised of AMPA and/or glyphosate coordinated to metal ions. Since chromatograms prior to and after complexation of metal with EDTA were comparable these attempts were proven not successful.

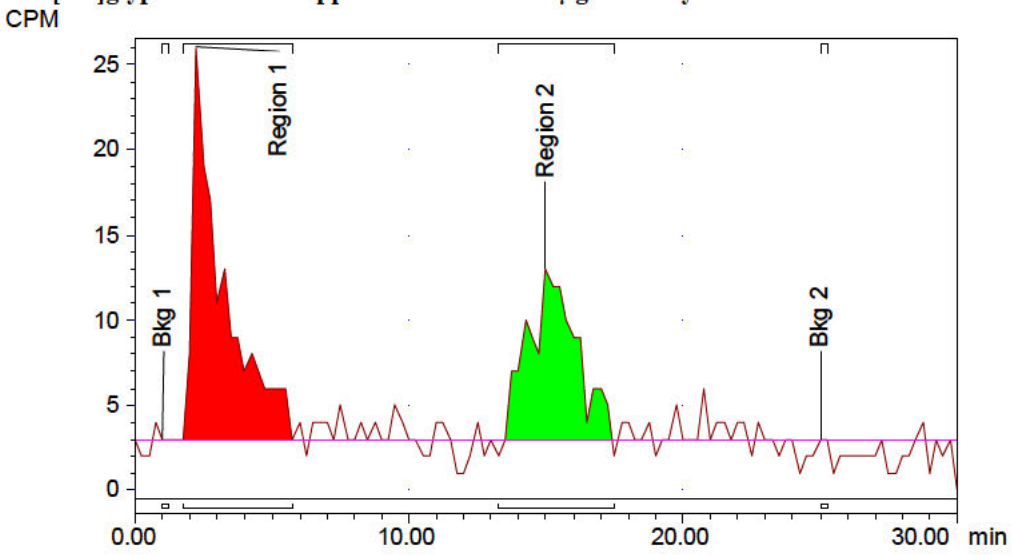
The unidentified peak was isolated by the secondary chromatographic method and the isolated fraction was analysed by the primary method. By the primary method, a peak was present at the correct retention time for AMPA, providing evidence that the unidentified peak co-eluted with AMPA by the primary method. The second region observed in this chromatogram has a retention time that does not relate to anything else seen in the second method analysis and is considered to be contamination rather than a metabolite (see Figures below).

Figure 8.2.2.2-1: Representative Secondary Method HPLC Chromatography of Isolated Sample Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62



Name	Retention (min)	%ROI (%)
Bkg 1	2.25	
Region 1	4.25	94.9
Region 2	14.00	5.1
Bkg 2	36.50	
2 Peaks		100.0

Figure 8.2.2.2-2: Representative Primary Method HPLC Chromatography of Isolated Sample Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62

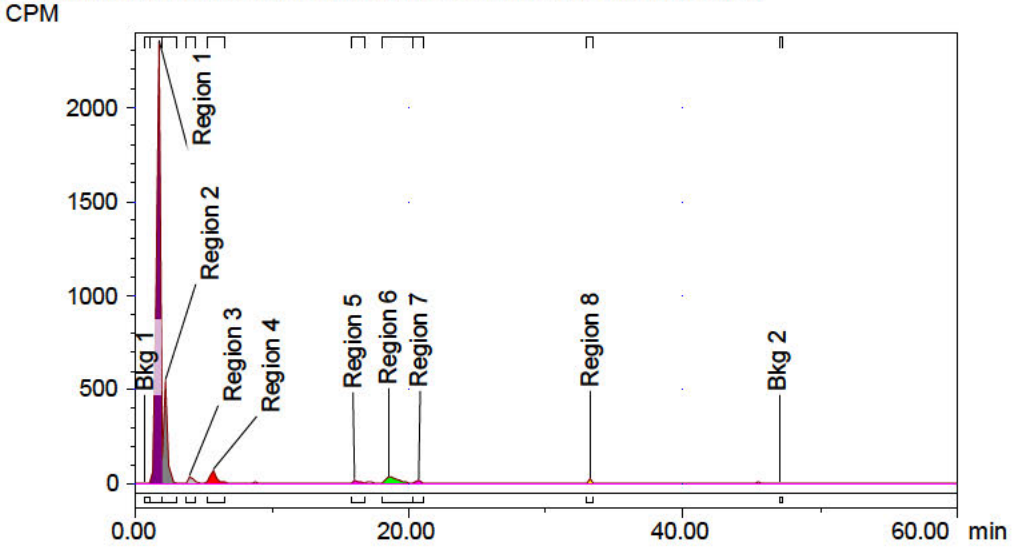


Name	Retention (min)
Bkg 1	1.00
Region 1	2.25
Region 2	15.00
Bkg 2	25.00
2 Peaks	

Afterwards, the isolated unidentified peak as well as a surface water samples (high dose, 62 DAT, replicate 1) were analysed by the tertiary method utilising a strong anion exchange (SAX) column. In the chromatograms of the tertiary method no region was observed that corresponds to the unidentified

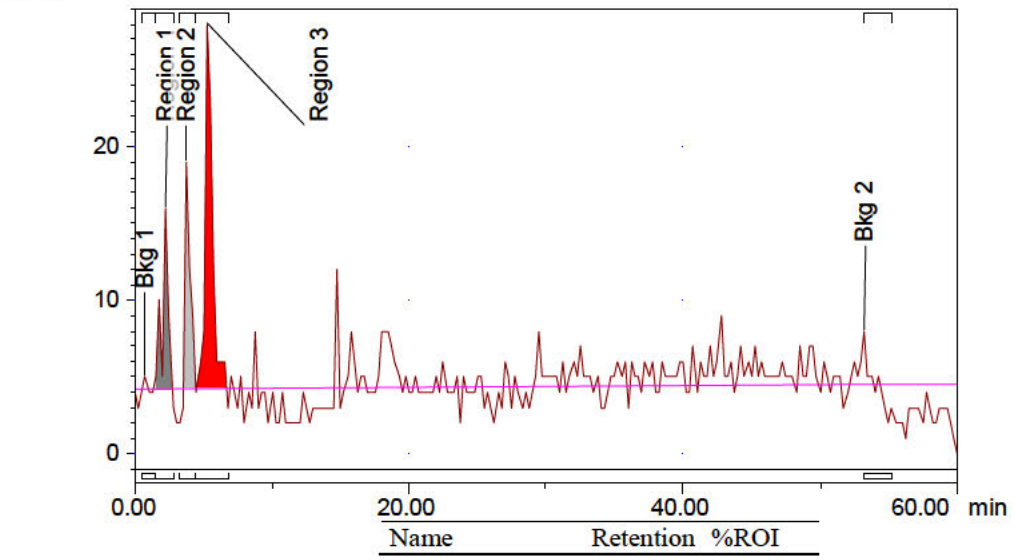
peak. Instead, there were several smaller regions, suggesting that the peak is comprised of multiple components (see Figures below).

Figure 8.2.2.2-3: Representative Tertiary Method HPLC Chromatography of Surface water Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62 (Rep 1)



Name	Retention (min)	%ROI (%)	%TRR (%)
Bkg 1	0.75		
Region 1	1.75	73.8	35.9
Region 2	2.25	16.9	8.2
Region 3	4.00	1.4	0.7
Region 4	5.75	3.1	1.5
Region 5	16.00	0.6	0.3
Region 6	18.50	3.1	1.5
Region 7	20.75	0.6	0.3
Region 8	33.25	0.5	0.2
Bkg 2	47.00		
8 Peaks		100.0	48.6

Figure 8.2.2.2-4: Representative Tertiary Method HPLC Chromatography of Isolated Sample Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62



	(min)	(%)
Bkg 1	0.75	
Region 1	2.25	22.0
Region 2	3.75	21.9
Region 3	5.25	56.1
Bkg 2	53.25	
3 Peaks		100.0

F. KINETIC EVALUATION

SFO, DFOP and FOMC models were applied to calculate degradation rates using CAKE version 3.3. The SFO model was selected as the best fit kinetic in all cases. The samples treated at 10 µg/L yielded a DT_{50} of 12.3 days and a DT_{90} of 41.0 days. The samples treated at 95 µg/L yielded a DT_{50} of 21.8 days and a DT_{90} of 72.4 days. The results of the kinetic evaluation are summarised in the tables below.

Table 8.2.2.2-10: Kinetic models and goodness-of-fit statistics of parent-only fits in the total system following application of 10 µg glyphosate/L

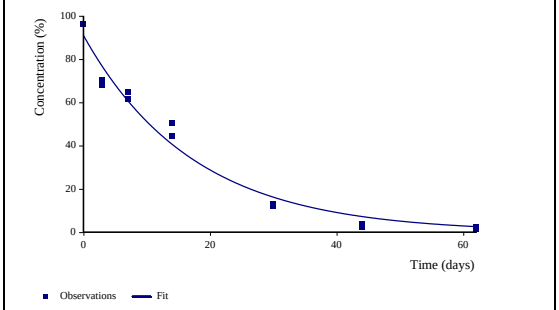
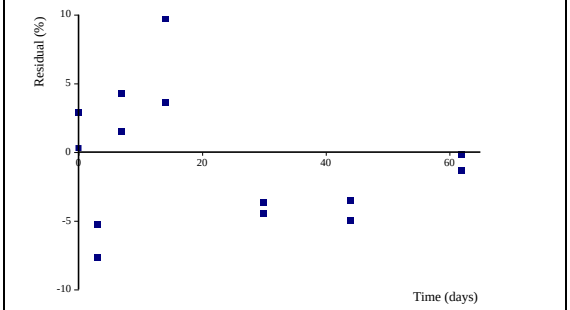
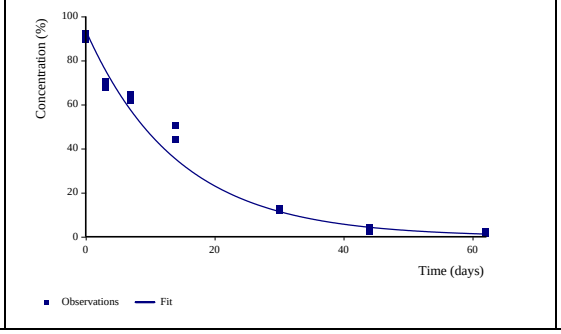
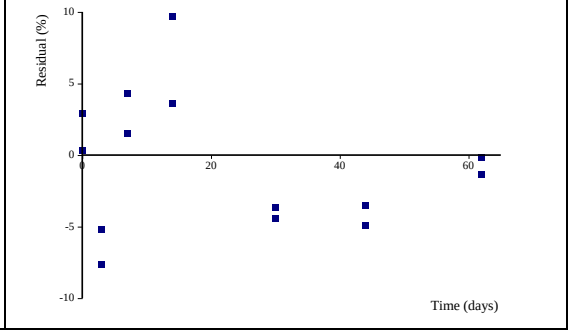
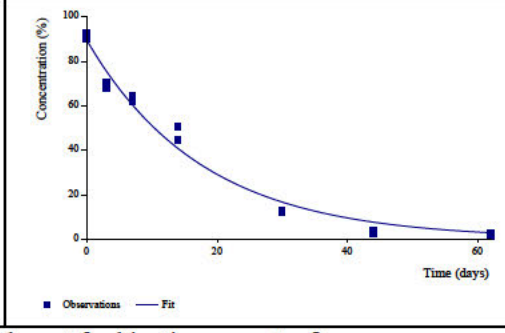
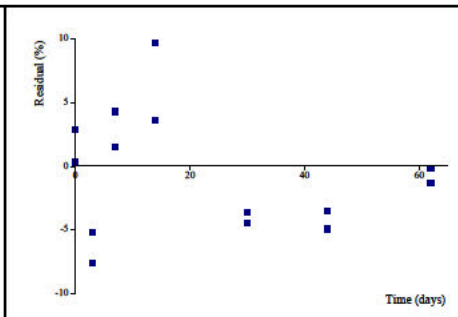
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	r^2	Prob > t (5 % level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	good	89.4	k: 0.0562	8.4	0.982	k: <0.001	k: 0.047	k: 0.066	12.3	41.0
FOMC	good	93.7	α: 5150 β: 73500	9.0	0.982	-[a]	β: nd	β: nd	9.89	32.9
DFOP	good	89.4	k_1: 0.0562 k_2: 0.0562 g: 0.971	10.0	0.982	k_1: 0.5 k_2: 0.5	k_1: -1631 k_2: -53840	k_1: 1630 k_2: 53800	12.3	41.0
The visual and statistical fits from the SFO model are good and describe the best fit. Conclusion: SFO will be used for determination of trigger endpoints.										
SFO										
FOMC										

Table 8.2.2.2-10: Kinetic models and goodness-of-fit statistics of parent-only fits in the total system following application of 10 µg glyphosate/L

DFOP		

[a] t-test not relevant for kinetic parameter β

Table 8.2.2.2-11: Kinetic models and goodness-of-fit statistics of parent-only fits in the total system following application of 95 µg glyphosate/L

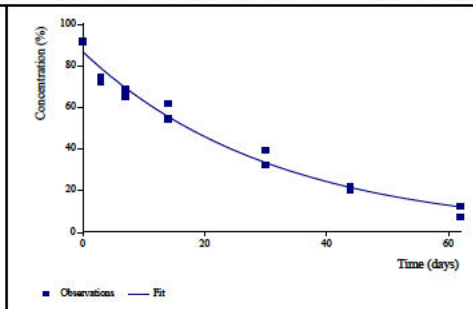
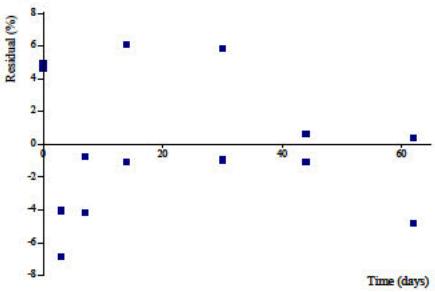
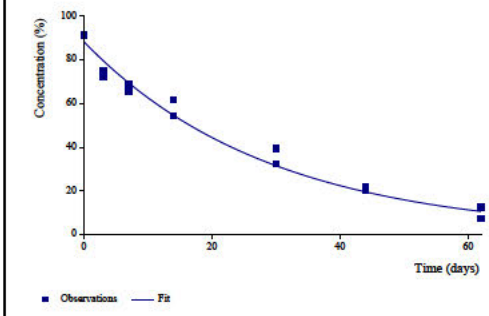
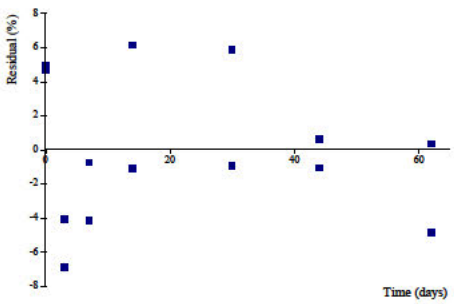
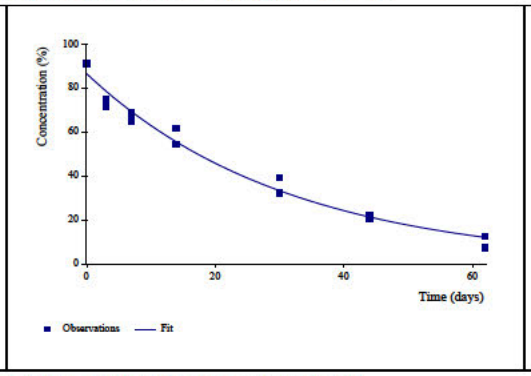
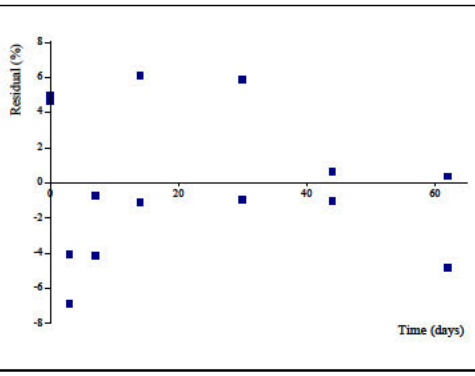
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	r^2	Prob > t (5 % level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	acceptable	86.7	k: 0.0318	5.2	0.979	k: <0.001	k: 0.027	k: 0.036	21.8	72.4
FOMC	acceptable	88.1	α: 587.9 β: 17100	5.6	0.979	-[a]	β: 12700	β: 21500	20.2	67.1
DFOP	acceptable	86.7	k_1: 0.0382 k_2: 0.0382 g: 0.114	6.1	0.979	k_1: 0.5 k_2: 0.5	k_1: -448.3 k_2: -57.5	k_1:448.4 k_2: 57.6	21.8	72.4
The visual and statistical fits from the SFO model are good and describe the best fit. Conclusion: SFO will be used for determination of trigger endpoints.										
SFO										
FOMC										

Table 8.2.2.2-11: Kinetic models and goodness-of-fit statistics of parent-only fits in the total system following application of 95 µg glyphosate/L

DFOP		

[a] t-test not relevant for kinetic parameter β

III. CONCLUSIONS

The aerobic mineralisation of glyphosate in a surface water system containing suspended sediment was studied at two concentrations, 10 µg/L and 95 µg/L. Dissipation of glyphosate in the surface water system occurred through a combination of microbial degradation and formation of non-extractable residues in the suspended sediment.

The major degradation product observed in the water phase was AMPA reaching a maximum mean level of 42.7 % AR. Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method finally showed that this peak was comprised of three individual peaks.

Mineralisation to $^{14}CO_2$ was significant in both test systems reaching mean values of 26.5 and 23.1 % AR by the end of the study in the 10 µg/L and 95 µg/L systems, respectively. Formation of non-extractable residues also contributed to the dissipation of glyphosate residues reaching a mean maximum level of 14.0 % AR in the 10 µg/L system by the end of the study and a maximum level of 9.1 % AR on Day 44 in the 95 µg/L system before declining slightly to 8.8% AR at the end of the study.

The dissipation rate of glyphosate in the total system (water + sediment) was evaluated using CAKE v. 3.3 software. The best-fit kinetics were obtained using an SFO kinetic model giving DT_{50} values of 12.3 and 21.8 days and DT_{90} values of 41.0 and 72.4 days for the 10 µg/L and 95 µg/L concentrations, respectively.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing only minor deviations.

The mass balance shows values below 90 % for several sampling points, this might be caused by the formation and loss of carbon dioxide during processing. In conclusion, the deviations do not influence the overall results and general outcome of the study.

Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method showed that this peak was comprised of three individual peaks. Further attempts to characterize this radioactivity will be reported in an amendment to this study report.

The study is considered valid to cover this data point and is in full compliance with the current guidances including the presented kinetic evaluation.

Assessment and conclusion by RMS:

The study consists in a suspended solids test and is overall well performed.

During the preparation of extracts for chromatography, the procedural recovery of radioactivity from filter membrane extracts following concentration and reconstitution in mobile phase A was determined for all samples. The recovery of radioactivity was in the range of 83 % and 101.5 ¨%. Where recoveries were < 90 %, the %AR the procedural loss translated to was calculated, and losses of < 5% AR were accepted as a procedural loss and not the loss of a metabolite.

Material balance was slightly below 90% AR for some samples, but RMS agrees that these losses are likely to be explained by incomplete trapping of carbon dioxide.

The kinetic analysis is based on concentrations determined by the primary chromatographic method. Since concentrations of glyphosate are higher using this method, this is considered as conservative for degradation rates determination.

As explained by the notifier, further data should be provided for the characterization of the unknown fraction observed in HPLC analyses 2 and 3. Therefore, a data gap is identified for the notifier to provide the amended report with information of the characterization of the unknown radioactivity when available. However this has no impact on the general outcome of the study nor on the risk assessment.

The study is considered acceptable.

B.8.2.2.3. Water/sediment study

The route and rate of degradation of glyphosate was investigated in 13 existing studies. In addition, the route and rate of degradation of AMPA was investigated in 4 studies where AMPA was applied. No new water/sediment study was provided in this renewal dossier.

An updated kinetic evaluation of the reliable data was provided in [redacted] 2020.

An existing frozen storage stability study is also available.

Laboratory studies with glyphosate

Table 8.2.2.3-1: List of existing and new water/Sediment studies on glyphosate

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.2.2.3/002	[redacted], 1999	Accepted in RAR 2015	Acceptable
CA 7.2.2.3/005 CA 7.2.2.3/006	[redacted], 1993 [redacted], 1995	Accepted in RAR 2015	Acceptable
CA 7.2.2.3/003	[redacted], 1997	Not mentionned in RAR (2015), mentionned in DAR (2001) but not evaluated.	Not acceptable
CA 7.2.2.3/004	[redacted], 1996	Accepted in RAR 2015	Not acceptable
CA 7.2.2.3/007	[redacted], 1993	Accepted in RAR 2015	Not acceptable
CA 7.2.2.3/008	[redacted] 1991	No, old study but not found in RAR 2015 nor in DAR (2001), considered as new study	Not acceptable
CA 7.2.2.3/009 CA 7.2.2.3/010	[redacted] [redacted], 1992	Not accepted in RAR 2015	Not acceptable
CA 7.2.2.3/011 CA 7.2.2.3/012	[redacted] [redacted], 1992	Not accepted in RAR 2015	Not acceptable
CA 7.2.2.3/013	[redacted], 1992 *Addendum to* [redacted], *1990, CA 7.2.2.3/009 and* [redacted], *1990, CA 7.2.2.3/011*	Not accepted in RAR 2015	Not acceptable
CA 7.2.2.3/014	[redacted], 1988	Not accepted in RAR 2015	Not acceptable
CA 7.2.2.3/015	[redacted], 1979	Not accepted in RAR 2015	Not acceptable

Table 8.2.2.3-1: List of existing and new water/Sediment studies on glyphosate

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.2.2.3/016	[redacted], 1978	Not accepted in RAR 2015	Not acceptable
CA 7.2.2.3/017	[redacted], 1972	Not accepted in RAR 2015	Not acceptable

[redacted], *1999*

Data point:	CA 7.2.2.3/002
Report author	[redacted]
Report year	1999
Report title	Glyphosate-Trimesium: Degradation of 14C-PMG Labelled Compound in Natural Water-Sediment Systems Under Laboratory Conditions
Report No	RR 99-039B
Guidelines followed in study	BBA Guideline Part IV, 5-1 SETAC "Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides", 8.2
Deviations from current test guideline	From OECD 308: - samples were incubated in an desiccator and air was drawn into the desiccator and not to each vessel individually. - water:sediment ratio about 2:1 instead of 3:1 to 4:1. - low organic carbon content (around 0.3%) for Cache system, below the recommended lowest level of 0.5%. - CO_2-free air was used. - mass balance < 90% at 2 sampling dates for Cache system. - residues of glyphosate and AMPA reported for water and total system, but not for sediment.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate-trimesium (radiolabelled phosphonomethyl-glycine anion)
Lot No.: 3350-149
Specific activity: 1927.7 MBq/mmol (52.1 mCi/mmol)
Radiochemical purity: >99 %

2. Test System:

The sediments were prepared for use in the study by sieving to 2 mm and by thorough mixing to provide homogeneous samples. The water was sieved through a 0.2 mm sieve and stored in polypropylene buckets lined with plastic bags. The water and sediment samples were stored at approximately 4 °C for about 4 weeks until all the water/sediment incubation jars had been set-up. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-2: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Cache	Putah
Country	United States of America	United States of America
Sediment:		
Textural Class (USDA)	Loamy sand	Silt loam
Sand [50 µm – 2 mm] (%)	76	26
Silt [2 µm – 50 µm] (%)	22	54
Clay [< 2 µm] (%)	2	20
pH [1]	8.1	7.5

Organic matter (%)	0.6	2.1
Organic carbon (%)	0.3	1.2
Cation exchange capacity (meq/100 g)	11.7	22.0
Microbial biomass (mg C/100g)		
Before application	20.3	29.7
Study end (100 DAT)	15.1	13.9
Water:		
pH	8.2	8.4
Dissolved O_2 at surface (mg/L)	10.2	10.0
Dissolved O_2 5 cm above sediment (mg/L)	10.2	9.8
Redox potential (mV)	587	608

DAT = days after treatment, USDA: United States Department for Agriculture
[1] medium not reported

B. STUDY DESIGN

1. Experimental conditions

The wet sediments were dispensed into cylindrical glass jars (237 mL) and the associated natural waters were added, 120 mL of Cache water and 130 mL of Putah water. The Cache test systems contained 75.7 g sediment (dry weight) and Putah test systems contained 58.9 g sediment (dry weight). In both the Cache and Putah system the average depth of settled sediment was 3.0 cm and the average depth of the surface water was 6.0 cm.

The test vessels were placed in a desiccator and CO_2-free air was drawn slowly into the desiccator over the surface in the jars to maintain the aerobic status of the water. Air entering the system was passed through a water hydrator and 1 N NaOH scrubber. After leaving the test vessels the air was passed through two traps containing 100 mL of 1 N NaOH to collect carbon dioxide.

The test systems were incubated in the dark in a constant temperature room at 20 ± 2°C. The water/sediment systems were pre-incubated at 20°C ± 2°C for 19 days (Putah) and 20 days (Cache) prior to treatment to allow equilibration.

The test substance was applied to the surface water in each jar to give a nominal initial concentration of 3.3 mg/L of glyphosate-trimesium in the water column, equivalent to a single surface application of 9 kg/ha of glyphosate-trimesium being evenly distributed to a depth of 30 cm. After application the test vessels (except 0 DAT), were closed with trap attachments.

Test systems were incubated under aerobic conditions in the dark for 100 days at 20°C. During acclimatization and incubation, pH value, oxygen saturation and redox potential of the water layer and the redox potential of the sediment layer were monitored in additional untreated test vessels.

2. Sampling

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 3, 7, 14, 30, 58 and 100 days after treatment (DAT). The surface waters were analysed by LSC and HPLC on the day they were sampled (except 14 DAT HPLC analysis, which was run within 7 days). All sediment samples were extracted on the designated sampling day and analysed by LSC within 2 days and by HPLC within 15 days. The NaOH traps were assayed and changed at each sampling interval, or approximately every two weeks, which ever was the sooner.

3. Analytical procedures

For each system the water column from above the sediment was transferred by suction to a 250 ml polypropylene centrifuge bottle without disturbing the sediment. Afterwards, the water was acidified with 50 mL of 0.5 M KH_2PO_4 and sparged for 30 minutes by pulling air through the water and on through two 1N NaOH traps to remove and trap volatile degradates and carbonate/carbon dioxide. Following the sparging, the volume of the acidified and CO_2-free water was measured and an aliquot was analysed by LSC. Small volumes (about 1 mL) of the acidified water samples were filtered and analysed by HPLC and TLC. Prior to acidification, small aliquots of selected water samples were removed for HPLC analysis.

The sediment was also acidified (and extracted) with 50 mL of 0.5 M KH_2PO_4 and sparged for 30 minutes to purge and trap volatile degradates and carbonate/carbon dioxide in a manner similar to the water. The acidified sediment was transferred into polypropylene centrifuge bottles and extracted by shaking for half an hour on a wrist action shaker. The extract was separated from the sediment by centrifugation, the volume measured and an aliquot analysed by LSC. The sediment was extracted 3-5 times and the extracts were combined for further analyses by HPLC. Selected extracts were analysed by TLC.

To quantify non-extractable residues (NER), extracted sediments were dried with acetone (50 mL) by shaking and centrifugation. The acetone layer was decanted, the volume measured and an aliquot analysed by LSC. The extracted sediments were left in the fume hood in open centrifuge bottles to dry. The radioactivity in the dry sediment was quantified by combustion/LSC. All sample calculations were corrected for combustion efficiency. Mean combustion efficiency was 98.0% for the study samples.

The limit of detection (LOD) for both the LSC and LSC/combustion methods was twice the background signal, corresponding to 0.001 ppm. The limit of quantitation for HPLC/RAM is twice the background signal, equalling a peak height greater than 20 cpm above background.

The sodium hydroxide trap solutions generated during sample sparging were analysed by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

Glyphosate and its metabolite were identified by co-chromatography with reference items.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 8.0 and 8.9 in system Cache and between 7.3 and 7.7 for system Putah. The oxygen saturation in the water phase ranged between 51 and 84 % in system Cache and between 33 and 48 % in system Putah. The redox potential of the water was between 55 and 198 mV for system Cache and between 155 and 282 mV for system Putah. The redox potential of the sediment was between 57 and 187 mV in system Cache and between -132 and 14 mV for system Putah.

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in water/sediment systems are summarised in the tables below.

Table 8.2.2.3-3: Distribution of radioactivity in Cache water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

Fraction	Replicate	DAT									
		0	0.25	1	2	3	7	14	30	58	100
$^{14}CO_2$ (Aq NaOH)	Mean	0.0 [1]	0.0 [1]	2.3	5.2	6.5	15.3	24.6	27.5	37.9	48.0
Surface water	A	98.2	88.4	75.7	67.9	62.1	44.7	30.2	18.4	10.2	5.1
	B	100.9	88.0	72.8	69.3	63.7	45.1	31.4	18.8	10.3	5.1
	Mean	99.6	88.2	74.3	68.6	62.9	44.9	30.8	18.6	10.3	5.1
	A	0.6	7.3	11.4	14.9	20.1	21.6	21.8	27.2	23.9	23.3

Sediment extract	B	0.5	8.1	15.4	17.4	17.8	21.0	23.1	28.6	24.0	24.1
	Mean	0.50	7.7	13.4	16.2	18.9	21.4	22.5	27.9	23.9	23.7
Acetone (drying)	A	<0.1	0.1	0.2	0.2	0.2	0.5	0.7	0.6	0.7	0.8
	B	0.1	0.1	0.2	0.2	0.2	0.4	0.5	0.6	0.6	0.6
	Mean	<0.1	0.1	0.2	0.2	0.2	0.4	0.6	0.6	0.7	0.7
Non-extractable residues (NER)	A	0.2	2.2	5.7	7.0	8.2	11.8	11.7	12.4	12.5	13.9
	B	0.2	2.2	7.7	7.1	9.5	12.2	12.4	11.7	14.6	13.1
	Mean	0.2	2.2	6.7	7.1	8.9	12.0	12.1	12.1	13.5	13.5
Mass balance	A	99.0	98.0	95.1	95.6	97.2	94.4	89.1	89.4	85.0	89.3
	B	101.7	98.4	98.5	98.9	97.5	93.5	91.9	84.0	87.6	92.5
	Mean	100.4	98.2	96.9	97.3	97.4	94.0	90.6	86.7	86.3	91.0

DAT: days after treatment

[1] Sparging and trapping was not performed on the 0 and 0.25 DAT samples.

$^{14}CO_2$ consists of both radioactivity trapped during incubation and radioactivity from the water/sediment compartments that was volatilized on acidification of water and sediment samples. The amount of radioactivity recovered in the post-desiccator NaOH traps was divided by the number of test vessels in the desiccator over the trapping period to determine the radioactivity evolved as CO_2 per jar.

Table 8.2.2.3-4: Distribution of radioactivity in Putah water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

		DAT									
Fraction	Replicate	0	0.25	1	2	3	7	14	30	58	100
$^{14}CO_2$ (Aq NaOH)	Mean	0.0 [1]	<0.1 [1]	3.8	0.8	2.2	2.0	3.9	5.2	5.7	5.9
Surface water	A	102.6	91.9	75.0	77.5	64.2	61.3	35.1	20.0	13.2	5.8
	B	100.4	92.9	66.7	76.6	64.2	61.6	33.6	22.7	10.2	5.5
	Mean	101.5	92.4	70.8	77.1	64.2	61.5	34.3	21.3	11.7	5.6
Sediment extract	A	0.7	5.4	12.8	12.6	20.0	22.5	37.9	57.0	59.7	60.5
	B	0.7	5.9	14.3	14.2	21.5	21.6	36.0	60.8	64.7	64.2
	Mean	0.7	5.7	13.6	13.4	20.7	22.1	37.0	58.9	62.2	62.3
Acetone (drying)	A	<0.1	<0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.2
	B	<0.1	<0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.3
	Mean	<0.1	<0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.3
Non-extractable residues (NER)	A	0.6	2.9	8.0	6.6	11.6	12.5	17.7	15.4	19.4	17.1
	B	0.5	2.95	8.9	6.9	10.4	10.0	15.8	15.0	21.1	16.2
	Mean	0.5	2.9	8.4	6.7	11.0	11.2	16.7	15.2	20.3	16.7
Mass balance	A	103.9	100.2	98.2	97.5	98.3	98.5	94.1	98.2	98.7	89.5
	B	101.6	101.8	95.2	98.6	98.1	95.5	90.3	103.5	101.4	92.0
	Mean	102.7	101.0	96.7	98.1	98.2	97.0	92.2	100.8	100.1	91.1

DAT: days after treatment

[1] Sparging and trapping was not performed on the 0 and 0.25-DAT samples.

$^{14}CO_2$ consists of both radioactivity trapped during incubation and radioactivity from the water/sediment compartments that was volatilized on acidification of water and sediment samples.

The amount of radioactivity recovered in the post-desiccator NaOH traps was divided by the number of test vessels in the desiccator over the trapping period to determine the radioactivity evolved as CO_2 per jar.

Table 8.2.2.3-5: Degradation of [^{14}C]glyphosate in Cache water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

			DAT									
Compound	Compartment	Replicate	0	0.25	1	2	3	7	14	30	58	100
Glyphosate	Water	A	97.63	87.38	74.26	66.24	59.9	39.51	21.98	7.34	1.53	0.79
		B	100.06	87.17	71.54	67.13	61.27	39.82	22.22	8.3	1.61	0.87
		Mean	*98.85*	*87.28*	*72.90*	*66.69*	*60.59*	*39.67*	*22.10*	*7.82*	*1.57*	*0.83*
	Sediment	A	*0.54*	*6.71*	*10.68*	*12.8*	*16.98*	*14.1*	*11.27*	*9.45*	*3.03*	*4.06*
		B	*0.54*	*7.48*	*14.38*	*15.31*	*14.77*	*14.36*	*12.35*	*10.3*	*3.76*	*3.3*

		Mean	*0.54*	*7.10*	*12.53*	*14.06*	*15.88*	*14.23*	*11.81*	*9.88*	*3.40*	*3.68*
	Total system	A	98.17	94.09	84.94	79.04	76.88	53.61	33.25	16.79	4.56	4.85
		B	100.6	94.65	85.92	82.44	76.04	54.18	34.57	18.6	5.37	4.17
		Mean	*99.39*	*94.37*	*85.43*	*80.74*	*76.46*	*53.90*	*33.91*	*17.70*	*4.97*	*4.51*
AMPA	Water	A	0.24	0.33	1.30	1.62	2.19	5.24	8.07	10.52	8.07	3.69
		B	0.66	0.46	1.30	2.17	2.45	5.30	8.93	10.10	8.08	3.97
		Mean	*0.45*	*0.40*	*1.30*	*1.90*	*2.32*	*5.27*	*8.50*	*10.31*	*8.08*	*3.83*
	Sediment	A	*0.00*	*0.58*	*0.72*	*1.85*	*2.99*	*7.08*	*9.86*	*16.45*	*19.19*	*17.02*
		B	*0.00*	*0.62*	*0.98*	*1.86*	*2.67*	*6.26*	*9.99*	*17.08*	*18.20*	*18.92*
		Mean	*0.00*	*0.60*	*0.85*	*1.86*	*2.83*	*6.67*	*9.93*	*16.77*	*18.70*	*17.97*
	Total system	A	0.24	0.91	2.02	3.47	5.18	12.32	17.93	26.97	27.26	20.71
		B	0.66	1.08	2.28	4.03	5.12	11.56	18.92	27.18	26.28	22.89
		Mean	*0.45*	*1.00*	*2.15*	*3.75*	*5.15*	*11.94*	*18.43*	*27.08*	*26.77*	*21.80*

DAT: days after treatment
Values given in *italics* are not directly available in the study report but were calculated by the applicant during dossier preparation

Table 8.2.2.3-6: Degradation of [^{14}C]glyphosate in Putah water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

			DAT									
Compound	Compartment	Replicate	0	0.25	1	2	3	7	14	30	58	100
Glyphosate	Water	A	101.59	90.68	74.05	76.63	63.52	60.24	34.02	18.64	11.45	5.26
		B	99.64	91.77	65.68	75.39	63.28	60.74	32.47	22.11	9.04	4.97
		Mean	*100.62*	*91.23*	*69.87*	*76.01*	*63.40*	*60.49*	*33.25*	*20.38*	*10.25*	*5.12*
	Sediment	A	*0.68*	*5.43*	*12.08*	*12.59*	*18.88*	*21.18*	*36.29*	*52.18*	*54.28*	*56.51*
		B	*0.69*	*5.9*	*13.36*	*13.78*	*20.17*	*20.69*	*34.56*	*57.77*	*60.15*	*59.93*
		Mean	*0.69*	*5.67*	*12.72*	*13.19*	*19.53*	*20.94*	*35.43*	*54.98*	*57.22*	*58.22*
	Total system	A	102.27	96.11	86.13	89.22	82.4	81.42	70.31	70.82	65.73	61.77
		B	100.33	97.67	79.04	89.17	83.45	81.43	67.03	79.88	69.19	64.9
		Mean	*101.30*	*96.89*	*82.59*	*89.20*	*82.93*	*81.43*	*68.67*	*75.35*	*67.46*	*63.34*
AMPA	Water	A	0.41	0.8	0.96	0.81	0.64	1.1	1.08	1.32	1.78	0.54
		B	0.37	0.69	0.89	0.9	0.86	0.82	1.11	0.58	1.12	0.5
		Mean	*0.39*	*0.75*	*0.93*	*0.86*	*0.75*	*0.96*	*1.10*	*0.95*	*1.45*	*0.52*
	Sediment	A	*0.00*	*0.00*	*0.75*	*0.00*	*0.89*	*0.91*	*1.6*	*4.01*	*4.37*	*3.13*
		B	*0.00*	*0.00*	*0.9*	*0.49*	*0.98*	*0.61*	*1.04*	*2.44*	*3.25*	*2.96*
		Mean	*0.00*	*0.00*	*0.83*	*0.25*	*0.94*	*0.76*	*1.32*	*3.23*	*3.81*	*3.05*
	Total system	A	0.41	0.8	1.71	0.81	1.53	2.01	2.68	5.33	6.15	3.67
		B	0.37	0.69	1.79	1.39	1.84	1.43	2.15	3.02	4.37	3.46
		Mean	*0.39*	*0.75*	*1.75*	*1.10*	*1.69*	*1.72*	*2.42*	*4.18*	*5.26*	*3.57*

DAT: days after treatment
Values given in *italics* are not directly available in the study report but were calculated by the applicant during dossier preparation

B. MASS BALANCE

Mean material balances ranged from 86.3 to 100.4 % of applied radioactivity (% AR) for the Cache water/sediment system, and from 91.1 to 102.7 % AR for the Putah water/sediment system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 100 DAT from 99.6 to 5.1 % AR in the Cache water/sediment system, and from 101.5 to 5.6 % AR in the Putah water/sediment system.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 100 DAT from 0.5 to 23.7 % AR in the Cache water/sediment system, and from 0.7 to 62.3 % AR in the Putah water/sediment system.

The amount of radioactivity in the total system decreased from 0 DAT to 100 DAT from 100.1 to 28.8 % AR in the Cache water/sediment system, and from 102.2 to 67.9 % AR in the Putah water/sediment system.

Levels of non-extractable residues (NER) in the sediment increased gradually to maxima of 13.5 % in the Cache system and 20.3 % in the Putah system at 58 DAT. The levels remained similar by 100 DAT in Cache system, but lower (16.7 %) in Putah system.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (100 DAT) were 48.0 and 5.9 % AR in the Cache and Putah systems, respectively. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in the water decreased from 0 DAT to 100 DAT from 98.85 to 0.83 % AR in system Cache and from 100.62 to 5.12 % AR in system Putah.

The amount of glyphosate in sediment extracts of system Cache increased from 0.54 % AR at 0 DAT to 15.88 % AR at 3 DAT and decreased to 3.68 % AR at 100 DAT. The amount of glyphosate in sediment extracts of system Putah increased from 0.69 % AR at 0 DAT to 58.22 % AR at 100 DAT.

The amount of glyphosate in the total system decreased from 0 DAT to 100 DAT from 99.39 to 4.51 % AR in system Cache and from 101.30 to 63.34 % AR in system Putah.

One major degradation product, aminomethylphosphonic acid (AMPA), formed primarily by microbial degradation of the parent, was found in both water/sediment systems over the course of the incubation. In the Cache total system, levels of AMPA were found to be highest at 30 to 58 DAT reaching up to 27.1 % AR (30 DAT) and decreased to 21.8 % AR at 100 DAT. Maximum amounts of AMPA in water and sediment extracts of system Cache were 10.3 % AR (30 DAT) and 18.7 % AR (58 DAT), respectively.

In the Putah total system, levels of AMPA were also found to be highest at 30 to 58 DAT, reaching 5.26 % AR at 58 DAT and decreased to 3.57 % AR at 100 DAT. Maximum amounts of AMPA in water and sediment extracts of system Putah were 1.45% AR (58 DAT) and 3.81 % AR (58 DAT), respectively.

No other metabolites were detected above 3 % AR at any time.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted], 2020, CA 7.2.2.3/001.

III. CONCLUSIONS

Glyphosate dissipated rapidly from surface water in natural water/sediment systems incubated in the dark at 20°C. The rapid initial loss of glyphosate from the surface waters was most likely due to binding to the sediment. This behaviour is consistent with the adsorptive properties of glyphosate.

The binding property of glyphosate was particularly evident in Putah sediment which was higher in the organic matter content. The strong absorptive property of glyphosate rendered it unavailable for the microbial degradation in the Putah system. The majority of the ^{14}C residue recovered from the multiple extractions of Putah sediment was determined to be glyphosate.

The only major metabolite of glyphosate detected in the water/sediment systems was aminomethylphosphonic acid (AMPA). In the Cache systems, AMPA reached maximum levels of 27.0 % of applied radioactivity by 30 DAT and declined to 21.8 % of the applied radioactivity at 100 DAT. In the Putah system, AMPA reached the maximum level of 5.3 % of the applied radioactivity by 58 DAT and declined to 3.6 % by 100 DAT.

A total of 48.0 % of the applied radioactivity in the Cache water/sediment system and 5.9 % in the Putah water/sediment system was mineralised to ^{14}C-carbon dioxide during the course of the incubation. No other individual radiolabelled compound amounted to more than 3 % of the applied radioactivity.

Assessment and conclusion by applicant:

The study was conducted consistent with the current guideline with minor deviations.

No detailed information on further degradates is given beyond the statement that 'no other radiolabelled compounds amounted to more than 3% of the applied radioactivity at any time during the incubation.' This is supported by the fact that the sum of glyphosate and AMPA in terms of % AR is nearly the same as the total radioactivity.

Samples were incubated in a desiccator and CO_2-free air was drawn into the desiccator.

Residues of glyphosate and AMPA are reported for water and total system with no separate values for sediment. Values for sediment were calculated upon dossier preparation and do not differ significantly from the amount of radioactivity extracted from the sediment.

Mass balance for Cache samples was below 90 % for some samples (85 and 87 % on 58 DAT, one replicate on 14, 30 and 100 DAT). Since the mass balance was slightly below 90 % and only for a few samples, this is considered negligible.

These deviations are considered to not influence the overall outcome of the study.

Therefore, the study and its data are considered valid to address the data point.

Assessment and conclusion by RMS:

The study is overall well performed.

RMS agrees with the deviations identified by the notifier and mentioned above.

According to OECD 308, CO2-free air should not be used as this can result in increases in the pH of the water. For both systems, RMS considers that there is no unusual increase of pH; then this deviation should not significantly impact the results of the study.

Moreover, it should be noticed that the water:sediment ratio used is 2:1 instead of 3:1 to 4:1 recommended in OECD guideline 308. Since glyphosate has a relatively high Kfoc, this ratio may have enhanced the adsorption of glyphosate on sediment. However, from the kinetic assessment presented in [redacted] 2020, total system DT_{50} obtained for these 2 water/sediment systems are in the range of other available values. Therefore RMS considers that can be used further for risk assessment.

The study is considered acceptable.

[redacted],*1993*

Data point:	CA 7.2.2.3/005
Report author	[redacted]
Report year	1993
Report title	Determination of the Degradability and Persistence of 14C-Glyphosate in the Water/Sediment-System.
Report No	ET01SE01
Guidelines followed in study	BBA Guideline Part IV, 5-1
Deviations from current test guideline	From OECD 308: - Mean material balances lower than 90% AR starting for all samplings from 14 DAT onwards (losses explained by insufficient trapping of volatiles). - processing recovery for some water samples below 90%, but more than 80%.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes (pending clarification from the applicant)

Data point:	CA 7.2.2.3/006
Report author	[redacted]
Report year	1995
Report title	Amendment to the final report - Determination of the Degradability and Persistence of ^{14}C-Glyphosate in the Water/Sediment-System - Report on the additional metabolite identification
Report No	ET01SE01
Guidelines followed in study	None
Deviations from current test guideline	- following re-analysis of water phases for metabolite identification, storage conditions were not reported for the approx. 6 months period between experimental completion including reporting and issue of the amendment
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate (labelled in the phosphonomethyl-position)
Lot No.: 1071-83-6
Specific activity: 12.3 MBq/mg
Radiochemical purity: 98.9 % by HPLC, >97.7 % by TLC

2. Test System:

The sediment was sieved to ≤2 mm and the water was sieved to ≤0.2 mm. Water and sediment were stored at 4 ± 2 °C for 8 days. During this time the sediment was shaken periodically and the water was purged with air to avoid anaerobic conditions. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-7: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Water/Sediment I	Water/Sediment II
Location	Bickenbach	Unter Widdersheim
Country	Germany	Germany
Sediment:		
Textural Class (DIN)	Sand	Loam
Sand (%)	82.3	15.0
Silt (%)	11.8	75.0
Clay (%)	5.9	10.0
pH [1]	7.80	7.68
Organic matter (%)	1.17	7.24
Organic carbon [2] (%)	0.68	4.20
Cation exchange capacity (mval/kg dry weight)	762	1030
Redox Potential (mV)	331	162
Microbial biomass (mg C/100 g dry weight)		
Study begin (0 DAT)	21.7	80.2
Study end (100 DAT)	2.8	10.1
Water:		
pH at sampling	8.65	8.47
pH at day 0	8.6	8.6
Total organic carbon (mg/L)	5.52	3.90
Redox Potential (mV)	527	493
Oxygen saturation (%)	131	104

DAT = days after treatment, DIN: Deutsches Institut für Normung e.V. (German Institute for Standardization)
[1] medium not reported

[2] calculated during dossier preparation using the equation: OC = OM/1.724

B. STUDY DESIGN

1. Experimental conditions

The test was performed in static test systems, consisting of 250-mL glass flasks filled with water and sediment in a way that the thickness of the sediment was 2 to 2.5 cm and the thickness of the water layer was 6 cm with a total volume of 190 mL. Glass tubes filled with two layers of soda lime and glass wool were used to collect carbon dioxide and other volatiles. After set-up of the test systems they were acclimatized at the experimental conditions (shaken at 20 ± 2 °C) for 5 days, until an equilibrium of oxygen content, redox potential and pH value had set.

Additionally, sterile samples were prepared by autoclaving and analysed after 100 days.

The study application rate corresponded to the highest recommended use rate of 3600 g a.s./ha. 230 µg of [^{14}C]glyphosate was applied to each test system. Immediately after the application of the test chemical, small glass tubes, filled with paraffin covered glass wool, were put up on top of the test container. During incubation, samples were shaken without mixing water and sediment.

Test systems were incubated under aerobic conditions in the dark for 100 days at 20 ± 2 °C.

2. Sampling

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 7, 14, 30, 61 and 100 days after treatment (DAT).

3. Analytical procedures

The determination of radioactivity was performed with the liquid scintillation counters (LSC). For each type of sample (e.g. water, sediment, extract of sediment) the blank value was subtracted. All analyses were conducted in triplicate.

At each sampling interval, water and sediment were separated by decantation without centrifugation. The decanted water phase was adjusted with deionized water to a final volume of 200 mL. For the determination of radioactivity by LSC, aliquots between 100 µL and 1000 µL were used. A suitable sample volume within the above mentioned range was used in order to minimize the error according to the "2-Sigma method". For the determination of the blank value, deionized water corresponding to the sample volumes, was mixed with 12 mL of scintillator.

The sediment samples were extracted four times with 150 mL of 0.5 N sodium hydroxide solution each for a period of 10 minutes. Afterwards the samples were centrifuged for 10 minutes at 4000 rpm and the combined extracts were adjusted to a final volume of 650 mL with deionized water. Aliquots of between 500 µL and 1000 µL were mixed with 12 mL scintillator and the total radioactivity was measured. A suitable sample volume within the above mentioned range was used in order to minimize the error according to the "2-Sigma method". Aliquots of 0.5 N sodium hydroxide solution corresponding to the sample volumes, were mixed with 12 mL of scintillator and used as controls.

The amount of non-extractable residues from sediment was determined by combustion. For the determination of the non-extractable amount of radioactivity from water, 3 mL of the water phase were extracted with 3 mL ethylacetate and measured by LSC.

Extracts and sediment were stored at -25 ± 15 °C until analysis.

For preparation of analysis water, aliquots were evaporated to dryness. In pre-experiments it could be shown, that the recoveries for this work-up step were >80 %. The residue was dissolved in a mixture of 750 µL methanol and 500 µL deionized water and 200 µL of 1 M disodiumphosphate buffer were added. After centrifugation at 3000 rpm for 5 min, an aliquot of the liquid phase was evaporated to a final volume of 100 µL. An aliquot of 10 µL was spotted onto a TLC plate. The mobile phase for the TLC was methanol/water/trichloroacetic acid/ammonia/glacial acetic acid (40 mL/ 60 mL/ 3.5 g/ 5 mL/ 2 mL).

For sediment extracts an aliquot was acidified with 150 µL glacial acetic acid and 50 µL were spotted on a TLC plate.

[^{14}C]glyphosate and metabolite AMPA were initially identified in study samples by thin layer chromatography (TLC) with reference items. In the course of the addendum, subsequent identification of an unknown metabolite was performed on selected concentrated water samples (30 DAT of system Unter Widdersheim and 61 DAT of system Bickenbach) by one-dimensional thin layer chromatography (1D-TLC) and two-dimensional thin layer chromatography (2D-TLC) co-spotted with reference standards.

The hydrophobized glass wool was removed from the glass tube and was extracted one after another with 5 mL hexane, 5 mL chloroform and 5 mL methanol for one minute using a vibro-fix. The extracts were combined and adjusted with a mixture of hexane/chloroform and methanol (1/1/1 v /v /v) to a final volume of 15 mL. This solvent mixture was also used for measuring blank values. After adding the scintillator aliquots of 1 mL of the combined extracts were measured. 1 mL of the solvent mixture was used as control value.

The two soda lime layers were removed from the glass tube and transferred quantitatively into a liberation apparatus for the determination of the CO_2 absorbed. Hydrochloric acid was added through a dropping funnel to slowly liberate the CO_2 from the soda lime. The liberated CO_2 was carried by a nitrogen stream into a vessel, which was filled with a cocktail of scintillator and absorber. The total radioactivity was determined by LSC.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 8.6 and 9.2 for the sandy system and between 8.6 and 8.9 for the loamy system. The oxygen content in the water phase ranged between 8.1 and 8.5 mg/L in the sandy system and between 7.8 and 8.8 mg/L in the loamy system. The redox potential of the water was in the highly positive range with values between 300 and 351 mV for both test systems. The redox potential of the sediment (mean) of the sandy system was 6 mV at 0 DAT, dropped to approx. -84 mV at 2 DAT and increased then to approx. 100 mV at 100 DAT. The redox potential of the sediment of the loamy system was -98 mV during the total incubation time and increased to approx. 92 mV at 100 DAT.

Radioactive mass balance and distribution of glyphosate and metabolites in water/sediment systems are summarised below.

Table 8.2.2.3-8: Amount of radioactivity in water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Water	A	91.51	81.04	66.04	51.89	35.96	34.06	24.24	17.31	8.22
	B	93.42	80.29	66.30	57.33	35.69	31.20	25.78	15.52	8.31
	Mean	92.47	80.69	66.17	54.61	35.83	32.63	25.01	16.42	8.27
Sediment extract	A	5.29	14.75	33.23	39.16	52.83	38.60	34.46	35.65	29.73
	B	5.23	16.04	30.56	39.62	53.32	36.14	32.86	34.05	28.74
	Mean	5.26	15.40	31.90	39.39	53.08	37.37	33.66	34.85	29.24
Non-extractable residues (NER)	A	0.07	0.18	0.63	0.98	2.76	4.56	8.71	16.37	26.03
	B	0.06	0.19	0.60	0.96	2.80	4.86	8.72	17.77	17.99
	Mean	0.07	0.19	0.62	0.97	2.78	4.71	*8.72* [1]	17.07	22.01
Organic volatiles	A	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01
	B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
	Mean	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01
CO_2	A	0.06	0.04	0.16	0.22	3.05	6.27	12.34	19.84	21.53
	B	0.05	0.04	0.10	0.22	3.34	5.89	11.13	20.63	25.42
	Mean	0.06	0.04	0.13	0.22	3.20	6.08	11.74	20.24	23.48
Mass Balance	A	96.93	96.01	100.1	92.25	94.60	83.49	79.75	89.18	85.52

	B	98.76	96.56	97.56	98.13	95.15	78.09	78.49	87.98	80.47
	Mean	97.86	96.32	98.83	95.19	94.89	80.79	*78.92* [1]	88.59	83.01

DAT: days after treatment

[1] These values were calculated during summary preparation, as the values given in the report (16.31 and 86.72%) were obviously not the mean values of the two corresponding replicates.

Table 8.2.2.3-9: Amount of radioactivity in water/sediment system Unter Widderheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Water	A	88.29	80.38	50.51	37.41	21.23	20.94	13.28	3.89	2.67
	B	87.33	74.88	51.92	33.32	30.81	23.85	12.79	4.38	3.51
	Mean	87.81	77.63	51.22	35.37	26.02	22.40	13.04	4.14	3.09
Sediment extract	A	5.01	21.29	39.52	52.83	66.09	46.38	43.37	54.76	44.14
	B	8.29	23.08	44.28	57.31	56.62	42.84	44.22	55.02	44.15
	Mean	6.65	22.19	41.90	55.07	61.36	44.61	43.80	54.89	44.15
Non-extractable residues (NER)	A	0.10	0.65	1.51	2.80	4.92	6.12	10.78	11.51	13.30
	B	0.30	0.60	1.93	1.88	5.61	6.66	10.10	11.40	13.91
	Mean	0.20	0.63	1.72	2.34	5.27	6.39	10.40	11.46	13.61
Organic volatiles	A	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.01	0.01
	B	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.01
	Mean	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.01	0.01
CO_2	A	0.08	0.05	0.20	0.42	2.69	4.49	10.58	19.04	17.21
	B	0.00	0.06	0.13	0.41	2.27	5.10	8.78	19.57	18.46
	Mean	0.04	0.06	0.17	0.42	2.48	4.80	9.68	19.37	17.84
Mass Balance	A	93.48	102.4	91.84	93.46	94.93	77.93	78.01	89.21	77.33
	B	95.92	98.62	98.39	92.92	94.81	78.45	75.89	90.37	80.04
	Mean	94.70	100.5	95.13	93.20	95.13	78.20	76.92	89.87	78.70

DAT: days after treatment

Table 8.2.2.3-10: Degradation of [14C]glyphosate in water of water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Glyphosate	A	91.51	81.04	63.18	47.68	21.52	14.92	5.86	1.10	0.20
	B	93.42	80.29	64.22	51.68	24.37	12.10	9.39	0.62	0.33
	Mean	92.47	80.67	63.70	49.68	22.95	13.51	7.63	0.86	0.27
AMPA	A	nd	nd	2.86	4.21	12.45	15.39	11.41	4.83	0.39
	B	nd	nd	2.08	5.65	8.98	16.10	11.61	5.23	0.56
	Mean	nd	nd	2.47	4.93	10.72	15.74	11.51	5.03	0.48
HMPA [1]	A	nd	nd	nd	nd	nd	3.75	2.67	11.37	7.63
	B	nd	nd	nd	nd	nd	3.01	4.78	8.58	7.41
	Mean	nd	nd	nd	nd	nd	3.38	3.72	9.97	7.52

DAT: days after treatment
nd: not detected
AMPA: Aminomethyl-phosphoric acid
HMPA: (Hydroxymethyl)-phosphonic acid
[1] The metabolite HMPA was identified by TLC co-chromatography in the course of the addendum.

Table 8.2.2.3-11: Degradation of [^{14}C]glyphosate in water of water/sediment system Unter Widderheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Glyphosate	A	83.92	78.03	47.17	34.41	16.77	14.78	8.30	3.31	1.83
	B	83.68	73.24	50.74	31. 06	25.43	17.07	8.25	3.66	3.02
	Mean	83.80	75.64	48.95	32.74	21.10	15.92	8.27	3.48	2.42

AMPA	A	4.37	2.35	2.95	2.77	3.91	5.41	3.22	0.47	0.39
	B	3.65	1.64	1.18	2.11	4.88	6.14	2.45	0.51	0.39
	Mean	4.01	1.99	2.07	2.44	4.40	*5.78* [1]	2.83	0.49	0.39
HMPA [2]	A	nd	nd	nd	0.24	0.63	0.81	1.76	0.11	0.12
	B	nd	nd	nd	0.15	0.51	0.77	2.09	0.21	0.10
	Mean	nd	nd	nd	0.20	0.57	0.79	1.93	0.16	0.11

DAT: days after treatment
AMPA: Aminomethyl-phosphoric acid
HMPA: (Hydroxymethyl)-phosphonic acid
[1] This value was calculated during summary preparation, as the value given in the report (8.84%) was obviously not the mean values of the two corresponding replicates.
[2] The metabolite HMPA was identified by TLC co-chromatography in the course of the addendum.

Table 8.2.2.3-12: Percentage radioactivity of the parent compound in extract samples of system Bickenbach and system Unter Widderheim (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Bickenbach										
Glyphosate	A	5.29	14.75	33.23	39.16	52.83	38.60	34.46	35.65	29.73
	B	5.23	16.04	30.56	39.62	53.32	36.14	32.86	34.05	28.74
	Mean	5.26	15.40	31.90	39.39	53.08	37.37	33.66	34.85	29.24
Unter Widderheim										
Glyphosate	A	5.01	21.29	39.52	52.83	66.09	46.38	43.37	54.76	44.14
	B	8.29	23.08	44.28	57.31	56.62	42.84	44.22	55.02	44.15
	Mean	6.65	22.19	41.90	55.07	61.36	44.61	43.80	54.89	44.15

DAT: days after treatment

Table 8.2.2.3-13: Percentage radioactivity of the parent compound in the total system (sum of sediment extracts and water) of system Bickenbach and system Unter Widderheim (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Bickenbach										
Glyphosate	A	96.80	95.79	96.41	86.84	74.35	53.50	40.32	36.75	29.93
	B	98.65	96.33	94.78	91.30	77.69	48.24	42.25	34.67	29.07
	Mean	97.73	96.06	95.59	89.07	76.02	50.87	41.29	35.71	29.50
Unter Widderheim										
Glyphosate	A	88.93	99.32	86.69	82.86	82.86	61.16	51.67	58.07	45.97
	B	91.97	96.32	95.02	82.05	76.30	59.91	52.47	58.68	47.17
	Mean	90.41	97.82	90.86	82.46	79.47	60.54	52.07	58.38	*46.57* [1]

DAT: days after treatment
[1] This value was calculated during summary preparation, as the value given in the report (51.07%) was obviously not the mean value of the two corresponding replicates.

B. MASS BALANCE

Mean material balances ranged from 80.79 to 98.83 % of applied radioactivity (% AR) for the sandy water/sediment system Bickenbach and from 76.92 to 100.5 % AR for the loamy water/sediment system Unter Widdersheim. Material balances below 90% may be caused by the formation of volatile metabolites.

The material balance for sterile samples at day 100 was 94.1 % for system Bickenbach and 93.5 % for system Unter Widdersheim.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 100 DAT from 92.47 to 8.27 % AR for the sandy water/sediment system Bickenbach and from 87.81 to 3.09 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 100 DAT from 5.26 to 29.24 % AR for the sandy water/sediment system Bickenbach and from 6.65 to 44.15 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of non-extractable residues (NER) increased from 0 DAT to 100 DAT from 0.07 to 22.01 % AR for sandy water/sediment system and from 0.20 to 13.61 % AR for the loamy water/sediment system Unter Widdersheim.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide were 23.48 % AR at 100 DAT in the sandy water/sediment system Bickenbach and 19.37 % AR at 61 DAT in the loamy water/sediment system Unter Widdersheim. Organic volatiles determined were ≤0.1 % AR for both test systems at all sampling points.

E. TRANSFORMATION OF THE TEST ITEM

The amount of [^{14}C]glyphosate in water decreased from 0 DAT to 100 DAT from 92.47 to 0.27 % AR for water/sediment system Bickenbach and from 83.80 to 2.42 % AR for water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in sediment extracts increased from 0 DAT to 7 DAT from 5.26 to 53.08 % AR, before declining to 29.24 % AR at 100 DAT for water/sediment system Bickenbach and increased from 0 DAT to 7 DAT from 6.65 to 61.36 % AR, before declining to 44.15 % AR at 100 DAT for water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in the total system decreased from 0 DAT to 100 DAT from 97.73 to 29.50 % AR for water/sediment system Bickenbach and from 90.41 to 46.57 % AR for water/sediment system Unter Widdersheim.

Two metabolites were identified in the water phase of both test systems. AMPA was detected with a maximum amount of 15.74 % AR at 14 DAT in water/sediment system Bickenbach, decreasing to 0.48 % AR at 100 DAT. HMPA was detected with a maximum amount of 9.97 % AR at 61 DAT in water/sediment system Bickenbach and decreased to 7.52 % AR at 100 DAT. No other metabolites were detected in water above 5 % AR at any time. No metabolites were detected at any timepoint in sediment extracts of both test systems.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted], 2020, CA 7.2.2.3/001.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing minor deviations.

The mass balance shows values below 90 % for several sampling points, explained by the formation and loss of volatile metabolites. The exact amount of sediment per test vessel is not provided, but the relative ratio of water to sediment (2-2.5 cm sediment layer and 6 cm water layer). Although the storage conditions for re-analysis of water samples are not provided in the amendment, the 1D-TLC results show that the chromatographic pattern is the same as in the main study and no additional spots were observed. In conclusion, the deviations do not influence the overall results and general outcome of the study.

Therefore, the study is considered valid.

Assessment and conclusion by RMS:

Some deviations from OECD 308 are identified. For both systems, incomplete mass balances are observed from 14 DAT to the study end. The study author explains that these material losses are due to formation and degradation of volatile metabolite. RMS notes that volatile metabolites were not observed in any other studies, however losses of entrained $^{14}CO2$ in the test systems waters and

sediments were identified. This might be an explanation, but further data should be provided by the applicant to explain these losses.

This study is considered acceptable pending that the low mass balance is further justified by the applicant (data gap identified).

[redacted], *1997*

Data point:	CA 7.2.2.3/003
Report author	[redacted]
Report year	1997
Report title	[14C-PMG]Glyphosate-trimesium: Aquatic sediment degradation
Report No	RR97-066B
Guidelines followed in study	US EPA 162-4
Deviations from current test guideline	From OECD 308: - Pre-equilibration time of test systems > 4 weeks - Mass balances below 90% AR (68 - 83 %) for all sampling intervals except day zero. - Low organic carbon content (around 0.3%) for Cache system, below the recommended lowest level of 0.5%. - Traps only for CO2 and not for other volatile compounds. - For samples processing, water and sediment were transferred into centrifuge bottles and centrifuged; according to the current guideline water should be decanted without disturbing the sediment - Inconsistencies with peak identification. - Results for individual replicates not reported
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not mentionned in RAR (2015), mentionned in DAR 1998 but not evaluated.
Acceptability/Reliability:	No

MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate-trimesium (labelled in the phosphonomethyl-position)

Lot No.: 3048-281

Specific activity: 51 Ci/mol (674,000 dpm/µg of glyphosate)

Radiochemical purity: 99 % by HPLC

2. Test System:

The sediments were sieved to ≤2 mm. The water and sediment were stored at 4 °C under aerobic conditions for eight weeks before the start of the experiment. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-14: Characteristics of water/sediment test systems

Parameter		Results	
System		Cache Creek	Putah Creek
Location		United States of America	United States of America
Sampling depth for	water	Mid-stream	Mid-stream
	sediment	1–3 meters from the bank	1–3 meters from the bank
Water			

pH	8.4/8.4	8.3/8.3
Total hardness (mg/L)	134	240
Total alkalinity (mg/L)	100/100	203/202
Chemical oxygen demand (mg/L)	5/4	15/12
Sediment		
Textural Class [1]	Loamy sand/Sand	Clay loam
Sand (%)	87.0/89.1	30.7/30.4
Silt (%)	4.2/4.1	34.5/36.8
Clay (%)	8.8/6.8	34.8/32.8
pH [2]	8.1/8.0	8.0
Organic matter (%)	0.49	1.41/1.36
Organic carbon (%)	0.3	0.8
Cation exchange capacity (meq/100 g)	9.14/8.78	23.83/24.34
Dry matter content (%)	76.1	53.8

[1] Classification system not reported
[2] Medium not stated
Two aliquots of both test systems were characterized

Biomass results indicated that the two water-sediment systems were microbially active at the start of the test incubation period, and that a similar pattern of activity remained at the end of the test period.

B. STUDY DESIGN

1. Experimental conditions

The flow-through test system consisted of a glass vessel connected via tubing to a vacuum system. Air entering the system was first moistened by bubbling through a column of distilled water. The water-sediment systems were pre-incubated at 22 °C for 38 days prior to treatment with the test substance to allow equilibration, as determined by assessment of redox potential, pH and dissolved oxygen levels. Following application of the radiolabelled test compound, the effluent air from each series of water/sediment systems was drawn through a tube of sodium hydroxide to absorb any $^{14}CO_2$ produced.

The wet sediments were dispensed into cylindrical glass vessels and the associated natural waters were added to a total volume of 150 ml. The Cache Creek test systems contained 79 g sediment (dry weight basis) and the Putah Creek test systems contained 46 g sediment (dry weight basis). For each system, the depth of settled sediment was between 2 and 2.5 cm and the depth of the surface water was approximately 6 cm. Throughout the equilibration period water levels were maintained at 150 mL in the systems by the addition, as necessary, of the appropriate river water.

The application rate was 2 mg glyphosate-trimesium/L in the water phase, which is equivalent to a use rate of 9000 g glyphosate-trimesium/ha (6000 g glyphosate/ha) evenly distributed to a depth of 30 cm.

Test systems were incubated under aerobic conditions in the dark for 52 days at 20 ± 1.5 °C.

Sterile systems were prepared to distinguish between microbial (biotic) and abiotic degradation of the test substance.

2. Sampling

Duplicate samples from each system were processed and analyzed at 3, 10, 13, 17, 24, 32 and 52 days after treatment (DAT). The NaOH traps were assayed at each sampling time or about every week, whichever came first. Sterile samples were processed and analysed at 10 and 52 DAT. Only results of 52 DAT are presented in this summary. Although duplicate samples were analyzed, only mean values were reported.

3. Analytical procedures

At each sampling interval, the water/sediment systems were transferred into centrifuge bottles and centrifuged. Afterwards, the water was decanted. Water samples were analysed directly by liquid scintillation counting (LSC).

Sediment samples were extracted four times (3 DAT samples were extracted three times) with ammonium hydroxide for 30 minutes by shaking followed by centrifugation. Fine suspended solids formed in the ammonium hydroxide extracts. The ammonium hydroxide extracts containing suspended solids were combined. The resulting suspension was treated with 0.1 M potassium phosphate monobasic and pH was adjusted to pH 2 using concentrated phosphoric acid. Samples from 3, 10, and 52 DAT were made acidic to pH 3-4 with concentrated hydrochloric acid prior to treatment with phosphate buffer. Subsequently, these suspensions were shaken for one minute and centrifuged. The supernatants were decanted and aliquots were taken for determination of radioactivity by LSC. Subsamples of the precipitates were assayed by combustion followed by LSC. Residues in water and sediment extracts were quantified by HPLC/radiodetection.

On removal, the radioactivity in the sodium hydroxide traps was quantified by LSC. The amount of radioactivity recovered in the sodium hydroxide traps was divided by the number of water-sediment systems in-line over the trapping period to determine evolved radioactivity per vessel.

Radioactivity in extracted sediments were determined by combustion/LSC.

Glyphosate and metabolites in the surface water and sediment extracts were characterized by co-chromatography using HPLC and, for selected samples, TLC.

Samples were stored at approximately -20 °C.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study (including pre-equilibration) between 7.8 and 8.4 in system Cache Creek and between 7.9 and 8.4 for system Putah Creek. The oxygen saturation in the water phase ranged between 61.9 and 76.2 % in system Cache Creek and between 61.9 and 85.7 % in system Putah Creek. The redox potential of the water was between 278 and 427 mV for system Cache Creek and between 300 and 450 mV for system Putah Creek. The redox potential of the sediment was between 170 and 352 mV in system Cache Creek and between 231 and 403 mV for system Putah Creek.

Radioactive mass balance and distribution of glyphosate and metabolites in water/sediment system extracts are summarized in the tables below.

Table 8.2.2.3-15: Amount of radioactivity in Cache Creek under aerobic conditions (mean values of two replicates, expressed as percent of applied radioactivity)

Fraction	DAT								
	0	3	10	13	17	24	32	52	52 sterile
Surface water	98.7	14.6	22.5	22.6	21.3	22.9	18.6	9.8	23.8
Sediment extractable	0.0	56.1	42.1	39.9	35.0	24.8	27.4	22.5	37.3
Non-extractable residues	1.2	11.1	9.5	12.4	13.1	15.3	15.5	16.7	20.5
CO_2	0.0	0.1	1.5	1.6	8.2	11.1	6.7	19.3	2.2
Mass balance	99.9	81.9	75.6	76.5	77.5	74.1	68.3	68.3	83.8

DAT: days after treatment

Table 8.2.2.3-16: Amount of radioactivity in Putah Creek under aerobic conditions (mean values of two replicates, expressed as percent of applied radioactivity)

Fraction	DAT								
	0	3	10	13	17	24	32	52	52 sterile
Surface water	98.7	2.3	9.7	8.6	7.5	13.6	16.5	10.3	1.2
Sediment (extractable)	0.0	63.3	53.3	53.4	45.1	46.9	43.3	33.7	62.8

Non-extractable residues	1.2	17.0	15.9	16.0	19.8	13.2	10.7	15.6	27.0
CO_2	0.0	0.0	1.0	2.5	2.5	2.8	3.5	12.9	3.1
Mass balance	99.9	82.6	79.9	80.5	74.9	76.4	74.0	72.4	94.3

DAT: days after treatment

Table 8.2.2.3-17: Degradation of [^{14}C]glyphosate in Cache Creek under aerobic conditions (of two replicates, expressed as percent of applied radioactivity)

Component	DAT								
	0	3	10	13	17	24	32	52	52 sterile
Glyphosate									
Surface water	90.1	4.4	4.3	2.0	1.1	0.4	0.5	0.0	15.2
Sediment (extractable)	NA	39.0	26.2	20.4	13.8	5.1	7.9	1.2	29.9
Total system	90.1	43.5	30.4	22.4	14.9	5.5	8.4	1.2	45.1
AMPA									
Surface water	NA	2.6	5.3	6.3	6.5	9.3	7.4	4.0	3.9
Sediment (extractable)	0.0	10.9	12.7	15.9	18.4	13.2	17.9	19.9	6.3
Total system	NA	13.5	18.1	22.2	24.9	22.5	25.3	24.0	10.2
N-methyl AMPA[1]									
Surface water	0.2	7.1	12.3	13.5	13.0	12.3	9.9	5.1	3.6
Sediment (extractable)	NA	0.6	0.8	1.0	0.9	0.8	1.0	0.5	0.3
Total system	0.2	7.7	13.2	14.5	13.9	13.0	10.9	5.5	3.9

[1] The peak assigned to N-methyl-AMPA was more likely due to $14CO_2$ not accounted for by the trapping system as discussed in [redacted] (1999), CA 7.2.2.3/002 and Expert Statement on this summary
DAT: days after treatment; NA: extracts were below 1 % AR and not analysed

Table 8.2.2.3-18: Degradation of [^{14}C]glyphosate in Putah Creek under aerobic conditions (mean values of two replicates, expressed as percent of applied radioactivity)

Component	DAT								
	0	3	10 [1]	13	17	24	32	52	52 sterile
Glyphosate									
Surface water	98.1	0.2	0.0	0.0	0.0	0.1	0.3	0.0	0.2
Sediment (extractable)	NA	54.7	36.7	37.4	27.1	28.2	21.3	8.3	51.2
Total system	98.1	54.9	36.7	37.4	27.1	28.3	21.6	8.3	51.4
AMPA									
Surface water	0.0	0.1	3.0	0.6	0.6	1.3	4.0	1.8	0.1
Sediment (extractable)	NA	3.3	11.2	13.0	14.6	17.2	20.3	23.5	8.9
Total system	0.0	3.4	14.2	13.6	15.1	18.5	24.3	25.3	9.0
N-methyl AMPA[2]									
Surface water	0.4	1.9	6.7	7.7	6.7	11.8	11.7	8.1	0.8
Sediment (extractable)	NA	1.5	1.3	1.0	1.2	0.6	1.3	0.6	1.5
Total system	0.4	3.4	8.0	8.7	7.9	12.3	13.0	8.7	2.3

[1] Calculations based on a single replicate due to sample loss.
[2] The peak assigned to N-methyl-AMPA was more likely due to $^{14}CO_2$ not accounted for by the trapping system as discussed in [redacted] (1999), CA 7.2.2.3/002 and Expert Statement on this summary
DAT: days after treatment; NA: extracts were below 1 % AR and not analyzed

B. MASS BALANCE

Mean mass balances ranged from 68.3 to 99.9 % of applied radioactivity (% AR) for system Cache Creek location and from 72.4 to 99.9 % AR for system Putah Creek.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the surface water decreased from 0 DAT to 52 DAT from 98.7 to 9.8 % AR in system Cache Creek system and from 98.7 to 10.3 % AR in system Putah Creek.

The amount of radioactivity extractable from the sediment increased from 0.0 % AR at 0 DAT to a maximum of 56.1 % AR at 3 DAT then decreased to 22.5 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of radioactivity extractable from the sediment increased from 0.0 % AR at 0 DAT to a maximum of 63.3 % AR at 3 DAT then decreased to 33.7 % AR at 52 DAT.

The amount of non-extractable residues (NER) increased from 1.2 % AR at 0 DAT to 16.7 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of NER increased from 1.2 % AR at 0 DAT to a maximum of 19.8 % AR at 17 DAT then decreased to 15.6 % AR at 52 DAT.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (52 DAT) were 19.3 and 12.9 % AR in the Cache Creek and Putah Creek systems, respectively.

E. TRANSFORMATION OF THE TEST ITEM

In the Cache Creek system, the amount of glyphosate in the total test system (water and sediment) decreased from 90.1 % AR at 0 DAT to 1.2 % AR at 52 DAT. In the water layer, it decreased from 90.1 % AR at 0 DAT to 0.0 % AR at 52 DAT. In the sediment, it decreased from 39.0 % AR at 3 DAT to 1.2 % AR at 52 DAT. In the Putah Creek system, the amount of glyphosate in the total test system (water and sediment) decreased from 98.1 % AR at 0 DAT to 8.3 % AR at 52 DAT. In the water layer, it decreased from 98.1 % AR to 0.0 % AR at 10 DAT. In the sediment, it decreased from 54.7 % AR at 3 DAT to 8.3 % AR at 52 DAT.

Besides carbon dioxide, two metabolites, aminomethylphosphonic acid (AMPA) and N-methylaminophosphoic acid (N-methyl AMPA), were detected in the water/sediment systems.

AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 32 DAT then decreased to 24.0 % AR at 52 DAT in the Cache Creek total system. In the water layer, it increased to 9.3 % AR at 24 DAT and decreased to 4.0 % AR at 52 DAT. In the sediment layer, it increased to 19.9 % AR at 52 DAT. In the Putah Creek system, AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 52 DAT. In the water layer, it increased from 0.0 % AR at 0 DAT to 4.0 % AR at 32 DAT and decreased to 1.8 % AR at 52 DAT. In the sediment layer, it increased to 23.5 % AR at the end of the study at 52 DAT.

Levels of a metabolite assigned to N-methyl-AMPA increased from 0.2 % AR at 0 DAT to a maximum of 14.5 % AR at 13 DAT then decreased to 5.5 % AR at 52 DAT in the Cache Creek total system. In the water layer it increased to 13.5 % AR at 13 DAT and decreased to 5.1 % AR at 52 DAT. In the sediment layer it increased to 1.0 % AR at 13 DAT and decreased to 0.5 % AR at 52 DAT. In the Putah Creek total system, N-methyl AMPA levels increased from 0.4 % AR at 0 DAT to a maximum of 13.0 % AR at 32 DAT then decreased to 8.7 % AR at 52 DAT. In the water layer, it increased to 11.8 % AR at 24 DAT and decreased to 8.1 % AR at 52 DAT. In the sediment, it increased to 1.3 % AR at 32 DAT and decreased to 0.6 % AR at 52 DAT.

Comparison with sterile samples shows that the degradation of glyphosate under the study conditions was primarily microbially mediated.

III. CONCLUSIONS

Glyphosate dissipated rapidly from surface water in natural water/sediment systems incubated in the dark at 20 °C. More than 90 % of the applied [^{14}C]glyphosate-trimesium is lost from the surface water in less than three days. The rapid initial loss of glyphosate from the surface waters was probably due to binding to the sediment and is consistent with the adsorption properties of glyphosate. Levels of glyphosate in the surface waters had fallen to below the detection limit after incubation for 52 days, in both water-sediment systems under the study conditions.

Besides carbon dioxide, aminomethylphosphonic acid (AMPA) was detected in the water/sediment systems. AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 32 DAT then decreased to 24.0 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 52 DAT.

Levels of a metabolite assigned to N-methyl-AMPA increased from 0.2 % AR at 0 DAT to a maximum of 14.5 % AR at 13 DAT then decreased to 5.5 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, N-methyl AMPA levels increased from 0.4 % AR at 0 DAT to a maximum of 13.0 % AR at 32 DAT then decreased to 8.7 % AR at 52 DAT.

No other individual radiolabelled degradate accounted for more than 1 % of the applied dose in either system.

Maximum amounts of carbon dioxide reached at study end (52 DAT) were 19.3 and 12.9 % AR in the Cache Creek and Putah Creek system, respectively.

Assessment and conclusion by applicant:

Pre-equilibration of the test systems was 38 days thus slightly exceeding 4 weeks as given by the guideline. Nevertheless, pH, oxygen content and redox potential were monitored throughout the study and thus, the validity is not affected.

Mass balances were below 90% AR (i.e. 68 - 83 %) for all samples except day zero.

Early sampling points like 1 and 2 DAT were not sampled. This limits the possibility of kinetic evaluation of the data. Additionally, mean values of two replicates are reported and no individual values are available.

For sample processing, water and sediment were transferred into centrifuge bottles and centrifuged; according to the current guideline water should be decanted without disturbing the sediment. Thus, the distribution residues between water and sediment may be affected.

The study by [redacted] (1999, CA 7.2.2.3/002) used the same sediments and thus repeated the study performed by [redacted] (1997, CA 7.2.2.3/003). In [redacted] (1999, CA 7.2.2.3/002), there is a comment that the identity of the peak assigned to N-methyl-AMPA in this study was more likely to be $^{14}CO_2$ not accounted for. In [redacted] (1999, CA 7.2.2.3/002), the potential presence of $^{14}CO_2$ in water and sediment was taken care for in work-up by acidification/additional trapping by NaOH and significant amounts $^{14}CO_2$ were released this way from water/sediment systems.

Labelling in original report (HPLC chromatogram of 10 DAT in Putah Creek water; see Figure 8.2.2.3-1 below in the expert statement) is inconsistent with the findings above.

The study is considered invalid.

The applicant also provided the following statement regarding the validity of the study.

Expert Statement – Assessment on validity

The Glyphosate Renewal Group found that the study ([redacted], 1997, CA 7.2.2.3/003) has major shortcomings and should not be considered for use in environmental risk assessments. The reasoning is based on the following:

Poor mass balances in both test systems make data unacceptable for rate of glyphosate dissipation determinations.

The metabolite reported as N-methyl AMPA in both systems is actually carbonate, and is therefore not to be considered a metabolite for risk assessment.

Inconsistencies with peak identification.

Rationales supporting these points are discussed below.

The mass balances in the 1997 study ([redacted], 1997, CA 7.2.2.3/003) for all time intervals in both test systems from Day 3 through the end of the study on Day 52 are well below current guidance regarding mass balance acceptance criteria; thus making data from the study unacceptable for rate of glyphosate dissipation determinations. The OECD 308 Aerobic and Anaerobic Transformation in

Aquatic Sediment Systems guideline states, "Recoveries should range from 90% to 110% for labelled chemicals (6) and from 70% to 110% for non-labelled chemicals." The study was conducted with ^{14}C-labelled glyphosate and except for the Day 0 samples, which averaged 99.9% in both test systems, mass balances were significantly below 90% from Day 3 through the end of the study on Day 52. In addition, mass balances generally decreased during the study. For the Putah Creek system, the average mass balance was 82.6% on Day 3 and 72.4% on Day 52. A similar result was obtained for the Cache Creek system with an average mass balance of 81.9% on Day 3 and 68.3% on Day 52.

No explanation for the low mass balances is reported. Because glyphosate, AMPA, and any other likely metabolites are highly water soluble, significant losses of radioactive residues to the test vessels is highly unlikely and has not been observed in other environmental fate studies. The only other reasonable explanation for the low mass balances is that $^{14}CO_2$ was not fully accounted for in the test systems. There are three primary ways $^{14}CO_2$ might not have been fully accounted for in the study: leaks in both systems, inefficient trapping of $^{14}CO_2$ in the NaOH traps, or $^{14}CO_2$ entrained in the waters and/or sediment as carbonate that was partially or completely lost during processing of samples for analysis. It seems unlikely that losses would have occurred through leaks or inefficient trapping, but neither possibility can be completely ruled out.

Because no efforts appear to have been taken, losses of entrained $^{14}CO_2$ also cannot be ruled out. Zeneca clearly recognized that $^{14}CO_2$ could have been entrained in the waters and sediments as they conducted a follow up study two years later. The study involved test systems from the same sources as the original study, but the study design was modified from the original study to account for entrained $^{14}CO_2$ in both the waters and sediments ([redacted], 1999, CA 7.2.2.3/002). The waters were acidified with 0.5 M KH_2PO_4 and sparged by pulling air through them into two 1 M NaOH traps to trap evolved $^{14}CO_2$. The sediments were extracted with aqueous 0.5 M KH_2PO_4 and evolved $^{14}CO_2$ was trapped in the same manner as the acidified water samples. Incorporating these precautions, mass balances were >90% for all time intervals except for the Day 30 and Day 58 intervals in the Cache test system which were 86.7% and 86.3%, respectively ([redacted] 1999, CA 7.2.2.3/002).

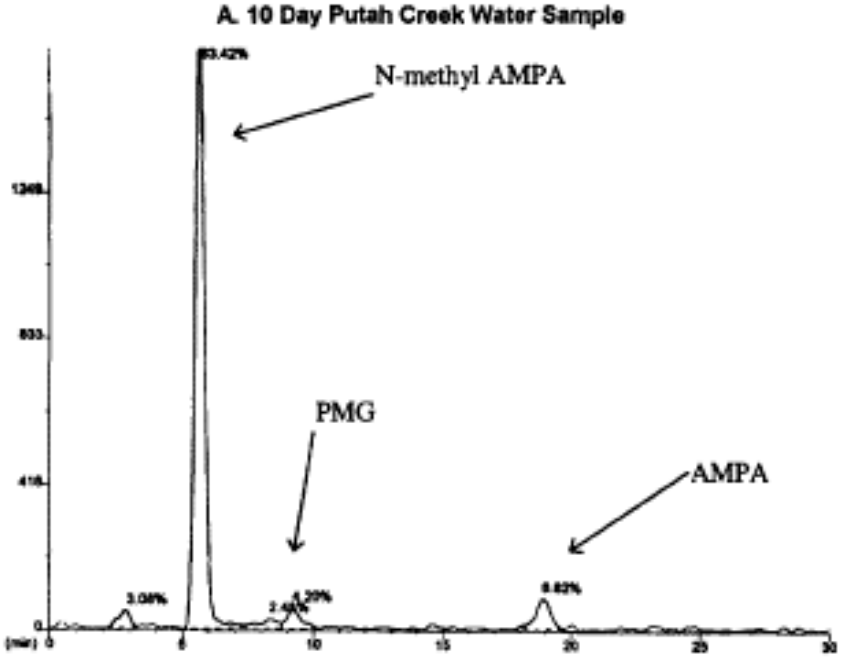
To show that the radioactive material sparged from waters and extracts was $^{14}CO_2$, Zeneca applied a standard approach used to test for $^{14}CO_2$. Aliquots of the NaOH traps were treated with $BaCl_2$ to precipitate $BaCO_3$. Analysis of the supernatants by LSC showed levels of radioactivity just above background. This provided clear evidence that $^{14}CO_2$ was entrained in the waters and sediment extracts.

The poor mass balances obtained in [redacted] (1997, CA 7.2.2.3/003) can clearly be attributed in large part, if not completely, to losses of entrained $^{14}CO_2$ in the test systems waters and sediments based on results from [redacted] (1999, CA 7.2.2.3/002). However, even with this explanation for the poor mass balances, the data are not appropriate for dissipation rate determinations of glyphosate in either test system in the 1997 study.

The Metabolite Identified as *N*-methyl AMPA is Actually Carbonate

The peak eluting at approximately 5.5 minutes in the chromatogram in Figure 8.2.2.3-1 (Figure 11 in original study) from [redacted] (1997, CA 7.2.2.3/003) was misidentified as *N*-methyl AMPA based on misinterpretation of the available data. Instead, the Glyphosate Renewal Group concludes that the peak actually corresponds to carbonate based on results from [redacted] (1999, CA 7.2.2.3/002) as well as an assessment of chromatographic properties obtained in [redacted] (1997, CA 7.2.2.3/003). In addition, the labelling in Figure 8.2.2.3-1 is inconsistent with the percentages of % total radioactivity in the tables (summary of [redacted], 1997, CA 7.2.2.3/003).

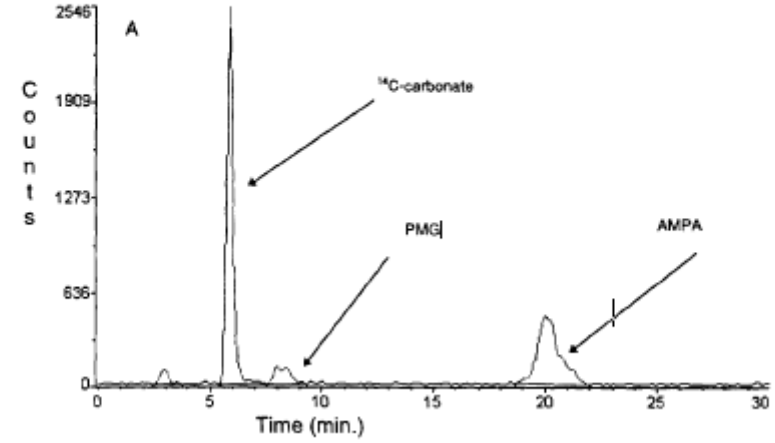
Figure 8.2.2.3-1: HPLC Chromatogram of Day 10 Putah Creek Water ([redacted], 1997, CA 7.2.2.3/003)

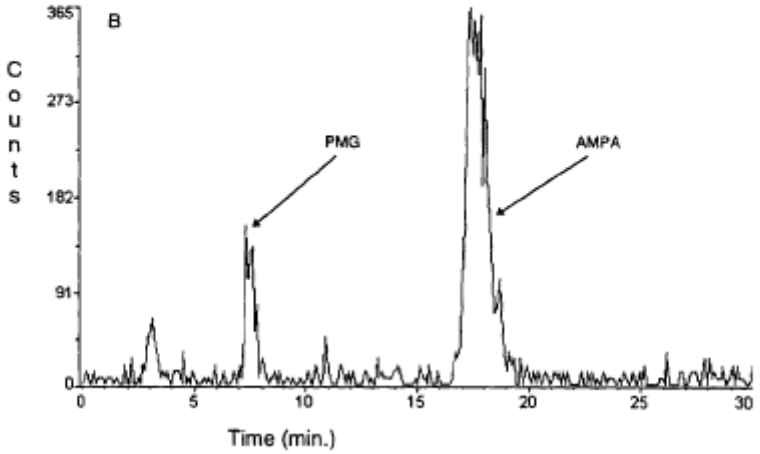


As described in the previous section, [redacted] (1999, CA 7.2.2.3/002) showed that $^{14}CO_2$ was entrained in the waters and sediment extracts of both test systems. To provide additional evidence for the presence of $^{14}CO_2$ in waters, a sample of the Day 58 Cache Creek water was analyzed by HPLC before and after acidification (Figure 8.2.2.3-2, Figure 11A in original report). The chromatogram obtained before acidification (Figure 8.2.2.3-2) contains a 5.5-minute peak along with peaks identified as PMG and AMPA. The chromatogram obtained after acidification (Figure 8.2.2.3-2, Figure 11B) does not contain the 5.5-minute peak and only shows PMG and AMPA as identified peaks. This result provides compelling information that the 5.5-minute peak is carbonate. Furthermore, because the chromatograms (Figure 8.2.2.3-1) from the [redacted] (1997, CA 7.2.2.3/003) were essentially obtained under the same HPLC conditions (flow rates differed by 0.1 mL/min between the two studies), it can be concluded that the 5.5-minute peak in that study is also carbonate.

Figure 8.2.2.3-2 HPLC Chromatograms of Day 58 Cache Creek Water from the 1999 Aquatic Sediment Study Before and After Acidification ([redacted], 1999, CA 7.2.2.3/002)

Figure 11 HPLC chromatograms of 58 Days Cache Creek surface water before (A) and after acidification and sparging (B)

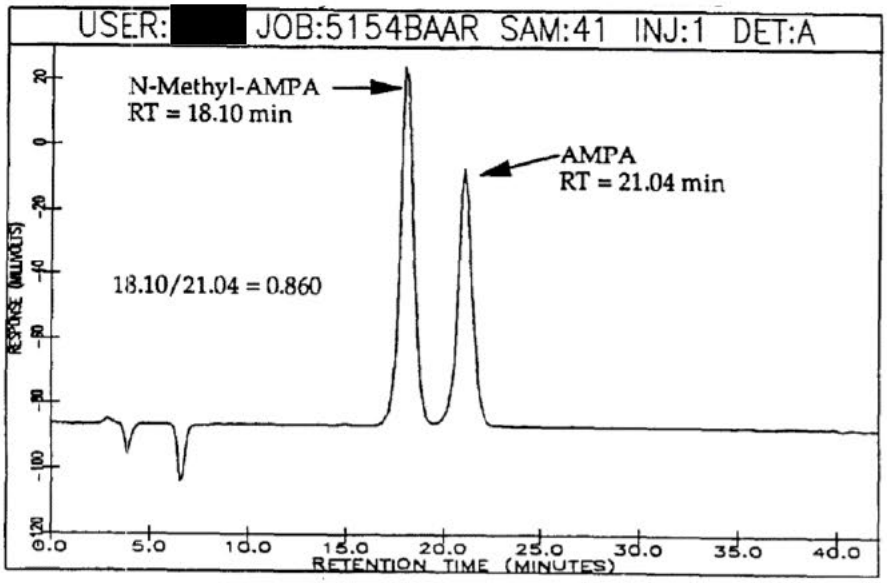




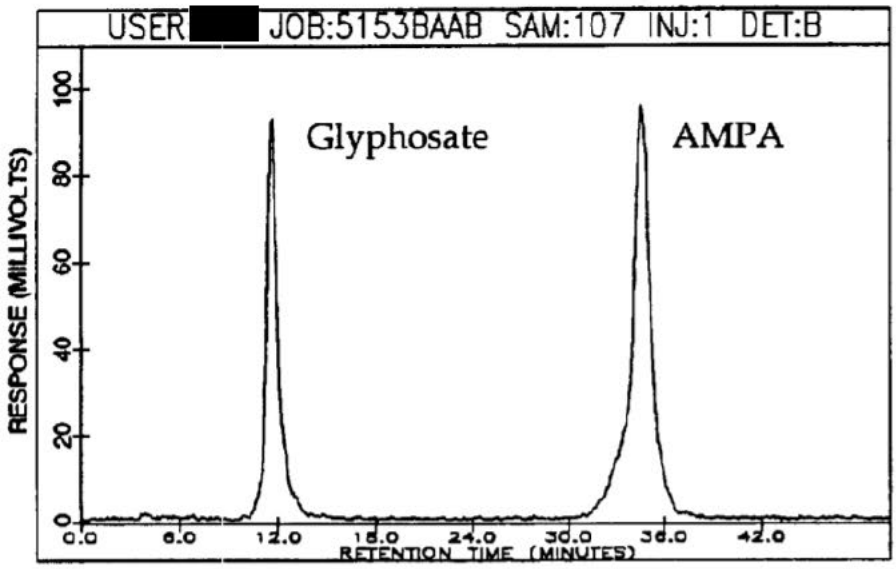
The chromatographic properties expected for *N*-methyl AMPA and carbonate under the strong cation exchange column conditions used in [redacted] (1997, CA 7.2.2.3/003) are another consideration. A retention time of 5.5 minutes is unreasonable for *N*-methyl AMPA, but it is reasonable for the retention time of carbonate. *N*-Methyl AMPA is structurally similar to AMPA with the only difference being a methyl group on nitrogen. Based on the structural similarities, one would expect comparable retention times. Evidence supporting this expectation is found in the top chromatogram in Figure 8.2.2.3-3 from the metabolism study with glyphosate-tolerant soybeans ([redacted], 1994, *Monsanto Report MSL-13520,* see M-CA Section 6, CA 6.2.1/022). The chromatogram was obtained on a cation exchange column using a mobile phase comparable to the one used in the two aquatic sediment studies. As can be seen, the two analytes are well retained with *N*-methyl AMPA eluting at 18.1 min and AMPA eluting at 21.0 min. In contrast, the bottom chromatogram in Figure 8.2.2.3-3 shows that glyphosate elutes at a much earlier time (~12 min) than AMPA (~34 min), but with the same relative elution order as in the aquatic sediment studies.

Figure 8.2.2.3-3 Analysis of N-Methyl AMPA, AMPA, and Glyphosate Reference Standards on a Cation Exchange Column

Analysis of N-Methyl AMPA and AMPA Reference Standards on a Cation Exchange Column Using a 0.005 M KH_2PO_4 / 4% Methanol Mobile Phase Adjusted to pH 2 ([redacted], 1994, Monsanto Report MSL 13520, see M-CA Section 6, CA 6.2.1/022)



Analysis of [^{14}C]AMPA and [^{14}C]Glyphosate Reference Standards on a Cation Exchange Column Using a 0.005 M KH_2PO_4 / 4% Methanol Mobile Phase Adjusted to pH 2 ([redacted], 1994, Monsanto Report MSL 13520, see M-CA Section 6, CA 6.2.1/022)



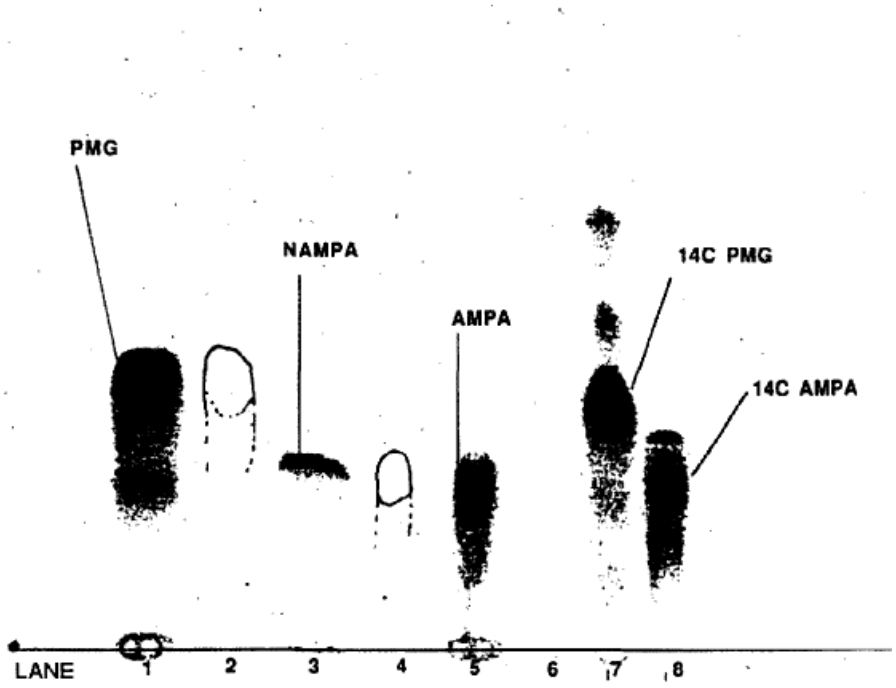
Lastly, the two different conditions used for TLC analyses in [redacted] (1997, CA 7.2.2.3/003) do not provide convincing evidence supporting the identification of *N*-methyl AMPA (Figure 8.2.2.3-4). In fact, a reasonable case can be made that regions associated with *N*-methyl AMPA in the analyses conducted are actually due to AMPA.

The TLC result in Figure 8.2.2.3-5 shows a radioactive region in lane 3 designated as *N*-methyl AMPA from a Day 32 water sample, a region in lane 4 corresponding to the *N*-methyl AMPA reference standard, and a radioactive region in lane 5 corresponding to AMPA from a Day 24 sediment extract sample. As can be seen, the leading front of each region migrated to the same extent, which means there was essentially no separation of *N*-methyl AMPA and AMPA under the conditions used for elution. In addition, the shape of the radioactive region in lane 3 assigned as *N*-methyl AMPA is much different

than that for the *N*-methyl AMPA reference standard in lane 4. A reasonable explanation for this is that the radioactive material actually corresponds to the low level of AMPA (~4.0%) in the sample, while the more predominant ^{14}C-carbonate residue in the sample (~11.7%) was lost during TLC under acidic conditions in the open system.

Figure 8.2.2.3-4 TLC on Silica Gel Using MeOH/H_2O/NH_4OH/ Trichloroacetic Acid (65/21/14/0.45) ([redacted], 1997, CA 7.2.2.3/003)

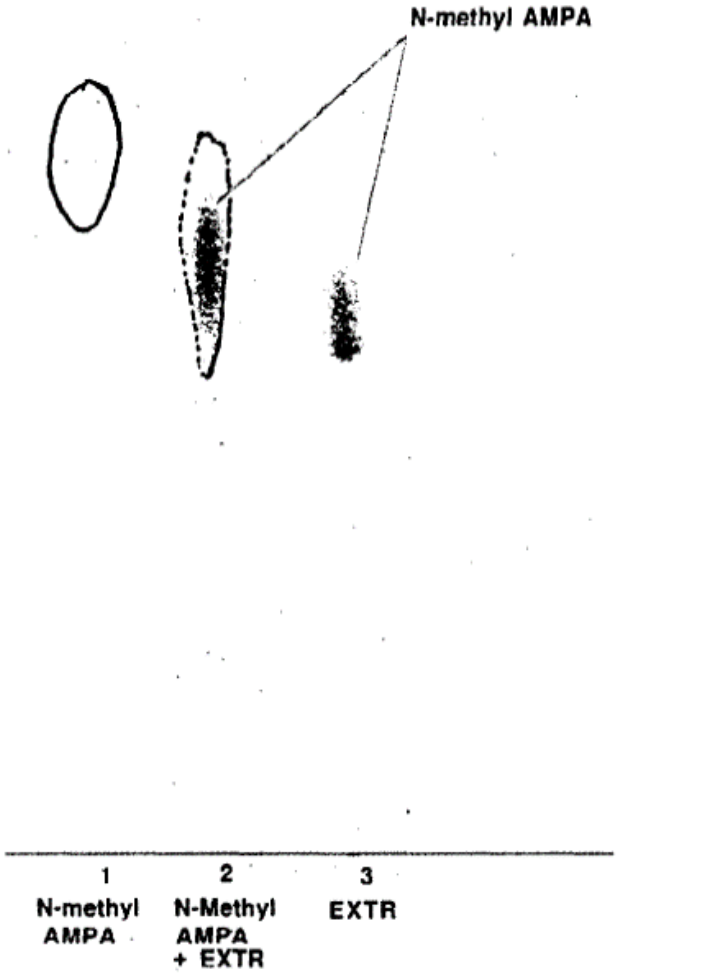
Figure 9. Co-chromatography of Selected Water Samples and Sediment Extracts with cold PMG and N-Methyl AMPA standards.



Lane 1. Co Chromatography of 72Hr Putah Creek Sediment Extract with Cold PMG Standard
Lane 2 Cold PMG Standard
Lane 3 Co-Chromatography of 32 Day Putah Creek Water Sample with Cold N-Methyl-AMPA.
Lane 4. Cold N-Methyl-AMPA
Lane 5 Co-Chromatography of 24 Day Cache Creek Sediment Extract with Cold AMPA Standard.
Lane 6. Cold AMPA Standard, low concentration, not visualized. AMPA identified above
Lane 7. ^{14}C PMG Standard
Lane 8. ^{14}C AMPA Standard

Figure 8.2.2.3-5 TLC on Silica Gel Using MeOH/50 mM NH_4HCO_3 at pH 3.7 (40/60) ([redacted], 1997, CA 7.2.2.3/003)

Figure 10. **TLC Co-Chromatography of 32 Day Water Sample with N-Methyl-AMPA standards in Method 2.**



lane 1 Cold N-Methyl-AMPA standard
lane 2 32 Day Water Sample and Cold N-Methyl-Ampa Standard
lane 3 32 Day Water Sample

The TLC result for the Day 32 water in Figure 8.2.2.3-5, which involved a different mobile phase than the one in Figure 8.2.2.3-4, is also generally consistent with the presence of AMPA instead of *N*-methyl AMPA. Lanes 2 and 3 contain a radioactive component that does not migrate to the same extent as the *N*-methyl AMPA reference standard in lane 1. Furthermore, the *N*-methyl AMPA reference standard cospotted with the Day 32 water sample in lane 2 migrated well beyond the radioactive component in the water sample. As with the explanation for the TLC result in Figure 8.2.2.3-4, a reasonable explanation for the results in Figure 8.2.2.3-5 is that the radioactive material actually corresponds to the AMPA in the sample, while the more predominant ^{14}C-carbonate residue was lost during TLC under acidic conditions.

The poor mass balance recoveries in the 1997 aquatic sediment study make the data unacceptable for rate of glyphosate dissipation determinations. The poor mass balances are due to losses of entrained $^{14}CO_2$ during sample processing. The peak identified as *N*-methyl AMPA in the 1997 study is actually carbonate.

Assessment and conclusion by RMS:

As mentioned in the conclusion from the applicant and in the expert statement, major deviations are identified for this study and in particular : incomplete mass balance for all samples, sampling method not in line with current guideline OECD 308 (water phase was not decanted and disturbance of water and sediment compartments may have occurred) and problems of metabolite identification.

RMS agrees with the justifications provided in the expert statement that the study is not considered acceptable. As explained by the applicant, the study was repeated by [redacted] (1999, CA 7.2.2.3/002), in which the design was adapted. Results from [redacted] (1999, CA 7.2.2.3/002) are therefore considered as more reliable.

[redacted], *1996*

Data point:	CA 7.2.2.3/004
Report author	[redacted].
Report year	1996
Report title	Degradation and metabolism of glyphosate in two water/sediment systems under aerobic conditions - laboratory test
Report No	96138/01-CUWS
Guidelines followed in study	BBA Guideline Part IV, 51
Deviations from current test guideline	From OECD 308: - Acclimation time prior to application not stated. - Water:sediment ratio between 3:1 and 2:1. - Mass balances were <90 % AR at the last 3 sampling times for the Creek system. - Low organic carbon content (0.11%) for Creek system, below the recommended lowest level of 0.5%. - System discontinuously ventilated with CO2 free air - Oxygen concentration of the water phase not in the recommended range of 7-10 mg/L - Experiment duration of 120 days, exceeding slightly the guideline recommendations of 100 days. - LOD of the chromatographic method not reported. - Non chromatographable residues: loss of activities at different steps of the experiment.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate, free acid (labelled in the phosphonomethyl-position)
GAB No.:	95138
Batch. No.:	25A
Code:	CFA.745
Specific activity:	2000 MBq/mmol (54 mCi/mmol; 11.7 MBq/mg)
Radiochemical purity:	98.3 %

Identification:	glyphosate technical (non-radiolabelled)
GAB No.:	96159
Batch. No.:	80240496
Code:	96/N-272
Chemical purity:	99 %

2. Test System:

Water and sediment were sampled from two different locations, *e.g.* pond and river, known not to be submitted to discharges of effluents or near human activity. Water was sampled down to a depth of 10 to 30 cm and the sediment was sampled from the top 20 cm of each system. The water/sediment systems were stored for approximately 14 days at approximately 4°C and afterwards prepared for acclimation.

The sediments were sieved to ≤2 mm and the water was sieved through a 0.2 mm sieve. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-19: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Pond (Bauschlott)	Creek (Ottenhofen)
Country	Germany	Germany
Sediment		
Textural Class	Loamy silt	Sand
Sand (%)	9.8	97.2
Silt (%)	79.1	1.7
Clay (%)	11	1.1
pH [1]	6.64	7.85
Organic carbon (%)	3.31	0.11
Organic matter [2] (%)	5.69	0.20
Cation exchange capacity (meq/100 g)	22.1	4.3
Redox potential (mV)	-192	208
Microbial biomass (µg C/ g dry matter)		
Before application	1017 ± 25	121 ± 3
Study end	1024 ± 24	214 ± 5
Water phase (at the time of sampling)		
pH	8.26	7.85
Oxygen concentration (mg/L)	15.6	11.3
Redox potential (mV)	88	90

[1] medium not reported
[2] calculated from organic carbon according to OM = OC x 1.72
DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

The study was performed with a closed gas flow system using 1000 mL all-glass metabolism flasks containing about 500 mL ± 40 mL water and approx. 230 g wet pond sediment (dry weight approx. 130 g) and 360 g creek sediment (dry weight approx. 308 g), respectively. In both systems, the height of the water column was about 6 cm and the sediment layer was approximately 2.5 cm thick. The systems were ventilated discontinuously for at least 60 min per day with CO_2 free, moistened air. After leaving the test vessels the air was passed through a trapping system for organic volatiles (ethylene glycol), two solid phase traps (soda lime) and one liquid trap (NaOH) to collect $^{14}CO_2$.

The test systems were incubated in the dark in a constant temperature room at 20 ± 2°C. The water/sediment systems were pre-incubated until an equilibrium based on measured variables in the water layer was reached.

The test item was applied to the water surface in each flask to give a nominal initial application of 691.2 µg glyphosate/ 80 cm^2, equivalent to 4.32 kg/ha. The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate, resulting in 185 kBq [^{14}C]glyphosate (15.6 µg) and 0.676 mg unlabelled glyphosate per test system.

Test systems were incubated under aerobic conditions in the dark for 120 days at 20±2°C.

2. Sampling

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 7, 14, 29, 58, 98 and 120 days after treatment (DAT). Traps for volatiles were exchanged at the date of sampling or after 28±2 days, whichever was shorter.

3. Analytical procedures

During acclimation and at each sampling point pH value, oxygen saturation and redox potential of the water layer and the redox potential of the sediment layer were monitored.

For each system the water column from above the sediment was poured out and filtered. The radioactivity in the water was analyzed by LSC. The sediment was extracted at ambient temperature three times with 1 M NH_4OH for 1 hour and one further time with acetone/water (50/50, v/v). The radioactivity in the water/acetone extracts was analysed by LSC and the extracts were discarded as they contained <5 % AR at all sampling intervals. The water and NH_4OH extracts were worked-up as described in "Rückstandsanalytik von Pflanzenschutzmitteln" (GAB SOP 12.3.2-1). The radioactivity in the sample that did not remain on the columns or precipitate during sample preparation was determined by LSC. The solution was concentrated by evaporation under vacuum and otherwise prepared for an analytical determination of the radioactivity.

The amounts of glyphosate and AMPA in water and sediment extracts were quantified by HPLC.

The recovery rates for the extraction of the sediment, the overlaying water phases and the sample preparation via ion exchangers were determined prior to sample analysis for both water/sediment systems were determined by spiked sediment samples taken through the entire work-up and clean-up procedure. Recoveries obtained for analysis of spiked sediment extracts were 72 % for Pond sediment and 97 % for Creek sediment. Recoveries obtained for analysis of spiked water samples were 88 % for Pond sediment and 100 % for Creek sediment.

To quantify non-extractable residues (NER), extracted sediments were combusted and the radioactivity was determined by LSC.

The sodium hydroxide and ethylene glycol trap solutions were analysed by LSC. The $^{14}CO_2$ collected in the solid soda lime traps was stripped by leading in of acidic gas and collection in a NaOH trap solution, which was analysed by LSC afterwards.

Extracts containing more than 5 % AR were characterised by HPLC and co-chromatography of available reference compounds.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water during the study was between 7.26 and 7.88 in the pond system and between 7.45 and 9.05 for creek system. The oxygen concentration in the water phase ranged between 0.9 and 4.0 mg/L in the pond system and between 0.9 and 8.2 mg/L in creek system. The redox potential of the water was between 80 and 222 mV for the pond system and between 80 and 224 mV for the creek system. The redox potential of the sediment was between -180 and -70 mV in the pond system and between -18 and 202 mV for the creek system.

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in water/sediment systems are summarized below.

Table 8.2.2.3-20: Mean distribution of radioactivity in pond water/sediment system (expressed as percent of applied radioactivity)

Compound	DAT									
	0	0.25	1	2	7	14	29	58	97	120
Water	84.00	73.01	52.78	41.55	37.23	19.27	12.43	1.55	3.08	3.79
Sediment	14.16	26.77	52.89	62.32	71.87	86.35	91.67	93.13	77.95	77.90
Volatiles	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03
Carbon dioxide	0.00	0.04	0.04	0.06	0.19	0.55	0.70	6.70	9.63	14.77
Total recovery	98.16	99.83	105.72	103.94	109.30	106.18	104.81	101.40	90.69	96.50

Table 8.2.2.3-21: Mean distribution of radioactivity in creek water/sediment system (expressed as percent of applied radioactivity)

Compound	DAT									
	0	0.25	1	2	7	14	29	58	97	120
Water	92.31	91.71	86.09	81.42	72.11	66.19	49.91	38.75	22.99	20.57
Sediment	8.74	9.33	12.99	19.88	25.55	26.36	36.20	35.78	30.54	31.76
Volatiles	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.04	0.04
Carbon dioxide	0.00	0.03	0.08	0.26	1.26	2.57	7.99	12.33	26.32	30.08
Total recovery	101.05	101.08	99.17	101.57	98.93	95.13	94.11	86.88	79.89	82.45

Table 8.2.2.3-22: Content of Glyphosate and its metabolites in the water phase in the pond water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
NCR [1]	%	11.84	16.91	11.72	8.82	7.85	3.29	2.44	1.13	1.55	1.32
	µg	81.8	116.9	81.0	60.9	54.2	22.7	16.9	7.9	10.7	9.1
Glyphosate	%	70.57	55.18	39.57	31.36	27.42	14.32	8.20	0.24	1.04	1.83
	µg	487.6	381.3	273.5	216.7	189.5	99.0	56.7	1.6	7.2	12.7
AMPA	%	1.59	0.93	1.49	1.38	1.97	1.67	1.79	0.12	0.49	0.64
	µg	11.0	6.4	10.3	9.5	13.6	11.5	12.4	0.8	3.4	4.4
Metabolite 2	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
	µg	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Sum	%	84.00	73.02	52.78	41.56	37.24	19.28	12.43	1.56	3.08	3.79
	µg	580.4	504.6	364.8	287.1	257.3	133.2	86.0	10.8	21.3	26.2

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

Table 8.2.2.3-23: Content of Glyphosate and its metabolites in the sediment in the pond water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
Bound Residues	%	2.60	2.84	10.06	11.62	19.85	25.12	24.46	29.46	16.13	17.15
	µg	18.0	19.6	69.5	80.3	137.2	173.6	169.0	203.6	111.5	118.5
NCR [1]	%	6.80	12.30	18.10	11.40	14.90	19.40	16.60	20.00	20.80	15.20
	µg	47.0	84.8	124.8	78.5	103.2	134.1	115.1	137.9	143.4	105.0
Glyphosate	%	3.60	8.80	19.90	33.40	31.60	35.50	40.00	33.00	27.10	29.80
	µg	24.9	60.8	137.4	231.1	218.2	245.6	276.7	228.1	187.6	206.0
AMPA	%	1.20	2.90	4.90	5.90	5.50	6.30	10.50	10.70	13.90	15.70
	µg	8.1	19.8	33.8	40.8	38.1	43.4	72.6	74.0	95.2	108.8
Sum	%	14.20	26.84	52.96	62.32	71.85	86.32	91.56	93.16	77.93	77.85
	µg	98.0	185.0	365.5	430.7	496.7	596.7	633.4	643.6	538.7	538.3

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

Table 8.2.2.3-24: Content of Glyphosate and its metabolites in the water phase in the creek water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
NCR [1]	%	6.42	14.20	10.68	7.82	12.99	16.20	24.83	22.39	16.62	12.47
	µg	44.4	98.1	73.8	54.0	89.8	112.0	171.6	154.7	114.9	86.2
Glyphosate	%	85.90	77.51	72.78	69.89	52.97	42.54	16.69	6.03	1.43	0.00
	µg	593.5	535.6	502.9	483.0	366.0	294.0	115.3	41.7	9.9	0.0
AMPA	%	0.00	0.00	2.63	3.71	6.16	7.45	8.39	10.34	4.95	8.10
	µg	0.0	0.0	18.2	25.6	42.6	51.5	58.0	71.5	34.2	56.0
Sum	%	92.32	91.71	86.09	81.42	72.12	66.19	49.91	38.76	23.00	20.57
	µg	637.9	633.7	594.9	562.6	498.4	457.5	344.9	267.9	159.0	142.2

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

Table 8.2.2.3-25: Content of Glyphosate and its metabolites in the sediment in the creek water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
Bound Residues	%	1.10	1.30	2.05	2.65	4.83	4.12	9.81	10.43	8.02	9.49
	µg	7.6	9.0	14.2	18.3	33.4	28.5	67.8	72.1	55.4	65.6
NCR [1]	%	1.40	1.50	2.10	4.20	4.90	6.90	7.10	6.30	7.40	6.40
	µg	10.0	10.4	14.2	28.7	33.5	47.4	48.8	43.5	51.4	43.9
Glyphosate	%	5.60	5.10	6.90	9.40	8.90	6.60	7.20	6.30	0.00	0.00
	µg	38.7	35.5	47.8	64.7	61.2	45.8	49.6	43.4	0.0	0.0
AMPA	%	0.60	1.40	2.00	3.70	7.00	8.70	12.20	12.80	15.10	15.90
	µg	4.2	9.6	13.7	25.7	48.5	60.5	84.0	88.3	104.3	110.0
Sum	%	8.70	9.30	13.05	19.95	25.63	26.32	36.31	35.83	30.52	31.79
	µg	60.5	64.5	89.9	137.4	176.6	182.2	250.2	247.3	211.1	219.5

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

B. MASS BALANCE

Mean material balances ranged from 90.69 to 109.30 % of applied radioactivity (% AR) for the pond water/sediment system and from 79.89 to 101.08 % AR for the creek water/sediment system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 58 DAT from 84.00 to 1.55 % AR and increased to 3.79 % AR at 120 DAT in the pond water/sediment system. In the creek water/sediment system, the amount of radioactivity in the water decreased from 92.31 % AR at 0 DAT to 20.57 % AR at 120 DAT.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 58 DAT from 14.16 to 93.13 % AR and decreased to 77.90 at 120 DAT in the pond water/sediment system. In the creek water/sediment system, the amount of radioactivity extractable from the sediment increased from 0 DAT to 29 DAT from 8.74 to 36.20 % AR and decreased to 31.76 at 120 DAT.

Levels of non-extractable residues (NER) in the sediment increased gradually to maxima of 29.46 % at 58 DAT in the pond system and 24.83 % at 29 DAT in the creek system. The levels dropped to 17.15 and 12.47 % by 120 DAT in the pond and creek system, respectively.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (120 DAT) were 14.77 and 30.08 % AR in the pond and creek systems, respectively.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in the water decreased from 0 DAT to 58 DAT from 70.57 to 0.24 % AR and showed a slightly higher amount of 1.83 % AR at 120 DAT in the pond system. The amount of glyphosate in the water decreased from 0 DAT to 120 DAT from 85.90 to 0.00 % AR in the creek system.

The amount of glyphosate in sediment extracts of pond system increased from 3.60 % AR at 0 DAT to 40.00 % AR at 29 DAT and decreased to 27.10 % AR at 97 DAT (29.80 at 120 DAT). The amount of glyphosate in creek system sediment extracts increased from 5.60 % AR at 0 DAT to 9.40 % AR at 2 DAT and decreased to 0.00 % AR at 97 DAT.

The amount of glyphosate in the total system decreased from 0 DAT to 97 DAT from 74.17 to 28.14 % AR at 97 DAT in the pond system and from 91.5 to 0.0 % AR at 120 DAT in the creek system.

The major degradation product, aminomethylphosphonic acid (AMPA), was found in both water/sediment systems over the course of the incubation. In the pond total system, the level of AMPA was found to be highest at 120 DAT with 16.34 % AR. Maximum amounts of AMPA in water and sediment extracts of pond system were 1.97 % AR (7 DAT) and 15.7 % AR (120 DAT), respectively.

In the creek total system, the level of AMPA was found to be highest at 120 DAT, with 24.0 % AR. Maximum amounts of AMPA in water and sediment extracts of the creek system were 10.34% AR (58 DAT) and 15.9 % AR (120 DAT), respectively. No other metabolites were detected above 0.1 % AR at any time in water or sediment extracts of both test system.

Additionally, non-chromatographable residues (NCRs) were quantified. This radioactivity is defined as activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatographic columns or not redissolvable precipitates. These NCRs were formed with an extend to 24.83 % AR in the water of the creek system and 16.91 % AR in the water of the pond system. In sediment extracts these NCR amounted to 7.40 and 20.80 % AR in creek and pond systems, respectively.

F. KINETICS

Glyphosate degraded with a total system DT_{50} of 71±24 days in the pond system and 10±2 days in the creek system, calculated using the Timme, time and Frehse method. The DT_{50} in the water phase was 2 days in the pond system and 10 days in the creek system.

III. CONCLUSIONS

The parent compound is degraded in the creek water phase and in the pond and creek sediment phase. Disappearance in the pond system was primarily caused by a continuous transfer from the water phase to the sediment phase, probably caused by sorption processes.

In the sediment, rising amounts of degradation products indicated that the degradation process was still in progress after 120 d in both systems. The main fractions were the uncharacterized group of bound residues (pond system), the soluble or extractable group of non-chromatographable residues and AMPA. The nature of the possible structures in the uncharacterised groups are given by the structure of the glyphosate itself. Glyphosate and its metabolites may be transferred to biological substrates as proteins, sugars and humidic acids.

Total mineralisation to carbon dioxide was important for pond (15 % AR) and creek (30 % AR), volatiles were negligible. In the pond system the pure degradation leads to a long degradation time for 90 % of the parent compound up to approx. Two years in the water/sediment system. Nevertheless, degradation and metabolisation were still in progress.

Two metabolites were detected. Metabolite 1 was identified as Aminomethylphosphonoic acid (AMPA). Metabolite 2 was not identified as it only appeared in samples taken from the pond system (58 d) with an amount of less than 0.1 % of the applied activity. The amount of organic volatiles was in any case below 0.1 % of the applied activity. No other metabolites were found except to the bound residues, the non-chromatographable part of the extracts and carbon dioxide.

Assessment and conclusion by applicant:

The study showed some deviations to the current guidelines. Mass balances were below 90 % AR for a number of sampling points in both systems. The acclimation period prior to application was not reported. The water/sediment ratio was between 3:1 and 2:1.

Additionally, non-chromatographable residues (NCRs) were quantified. This radioactivity is defined as activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatographic columns or not redissolvable precipitates. These NCRs were formed with an extend to 24.83 % AR in the water of the creek system and 16.91 % AR in the water of the pond system. In sediment extracts these NCR amounted to 7.40 and 20.80 % AR in creek and pond systems, respectively.

In conclusion, the study was considered invalid.

Assessment and conclusion by RMS:

Several deviations from OECD 308 are identified.

Acclimatation time prior to application of test item is not stated, the organic carbon content for the Creek system (0.11%) is significantly lower than the lowest content recommended (0.5%), the water:sediment ratio used is 2:1 instead of 3:1 to 4:1, the systems were discontinuously ventilated with CO2 free moistened air (the use CO2-free air is not recommended), oxygen concentration of the water phase is not in the recommended range of 7-10 mg/L, for the creek system, mass balances at the three last samplings are below 90% (from 80 %AR to 87 %AR), LOD of the chromatographic method is not reported, and a significant part of radioactivity is defined as "non chromatographable residues" and is reported to correspond to loss of activities at different steps of the experiment. Based on all these deviations, the study is not considered acceptable.

[redacted], *1993*

Data point:	CA 7.2.2.3/007
Report author	[redacted]
Report year	1993
Report title	Water/sediment biodegradation of [14C] glyphosate
Report No	IMW R93/033
Guidelines followed in study	Dutch Regulations for Biocides G.2.1.
Deviations from current test guideline	From OECD 308: - Particle size for sieving of sediment and water is not reported. - Temperature was between 22 and 24 °C for 75 h during study. - Microbial biomass characterised by toxicity and viability test. - Study duration slightly below 100 d (13 weeks), only 5 sampling time points. - The redox potential of the water was not determined during the study. - No parameters (pH, oxygen and redox) of the sediment were determined during the study - Water/sediment ratio not reported - Procedural recoveries were low for water and sediment extracts following freeze drying prior to TLC analysis (i.e. 51 to 98%, mean about 73%, as calculated from tables in report). Following freeze-drying and attempts to re-suspend residues, "radioactivity adhered irreversibly to plastic storage bottles". - Low recovery of test item at 0 DAT of 56 % AR in total TNO systems. In TNO systems, an unidentified compound occurred at about 46 % AR at 0 DAT. In the Kromme Rijn system, 92 % AR corresponded to glyphosate and 11 % AR of that unidentified compound. Unknown occurrence was explained in the report by "potential formation of complexes between glyphosate and water soluble humic acids". - Mass blance < 90% AR in Kromme Rijn system after 13 weeks
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	No

MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate (labelled in the phosphonomethyl-position)
Lot No.: Code CFA.745, batch 17
Specific activity: 12.3 MBq/mg
Radiochemical purity: 98.6 % by TLC
Identification: glyphosate (non-radiolabelled)
Lot No.: F92/-/086
Chemical purity: 99 %

2. Test System:

The sediments were allowed to settle and then sieved to remove coarse particles. Water samples were filtered through a paper filter to remove water fleas and large particles. Water and sediment were stored refrigerated until used. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-26: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Water/Sediment I	Water/Sediment II
Location	TNO Zuidpolder	Kromme Rijn
Country	The Netherlands	The Netherlands
Sediment:		
Sand (>50 µm) (%)	39.0	79.6
Silt (2 µm – 50µm) (%)	34.2	11.1
Clay (< 2 µm) (%)	26.8	9.3
pH (KCl)	7.3	7.4
Organic matter (%)	12.4	2.5
Organic carbon [1] (%)	7.19	1.45
Cation exchange capacity (meq/100 g dry weight)	35.0	7.8
Total N (g/100 g dry weight)	0.509	0.118
Total P_2O_5 (g/100 g dry weight)	440	242
Water:		
pH [2]	9.3	7.7
Oxygen content (mg/L) [2]	15.3	7.0

[1] calculated during dossier preparation using the equation: OC = OM/1.724
[2] measured in lab after sampling

B. STUDY DESIGN

1. Experimental conditions

The test was performed in static test systems, consisting of 250-mL cylindrical flasks (biometer flasks) filled with water and sediment in a way that the thickness of the sediment was about 2 cm. For the TNO system, 54.2 g of wet sediment (20 g dry solids) and 166 mL water were used. For the Kromme Rijn system, 48.4 g of wet sediment (30 g dry solids) and 182 mL water were used. The flasks were closed with a screw cap from which a carbon dioxide trap, filled with 10 M NaOH, was suspended.

After set-up of the test systems they were pre-incubated on a rotary shaker for 14 days at 20 ± 2 °C in the dark.

The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate in 100 µL aqueous solution, resulting in 170.7 kBq [^{14}C]glyphosate and 0.20 mg unlabelled glyphosate per test system.

Samples were incubated for 13 weeks on a rotary shaker in the dark at 20 ± 2°C. Thereby an aerobic environment in the upper section was achieved while maintaining an undisturbed anaerobic sediment.

Additionally, four flasks for toxicity and viability test of each sediment were prepared. To two flasks of each sediment glyphosate was added at a concentration of 1 mg/L. To all flasks about 40 kBq radiolabelled and unlabelled sodium acetate was added to reach a final concentration of 100 mg/L.

2. Sampling

Duplicate test systems were processed and analysed 0, 2, 4, 8 and 13 weeks after treatment. The contents of the flasks of week 0 were analysed about one hour after addition of the test compound. Carbon dioxide traps were collected after 2, 4, 8 and 13 weeks after treatment. At the same time the trapping solution was replaced with fresh NaOH in the biometer flasks which were not sacrificed.

3. Analytical procedures

The pH and oxygen concentrations were measured in the flasks that were sacrificed for analysis.

At each sampling interval, water and sediment were separated by decantation of the water through a plug of cotton wool in a glass funnel. If the aqueous phase contained >2.5 % AR they were freeze-dried.

$^{14}CO_2$ dissolved in the aqueous phase was determined by adding 18 % hydrochloric acid to an aliquot (10 mL) of the sample in a closed system.

Sediment samples were extracted by shaking for 5 min with 0.5 M ammonium hydroxide solution. Cotton wool plug and the funnel, used for decanting the water phase, were rinsed with 0.5 M ammonium hydroxide and the plug was squeezed out. This ammonium hydroxide was added to the sediment and the solvent was removed from the sediment by centrifugation. Sediments were extracted with varying amount of 0.5 M ammonium hydroxide, until the extract contained <5 % AR. All extracts were pooled. Extracts were freeze-dried if they contained >2.5 % AR.

The determination of radioactivity in liquid samples (water, sediment extracts, volatile traps) was performed by liquid scintillation counter (LSC).

Radioactivity in the solids (non-extractable residues) after drying at room temperature were determined by combustion of the samples.

Freeze-dried residues of the aqueous phases and the extracts of the solids were extracted with 0.5 M ammonium hydroxide and 18 % hydrochloric acid. The recoveries after freeze-drying of the aqueous phases and the extracts of the solids were not very high, because part of the radioactivity adhered irreversibly to the plastic bottles used for freeze-drying the fractions.

The amounts of glyphosate and its metabolites were determined by TLC in the various concentrated phases with the use of reference compounds. Plates were developed in isobutyric acid:water:1-propanol:concentrated ammonium hydroxide:2-propanol:1-butanol (500:95:70:20:15:15) with 0.24 g of sodium-EDTA.

II. RESULTS AND DISCUSSION

A. DATA

Only small differences between the blank and the flasks with addition of 1 mg/L glyphosate were measured in the toxicity and viability test. Mean values differed by 1.8 % AR for the TNO system and by 0.5 % AR for the Kromme Rijn system. Radioactive mass balances of the carbon dioxide traps in the toxicity and viability test are summarised below.

Table 8.2.2.3-27: Amount of carbon dioxide evolved in the toxicity and viability test incubated with sodium acetate (expressed as percent of applied radioactivity)

System		Replicate	Time (weeks)						
			0	2	4	8	13	In H_2O	Sum
TNO	Blank	A	8.1	8.3	14.0	5.5	7.6	0.8	44.3
		B	9.2	8.0	13.2	6.1	6.3	0.9	43.6
		Mean	*8.7*	*8.2*	*13.6*	*5.8*	*7.0*	*0.9*	*44*
	+GLY	A	9.0	6.9	11.3	6.5	6.6	1.1	41.4
		B	8.7	7.0	13.5	5.7	7.3	0.9	43.1
		Mean	*8.9*	*7.0*	*12.4*	*6.1*	*7.0*	*1*	*42.3*
Kromme Rijn	Blank	A	14.6	17.9	24.3	6.7	2.3	0.9	66.8

		B	16.5	19.6	23.4	4.9	2.0	0.7	67.1
		Mean	*15.6*	*18.8*	*23.9*	*5.8*	*2.2*	*0.8*	*66.9*
	+GLY	A	18.1	19.1	25.8	5.8	1.3	1.1	71.2
		B	14.9	16.2	21.7	5.3	2.3	1.2	61.7
		Mean	*16.5*	*17.7*	*23.8*	*5.6*	*1.8*	*1.2*	*66.4*

Blank = nothing added
+GLY = 0.198 mg of glyphosate added in 30 µL of water
In H_2O = Carbon dioxide remaining in the aqueous phases after 13 weeks
Values calculated in the course of this summary are given in *italics*

During the biodegradation test the pH varied between 7.4 and 9.1 in the TNO system and between 7.1 and 8.6 in the Kromme Rijn system (individual values of replicates). The oxygen content in the water phase ranged between 7.5 and 8.7 mg/L in the TNO system and between 6.8 and 8.8 in the Kromme Rijn system (individual values of replicates).

Radioactive mass balance and distribution of glyphosate and metabolites in water/sediment systems extracts are summarised below.

Table 8.2.2.3-28: Amount of radioactivity in water/sediment system TNO (expressed as percent of applied radioactivity)

Compound	Replicate	Time (weeks)				
		0	2	4	8	13
CO_2 trap	A	0.0	0.2	0.6	2.9	4.8
	B	0.0	0.1	0.5	2.1	4.2
	Mean	0.0	0.2	0.6	2.5	4.5
CO_2 in H_2O	A	0.0	1.2	1.7	0.6	0.6
	B	0.0	1.0	1.2	0.8	2.0
	Mean	0.0	1.1	1.4	0.7	1.3
CO_2 Sum	A	0.0	1.4	2.2	3.5	5.4
	B	0.0	1.1	1.7	2.9	6.2
	Mean	0.0	1.2	2.0	3.2	5.9
H_2O	A	97.2	13.5	5.1	0.1	0.2
	B	96.6	17.0	3.8	0.2	0.0
	Mean	96.9	15.2	4.4	0.2	0.1
Solid	A	6.7	52.0	49.7	56.5	53.6
	B	5.8	52.5	54.5	51.6	51.6
	Mean	6.2	52.2	52.1	54.0	52.6
Non-extractable residues	A	1.7	28.6	35.0	34.7	33.4
	B	1.4	24.7	33.7	38.1	36.7
	Mean	1.6	26.6	34.4	36.4	35.0
Recovery	A	105.7	95.5	92.1	94.7	92.5
	B	103.8	95.3	93.7	92.8	94.5
	Mean	104.8	95.4	92.9	93.8	93.5

CO_2 trap = Results of carbon dioxide measurements in the trap
CO_2 in H_2O = Carbon dioxide in the aqueous phase
CO_2 sum = Sum of the carbon dioxide measurements
H_2O = Radioactivity in the aqueous phase (excluding CO_2)
Solids = Extractable radioactivity in the solids (sediment)

Table 8.2.2.3-29: Amount of radioactivity in water/sediment system Kromme Rijn (expressed as percent of applied radioactivity)

Compound	Replicate	Time (weeks)				
		0	2	4	8	13
CO_2 trap	A	0.0	5.9	10.5	19.7	22.5
	B	0.0	6.0	10.3	19.0	24.9
	Mean	0.0	6.0	10.4	19.4	23.7

CO_2 in H_2O	A	0.0	8.2	8.2	2.6	1.8
	B	0.0	9.6	5.1	2.3	2.1
	Mean	0.0	8.9	6.6	2.4	2.0
CO_2 Sum	A	0.0	14.1	18.7	22.2	24.3
	B	0.0	15.6	15.5	21.4	27.0
	Mean	0.0	14.8	17.1	21.8	25.6
H_2O	A	95.9	2.5	0.3	0.5	0.8
	B	92.5	5.7	2.3	0.6	0.4
	Mean	94.2	4.1	1.3	0.6	0.6
Solid	A	8.4	61.9	50.9	42.3	30.8
	B	10.2	64.3	51.0	43.0	30.0
	Mean	9.3	63.1	51.0	42.6	30.4
Non-extractable residues	A	0.7	20.5	24.8	26.9	32.3
	B	1.0	11.7	19.9	25.6	28.6
	Mean	0.8	16.1	22.4	26.2	30.4
Recovery	A	105.1	98.9	94.7	92.0	88.2
	B	103.8	97.4	88.6	90.6	86.0
	Mean	104.4	98.2	91.6	91.3	87.1

CO_2 trap = Results of carbon dioxide measurements in the trap
CO_2 in H_2O = Carbon dioxide in the aqueous phase
CO_2 sum = Sum of the carbon dioxide measurements
H_2O = Radioactivity in the aqueous phase (excluding CO_2)
Solids = Extractable radioactivity in the solids (sediment)

Table 8.2.2.3-30: Distribution of radioactivity in water/sediment system TNO (mean values of two replicates, expressed as mean percent of applied radioactivity)

Phase	Rf	Time (weeks)				
		0	2	4	8	13
Aqueous phase (freeze-dried)	0.0 (Glyphosate)	52	14	4	nd	nd
	0.1	-	-	<1	nd	nd
	0.2	-	<1	-	nd	nd
	0.4	-	<1	-	nd	nd
	0.5	-	<1	-	nd	nd
	0.9	44	-	-	nd	nd
Extracts of the solids (freeze-dried)	0.0 (Glyphosate)	4	51	52	54	53
	0.9	2	1	-	-	-
Sum of non-volatile radiolabelled compounds	0.0 (Glyphosate)	56	66	56	54	53
	0.1	-	-	<1	-	-
	0.2	-	<1	-	-	-
	0.4	-	<1	-	-	-
	0.5	-	<1	-	-	-
	0.9	46	1	-	-	-

nd: not determined
- not detected

Table 8.2.2.3-31: Distribution of radioactivity in water/sediment system Kromme Rijn (mean values of two replicates, expressed as mean percent of applied radioactivity)

Phase	Rf	Time (weeks)				
		0	2	4	8	13
Aqueous phase freeze-dried	0.0 (Glyphosate)	84	5	nd	nd	nd
	0.4	-	<1	nd	nd	nd
	0.5	-	<1	nd	nd	nd
	0.8	-	<1	nd	nd	nd
	0.9	10	-	nd	nd	nd
Extracts of the solids (freeze-dried)	0.0 (Glyphosate)	8	63	51	42	30
	0.9	1	-	-	-	-
	0.0 (Glyphosate)	92	66	51	42	30

Sum of non-volatile radiolabelled compounds	0.4	-	<1	-	-	-
	0.5	-	<1	-	-	-
	0.8	-	<1	-	-	-
	0.9	11	-	-	-	-

nd: not determined
- not detected

B. MASS BALANCE

Mean material balances ranged from 92.9 to 104.8 % AR for the TNO water/sediment system and from 87.1 to 104.4 % AR for the Kromme Rijn water/sediment system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 weeks after treatment to 13 weeks after treatment from 96.9 to 0.1 % AR for the TNO water/sediment system and from 94.2 to 0.6 % AR for the Kromme Rijn water/sediment system.

The amount of radioactivity extractable from the sediment of the TNO water/sediment system increased from 0 weeks after treatment to 8 weeks after treatment from 6.2 to 54.0 % AR and decreased then to

52.6 % AR at 13 weeks after treatment. The amount of radioactivity extractable from the sediment of the Kromme Rijn water/sediment system increased from 0 weeks after treatment to 2 weeks after treatment from 9.3 to 63.1 % AR and decreased then to 30.4 % AR at 13 weeks after treatment.

The amount of non-extractable residues (NER) increased from 0 weeks after treatment to 13 weeks after treatment from 1.6 to 35.0 % AR for the TNO water/sediment system and from 0.8 to 30.4 % AR for the Kromme Rijn water/sediment system.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide were 5.9 % AR at 13 weeks after treatment in the TNO water/sediment system and 25.6 % AR at 13 weeks in the Kromme Rijn water/sediment system.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in water decreased from 52 % AR at 0 weeks to 4 % AR at 4 weeks after treatment to not detectable at 13 weeks after treatment for water/sediment system TNO and from 84 % AR at 0 weeks after treatment to 5 % AR at 2 weeks after treatment to not detectable at 4 weeks after treatment for water/sediment system Kromme Rijn water/sediment system.

The amount of glyphosate in sediment extracts increased from 4 % AR at 0 weeks after treatment to 54 % AR at 8 weeks after treatment and slightly decreased to 53 % AR at 13 weeks after treatment for water/sediment system TNO. For water/sediment system Kromme Rijn, the amount of glyphosate in the sediment extracts increased from 8 % AR at 0 weeks after treatment to 63 % AR at 2 weeks after treatment and declined to 30 % AR after 13 weeks after treatment.

The amount of glyphosate in the total system decreased from 56 % AR at 0 weeks after treatment to 53 % AR at 13 weeks after treatment for water/sediment system TNO and from 92 % AR at 0 weeks after treatment to 30 % AR at 13 weeks after treatment for water/sediment system Kromme Rijn.

One unknown metabolite, which was mostly present in the water phase, was detected in both test systems. This metabolite was only detected at 0 weeks after treatment and it was suggested, that this was an artefact caused by formation of a complex of glyphosate with water soluble humic acids which resulted in a different behaviour on the cellulose TLC plates. No other metabolite in water or sediment was detected with >1 % AR.

F. KINETICS

The DT_{50} in the TNO water/sediment system was reported to be 17.7 weeks, best described by a reaction of a root second order. In Kromme Rijn water/sediment system, the DT_{50} was reported to be 4.4 weeks, best described by a reaction of a root first order.

Assessment and conclusion by applicant:

Besides several minor deviations and shortcomings the study shows the following two major deficiencies.

Procedural recoveries were low for water and sediment extracts following freeze-drying prior to TLC analysis (i.e. 51 to 98%, mean about 73%, as calculated from tables in report). Following freeze-drying and attempts to re-suspend residues, "radioactivity adhered irreversibly to plastic storage bottles".

Low recovery of test item was observed at 0 DAT of 56 % AR in total TNO systems and 92 % AR for Kromme Rijn system. In TNO systems, an unidentified compound occurred at about 46 % AR at 0 DAT. In the Kromme Rijn system, 92 % AR corresponded to glyphosate and 11 % AR of that unidentified compound. Unknown occurrence was explained in the report by "potential formation of complexes between glyphosate and water soluble humic acids".

In consequence the amounts of glyphosate determined in this study are not considered reliable.

Therefore, the study is considered invalid.

The following information was also provided by the applicant to further justify the invalidity of the study.

Further information on justifying invalidity

Extracts with >2.5 % AR were freeze dried for further TLC analysis. The recovery of this workup was not reported within the report. Calculated recovery based on reported radioactivity before and after freeze-drying and re-suspending can be found below.

Table 8.2.2.3-32: Recovery after freeze-drying and re-suspending of the aqueous phases and the extracts of solids in the TNO water/sediment system

Phase		Time (weeks)				
		0	2	4	8	13
Aqueous phases	Replicate 1					
	H_2O	97.2	13.5	5.1	0.1	0.2
	After	79.7	10.0	5.0	nd	nd
	Recovery	*82.0*	*74.1*	*98.0*	*nd*	*nd*
	Replicate 2					
	H_2O	96.6	17.0	3.8	0.2	0.0
	After	81.8	15.0	3.7	nd	nd
	Recovery	*84.7*	*88.2*	*97.4*	*nd*	*nd*
Extracts of the solids	Replicate 1					
	H_2O	6.7	52.0	49.7	56.5	53.6
	Pool	3.8	32.0	32.7	38.2	29.9
	Recovery	*56.7*	*61.5*	*65.8*	*67.6*	*55.8*
	Replicate 2					
	H_2O	5.8	52.5	54.5	51.6	51.6
	Pool	3.0	32.7	37.0	33.6	29.9
	Recovery	*51.7*	*62.3*	*67.9*	*65.1*	*58.0*

H_2O = Aqueous phase
Pool = Sum of 0.5 M NH_4OH extracts of the solids
After = After freeze-drying and resuspending
nd = not determined
Values calculated in the course of this summary are given in *italics*

Table 8.2.2.3-33: Recovery after freeze-drying and re-suspending of the aqueous phases and the extracts of solids in the Kromme Rijn water/sediment system

Phase		Time (weeks)				
		0	2	4	8	13
Aqueous phases	Replicate 1					

	H_2O	95.9	2.5	0.3	0.5	0.8
	After	83.0	nd	nd	nd	nd
	Recovery	*86.6*	*nd*	*nd*	*nd*	*nd*
	Replicate 2					
	H_2O	92.5	5.7	2.3	0.6	0.4
	After	78.8	6.9	nd	nd	nd
	Recovery	*85.2*	*121.1*	*nd*	*nd*	*nd*
Extracts of the solids	Replicate 1					
	Pool	8.4	61.9	50.9	42.3	30.8
	After	6.3	43.7	39.0	31.7	22.6
	Recovery	*75.0*	*70.6*	*76.6*	*74.9*	*73.4*
	Replicate 2					
	Pool	10.2	64.3	51.0	43.0	30.0
	After	7.1	44.3	38.9	33.2	20.8
	Recovery	*69.6*	*68.9*	*76.3*	*77.2*	*69.3*

H_2O = Aqueous phase
Pool = Sum of 0.5 M NH_4OH extracts of the solids
After = After freeze-drying and resuspending
nd = not determined
Values calculated in the course of this summary are given in *italics*

Workup recovery of the freeze-drying ranged from 51.7 to 98.0 % and from 68.9 to 121.1 % in the TNO and Kromme Rijn water/sediment system, respectively. Recoveries for the freeze-drying ranged from 74.1 to 98.0 % for work up of the aqueous phase and from 51.7 to 77.2 % for the work up of the extracts of the solids. Overall mean workup recovery of the freeze-drying was 74.5 %.

Low recoveries, especially for the sediment workup, indicate a substantial loss of radioactivity during freeze drying. It cannot be clearly proven, that this loss can be attributed equally to the parent substance and its metabolites. The report itself did not discuss this issue.

Therefore, the poor procedural recoveries during workup justify to consider the study invalid.

Assessment and conclusion by RMS:

Several deviations from OECD 308 are identified. Particle size for sieving of sediment and water is not reported, temperature was between 22 and 24 °C for 75 h during study, microbial biomass was not characterised according to recommended method, there was only 5 sampling dates, some parameters were not determined during the study (redox potential in water, pH, oxygen level and redox potential in sediment), water / sediment ratio is not reported.

In addition, procedural recoveries were low for water and sediment extracts following freeze drying prior to TLC analysis (i.e. 51 to 98%, mean about 73%, as calculated from tables in report). Following freeze-drying and attempts to re-suspend residues, "radioactivity adhered irreversibly to plastic storage bottles". Recovery of test item at 0 DAT was very low (56 % AR) in total TNO systems. In TNO systems, an unidentified compound occurred at about 46 % AR at 0 DAT. In the Kromme Rijn system, 92 % AR corresponded to glyphosate and 11 % AR of that unidentified compound. Unknown occurrence was explained in the report by "potential formation of complexes between glyphosate and water soluble humic acids".

In RMS opinion, the results from this study cannot be considered as reliable.

The study is not acceptable.

[redacted], *1991*

Data point:	CA 7.2.2.3/008
Report author	[redacted]
Report year	1991
Report title	(^{14}C)-Sulfosate: Degradation in ditch waters and their associated hydrosoils

Report No	6589-38/127
Guidelines followed in study	Not reported
GLP/Officially recognised testing facilities	No
Previous evaluation	No, old study but not found in RAR 2015 nor in DAR 2001
Acceptability/Reliability:	No

Short description of study design and observations:	Study type: Water/sediment Test item: ^{14}C-PMG anion (glyphosate, radiochemical purity 97.1 %, specific radioactivity 974.77 Bq/µg) ^{14}C-TMS cation (radiochemical purity 98.2 %, specific radioactivity 1173 Bq/µg)) Test system: Old Basing and Carrick Hill Soil type: Silty clay loam (Old Basing) Sandy loam (Carrick Hill) pH: Old Basing: 7.4 Carrick Hill: 6.9 Organic matter: Old Basing: 26.3 % Carrick Hill: 1.6 % Sediment was sieved to 5 mm. Degradation of ^{14}C- PMG anion and ^{14}C-TMS cation was assessed in two water/sediment systems at 20°C, illuminated 12 h/dark 12°h for a duration of 91 days. Only results for the PMG anion (glyphosate) are considered here. Application rate:1.6 mg/L (^{14}C-PMG anion) Test design: static system with borosilicate glass cylinders Volatiles trapping: CO_2: Two ethanolamine trap Organic volatiles, non-polar: One trap containing 2 % liquid paraffin in xylene Organic volatiles, polar: One trap containing ethanediol Additional volatile trap: One Polyurethane foam bung Incubation: Exposed to a 12 h fluorescent lighting and 12 h dark regime at 20 °C Sampling: 0, 3, 7, 14, 30, 60 and 91 DAT (duplicate samples) Workup: The contents of each unit were mixed thoroughly by manual shaking (5 to 10 mins), then centrifuged (4300 x g, 20 mins) and supernatants were decanted. Sediment was extracted with 0.37 M ammonia. Samples (water or sediment extracts) containing insufficient radioactivity were concentrated by ultracentrifugation followed by freeze-drying of the supernatant for 48 h. Samples were reconstructed in 0.1 M formic acid. Storage: Loss of radioactivity during storage (ca. 4 months at ca. -18 °C) determined on one exemplary 7-day water sample per test sytem was 77.3 % for Old Basing and 52.9 % for Carrick Hill. Analysis of radioactivity: Water: LSC Extracts: LSC NER: Combustion/LSC Volatiles: LSC Identification of radioactive residues: TLC with reference standard Levels of radioactivity in associated water samples were often <5 %, so many samples were not analysed. For those samples that were analysed, significant losses of radioactivity (72 to 85%) occurred during sample concentration. A preliminary experiment showed that <5 % of ^{14}C-glyphosate (anion or cation labelled) was lost during this procedure, thus, the losses were presumably not

	^{14}C-glyphosate. Further losses (34 to 58%) occurred from the TLC plate between sample application and sample analysis. The result of the combined loss of radioactivity was that radioactivity on the TLC plate at the time of analysis accounted for only ca. 1 % of applied radioactivity for probably all but day 0 samples. For sediment extracts, the loss from the TLC plate between sample application and sample analysis was not analysed directly.
Short description of results:	^{14}C-PMG anion (glyphosate): Recovery of radioactivity: 68.04-97.29 % AR Losses of radioactivity may be due to the formation of volatile compounds (e.g. dimethyl sulphide or methane) which are not absorbed by the trapping reagents employed in this study. Mineralisation: Old Basing: 4.17 % AR at 91 DAT Carrick Hill: 22.12 % AR at 91 DAT Other volatiles: Polar organic volatiles: Old Basing: 0.2 % AR at 91 DAT Carrick Hill: 0.1 % AR at 91 DAT Non-polar organic volatiles: Old Basing: 0.06 % AR at 91 DAT Carrick Hill: 0 % AR at 91 DAT Extractable radioactivity: Old Basing: Water: 2.78 % AR at 0 DAT, 2.37 % AR at 91 DAT Sediment: 73.26 % AR at 0 DAT, 41.29 % AR at 91 DAT Carrick: Water: 13.86 % AR at 0 DAT, 0.93 % AR at 91 DAT Sediment: 57.09 % AR at 0 DAT, 29.71 % AR at 91 DAT Non-extractable radioactivity: Old Basing: 13.73 % AR at 0 DAT, max 37.00 % AR at 91 DAT Carrick Hill: 10.98 % AR at 0 DAT, max 17.92 at 14 DAT, 14.74 % AR at 91 DAT Transformation of test item: Due to the high losses of radioactivity during work-up of the water phase, the radioactivity on the TLC plate at the time of analysis accounted for only ca. 1% of applied radioactivity. Thus, degradation can only be assessed relatively as percentage of TLC plate radioactivity and not given in % of applied radioactivity. Old Basing: Water (7 DAT): Glyphosate: 27 % of plate radioactivity AMPA: 16 % of plate radioactivity Other degradates (two compounds): 51 % of plate radioactivity Sediment (7 DAT): Glyphosate: 80 % of plate radioactivity AMPA: 15 % of plate radioactivity Carrick Hill: Water: Glyphosate: 92 % of plate radioactivity at 0 DAT, 31 % of plate radioactivity at 7 DAT AMPA: 2 % of plate radioactivity at 0 DAT, 52 % of plate radioactivity at 7 DAT Other degradates (three compounds): 15 % of plate radioactivity at 7 DAT Sediment: Glyphosate: 91 % of plate radioactivity at 0 DAT, 31 % of plate radioactivity at 91 DAT AMPA: 3 % of plate radioactivity at 0 DAT, 65 % of plate radioactivity at 7 DAT

	DT_{50} for glyphosate was determined to be >100 days for Old Basing system and 35 days for Carrick Hill system.
Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid based on the following deficiencies: - incubation followed a 12 h fluroescent light and 12 h dark regime, i.e. not in full darkness, - mass balances below 75 % AR in System 2 and below 90 % in System 1, - work-up procedure disturbed distribution of radioactivity between sediment and water (water and sediment mixed, then centrifuged), - only samples with >5% AR were analysed by TLC, - procedural losses during extract concentration, frozen storage and between application to TLC plates and analysis, - for TLC analysis, significant (72 to 85 %) losses occurred during sample concentration; only ca. 1% AR on the TLC plate at the time of for probably all but day 0 samples, - TLC results not available for all sampling points, - degradation products not reported as % AR, - acclimation for eight weeks, - sediment was sieved to 5 mm.

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted], *1990a and Honegger, 1992a*

Data point:	CA 7.2.2.3/009
Report author	[redacted], S.B.
Report year	1990
Report title	Aerobic aquatic metabolism of [^{14}C]Glyphosate
Report No	MSL-10576
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Section 162-4
GLP	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Data point:	CA 7.2.2.3/010
Report author	[redacted]
Report year	1992
Report title	Addendum to MSL-10576 Aerobic aquatic metabolism of [^{14}C] Glyphosate
Report No	MSL-10576
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Section 162-4
GLP	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:	Study type: Water/sediment Test item: ^{14}C-labeled glyphosate (radiochemical purity 98.8 %, specific radioactivity 3.98 mCi/mMole) Test water: Pond water (Fayette County, Kentucky) Test sediment: Pond bottom (Fayette County, Kentucky) Soil type: Silty Clay Loam Organic matter: 0.9 % pH: Water: 7.3, sediment: 6.6 Test system: 20 g sediment (dry weight) and 100 mL pond water in Erlenmeyer flasks, equipped with inlet and outlet tubes

	Application: 1 mL aqueous solution, resulting concentration 4.1 mg/kg, flasks swirled to mix Test design: Incubation at approximately 25 °C, flushed with oxygen Volatiles trapping: CO_2: 10 % NaOH trapping solution Organic volatiles: ethylene glycol trapping solution Sampling: 0, 1, 3, 7, 10, 15, 20, 24 and 30 DAT, duplicate samples Work up: Water and sediment were transferred completely to centrifuge bottles, centrifugation, decantation; extraction of sediment with 0.5 N KOH two or three times (20 min), samples from 3, 7, 10, 15, 20, 24 and 30 DAT were subsequently extracted with 0.03 M EDTA one to three times Analysis of radioactivity: Water: LSC Extracts: LSC NER: combustion/LSC Volatiles: LSC Identification of radioactive residues: radio HPLC with reference standards
Short description of results:	Recovery of radioactivity: 78.3-104.8 % (single values) pH during study: 5.9-7.0 Dissolved oxygen during study: 5.0-19.5 mg/L Mineralisation (maximum CO_2 at 24 DAT, mean): 24.3 % AR Other volatiles (maximum at 24 DAT, mean): 4.8 % AR Radioactivity in water (mean): 1.2 at 0 DAT Radioactivity in KOH extracts (mean): 98.9 % AR at 0 DAT Radioactivity in EDTA extracts (mean): 4.0 % AR at 24 DAT Non extractable radioactivity (mean): 7.2 % AR at 30 DAT Transformation of the test item in total system (mean): 0 DAT: 93.0 % AR Glyphosate 3.3 % AR AMPA 0.4 % AR Unknown A 2.5 % AR Unknown B 1.1 % AR others 30 DAT: 22.2 % AR Glyphosate 22.7 % AR AMPA 1.5 % AR Unknown A 2.2 % AR Unknown B 1.0 % AR others Max values of metabolites: AMPA: 24.8% AR (20 DAT) Unknown A: 1.9% AR (20 DAT) Unknown B: 2.6% AR (24 DAT) Others: 1.1% AR (0 DAT) It was stated in the amendment that Unknown A and B may not be the product of microbial degradation but have been derived from AMPA by another mechanism such as radiolysis. The half-life of glyphosate was estimated to about 14.4 days.

Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The studies are considered invalid based on the following discrepancies: - Closed vessels with headspace of oxygen instead of atmospheric air. - Work-up procedure disturbed distribution between sediment and water (water and sediment transferred to centrifuge bottles and centrifuged). - Short test duration (30 d, 22 % of ^{14}C glyphosate still remaining). - After application, test vessels were swirled to mix. - Less than 50 g dry weight of sediment were used per sample. - Mass balance below 90 % for some sampling intervals (77-105%, 85% on 30 DAT). - No acclimation period. - Microbial biomass was not determined. - Sample storage time prior to analysis not reported. - Redox potential not measured during study.
Acceptability/Reliability	No

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted] *1990b* [redacted], *1992b*

Data point:	CA 7.2.2.3/011
Report author	[redacted]
Report year	1990
Report title	Anaerobic aquatic metabolism of [^{14}C] Glyphosate
Report No	MSL-10577
Guidelines followed in study	EPA Guidelines, Subdivision N, Section 162-3
GLP	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Data point:	CA 7.2.2.3/012
Report author	[redacted]
Report year	1992
Report title	Addendum to MSL-10577 Anaerobic aquatic metabolism of [^{14}C] Glyphosate
Report No	MSL-10577
Guidelines followed in study	EPA Guidelines, Subdivision N, Section 162-3
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:	Study type: water/sediment, anaerobic Test item: [^{14}C] Glyphosate (radiochemical purity 98.8 %) Test water: Pond water (Fayette County, Kentucky) Test sediment: Pond bottom (Fayette County, Kentucky) Soil type: Silty Clay Loam Organic matter: 0.9 % pH: Water:7.3, sediment: 6.6 An anaerobic water/sediment experiment was conducted for 365 days. Application rate: 3.87 mg/kg Test design: Static system with Erlenmeyer flasks flushed with nitrogen Volatiles trapping:

	CO_2: 10 % NaOH trap Organic volatiles: Ethylene glycol trap Incubation: In darkness at mean 25.4 ± 0.84 °C (20-27 °C) Sampling: 0, 1, 4, 7, 15, 29, 60, 90, 180, 270 and 365 DAT, duplicate samples Workup: Water and sediment were transferred completely to centrifuge bottles and centrifuged. Supernatant water was decanted and sediment extracted. 0-90 DAT: Extracted with 50 mL 0.5 N KOH (30 min) and 100 mL 0.5 N KOH (overnight) 180 DAT: Extracted three times with 50 mL 0.5 N NH_4OH (30 min), twice with 50 mL 0.5 N KOH (1 h) and 100 mL 0.5 N KOH (overnight) 270 and 365 DAT: Extracted twice with 50 mL 0.5 N KOH (30 min) and 100 mL 0.5 N KOH (overnight) All successive extractions for each sample were pooled. 15 and 29 DAT: Subsequently extracted with 0.03 M EDTA Analysis of radioactivity: Water: LSC Extracts: LSC (combined extracts) NER: Combustion/LSC Volatiles: LSC Identification of radioactive residue: HPLC
Short description of results:	Recovery of radioactivity: 69.6-104.3 % AR (single values) An additional test was performed to investigate the loss of radioactivity during incubation. Therefore, new test vessels were used and 4.15 ppm [^{14}C]glyphosate was incubated with 20 g sediment and 100 mL water for 6 months. Recoveries of the additional test were between 91.7-103.2 %. Thus, it is considered to be proven that the loss of radioactivity during the degradation was due to a loss of $^{14}CO_2$. pH during study: 5.7-6.2 Dissolved oxygen during study: 1.4-3.7 mg/L Mineralisation: max. 35.0 % AR at 365 DAT (single value) Other volatiles: max. 3.7 % AR at 270 DAT (single value) Radioactivity in water (mean values): 7.5 % AR at 0 DAT Radioactivity in KOH (mean values): 93.6 % AR at 0 DAT, 40.1 % AR at 365 DAT Radioactivity in EDTA extracts (mean values): 5.5 % AR at 15 DAT Non-extractable radioactivity (mean values): 2.3 % AR at 0 DAT, 3.9 % AR at 365 DAT Transformation of the test item in total system: 0 DAT (mean values): 95.3 % AR Glyphosate 3.8 % AR AMPA 0.5 % AR Unknown A 1.0 % AR Unknown B 1.0 % AR others 365 DAT (only one replicate available): 20.3 % AR Glyphosate 17.7 % AR AMPA 0.6 % AR Unknown A 1.0 % AR Unknown B 0.5 % AR others Max values of metabolites (mean values): AMPA: 25.3% AR (7 DAT) Unknown A: 1.1% AR (7 DAT) Unknown B: 3.8% AR (29 DAT) Others: 1.3% AR (180 DAT)

	It was stated in the amendment that Unknown A and B may not be the product of microbial degradation but have been derived from AMPA by another mechanism such as radiolysis. The half-life of glyphosate was estimated to about 208 days. [redacted] (1992) discussed and recalculated the half-life using nonlinear first order kinetics, due to poor fit of the data points in the original report. The half-life of glyphosate in the calculation in the addendum was estimated to be 8.1 days.
Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The studies are considered invalid based on the following discrepancies: - Anaerobic study; no data requirement. - Work-up procedure disturbed the sediment (water and sediment transferred to centrifuge bottles and centrifuged). - Water and sediment extracts were pooled prior to HPLC analysis. - Test vessels were sealed. - No acclimation prior to application. - Test vessels were swirled to mix after application. - Low mass balance (70-100 %): attributed to loss of $^{14}CO_2$. - Less than 50 g dry weight of sediment were used. - Incubation temperature not controlled (20-27 C). - Long test duration: 365 d, but 7 sampling intervals analysed till 90 DAT. - Sample storage time prior to analysis not reported. - Microbial biomass was not determined. - Redox potential not measured during study.

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted], *1992c*

Data point:	CA 7.2.2.3/013
Report author	[redacted]
Report year	1992
Report title	Review of the aquatic metabolism of Glyphosate.
Report No	Addendum to PTRL 366 and PTRL 367
Guidelines followed in study	see CA 7.2.2.3/009 and CA 7.2.2.3/011
GLP	No
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:	This addendum discusses the half-lives of glyphosate in water/sediment systems calculated in the reports PTRL 366 (aerobic aquatic metabolism) and PTRL 367 (anaerobic aquatic metabolism). The DT_{50} was calculated assuming pseudo first order kinetics to be 14.4 days and 208 days in PTRL 366 and PTRL 367, respectively. The degradation rate of glyphosate was re-calculated in this addendum, as the degradation was found to be better described by non-linear first order kinetics.
Short description of results:	Using non-linear first order kinetics, the DT_{50} was determined to be 6.48 and 8.12 days and DT_{90} was determined to be 107 and 6630 days in PTRL

	366 and PTRL 367, respectively. However, the DT_{90} value for the anaerobic aquatic metabolism study was extrapolated from the data and the confidence interval for this value (6630 days) was quite large (0-24,400 days).
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	Addendum to two invalid studies

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted], *1988*

Data point:	CA 7.2.2.3/014
Report author	[redacted]
Report year	1988
Report title	Aquatic dissipation of glyphosate and AMPA in water and soil sediment following application of glyphosate in irrigated crop and forestry uses
Report No	MSL-8332
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Reference Number 164-2 of Subdivision N.
GLP	Yes
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:

Study type: Water/sediment field study
Test item: Rodeo® on irrigation water
Accord® on forestry sites
Two experiments were conducted: An application of the test item to two irrigation sources and an aerial application of the test item to a forestry site.

Test systems:
Irrigation water:
Non-flowing farm pond Clarence, Missouri:
pH sediment: 4.8-7.2
organic matter sediment: 1.5-2.1 %
sediment texture: loam – clay loam
Flowing irrigation ditch Ephrata, Washington:
pH sediment: 6.3-6.8
organic matter sediment: 0.9-1.7 %
sediment texture: sandy loam
Forestry sites (8.1 ha each and each containing a flowing stream and a pond water source):
Chassell, Michigan:
pH sediment: 4.8-5.0
organic matter sediment: 2.5-2.6 %
sediment texture: sandy loam
Corvallis, Oregon:
pH sediment: 5.6-5.8
organic matter sediment: 4.1-7.2 %
sediment texture: clay loam – sandy clay loam
Cuthbert, Georgia:

pH sediment: 5.4-5.6
organic matter sediment: 0.4-0.8 %
sediment texture: sandy loam

Irrigation water experiment:
Application rate: not stated
Test design: Rodeo® was applied as a 1.5 % v/v solution to the edge of the irrigation source with backpack or tractor-mounted sprayer. Water from these sources was used to irrigate alfalfa, corn, grass and lettuce. Irrigation water and sediment located under treated areas was analysed. Water samples were collected from the treated area, the sprinkler pump and the sprinkler head.
Sampling:
Water: 0, 1, 3, 7, 14, 30, 49 (only Clarence), 55 (only Ephrata) DAT
Sediment: 0, 1, 3, 7, 14, 30, 60, 120, 180, 365 DAT

Forestry site experiment:
Application rate: 4.2 kg/ha
Test design: Accord® was sprayed over the forest by helicopter. Pond and stream water samples and pond and stream sediments samples were analysed.
Sampling:
Water: 0, 1, 3, 7, 14, 28/30 DAT
Sediment: approx. 0, 1, 3, 7, 14, 30, 60, 120, 180, 365 DAT

Analytical procedures for both experiments:
Workup: water samples were acidified and evaporated
sediment was extracted with 0.5 N KOH, centrifuged, acidified with HCl to pH 2 and filtered, chelated with Chelex 100 resin in the Fe(III) form, eluted with HC, Iron was removed using anion exchange resin; concentration to dryness, samples redissolved in HPLC mobile phase containing EDTA
Analysis: analysis by HPLC-PCR using fluorometric detection

Short description of results:

Irrigation water experiment:
Maximum glyphosate:
In Water:
Clarence (Non-flowing):
Treated area: 21.3 ppm at 0 DAT; 0.46 ppm at 1 DAT
Intake area: 0.318 ppm at 1 DAT
Sprinkler head: 0.125 ppm at 7 DAT
Ephrata (Flowing):
Treated area: <0.001 ppm
Intake area: <0.001 ppm
Sprinkler head: <0.001 ppm
In Sediment:
Clarence: 11.20 ppm at 0 DAT; 1.17 ppm at 1 DAT
Ephrata: <0.05 ppm at all samplings
Maximum AMPA:
In Water:
Clarence (Non-flowing):
Treated area: 0.134 ppm at 0 DAT; 0.049 ppm at 1 DAT
Intake area: 0.019 ppm at 14 DAT
Sprinkler head: 0.021 ppm at 15 DAT
Ephrata (Flowing):
Treated area: <0.001 ppm
Intake area: <0.001 ppm
Sprinkler head: <0.001 ppm at all samplings
In Sediment:
Clarence: 1.23 ppm at 14 DAT
Ephrata: <0.05 ppm
Half-lives for Clarence were estimated as 6.3-9.26 days for pond water and 72.72-346.99 days for pond sediment.

	Forestry site experiment: Pond water samples: Maximum glyphosate: In Water: Chassell: 1.68 ppm at 0 DAT Corvallis: 0.091 ppm at 0 DAT Cuthbert: 0.985 ppm at 0 DAT In Sediment: Chassell: 2.11 ppm at 60 DAT Corvallis: 20.19 ppm at 28 DAT Cuthbert: 0.26 ppm at 0 DAT Maximum AMPA: In Water: Chassell: 0.035 ppm at 3 DAT Corvallis: 0.002 ppm at 0 DAT Cuthbert: 0.014 ppm at 0 DAT In Sediment: Chassell: 1.53 ppm at 30 DAT Corvallis: 1.95 ppm at 28 DAT Cuthbert: 0.13 ppm at 321 DAT Flowing stream water samples: Maximum glyphosate: In Water: Chassell: 1.237 ppm at 0 DAT Corvallis: 0.035 ppm at 0 DAT Cuthbert: 0.031 ppm at 0 DAT In Sediment: Chassell: 0.69 ppm at 7 DAT Corvallis: 0.11 ppm at 180 DAT Cuthbert: 0.18 ppm at 1 DAT Maximum AMPA: In Water: Chassell: 0.01 ppm at 0 DAT Corvallis: 0.002 ppm at 1 DAT Cuthbert: <0.001 ppm In Sediment: Chassell: 0.38 ppm at 14 DAT Corvallis: 0.18 ppm at 63 DAT Cuthbert: <0.05 at all samplings
Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid based on the following discrepancies: - By its design, the study is not a water/sediment study but an outdoor study investigating the dissipation of glyphosate in water and sediment following residues from irrigation (farm pond & irrigation ditch), forest pond water & stream sources water at different locations in the US after edge of field application (irrigation sources) or forestry treatment. - By the used study design it cannot be distinguished between processes of dilution, adsorption and degradation. - No information of actual application rate (e.g. trough quantification of losses during application), thus the detected amount of glyphosate and AMPA cannot be related to the applied amount. - Pesticide history of test systems not reported. - Application of the formulated product (Rodeo® or Accord®) and not the active substance. - Samples were deep-frozen prior start of analytical procedures; storage length not reported.

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted]

Data point:	CA 7.2.2.3/015
Report author	[redacted]
Report year	1979
Report title	Glyphosate dissipation in water following aquatic use of Roundup® in the U.K.
Report No	MLL-30038
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:	Study type: Water/sediment field study with overspray application to water Test item: Roundup® Two experiments were conducted with a duration of 32 days: Roundup® was applied to flowing water, near Wessex, Chippenham (UK) and to non-flowing water near Boston, Lincolnshire (UK) Test systems: Wessex (flowing water): canal was approximately 15 m wide with a depth of 1.5 m in the center and 0.5 m near the sides; water flow between sampling stations 1 and 2 was 2.25 min, 1.75 min between stations 3 and 4 and 2.5 min between stations 5 and 6. Boston (non-flowing water): two non-flowing farm drainage canals; water depth varied from 0.25 to 0.375 m pH: Wessex: Water: 6.9-7.5 (mean 7.2) Hydrosoil: 7.1 Boston: Water: 6.4-7.7 (mean 6.7) Hydrosoil: 6.6-7.4 (mean 6.9) Water temperature: Wessex: 14-16°C (mean 14.9°C) Boston: 7.2-15.5°C (mean 13.2°C) % Dry matter: Wessex: 0.3-1.0 % (mean 0.5 %) Boston: 0.4-1.4 % (mean 0.8 %) Application rate: 3.6 kg glyphosate/ha Test design: Flowing water: Roundup® sprayed over the channel with a knapsack sprayer Non-flowing water: Roundup® sprayed over the channel using a tractor mounted sprayer Sampling: 0.5, 1, 4, 8 h after treatment and 1, 2, 4, 8, 16, 32 DAT Collected by scooping top water layer and by using a vacuum system for sampling hydrosoils from canal bottom Samples were obtained from three replicate sampling points Samples were deep-frozen prior start of analytical procedures Workup: Hydrosoil samples were mixed with 30 mL of 0.5 M NH_4OH prior to filtration, glyphosate and aminomethylphosphonic acid (AMPA) recovered from samples by concentration on an anion exchange

	column, fractionation an a cation exchange column, derivatization to the N-trifluoroacetyl methyl esters Analysis: Gas-liquid chromatography using a phosphorous specific flame photometric detector; the detection limit was 0.005 mg/kg
Short description of results:	Flowing water: Recovery of the test item: 85-100 % Maximum glyphosate: In water: 0.24 mg/kg at 30 min after treatment In hydrosoil: 0.006 mg/kg at 1 h after treatment Maximum AMPA: In water: <LOD In hydrosoil: <LOD 8 hours after application, no more glyphosate could be detected neither in the hydrosoils nor in the water samples taken at the application point Non-flowing water: Recovery of the test item: 80-100 % Maximum glyphosate: In water 1.7 mg/mg at 4 h after treatment In hydrosoil: 0.03 mg/kg at 4 h after treatment Maximum AMPA: In water: 0.07 mg/kg at 4 DAT In hydrosoil: <LOD Glyphosate content dissipated below detection limit in less than 8 days in those hydrosoils; AMPA fully dissipated 16 days after treatment; the half-life for the dissipation of glyphosate in water was calculated to be 0.36 days using the nonlinear model of Gustafson and Holden.
Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid based on the following discrepancies: - field study with overspray application to water. - with this setup it cannot be distinguished between dilution, adsorption and degradation. - no information of actual application rate (e.g. trough quantification of losses during application), thus the detected amount of glyphosate and AMPA cannot be related to the applied amount. - pesticide history of test systems not reported. - application of the formulated product (Roundup®) and not the active substance. - samples were deep-frozen prior start of analytical procedures; storage length not reported.

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted], *1978*

Data point:	CA 7.1.1.3/016
Report author	[redacted]
Report year	1978
Report title	Photodegradation and anaerobic aquatic metabolism of Glyphosate, N-phosphonomethylglycine
Report No	MSL-0598
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:	Study type: Water/sediment (anaerobic) Test item: [^{14}C]-labelled glyphosate (specific activity 10.12 mC/mM, 98-99 % radiochemical purity) Test water and sediment: Natural water and sediment from lake number 34, Busch Wildlife Area, Weldon Springs, Missouri pH (water): 6.6 pH (sediment): 7.3 (medium not stated) Organic matter: 1.4 % Sediment was sieved with a 4 mesh sieve. One anaerobic water sediment experiment was conducted with natural water and sediment. Application rate: 150 µg was added to each flask (0.1 ppm) Test design: Anaerobic metabolism flasks were filled with 100 mL water and 50 mL of sediment, flushed with with nitrogen for 10 min, closed and incubated in the dark at 30 °C. The test substance was applied after 35 days. After application of glyphosate the flasks were flushed with nitrogen again and fitted with a carbon dioxide trap. Volatiles trapping: CO_2: ascarite trap Organic volatiles: no trapping Incubation: 30°C, gassed with nitrogen Sampling: 0, 1, 2, 3, 4 and 6 weeks after treatment Workup: Flasks were terminated by separating the sediment and water by centrifugation and subsequently extracting the sediment two times with 0.5 N NH_4OH. Analysis of radioactivity: Water: LSC Extracts: LSC/HPLC NER: combustion/LSC Volatiles: LSC Identification of radioactive residues: HPLC/radiodetection co-chromatography with reference items
Short description of results:	Recovery of radioactivity: 80.3-104.5 % Mineralisation: 10.4 % AR after 6 weeks Other volatiles: not measured Extractable radioactivity: 28.5 % AR after 6 weeks Radioactivity in water: 8.1 % AR after 0 weeks, 2.3 % AR after 6 weeks Non-extractable radioactivity: 29.4 % AR after 0 weeks, 40.7 % AR after 6 weeks Transformation of test item in sediment extracts (HPLC analysis): Glyphosate: 44.0 % AR after 0 weeks, 9.8 % AR after 6 weeks AMPA: 23.0 % AR after 0 weeks, 18.7 % AR after 6 weeks
Reasons why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid based on the following discrepancies: - Anaerobic incubation (no data requirement). - Incubation at 30 °C. - Water was not analysed. - Recovery of radioactivity below 90% for several samplings. - Water and sediment were separated by centrifugation which disturbed the sediment. - Only 8% of radioactivity in water at time zero and 29% non-extractable residues, indication of work-up issues resulting in fast dissipation to the sediment. - No proof of stability during application. - Only single samples were incubated. - Recovery of glyphosate at time zero far below 90%.

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

[redacted], *1972*

Data point:	CA 7.2.2.3/017
Report author	[redacted]
Report year	1972
Report title	The degradation and metabolism of MON-0573 in river and lake bottom sediments and surface water
Report No	276
Guidelines followed in study	US EPA guidelines for registering pesticides, draft 5-1-71, Section II – Degradation studies in water containing suspended solids, and Section III – Degradation studies in bottom sediments, draft 5-1-72
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, not accepted in RAR (2015)
Acceptability/Reliability	No

Short description of study design and observations:

Study type: Water/sediment
Test item: methane-^{14}C-labeled MON-0573 (N-(phosphonomethyl)glycine; glyphosate), radiochemical purity 96.5 %, specific radioactivity 8.51 mCi/mMol
Test water/sediment:
Mississippi River (75 feet from the shore, swift current)
Illinois River (3 feet from the shore, moderate current)
Missouri River (close to the shore, slow current)
Springfield Lake, Illinois (30 feet from the shore)

pH: Water samples: 8.20-8.55
Mississippi Sediment: 7.75
Illinois Sediment: 7.65
Missouri Sediment: 7.85
Springfield Sediment: 7.60

Degradation in collected water and sediment was assessed in separated experiments. A degradation study containing sediment, distilled water and the test substance and a degradation study containing the test water and the test substance were performed. Experiments were carried out with a duration of 14 days for the sediment experiment and 45 days for the water experiment in a shaker at 30 °C. In parallel control vessels were incubated with ^{14}C-sucrose to determine the evolution of $^{14}CO_2$ without presence of the test item.

<u>Sediment experiment:</u>
Test system: bottom sediment was mixed for 20 min with a Hobart mixer, aliquots (10 g dry weight) were weighed into a funnel and flushed into the flasks with distilled water (95/100 mL)

Application: 1 mL NH_4CO_3 solution containing 0.5 mg of ^{14}C-glyphosate
Test design: closed static system with sealed Erlenmeyer flasks shaken at 180 rpm
at each sampling point, CO_2 collection apparatus was attached, and air was flushed through the systems
Volatiles trapping:
CO_2: apparatus containing ascarite (NaOH, glass wool and drierite ($CaSO_4$) attached to the flask by glass ground joints

Organic volatiles: none
Incubation: at 30°C
Sampling: 0, 4, 7 and 14 DAT
Work up: water was separated from sediment by centrifugation; sediment was washed with 25 mL water, suspended by vigorous shaking followed by centrifugation; after lyophilisation, sediment was extracted three times with 40 mL of 0.5 N NH_4OH; samples were combusted prior to and after extraction with NH_4OH
Analysis of radioactivity:
Extracts: LSC
NER: combustion/LSC
Volatiles: LSC
Identification of radioactive residues: TLC/Beta Camera with reference standards

Water experiment:
Test system: 100 mL of test water were filled into the flasks
Application: 1 mL NH_4CO_3 solution containing 0.5 mg of ^{14}C-glyphosate
Test design: static system with sealed Erlenmeyer flasks
CO_2: apparatus containing ascarite (NaOH, glass wool and drierite ($CaSO_4$) attached to the flask by glass ground joints
Organic volatiles: none
Incubation: at 30°C
Sampling: 0, 4, 7, 14, 21, 28, 35 and 45 DAT
Work up: None
Analysis of radioactivity:
Supernatant: LSC
Volatiles: LSC
Identification of radioactive residues: TLC/Beta Camera with reference standards

Short description of results:

Sediment test:
Recovery of radioactivity: 82.6-94.7 %
Mineralisation (cumulative CO_2 after14 days):
- Missouri Sediment: 41.0°%°AR
- Illinois Sediment: 44.6 % AR
- Mississippi Sediment: 41.5 % AR
- Springfield Sediment: 43.6 % AR

Other volatiles: none
Radioactivity in supernatant at 14 DAT:
- Missouri Sediment: 12.3 % AR
- Illinois Sediment: 6.9 % AR
- Mississippi Sediment: 10.3 % AR
- Springfield Sediment: 10.7 % AR

Radioactivity in NH_4OH extracts at 14 DAT:
- Missouri Sediment: 23.8 % AR
- Illinois Sediment: 16.6 % AR
- Mississippi Sediment: 20.4 % AR
- Springfield Sediment: 20.0 % AR

Non extractable radioactivity at 14 DAT:
- Missouri Sediment: 11.3 % AR
- Illinois Sediment: 12.7 % AR
- Mississippi Sediment: 20.3 % AR
- Springfield Sediment: 16.5 % AR

Distribution of residues at 14 DAT (water/ sediment extract/ total system in % AR):
Missouri Sediment:
- Glyphosate: 1.2 / 8.6/ 9.8
- AMPA: 11.1 / 15.2 / 26.3

Illinois Sediment:

	Glyphosate: 0.7 / 2.6 / 3.3 AMPA: 6.2 / 14.0 / 20.2 Mississippi Sediment: Glyphosate: 0.5 / 5.9 / 6.4 AMPA: 8.4 / 14.5 / 22.9 Unknown I: 0.7 / - / 0.7 Unknonwn II: 0.7 / - / 0.7 Springfield Sediment: Glyphosate: 1.6 / 3.0 / 4.6 AMPA: 7.2 / 13.4 / 20.6 Unknown I: 0.3 / 0.6 / 0.9 Unknown II: 0.5 / 1.0 / 1.5 Water test: Recovery of radioactivity: 90.8-95.3 % Mineralisation(cumulative CO_2 after14 days): Missouri Water: 1.82 % AR Illinois Water: 1.55 % AR Mississippi Water: 1.49 % AR Springfield Water: 5.76 % AR Other volatiles: none Transformation of the test item at 45 DAT (in % AR): Missouri Water: glyphosate: 82.1 AMPA: 9.2 Illinois Water: glyphosate: 86.6 AMPA: 7.1 Mississippi Water: glyphosate: 86.9 AMPA: 6.9 Springfield Water: glyphosate: 70.7 AMPA: 14.3
Reasons for why the study is not considered relevant/reliable or not considered as key study by the applicant:	The study is considered invalid based on the following discrepancies: - Separate incubation in water and sediment, i.e. no 'systems'. - Incubation of sediment by adding distilled water. - Test was performed at 30 °C. - Sediment was extracted with NH_4OH after lyophilisation. - Only 4 instead of the recommended six sampling times were processed in the sediment experiment. - Distribution into components only reported for the last sampling. - Test duration was 14 days for sediment and 45 days for water. - Oxygen saturation, pH value and redox potential during study were not reported. - After sampling sediment was mixed for 20 min using a Hobard mixer. - Characterisation data (pH, organic carbon, texture) of test systems not available. - Recovery in the sediment experiment <90 % for one system. - No LOD/LOQ reported. - No acclimation period of test systems prior application. - 10 g dry weight of sediment used and thus less than recommended 50 g. - Range of temperature during study not reported. - Single samples were investigated per sampling interval. - No information whether samples were incubated in the dark.

Assessment and conclusion by RMS:

Considering all the deviations mentioned above by the applicant, the study is not considered acceptable. The short study summary reported by the applicant was not completed by the RMS.

Water/sediment studies with AMPA as test item

Table 8.2.2.3-34: List of existing and new water/Sediment studies on AMPA

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.2.2.3/018	[redacted], 2004	Accepted in RAR 2015	Acceptable
CA 7.2.2.3/019	[redacted], 2003	Accepted in RAR 2015	Acceptable
CA 7.2.2.3/020	[redacted], 2002	Accepted in RAR 2015	Acceptable
CA 7.2.2.3/021	[redacted], 1991	Accepted in RAR 2015	Acceptable

[redacted], *2004*

Data point:	CA 7.2.2.3/018
Report author	[redacted]
Report year	2004
Report title	[14C]-AMPA: Degradation and fate in water/sediment systems
Report No	SNN/03
Guidelines followed in study	Guidelines concerning the inclusion of Active Substances in Annex I 91/414/EEC SETAC
Deviations from current test guideline	From OECD 308: - Water:sediment ratio of 2:1 by volume (instead of 3:1 to 4:1 as recommended by the guideline). - Single test systems processed at each timepoint. - No storage time at -15 °C reported for water phases prior to chromatographic analysis. - Mass balance below 90% AR for the last 2 or 3 sampling dates - Issues in analysis of sediment extracts of Sediment B, probably caused by co-extracted matrix disrupting the ion-exchange chromatography. - Unidentified radioactivity > 5%
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes, for sytem A only

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: [^{14}C]AMPA
Lot No.: RUS 0316
Specific activity: 17.65 mCi/mmol (159 µCi/mg)
Radiochemical purity: 97.8 %

2. Test System:

Water and sediment were obtained immediately from natural locations prior to the start of the study. Sediments were sieved to ≤ 2 mm. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-35: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Manningtree A	Manningtree B
Country	United Kingdom	United Kingdom
Sediment:		
Textural Class (UK)[1]	Clay loam	Clay loam
Sand (%)	48	48
Silt (%)	29	28
Clay (%)	23	24

Table 8.2.2.3-35: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Manningtree A	Manningtree B
pH [2]	7.6	6.3
Organic matter (%)	5.6	6.0
Organic carbon [3] (%)	3.2	3.5
Cation exchange capacity (meq/100 g)	14.7	17.0
Water content (% dry weight)	88.9	96.3
Water content (% wet weight)	47.1	49.0
Microbial biomass (µg C/g)		
Study beginning (0 DAT)	338.6	316.3
Study end (103 DAT)	296.1	143.9
Water:		
Organic carbon (mg/L)	12.1	26.4
pH	7.2	7.1

DAT = days after treatment, USDA: United States Department for Agriculture
[1] no details on classification system (i.e. particle size) reported
[2] medium not reported
[3] calculated during dossier preparation using the equation: OC = OM/1.724

B. STUDY DESIGN

1. Experimental conditions

Flow-through test system were used, consisting of a dreschel bottle (with sintered stem for uniform gas dispersion) containing water to humidify the air-flow, connected to the test vessel containing the water/sediment test system (the end of the glass tube bringing air into the test vessel was positioned just below the water surface). The test system was connected to an empty dreschel bottle followed by a dreschel bottle containing ethyl digol (to trap organic volatile compounds) and two dreschel bottles containing 1 M aqueous potassium hydroxide (KOH) solution with phenolphthalein indicator (to trap $^{14}CO_2$).

Sediment, equivalent to 55 to 60 g dry weight (ca 100 mL equivalent to 120 g wet weight) was added to each test vessel and covered with approximately 200 mL of the corresponding water. The test systems were incubated at 20 ± 2°C in darkness with an air flow-rate sufficient to achieve as close as possible to the specified water oxygen content (20% saturation), until an equilibrium was reached with respect to the pH and oxygen content in the water and the redox potential in the water and sediment.

The application rate was 0.51 mg AMPA/test vessel.

Test systems were incubated under aerobic conditions in the dark for 103 days at 20 ± 2°C. During acclimation and incubation pH value, oxygen saturation and redox potential of the water layer and the redox potential of the sediment layer were monitored in additional untreated test vessels.

2. Sampling

Single samples from each system were processed and analysed at 0, 1, 7, 14, 29, 61, and 103 days after treatment (DAT). The ethyl digol and KOH traps were assayed and changed on a weekly basis for the first month of the study and about ten days thereafter.

3. Analytical procedures

The sediment and water in each test vessel were separated by decanting the water from the test vessel. At each sampling interval, the radioactivity associated with dosing formulations, water, air traps and sediment extracts was determined directly by liquid scintillation counting (LSC). Water samples were stored at -15 °C prior to chromatographic analyses.

Sediments were extracted three times at room temperature with 0.5 M ammonium hydroxide for 2 hours using a shaker. Afterwards, sediments were extracted by shaking twice at room temperature for 2 hours using 1 M hydrochloric acid. Each extract was separated by centrifugation and analysed by LSC in duplicates separately. Sediment residues were air-dried and analysed by combustion/LSC.

Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification (LOQ).

Residues in water and sediment extracts were quantified by HPLC. The limit of detection was not reported.

Selected samples of extracted sediments containing >10 % applied radioactivity were further extracted with 0.5 M NaOH solution for fractionation intro humins, fulvic acid and humic acid.

The identification of CO_2 in the potassium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 6.9 and 7.8 in system Manningtree A and between 7.0 and 8.1 for system Manningtree B. The oxygen saturation in the water phase ranged between 10 and 18 % in system Manningtree A and between 5 and 19 % in system Manningtree B. The redox potential of the water was between 32 and 210 mV for system Manningtree A and between -44 and 239 mV for system Manningtree B. The redox potential of the sediment was between -93 and 222 mV in system Manningtree A and between -129 and 248 mV for system Manningtree B.

Radioactive mass balance and distribution of AMPA and metabolites in two water/sediment systems are summarised below.

Table 8.2.2.3-36: Distribution of radioactivity in water/sediment system Manningtree A under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound	DAT						
	0	1	7	14	29	61	103
Water	94.7	38.4	11.3	6.8	5.3	2.8	1.8
Sediment Extracts	1.5	39.2	55.1	66.9	64.7	67.7	65.3
Non-extractable Residue	0.4	13.4	24.5	16.7	16.0	7.9	13.0
Carbon dioxide	n.s.	0.3	0.5	0.6	3.3	8.1	9.8
Mass balance	96.6	91.3	91.4	91.0	89.3	86.5	89.9

DAT: days after treatment
n.s. no sample
Radioactivity in ethyl digol traps was always <0.1 % AR.

Table 8.2.2.3-37: Distribution of radioactivity in water/sediment system Manningtree B under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound	DAT						
	0	1	7	14	29	61	103
Water	96.7	53.5	9.0	6.6	2.3	0.3	0.3
Sediment Extracts	0.3	25.8	48.0	50.8	47.4	62.9	56.2
Non-extractable Residue	0.2	13.0	35.3	31.6	40.7	10.7	23.0
Carbon dioxide	n.s.	0.2	0.6	2.4	3.0	8.0	8.2
Mass balance	97.2	92.5	92.9	91.4	93.4	81.9	87.7

DAT: days after treatment
n.s. no sample
Radioactivity in ethyl digol traps was always <0.1 % AR.

Table 8.2.2.3-38: Degradation of [14C]AMPA in water/sediment system Manningtree A under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound	DAT

		0	1	7	14	29	61	103
Water	P1a	<0.1	0.3	0.4	0.7	0.5	0.5	0.7
	AMPA	90.5	37.7	10.7	5.9	4.7	2.3	0.8
	Others [1]	4.2	0.5	0.2	0.2	0.1	0.1	0.4
Sediment	P1a	0.3	21.7	34.8	53.0	24.5	31.6	31.0
	AMPA	1.1	15.4	16.4	3.5	29.6	30.2	12.3
	Others [1]	0.1	2.1	3.9	10.4	10.6	5.9	22.0
Total system	AMPA	*91.6*	*53.1*	*27.1*	*9.4*	*34.3*	*32.5*	*13.1*

DAT: days after treatment
[1] Represents regions of radioactivity which cannot be assigned to a designated peak
Values calculated during dossier preparation are given in *italics*

Table 8.2.2.3-39: Degradation of [14C]AMPA in water/sediment system Manningtree B under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound		DAT 0	1	7	14	29	61	103
Water	P1a	<0.1	0.3	0.3	0.3	0.2	0.1	<0.1
	AMPA	91.2	52.7	8.6	6.2	1.9	0.1	0.2
	Others [1]	5.5	0.5	0.1	0.1	0.2	0.1	0.1
Sediment	P1a	0.1	1.4	44.1	49.2	26.6	41.8	18.0
	AMPA	0.1	22.3	2.6	0.2	<0.1	<0.1	<0.1
	P3	<0.1	<0.1	<0.1	<0.1	6.1	16.6	33.2
	Others [1]	0.1	2.1	1.3	1.4	14.7	4.5	5.0
Total system	AMPA	*91.2*	*52.7*	*8.6*	*6.2*	*1.9*	*0.1*	*0.2*

DAT: days after treatment
[1] Represents regions of radioactivity which cannot be assigned to a designated peak
Values calculated during dossier preparation are given in *italics*

Table 8.2.2.3-40: Fractionation of day 103 post extracted sediment (in percent of applied radioactivity)

Experiment	Fulvic acid	Humic acid	Humin
Manningtree A	38.8	59.5	1.8
Manningtree B	28.2	68.4	3.4

B. MASS BALANCE

Material balances ranged from 89.3 to 96.6 % of applied radioactivity (% AR) for Manningtree A (one exception being on 61 DAT, recovery 86.5 % AR) and from 87.7 to 97.2 % AR (one exception being on 61 DAT, recovery 81.9 % AR for Manningtree B).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

In system Manningtree A, radioactivity recovered in the water decreased from 94.7 % AR at 0 DAT to 1.8 % AR at 103 DAT. Correspondingly, radioactivity in the sediment extracts increased from 1.5 % AR at 0 DAT to 65.3 % AR at 103 DAT.

In system Manningtree B, radioactivity recovered in the water decreased from 96.7 % AR at 0 DAT to 0.3 % AR at 103 DAT. Correspondingly, radioactivity in the sediment extracts increased from 0.3 % AR at 0 DAT to 56.2 % AR at 103 DAT.

In system Manningtree A, non-extractable residues (NER) accounted for up to 24.5 % AR (7 DAT) and ranged between 0.4 and 24.5 % AR during the course of the study. Fractionation indicated that the majority of radioactivity was associated with the humic acid fraction.

In system Manningtree B, NER accounted for up to 40.7 % AR (29 DAT) and ranged between 0.2 to 40.7 % AR during the course of the study. Fractionation indicated that the majority of radioactivity was associated with the humic acid fraction.

D. VOLATILE RADIOACTIVITY

In both test systems, the majority of volatiles was carbon dioxide. Maximum amounts of volatiles reached at study end (103 DAT) were 9.8 and 8.2 % AR in systems Manningtree A and Manningtree B,

respectively. The barium precipitation test confirmed the identity of volatiles from the KOH traps as carbon dioxide. Radioactivity in ethyl digol traps was always <0.1 % AR.

E. TRANSFORMATION OF THE TEST ITEM

Analysis of water samples by HPLC showed that the majority of the radioactivity in samples from both sediments was associated with AMPA. In the water sample from Manningtree A, AMPA decreased from 90.5 % AR at 0 DAT to 0.8 % AR at 103 DAT. A minor unidentified peak chromatographically more acidic than AMPA (P1a) was detected at 5 minutes, but this accounted for less than 1 % AR at each time point.

Analysis of water samples from Manningtree B showed a decrease in AMPA from 91.2 % AR at 0 DAT to 0.2 % AR at 103 DAT. Similarly a minor unidentified peak (P1a) was detected at 5 minutes, but this accounted for less than 0.5 % AR at each time point.

Analysis of the Manningtree A sediment extracts showed that the amount of AMPA in the sediment increased from 1.1 % AR at 0 DAT to 30.2 % AR at 61 DAT and decreased to 12.3 % AR until 103 DAT. Peak P1a was also detected in the extract samples and accounted for approximately 53 % AR up to 14 DAT decreasing to ca 31 % AR by 103 DAT.

Analysis of the extracts from Manningtree B sediment, AMPA accounted for 22.3 % AR at 1 DAT but decreased to 0.2 % AR at 14 DAT. Peak P1a was also detected and accounted for ca 42% AR up to 61 DAT. Radioactivity associated with P1a accounted for 18 % AR at 103 DAT.
In this system severe problems were encountered in obtaining chromatography for these extracts. This is believed to be due to the presence of co-extracted endogenous material affecting the ion-exchange chromatography. There appears to be a further radioactive component present in this system (designated P3) and this accounted for 6.1 % AR at 29 DAT and 33.2 % AR at 103 DAT. This component was observed as a broad peak and could well be composed of several components that were unresolved due to chromatographic interference from endogenous material. Several attempts were made to improve the chromatography, including solid phase extraction dilution of the extracts with mobile phase and concentration/re-suspension in mobile phase. All attempts proved unsuccessful. Since the study was a metabolite study, not a parent glyphosate study, and the compounds were never detected in any of the available glyphosate water/sediment studies no further attempts were made to identify these breakdown products.

In system Manningtree A the amount of AMPA in the total system decreased from 91.6 % AR at 0 DAT to 9.4 % AR at 14 DAT, increased then to 34.3 % AR at 29 DAT and finally decreased to 13.1 % AR at 103 DAT. In system Manningtree B, the amount of AMPA in the total system decreased from 0 DAT to 103 DAT from 91.2 to 0.2 % AR.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted], 2020, CA 7.2.2.3/001.

Assessment and conclusion by applicant:

Material balances at 61 DAT and 103 DAT were between 81 and 89 % for both test systems. This can be attributed to a loss of volatiles or losses during combustion. Nevertheless, the time course of the radioactivity distribution in water and sediment is reasonable and consistent for both test systems. Thus, there is no effect on the understanding of the degradation behaviour of AMPA in this study.

Issues occurred in analysis of sediment extracts of Sediment B, probably caused by co-extracted matrix disrupting the ion-exchange chromatography. Attempts to improve the chromatography (e.g. solid phase extraction) were not successful. Therefore, the rate of AMPA was only calculated for water, sediment and total system of test system Manningtree A and for water of system Manningtree B. The degradation rate of AMPA in sediment and total system of Manningtree B was not calculated.

The study is considered valid.

Assessment and conclusion by RMS:

Some deviations from OECD 308 are identified. .

Due to problems analysing extracts obtained from sediment B, levels of AMPA in system B are uncertain. RMS considers that results from system B should not be considered further, especially since total system degradation rate cannot be derived.

Regarding System A, single test systems were processed at each timepoint, but this does not invalidate the results of the study. Water:sediment ratio of 2:1 instead of 3:1 to 4:1 was used. Since AMPA has a relatively high Kfoc, this may have enhanced the adsorption on sediment.

Mass balance is below 90% AR (86.5 to 89.9% AR) at the 3 last sampling points but this slight deviation is not expected to impact the reliability of the results.

It is stated that no further attempts were made to identify the breakdown products since the study was a metabolite study, not a parent glyphosate study. It would have been more suitable to characterize all the breakdown products exceeding 5 % AR, however this has no impact on the reliability of the results to derive degradation rates of AMPA.

It should be noticed that in system A, AMPA was observed at 3.5 % AR at 14 DAT in sediment whereas it corresponded to 16.4%AR and 39.6%AR respectively at 7 DAT and 29 DAT. This punctual decrease is not pointed out by the notifier and is quite unexpected, however results remain valid to derive endpoints.

The study is partly acceptable. Only results from system A can be relied on to derive degradation rates for AMPA.

[redacted], *2003*

Data point:	CA 7.2.2.3/019
Report author	[redacted]
Report year	2003
Report title	Aerobic aquatic degradation of aminomethylphosphonic acid according to SETAC, part 1.8.2 (March 1995)
Report No	IF-02/00005222
Guidelines followed in study	SETAC "Procedures for assessing the environmental fate of ecotoxity of pesticides", Part 1, 8.2
Deviations from current test guideline	From OECD 308: - Pre-equilibration of the test systems for 34 days. - Limit of detection and limit of quantification of the chromatographic methods is not reported. - Unidentified radioactivity > 5%.
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]Aminomethylphosphonic acid
Lot No.: Amersham Pharmacia CFQ12959 (Item Number BE9181)
Specific activity: 55 mCi/mmol
Radiochemical purity: 98.6 % by HPLC

2. Test System:

Sediments were sieved to ≤2 mm and water was filtered to ≤0.2 mm. Water and sediment were stored separately in the dark at 4 ± 2°C for approximately one week before acclimation of the test systems was started. Aerobic conditions of the aquatic test systems were maintained during the storage period. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-41: Characteristics of test water/sediment systems

Parameter		Results	
System		Bickenbach	Unter-Widdersheim
Description		Brook	Brook
Location		Bickenbach, Germany	Hungen, Germany
Sampling depth for	water	Not provided	Not provided
	sediment	15-30 cm below water surface	3-15 cm below water surface
Water			
pH		8.5	8.5
Total hardness (mmol/L)		1.88	3.22
Total organic carbon (mg/L)		1.78	2.50
Total phosphorus (mg/L)		0.06	<0.06
PO_4 (mg/L)		<0.18	<0.18
Total nitrogen (mg/L)		4.3	6.05
NO_3-N (mg/L)		3.38	4.99
NO_2-N (mg/L)		<0.02	0.04
Sediment			
Textural Class (USDA)		Not reported	Not reported
Sand (%)		94.3	35.9
Silt (%)		5.5	41.0
Clay (%)		0.2	23.1
Textural Class (DIN)		Sand	Slight sandy loam
Sand (%)		93.8	34.0
Silt (%)		6.2	42.9
Clay (%)		0.2	23.1
pH [1]		8.5	8.5
Maximum Water Holding Capacity (MWHC) (g water/(100 g))		41.4	75.4
Organic carbon (%)		0.64	2.96
Organic matter (%)		1.10	5.10
Cation exchange capacity (mval/kg)		28.7	123
$CaCO_3$ (%)		2.07	0.36
Total phosphorus (mg/kg)		459	1250
Total nitrogen (mg/kg)		400	1700
Microbial activity at 40 % MWHC (mg C/(100 g))			
After sampling		23	24
At 104 DAT		14	15

[1] Medium not stated
USDA: United States Department for Agriculture, DIN: Deutsches Institut für Normung

B. STUDY DESIGN

1. Experimental conditions

The flow-through test system consisted of six bottles connected via tubing to a vacuum system. The first bottle was a hydration flask containing reagent water. The next Woulff'sche flask containing the treated water/sediment was connected to a security bottle. The next bottle contained 50 mL 2 N NaOH with saturation indication by cresol red for the collection of CO_2. The last bottle contained 50 mL of 2-methoxy ethanol to trap volatile organics and was connected to a vacuum pump so that moist air could be pulled through all bottles. One series of metabolism flasks consisted of two replicates per sampling date.

75 g of water saturated sediment (dry weight equivalents) and 300 mL of reagent water were added to each test vessel, corresponding to a water:sediment ratio of 4:1 (on a weight basis). The oxygen concentration was at least greater than 20 % of its saturation during the experiment. The water/sediment systems were pre-incubated for 34 days at 20 ± 2 °C in the dark, until an equilibrium based on redox potential, pH-value of water and sediment and oxygen concentration of the water was reached.

The application rate was 0.958 mg AMPA/L which is equivalent to 2837 g AMPA/ha. A test solution of [^{14}C]AMPA was prepared in water and 110 µL of this solution were applied to the surface of the water phase in each test system.

Test systems were incubated under aerobic conditions in the dark for104 days at 20 ± 2 °C.

2. Sampling

Duplicate samples from each system were processed and analysed at 0, 0.25, 1, 2, 7, 14, 30, 62, and 104 days after treatment (DAT). The 2-methoxy ethanol and NaOH traps were assayed at each sampling time or at about every 14 days, whichever came first.

3. Analytical procedures

After removal of the water phase from the test system by decantation and radioactivity in the water was analysed by liquid scintillation counting (LSC). Water samples were analysed by HPLC and TLC after concentration, if needed.

Sediment samples were extracted several times with 1 M NH_3-solution for 1 hour by shaking followed by centrifugation, until the final extraction step resulted in <5 % of applied radioactivity. The sequential extractability of radioactivity of each individual extract as well as the combined extraction solutions were radioassayed by LSC. The combined extraction solutions were adjusted to pH 2 by the addition of HCl and centrifuged again. Processed specimen extracts were analysed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Residual radioactivity in sediments was assayed by combustion/LSC.

AMPA was identified by co-chromatography with reference items. Attempts to identify unknown fraction by LC/MS failed due to the presence of matric components.

Analysed extracts were stored in tightly closed glass storage containers at ≤ - 18 °C in the dark.

The extracted sediments of the 104 DAT samplings (air-dried and ground) were subjected to further characterization of sediment radioactivity, which remained bound to the humic and fulvic acids and the humin fraction.

Aliquots of the volatile traps were directly analysed by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by precipitation of $BaCO_3$ using barium chloride.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 7.6 and 8.0 in system Bickenbach and between 7.2 and 8.2 for system Unter-Widdersheim. The pH value of the sediment remained relatively constant during the study between 7.0 and 7.2 in system Bickenbach and between 7.2 and 7.4 for system Unter-Widdersheim. The oxygen saturation of the water ranged between 39 and 51 % for system Bickenbach and between 31 and 42 % for system Unter-Widdersheim. The redox

potential of the water ranged between 137 and 162 mV for system Bickenbach and between 99 and 116 mV for system Unter-Widdersheim. The redox potential of the sediment ranged between -142 and -196 mV in system Bickenbach and between -204 and -216 mV for system Unter-Widdersheim.

Radioactive mass balance and distribution of AMPA and its degradation products in water/sediment systems are summarised below. Fractionation of non-extractable residues into fulvic acid, humic acid, and humin fractions is also presented.

Water and sediment extracts were analysed by HPLC and TLC. In the report, the results of both methods were presented in tables. In the main text, only the results derived from HPLC analysis were discussed. Therefore, although not clearly stated, it is assumed that HPLC was considered the quantification system while the results obtained by TLC (which are very similar to HPLC results) were considered as confirmatory. Therefore, in this summary results of HPLC and TLC are presented in tables but only results determined by HPLC are discussed further.

Table 8.2.2.3-42: Distribution of radioactivity in water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Total water	1	94.4	88.4	69.7	65.3	41.5	27.4	13.2	12.3	6.8
	2	99.9	87.4	72.0	65.5	41.4	23.3	17.7	12.4	8.2
Sediment extractable	1	2.5	11.6	23.0	28.4	32.2	32.5	33.5	24.9	18.3
	2	1.4	10.5	25.4	29.7	33.5	33.1	33.0	25.4	15.3
Non-extractable residues (NER)	1	0.4	0.9	1.9	3.2	10.6	16.4	27.4	31.8	30.0
	2	0.4	0.8	2.2	2.7	10.7	19.4	20.9	31.6	32.5
CO_2	1	n.p.	0.1	0.2	1.7	10.1	18.0	24.4	32.7	40.1
	2	n.p.	0.1	0.2	1.7	10.1	18.0	24.4	32.7	40.1
Organic volatiles	1	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	1.5	1.5
	2	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	1.5	1.5
Mass balance	1	97.3	101.0	94.8	98.6	94.4	94.3	100.0	103.2	96.7
	2	101.7	98.8	99.8	99.6	95.7	93.8	97.5	103.6	97.6

DAT: days after treatment, n.p.: not performed

Table 8.2.2.3-43: Distribution of radioactivity in water/sediment system Unter-Widdersheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Total water	1	99.l	83.7	67.4	57.9	33.5	17.4	12.2	2.7	2.5
	2	98.9	85.5	71.0	58.4	37.0	19.4	12.0	3.0	2.2
Sediment extractable	1	1.1	14.3	30.2	40.3	55.5	65.1	64.4	60.9	39.4
	2	2.5	14.1	30.5	38.0	53.6	60.4	65.8	57.1	46.6
Non-extractable residues (NER)	1	0.5	2.9	3.2	6.3	8.0	15.5	14.6	26.9	36.0
	2	1.0	2.9	4.3	4.7	6.5	15.4	15.9	28.5	29.5
CO_2	1	n.p.	<0.1	0.1	0.3	1.6	3.4	9.3	15.9	21.2
	2	n.p.	<0.1	0.1	0.3	1.6	3.4	9.3	15.9	21.2
Organic volatiles	1	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	0.1
	2	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	0.1
Mass balance	1	100.7	100.9	100.9	104.8	98.6	101.4	100.6	106.5	99.2
	2	102.4	102.5	105.9	101.4	98.7	98.6	l03.1	104.6	99.6

DAT: days after treatment, n.p.: not performed

Table 8.2.2.3-44:Degradation of [^{14}C]AMPA in the water of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Bickenbach										
AMPA	1	92.6	86.2	61.5	45.5	25.8	16.6	9.4	7.0	4.7
	2	97.7	87.4	59.9	52.2	23.8	15.3	13.1	6.6	2.6
	Mean	*95.2*	*86.8*	*60.7*	*48.9*	*24.8*	*16.0*	*11.3*	*6.8*	*3.7*

Unknown 1	1	1.9	n.d.	3.1	10.0	4.6	5.9	1.7	3.1	1.9
	2	2.2	n.d.	7.0	4.2	7.6	3.3	2.0	2.7	5.7
Unknown 2	1	n.d.	2.2	3.1	7.1	11.2	5.0	2.3	2.3	0.3
	2	n.d.	n.d.	1.4	6.1	10.1	4.8	2.8	3.3	n.d.
Total Unknown	1	1.9	2.2	8.3	19.9	15.8	10.9	4.0	5.4	2.2
	2	2.2	n.d.	12.2	13.4	17.7	8.1	4.8	6.0	5.7
Unter-Widdersheim										
AMPA	1	97.4	79.8	62.3	51.4	28.3	10.8	5.6	1.3	0.6
	2	96.8	81.2	66.1	53.1	32.4	13.5	5.9	1.0	1.2
	Mean	*97.1*	*80.5*	*64.2*	*52.3*	*30.4*	*12.2*	*5.8*	*1.2*	*0.9*
Unknown 1	1	1.7	2.2	2.3	2.9	1.9	2.1	1.9	0.8	1.8
	2	2.2	1.8	2.5	2.4	2.1	1.9	1.7	1.3	0.8
Unknown 2	1	n.d.	1.8	2.9	3.7	3.4	4.6	4.8	0.6	0.2
	2	n.d.	2.5	2.5	2.9	2.6	4.1	4.0	0.7	0.2
Total Unknown	1	1.7	4.0	5.2	6.6	5.3	6.7	6.7	1.4	2.0
	2	2.2	4.3	5.0	5.3	4.7	6.0	6.2	2.0	1.0

DAT: days after treatment, n.d.: not detected, n.p.: not performed
Values calculated for this summary are given in *italics*.

Table 8.2.2.3-45: Degradation of [^{14}C]AMPA in the sediment of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT 0	0.25	1	2	7	14	30	62	104
Bickenbach										
AMPA	1	n.p.	11.6	23.0	26.0	30.6	30.0	31.7	23.5	18.3
	2	n.p.	10.5	25.4	28.4	30.3	31.4	31.2	22.5	14.0
	Mean	n.p.	*11.1*	*24.2*	*27.2*	*30.5*	*30.7*	*31.5*	*23.0*	*16.2*
Unknown 1	1	n.p.	n.d.	n.d.	1.7	1.6	2.5	1.8	1.4	n.d.
	2	n.p.	n.d.	n.d.	1.1	2.3	1.7	1.9	1.8	1.3
Unknown 2	1	n.p.	n.d.	n.d.	0.5	n.d.	n.d.	n.d.	n.d.	n.d.
	2	n.p.	n.d.	n.d.	n.d.	1.0	n.d.	n.d.	1.2	n.d.
Total Unknown	1	n.p.	n.d.	n.d.	2.5	1.6	2.5	1.8	1.4	n.d.
	2	n.p.	n.d.	n.d.	1.3	3.3	1.7	1.9	3.0	1.3
Unter-Widdersheim										
AMPA	1	n.p.	13.3	30.2	39.8	55.5	62.5	64.4	57.1	36.3
	2	n.p.	14.1	30.5	37.9	53.6	58.8	63.2	52.7	44.2
	Mean	n.p.	*13.7*	*30.4*	*38.9*	*54.6*	*60.7*	*63.8*	*54.9*	*40.3*
Unknown 1	1	n.p.	n.d.	n.d.	0.6	n.d.	2.6	n.d.	2.6	3.2
	2	n.p.	n.d.	n.d.	n.d.	n.d.	1.6	2.7	2.6	2.5
Unknown 2	1	n.p.	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	n.d.
	2	n.p.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.9	n.d.
Total Unknown	1	n.p.	1.1	n.d.	0.6	n.d.	2.6	n.d.	3.9	3.2
	2	n.p.	n.d.	n.d.	0.2	n.d.	1.6	2.7	4.5	2.5

DAT: days after treatment, n.d.: not detected, n.p.: not performed
Values calculated for this summary are given in *italics*.

Table 8.2.2.3-46: Degradation of [^{14}C]AMPA in the total system of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT 0	0.25	1	2	7	14	30	62	104
Bickenbach										
AMPA	1	92.6	97.8	84.5	71.5	56.4	46.6	41.l	30.5	23.0
	2	97.7	97.9	85.3	80.6	54.1	46.7	44.3	29.l	16.6
	Mean	*95.2*	*97.9*	*84.9*	*76.1*	*55.3*	*46.7*	*44.3*	*30.5*	*19.8*
Unknown 1	1	1.9	n.d.	3.1	11.7	6.2	8.4	3.5	4.5	l.9
	2	2.2	n.d.	7.0	5.3	9.9	5.0	3.9	4.5	7.0
Unknown 2	1	n.d.	2.2	3.1	7.6	11.2	5.0	2.3	2.3	0.3

	2	n.d.	n.d.	1.4	6.1	11.1	4.8	2.8	4.5	n.d.
Total Unknown	1	1.9	2.2	8.3	22.4	17.4	13.4	5.8	6.8	2.2
	2	2.2	n.d.	12.2	14.7	21.0	9.8	6.7	9.0	7.0
Unter-Widdersheim										
AMPA	1	97.4	93.1	92.5	91.2	83.8	73.3	70.0	58.4	36.9
	2	96.8	95.3	96.6	91.0	86.0	72.3	69.1	53.7	45.4
	Mean	*97.1*	*94.2*	*94.6*	*91.1*	*84.9*	*72.8*	*69.6*	*56.1*	*41.2*
Unknown 1	1	1.7	2.2	2.3	3.5	1.9	4.7	l.9	3.4	5.0
	2	2.2	l.8	2.5	2.4	2.1	3.5	4.4	3.9	3.3
Unknown 2	1	n.d.	2.9	2.9	3.7	3.4	4.6	4.8	1.9	0.2
	2	n.d.	2.5	2.5	2.9	2.6	4.1	4.0	2.6	0.2
Total Unknown	1	1.7	5.1	5.2	7.2	5.3	9.3	6.7	5.3	5.2
	2	2.2	4.3	5.0	5.5	4.7	7.6	8.9	6.5	3.5

DAT: days after treatment, n.d.: not detected
Values calculated for this summary are given in *italics*.

Table 8.2.2.3-47: Degradation of [^{14}C]AMPA in water and sediment of both water/sediment systems under aerobic conditions based on TLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT 0	0.25	1	2	7	14	30	62	104
Bickenbach										
Water AMPA	1	94.4	87.8	63.9	58.5	27.9	16.7	9.7	7.6	4.7
	2	99.9	87.4	62.5	59.5	28.6	15.4	13.8	7.6	5.8
	Mean	*97.2*	*87.6*	*63.2*	*59.0*	*28.3*	*16.1*	*11.8*	*7.6*	*5.3*
Sediment AMPA	1	n.p.	11.0	23.0	26.1	29.0	29.3	32.0	22.5	17.7
	2	n.p.	10.l	25.4	27.6	30.7	31.3	31.7	23.4	14.2
	Mean	n.p.	*11.0*	*24.2*	*26.9*	*29.9*	*30.3*	*31.9*	*23.0*	*16.0*
Unter-Widdersheim										
Water AMPA	1	99.1	80.6	60.2	52.7	28.9	12.2	7.2	1.3	1.8
	2	98.9	82.1	63.1	54.4	32.0	14.4	6.8	1.5	0.8
	Mean	*99.0*	*81.4*	*61.7*	*53.6*	*30.5*	*13.3*	*7.0*	*1.4*	*1.3*
Sediment AMPA	1	n.p.	13.7	30.2	37.9	53.7	62.7	61.3	55.2	37.0
	2	n.p.	13.2	30.5	35.9	51.5	58.3	61.8	50.7	43.2
	Mean	n.p.	*13.5*	*30.4*	*36.9*	*52.6*	*60.5*	*61.6*	*53.0*	*40.1*

DAT: days after treatment, n.p.: not performed
Values calculated for this summary are given in *italics*.

Table 8.2.2.3-48:Fractionation of 104 DAT post extracted sediment (in percent of AR)

Experiment	Fulvic acid	Humic acid	Humin
Bickenbach	4.4	8.5	17.1
	4.2	7.2	20.6
Unter-Widdersheim	2.9	6.2	26.5
	3.9	8.9	16.7

B. MASS BALANCE

Mass balances (single values) ranged from 93.8 to 103.6 % AR for system Bickenbach and from 98.6 to 106.5 % AR for system Unter-Widdersheim.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 104 DAT from 97.2 to 7.5 % AR in system Bickenbach and from 98.9 to 2.4 % AR in system Unter-Widdersheim.

The amount of radioactivity extractable from the sediment of system Bickenbach increased from 2.0 % AR at 0 DAT to a maximum of 33.3 % AR at 30 DAT and then decreased to 16.8 % AR at 104 DAT. In the Unter-Widdersheim system, the amount of radioactivity extractable from the sediment

increased from 1.8 % AR at 0 DAT to a maximum of 65.1 % AR at 30 DAT and then decreased to 43.0 % AR at 104 DAT.

The amount of non-extractable residues (NER) in system Bickenbach increased from 0.4 % AR at 0 DAT to 31.7 % AR at 62 DAT and slightly decreased to 31.3 % AR at 104 DAT. In the Unter-Widdersheim system, the amount of NER increased from 0 DAT to 104 DAT from 0.8 to 32.8 % AR. Most of the residual radioactivity (16.7 to 26.5 % AR) was found to be bound to the humin fraction in the sediments of both locations after 104 days of incubation and is not expected to be bioavailable.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (104 DAT) were 40.1 and 21.2 % AR in the Bickenbach and Unter-Widdersheim systems respectively. Organic volatiles were ≤1.5 % AR for both systems at all sampling points.

E. TRANSFORMATION OF THE TEST ITEM

The amount of AMPA in the water decreased from 0 DAT to 104 DAT from 95.2 to 3.7 % AR in system Bickenbach and from 97.1 to 0.9 % AR in system Unter-Widdersheim.

The amount of AMPA in the sediment of system Bickenbach increased from 11.1 % AR at 0.25 DAT to 31.5 % AR at 30 DAT and decreased then to 16.2 % AR at 104 DAT. The amount of AMPA in the sediment of system Unter-Widdersheim increased from 13.7 % AR at 0.25 DAT to 63.8 % AR at 30 DAT and decreased from to 40.3 % AR at 104 DAT.

The amount of AMPA in the total decreased from 0 DAT to 104 DAT from 95.2 to 19.8 % AR in system Bickenbach and from 97.1 to 41.2 % AR in system Unter-Widdersheim.

Besides carbon dioxide, two unknown compounds were detected in the total system by HPLC. Unknown 1 was detected with a maximum amount (mean value) of 8.5 % AR at 2 DAT in the Bickenbach system and 4.4 % AR at 30 DAT in the Unter-Widdersheim system. Unknown 2 was detected with a maximum amount (mean value) of 11.2 % AR at 7 DAT in the Bickenbach system and 4.4 % AR at 30 DAT in the Unter-Widdersheim system. Additional attempts to characterize the structure of the unknown by LC/MS failed due to the presence of matrix components. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, the compounds are not considered relevant for further evaluation.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted], 2020, CA 7.2.2.3/001.

In the report document available for the evaluation, the individual results of HPLC analysis for water and sediment phase were missing. Thus, the evaluation could only be based on results of TLC analysis. The missing data led to inconsistencies in the reporting of the amounts of AMPA in sediment extracts in the text of the study report compared to tabulated results from TLC analysis. Therefore, no kinetic evaluation was performed for the sediment phase as well as the total system of both systems and only a kinetic evaluation for the water phase is included in the current submission.

A complete report document including the results of HPLC analysis was received after completion of the kinetic evaluation. The complete data may be used to update the evaluation at a later time point.

III. CONCLUSIONS

AMPA degraded rapidly in the water phases of the two German aquatic test matrices. In the processed water of Bickenbach location, two unknown metabolites reached maximum levels of more than 10 % of the applied radioactivity. Both of the metabolites were of transient character. In the Unter-Widdersheim water phases, unknown components did not exceed 5 % of the applied radioactivity. In the processed sediment, extractable radioactivity of both test matrices, unknown components reached maximum levels of below 5 %.

AMPA was converted in both compartments, but predominately in the aerobic water phases, of the two test matrices into two unidentified degradates. The degradation of AMPA was reflected by the formation of residual residues and the formation of $^{14}CO_2$.

Assessment and conclusion by applicant:

Pre-equilibration of the test systems was 34 days and thus, slightly exceeded the 4 weeks period given by the guideline. Nevertheless, pH, oxygen content and redox potential were monitored throughout the study and thus, the validity is not affected.

Two unknown compounds were detected in the total system with a maximum amount (mean value) of 8.5 % AR at 2 DAT and 11.2 % AR at 7 DAT, respectively. Additional attempts to characterize the structure of the unknowns by LC/MS failed due to matrix effects. As indicated by the occurrence, the components showed transient character to decrease towards study end. Being a metabolite study, the components are not considered relevant for further evaluation or risk assessment.

The study is considered valid to evaluate the degradation of AMPA in water/sediment systems.

Assessment and conclusion by RMS:

The study is overall well performed.

RMS agrees that the pre-equilibration time of 34 days has no impact on the validity of the study.

Two unidentified breakdown products exceeding 10% AR or 5% AR at 2 consecutive time points were observed. It is stated that no further attempts were made to identify the breakdown products since the study was a metabolite study, not a parent glyphosate study. It would have been more suitable to characterize all the breakdown products exceeding 5 % AR. In this case, considering that metabolite AMPA is formed at a maximal level of 27% AR from parent in total water-sediment systems (based on studies with glyphosate applied), a rough estimation of the maximal occurrence level of these fractions indicate that they are not expected to be formed above 10% or above 5% of glyphosate at 2 consecutive timepoints. Therefore, RMS considers that there is no need to consider these fractions for risk assessment.

The metabolite identification issues have no impact on the reliability of the results to derive degradation rates of AMPA.

Since detailed results were missing in the initial study report for sediment phase as well as the total system of both systems for HPLC analysis, no kinetic evaluation was provided by the notifier except for results in water (based on TLC results). Updated kinetic evaluation should be provided considering that all the results for HPLC analysis are now available (data gap identified).

The study is considered acceptable.

[redacted], *2002*

Data point:	CA 7.2.2.3/020
Report author	[redacted]
Report year	2002
Report title	Aminomethylphosphonic acid: fate and behaviour in water-sediment
Report No	A&M01-106
Guidelines followed in study	BBA Guideline Part IV, 5-1
Deviations from current test guideline	From OECD 308: - Acclimation period not reported. - Sediment layer between 1.5-2 cm instead of 2.5±0.5 cm - Study duration (119 days) slightly longer than recommended (100 days).

	- No storage time reported for water and sediment extracts until analysis. - Unidentified radioactivity > 5%
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]AMPA
Lot No.: CFQ13045
Specific activity: 2.11 GBq/mmol
Radiochemical purity: 98.1 % (supplier), 98.7 % (determined at test facility)

2. Test systems:

Sediments were sieved to ≤2 mm, water was sieved to ≤0.1 mm. The aquatic systems were were taken and stored well ventilated at 2 to 8°C in the dark for 4 days after receipt. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-49: Characteristics of test sediments

Parameter	Results			
Sediment	Rückhaltebecken		Schaephysen	
Country	Germany		Germany	
Textural Class (DIN)	Not reported		Not reported	
Sand (63 µm – 2 mm) (%)	10.6		86.2	
Silt (2 µm – 63 µm) (%)	83.7		9.3	
Clay (< 2 µm) (%)	5.7		4.5	
pH [1]	7.64		7.34	
Total organic carbon (% dry weight) [2]	1.3 / 1.3 / 1.4		4.2 / 3.2 / 3.2	
Cation exchange capacity (µmol/g)	142.3		172.7	
Microbial biomass (mg C/100g dry weight)	Trial 1	Trial 2	Trial 1	Trial 2
Post handling (before acclimatisation period)	13.9	–	20.25	–
Start of test (after acclimatisation period)	11.21	10.89	15.85	11.70
End of test (119/123 days; incubation with AMPA)	11.81	11.71	13.05	14.04
End of test (122/124 days; control)	10.16	14.4	11.60	13.93
Water				
pH at sampling	7.1		7.3	
pH at 0 DAT [1]	8.7		8.0	
Total organic carbon (mg/L) [2]	<1 / 4 / 6		<1 / 5 / 6	

DAT = days after treatment, DIN: Deutsches Institut für Normung
[1] determined in test systems of day 0
[2] total organic carbon was determined at different time points (post handling / start of test /end of test)

B. STUDY DESIGN

1. Experimental conditions

The test systems consisted of 110 g of each sediment filled into 500-mL flasks up to a height of 1.5 to 2.0 cm and 220 g of the corresponding water phase were added to a height of about 6 cm. The systems were left at 20 ± 2°C to reach a steady state in pH, redox potential, oxygen content and clearing of the water phase.

Sterile test systems were prepared by heating the water/sediment systems on two consecutive days in an autoclave for 2 h.

The absorption/ventilation device consisted of a glass tube with a gas inlet tube filled with (from inside to outside): 1 g paraffin-covered glass wool for adsorption of volatile organic compounds (moistened with 2 % paraffin-oil in hexane), 0.2 g glass wool, 10 g soda lime for absorption of carbon dioxide from the incubation mixture, 0.2 g glass wool, 4 g soda lime for absorption of atmospheric carbon dioxide and 0.2 g glass wool.

The study application rate was calculated to be 0.197 mg AMPA/L water based on a field application rate of glyphosate of 1.8 kg/ha and the assumption that glyphosate was metabolised to AMPA to an extent of 50 %. [^{14}C]AMPA application solution was prepared in water to a final concentration of 0.438 mg/mL and dripped onto the water surface of the test systems.

Test systems were incubated under aerobic conditions in the dark for up to 119 days at 20 ± 2°C.

2. Sampling

Duplicate test systems were processed and analysed 0, 3, 7, 14, 31, 60, 89 and 119 days after treatment (DAT) for system Rückhaltebecken and 0, 3, 5, 13, 31, 60, 90 and 119 DAT for system Schaephysen. The sterile control was processed and analysed at 122 DAT. Volatile traps were assayed at each sampling interval.

3. Analytical procedures

After adding 1 mL 0.1 M NaOH to the test system, the water phase was decanted from the sediment using a folded filter. After measuring the volume, the water was filled into polyethylene flasks and stored in the dark at ≤-18°C and for further analysis. The radioactivity was determined by LSC. For HPLC analysis, samples were thawed, centrifuged and an aliquot was taken.

The formation of carbon dioxide in some samples from each test system were >20 %. According to the test protocol, the water phases from these samples were analysed for water dissolved carbon dioxide. The water phases were thawed and 50 mL were used for the liberation of carbon dioxide as described for the soda lime below.

The sediment was extracted by shaking for 5 minutes with 0.1 M NaOH followed by centrifugation. The supernatant was decanted using a folded filter. The sediment was extracted a second time using 0.1 M NaOH as described above. The NaOH-extracts were combined and analysed by LSC. The NaOH-extracted sediment was exhaustively extracted in a soxhlet apparatus with methanol for approximately 2 h. The radioactivity of the soxhlet-extract was determined by LSC. After air-drying, aliquots of the extracted sediment were combusted.

For HPLC analysis, thawed aliquots of the NaOH extracts were acidified with 32 % hydrochloric acid and centrifuged. The methanol extracts, obtained by Soxhlet extraction were evaporated and re-dissolved in water. Then, 32 % hydrochloric acid was added and an aliquot was analysed by HPLC.

The alkaline NaOH- and MeOH-soxhlet-extracts of the sediment were dark brown and contained also the fulvic acid-, humic acid- and humin-associated radioactivity. In order to avoid precipitation on the column by using an acidic eluent, these extracts had to be acidified resulting in the loss of the humic acid associated radioactivity. The supernatant which was used for HPLC analysis represented the fulvic acid-associated radioactivity. For the system Rückhaltebecken, 60% to 84% of the radioactivity of the NaOH-extracts and 65% to 95% of the MeOH-soxhlet-extract were available for HPLC analysis and for the system Schaephysen 52% to 95% and 47% to 95% of the radioactivity of the NaOH-extracts and MeOH-soxhlet-extracts. The remaining radioactivity was associated to the humic acid fraction. The water phase was analysed without acidification.

The lower limit of quantification (LLOQ) was 500 dpm/mL for radio HPLC corresponding to 0.4%, 0.3% and 0.8% (mean values) of the applied radioactivity for water, 0.1 M NaOH-extracts and MeOH-soxhlet-extracts, respectively. The recovery of the radioactivity from the HPLC-column was determined to 92%.

The carbon dioxide adsorbed to the inner soda lime compartment was liberated by hydrochloric acid and radioactivity determined by LSC. The paraffin oil-covered quartz wool was extracted with ethyl acetate, an aliquot of the extract was analysed by LSC.

At each processing time of the incubation period oxygen content of the water, pH of the water and sediment, redox potential of the water and redox potential of the sediment were determined.

[^{14}C]AMPA and metabolites were identified by HPLC-MS, flow injection MS analysis and radio HPLC of selected samples.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 8.40 and 9.28 in system Rückhaltebecken and between 7.70 and 8.72 for system Schaephysen. The pH value of the sediment remained relatively constant during the study between 7.57 and 8.10 in system Rückhaltebecken and between 6.90 and 7.64 for system Schaephysen. The oxygen saturation of the water ranged between 90 and 96 % for system Rückhaltebecken and between 78 and 96 % for system Schaephysen. The redox potential of the water ranged between 193 and 281 mV for system Rückhaltebecken and between 243 and 316 mV for system Schaephysen. The redox potential of the sediment ranged between -78 and -322 mV in system Rückhaltebecken and between -167 and -384 mV for system Schaephysen.

Radioactive mass balance and distribution of [^{14}C]AMPA and metabolites in water/sediment systems are summarised in the tables below.

Table 8.2.2.3-50: Degradation of [14C]AMPA in the Rückhaltebecken aquatic system under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Repli-cate	DAT								
		0	3	7	14	31	60	89	119	Sterile 122
Water	mean	94.8	45.6	30.4	23.9	16.9	12.1	8.3	5.6	4.7
Sediment extractables	mean	*5.1*	*46.6*	*55.5*	*57.8*	*54.1*	*46.2*	*36.7*	*39.0*	*61.5*
Non-extractable residues	mean	0.4	5.2	10.4	13.2	17.2	19.7	30.1	25.1	21.9
Carbon dioxide [1]	mean	n.t.	0.2	1.0	3.1	8.7	17.9	22.1	27.6	7.6
Volatile compounds	mean	n.t.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Recovery	1	100.2	97.3	97.2	97.6	96.0	95.0	97.7	102.4	96.8
	2	100.4	97.9	97.4	98.2	97.8	96.7	96.6	92.0	94.6
	mean	100.3	97.6	97.3	97.9	96.9	95.9	97.2	97.2	95.7
Distribution in water										
AMPA	1	93.36	39.27	24.96	19.17	7.36	4.04	0.88	2.06	2.40
	2	91.39	46.05	25.39	11.94	8.37	3.27	3.18	0.75	2.34
	mean	92.4	42.7	25.2	15.6	7.9	3.7	2.0	1.4	2.4
M 2.5	mean	1.0	1.1	0.6	0.7	0.7	0.6	1.0	0.6	1.5
M 3.3	mean	0.5	–	–	–	–	–	–	–	0.2
M 7	mean	– [3]	0.9	3.4	7.2	8.0	7.6	5.3	3.7	0.6
Non classified radioactivity [2]	mean	0.9	0.9	1.2	0.4	0.3	0.6	0.5	–	–
Distribution in sediment (sum of sodium hydroxide and Soxhlet extract)										
AMPA	1	2.85	28.82	34.46	32.73	32.04	26.62	27.91	17.80	40.72
	2	3.91	26.90	34.19	36.47	29.05	27.94	21.21	27.10	45.91
	mean	3.4	27.9	34.3	34.6	30.5	27.3	24.6	22.5	43.3
M 2.5	mean	–	3.2	3.9	4.3	4.1	3.3	2.2	1.5	4.4
M 3.3	mean	0.6	7.0	7.4	8.8	7.4	5.5	3.2	2.8	5.9
M 7	mean	–	0.3	–	0.6	1.8	0.6	1.2	0.4	1.8
Non classified radioactivity [2]	mean	1.1	8.4	9.9	9.7	10.3	9.5	5.5	11.6	7.0
Total system (water + sediment)										
AMPA	1	96.21	68.08	59.42	51.90	39.40	30.67	28.78	19.86	43.12
	2	95.30	72.94	59.59	48.41	37.43	31.21	24.38	27.86	48.25
	mean	95.8	70.5	59.5	50.2	38.4	30.9	26.6	23.9	45.7
M 2.5	mean	1.0	4.2	4.6	5.1	4.8	3.6	2.7	1.8	5.9

M 3.3	mean	1.1	7.0	7.4	8.8	7.4	5.5	3.2	2.8	6.0
M 7	mean	–	1.1	3.4	7.5	9.8	8.2	6.5	4.1	1.5
Non classified radioactivity [2]	mean	2.0	9.4	11.1	10.1	10.6	10.1	6.0	6.2	2.5

[1] the formation of carbon dioxide in the sterile controls may be caused by the use of non-sterilised water of the application solution

[2] non-classified radioactivity in water = non-classified radioactivity from the HPLC-analysis; non-classified radioactivity in sediment = sum of the non-classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was not available for HPLC-analysis; non-classified radioactivity in total system = sum of the non-classified radioactivity from the HPLC-analysis of water and sediment extracts and the humic acid associated radioactivity of sediment extracts which was not available for HPLC-analysis

[3] –: value <LLOQ, not detected or not tested

DAT: days after treatment, n.t.: not tested

Mean values were calculated from two replicates. Values calculated during dossier preparation are given in *italics*.

Table 8.2.2.3-51: Degradation of [14C]AMPA in the Schaephysen aquatic system under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Repli-cate	DAT								
		0	3	5	13	31	60	90	119	122 Sterile
Water	mean	84.4	24.4	16.5	5.4	1.3	1.8	1.3	0.4	1.2
Sediment extractables	mean	*11.8*	*55.7*	*63.3*	*72.4*	*61.1*	*56.9*	*53.0*	*47.4*	*41.8*
Non-extractable residues	mean	2.4	13.7	16.0	18.8	27.3	30.5	32.8	39.1	37.7
Carbon dioxide [1]	mean	n.t. [4]	0.1	0.5	2.5	6.1	9.3	10.6	11.8	16.8
Volatile compounds	mean	n.t.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Recovery	1	97.4	93.8	96.2	99.3	98.8	99.4	98.3	99.9	98.5
	2	99.7	94.0	96.4	99.0	92.6	95.7	97.1	97.7	96.5
	mean	98.5	93.9	96.3	99.1	95.7	97.5	97.7	98.8	97.5
Distribution in water										
AMPA	1	82.97	18.91	15.80	3.69	0.82	not detected	<LLOQ	0.24	0.61
	2	80.53	24.94	13.95	3.46	0.30	0.88	0.36	<LLOQ	0.85
	mean	81.8	21.9	14.9	3.6	0.6	0.9	0.4	0.2	0.7
M 2.5	mean	0.8	0.7	0.5	–	–	–	–	–	–
M 3.3	mean	– [3]	0.6	–	–	–	–	–	–	–
M 7	mean	0.9	1.0	1.6	1.5	0.5	1.9	1.3	0.2	0.3
Non classified radioactivity [2]	mean	1.4	0.5	0.4	0.4	0.3	0.5	0.5	–	–
Distribution in sediment (sum of sodium hydroxide and Soxhlet extract)										
AMPA	1	3.76	19.58	19.48	21.47	19.04	27.28	20.54	22.94	25.90
	2	6.51	19.73	19.43	23.49	18.35	23.48	19.18	23.55	22.13
	mean	5.1	19.7	19.5	22.5	18.7	25.4	19.9	23.2	24.0
M 2.5	mean	1.2	6.2	5.9	7.0	5.7	4.4	4.4	3.5	4.1
M 3.3	mean	0.5	13.7	19.3	22.9	20.8	11.8	13.9	8.5	6.1
M 7	mean	0.6	0.6	0.7	–	1.2	0.6	0.4	0.5	0.5
Non classified radioactivity [2]	mean	4.4	15.6	17.9	20.0	14.7	14.1	14.7	11.7	7.3
Total system (water + sediment)										
AMPA	1	86.73	38.49	35.28	25.16	19.87	27.28	20.54	23.18	26.51
	2	87.04	44.67	33.38	26.95	18.65	24.36	19.54	23.55	22.97
	mean	86.9	41.6	34.3	26.1	19.3	25.8	20.0	23.4	24.7
M 2.5	mean	2.0	6.9	6.4	7.0	5.7	4.4	4.4	3.5	4.1
M 3.3	mean	0.5	14.0	19.3	22.9	20.8	11.8	13.9	8.5	6.1
M 7	mean	1.0	1.5	1.5	1.5	1.7	1.2	1.8	0.6	0.6
Non classified radioactivity [2]	mean	5.8	16.1	18.3	20.4	15.0	14.5	15.1	11.5	6.3

[1] the formation of carbon dioxide in the sterile controls may be caused by the use of non-sterilised water of the application solution

[2] non-classified radioactivity in water = non-classified radioactivity from the HPLC-analysis; non-classified radioactivity in sediment = sum of the non-classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was not available for HPLC-analysis; non-classified radioactivity in total system = sum of the non-classified radioactivity from the HPLC-analysis of water and sediment extracts and the humic acid associated radioactivity of sediment extracts which was not available for HPLC-analysis

[3] –: value <LLOQ, not detected or not tested
DAT: days after treatment, n.t.: not tested
Mean values were calculated from two replicates. Values calculated during dossier preparation are given in *italics*.

B. MASS BALANCE

Mass balances (single replicates) ranged from 92.0 to 102.4 % of applied radioactivity (% AR) for system Rückhaltebecken and from 92.6 to 99.9 % AR for system Schaephysen.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 119 DAT from 94.8 to 5.6 % AR for system Rückhaltebecken and from 84.4 to 0.4 % AR for system Schaephysen.

The amount of radioactivity extractable from the sediment (sum of NaOH and Soxhlet extracts) increased for system Rückhaltebecken from 5.1 % AR at 0 DAT to 57.8 % AR at 14 DAT, before decreasing to 39.0 % AR at 119 DAT. The amount of radioactivity extractable from the sediment extracts increased for system Schaephysen from 11.8 % AR at 0 DAT to 72.4 % AR at 13 DAT, before decreasing to 47.4 % AR at 119 DAT.

The amount of non-extractable residues (NER) increased from 0.4 % AR at 0 DAT to 30.1 % AR at 89 DAT, before decreasing to 25.1 % AR at 119 DAT for system Rückhaltebecken. In system Schaephysen NER increased from 0 DAT to 119 DAT from 2.4 to 39.1 % AR.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (119 DAT) were 27.6 and 11.8 % AR or the Rückhaltebecken and Schaephysen aquatic sediment systems, respectively. Organic volatiles determined were ≤0.0 % AR for both test systemsat all sampling points.

E. TRANSFORMATION OF THE TEST ITEM

The amount of AMPA in the water decreased from from 0 DAT to 119 DAT from 92.4 to 1.4 % AR for system Rückhaltebecken and from 81.8 to 0.2 % AR for system Schaephysen.

The amount of AMPA in the sediment extract increased from 0 DAT to 14 DAT from 3.4 to 34.6 % AR before decreasing to 22.5 % AR at 119 DAT for system Rückhaltebecken. The amount of AMPA in the sediment extract of system Schaephysen increased from 0 DAT to 60 DAT from 5.1 to 25.4 % AR on before decreasing to 23.3 % AR at 119 DAT.

The amount of AMPA in the total system decreased from from 0 DAT to 119 DAT from 95.8 to 23.9 % AR for system Rückhaltebecken and from 86.9 to 23.4 % AR for system Schaephysen.

Up to three different degradation products of AMPA were detected in the water/sediment systems which were assigned to M2.5 (max. 7.0 % AR), M3.3 (max. 22.9 % AR) and M7 (max. 9.8 % AR). M3.3 was found mainly in the sediments, while the M7 occurred rather in the water phases. M3.3 could be characterised as 1-oxo-AMPA; M2.5 and M7 were not identified/characterised. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, the compounds are not considered relevant for further evaluation.

The non-classified radioactivity in water is equal to the non-classified radioactivity from the HPLC-analysis and does not exceed 1.4 % AR at any sampling interval for both test systems. The non-classified radioactivity in sediment is reported as the sum of the non-classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was removed from the NaOH and Soxhlet extracts by acidification prior to HPLC-analysis and reached a maximum of 20 % AR (13 DAT, system Schaephysen). As the HPLC method was able to separate compounds to <5 % AR as shown for the water samples, the majority of the non-classified radioactivity was associated to the humic acid fraction. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, no further attempts were made to identify this unclassified radioactivity.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted], 2020, CA 7.2.2.3/001.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing minor deviations. Sediments were filled into the test vessels to a height of 1.5 to 2.0 cm, being slightly below the actual requirement of 2.0 ± 0.5 cm. Since the water/sediment volume ratio was within the requirement of 3.1 and 4:1, variations regarding the height of the sediment layer are considered acceptable.

The duration of acclimation prior to application of the test item is not provided. The study duration of 119 days is slightly longer than the recommended duration of 100 days. The deviations are considered not to influence the overall outcome of the study.

The study is considered valid to evaluate the degradation of AMPA in water/sediment systems.

Assessment and conclusion by RMS:

The study is overall well performed.

The following minor deviations from OECD 308 are identified: length of the acclimation not reported, sediment layer is 1.5-2 cm instead of the recommended 2.5±0.5 cm (but water:sediment ratio is appropriate).

Study duration slightly exceeded that recommended 100 days, but microbial biomass was measured at start and end of the test and no significant decline was observed.

Storage time of water and sediment extracts until analysis is not reported. However, considering the available storage stability study [redacted] 1989, both glyphosate and AMPA are considered stable in water. Therefore, no impact is expected on the study results.

Three unidentified breakdown products exceeding 10% AR or 5% AR at 2 consecutive times were observed: fractions M2.5, M3.3 and M7.
For fractions M2.5 and M7, their maximal occurrence levels observed do not exceed 10% AR. It is stated that no further attempts were made to identify both breakdown products since the study was a metabolite study, not a parent glyphosate study. It would have been more suitable to characterize all the breakdown products exceeding 5 % AR. However, considering that metabolite AMPA is formed at a maximal level of 27% AR from parent in total water-sediment systems (based on studies with glyphosate applied), a rough estimation of the maximal occurrence levels for both fractions M2.5 and M7 would be below 5% of glyphosate. Therefore, there is no need to consider these fractions for risk assessment.
Fraction M3.3 was identified as 1-oxo-AMPA. Its maximal occurrence level reached 23%AR after 13 days in sediment of system Schaephysen. Moreover, the occurrence levels observed for this fraction are around 20% AR for three consecutive timepoints. Thus, considering that metabolite AMPA is formed at a maximal level of 27% AR from parent in total water-sediment systems, a rough estimation of the maximal occurrence levels for metabolite 1-oxo-metabolite would be above the trigger of 5% AR from parent for several timepoints. Therefore it should be considered in more details whether this metabolite 1-oxo-AMPA exceeds the trigger for further assessment. A data gap is identified for the applicant to further address this metabolite, quantitatively or qualitatively.

The metabolite identification issues have no impact on the reliability of the results to derive degradation rates of AMPA.

The study is considered acceptable.

[redacted], *1999*

Data point:	CA 7.2.2.3/021
Report author	[redacted]
Report year	1999

Report title	Aminomethylphosphonic acid: Water/Sediment Metabolism
Report No	MSL-19217
Guidelines followed in study	SETAC Guideline "Procedures of assessing the environmental fate and ecotoxicity of pesticides", part 1, 8.2
Deviations from current test guideline	From OECD 308: - Sediment history not given. - Sediment sampling from top 15 cm instead of top 5-10 cm. - CO_2-free air used. - Unidentified radioactivity > 5%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]aminomethylphosphonic acid (AMPA)
Lot No.: C-2266.4
Specific activity: 4.8 mCi/mmol (43.23 µCi/mg)
Radiochemical purity: ≥99 % (checked by HPLC and TLC during study conduct)

The study was conducted with a mixture of ^{13}C- and ^{14}C-labelled AMPA, diluted with analytical grade ^{12}C-AMPA.

2. Test System:

Sediment was sampled from 1 to 15 cm below the water/sediment surface. Sediments were sieved to ≤2 mm and water was filtered to ≤0.2 mm. Water and sediment were stored separately in the dark at 4 ± 2°C for approximately one week before acclimation of the test systems was started. Aerobic conditions of the aquatic test systems were maintained during the storage period. Characteristics of the test systems are presented in the table below.

Table 8.2.2.3-52: Characteristics of test systems

Parameter		Results	
Sediment		Bickenbach	Unter Widdersheim
Country		Germany	Germany
Textural Class		Sand	Silty-sandy loam
Sand (63 µm – 2 mm) (%)		99.3	38.5
Silt (2 µm – 63 µm) (%)		3.7	45.7
Clay (< 2 µm) (%)		0.6	17.5
pH		7.4	7.5
Organic carbon (%)		0.52	3.83
Organic matter (%)		0.90	6.60
Cation exchange capacity (mval/kg)		16.1	137
Maximum Water Holding Capacity (g/100 g)		17.0	69.5
Microbial biomass (mg C/100g)			
Within the course of the study		21	27
Study end (100 DAT)		10	11
Water			
pH	At sampling: After sampling: At experimental end:	8.1 8.3 7.9	8.4 8.2 7.6
Redox-potential (mV)	At sampling: After sampling: At experimental end:	452 564 495	409 602 450
Oxygen level (mg/L)	At sampling: After sampling:	9.7 -	9.0 -

	At experimental end:	10.1	7.7

DAT = days after treatment

B. STUDY DESIGN

1. Experimental conditions

The metabolism flasks were filled with a 2.5 cm thick sediment layer (approximately 250 g and 215 g water saturated sediment of systems Bickenbach or Unter Widdersheim, respectively) and the corresponding water at a water column height of about 6 cm, corresponding to approximately 300 mL of water. Flow-through systems, purged with moistened, CO_2 free air were used. To maintain aerobic conditions during the experiment, the oxygen concentration of water was above 20 % of its saturation. The test systems were connected to a security bottle, two gas washing bottles filled with 50 mL of 2 N NaOH (with saturation indication by cresol red) to absorb CO_2 from sediment respiration and $^{14}CO_2$ from the mineralisation of the test substance and two gas washing bottles filled with methoxy ethanol to collect volatile organic compounds. The sodium hydroxide trapping system was checked visually for CO_2 saturation (non-saturated: crimson/ saturated: yellow) on a weekly basis, in general. At no time did the indicator show CO_2 saturation.

Test systems were pre-incubated at 20 °C in the dark for 28 days until an equilibrium based on redox potential of water and sediment, oxygen concentration and pH-value of the water was reached.

AMPA was applied to the water surface at a rate of 470 µg/L corresponding to a rate of 1.42 kg/ha to represent a worst-case concentration based on the maximum field rate of 4.32 kg glyphosate acid/ha and a maximum formation from glyphosate of 50 %.

After application, test systems were incubated under aerobic conditions in the dark with gentle agitation of the water phase for 100 days at 20 ± 2°C.

2. Sampling

Duplicate samples from each system were processed and analysed at 0, 0.25, 1, 2, 7, 14, 30, 59 and 100 days after treatment (DAT). The volatile traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds. For analysis, the sediment and water from each metabolism flask were separated by decantation. Thereafter, water and sediment were analysed separately. Samples were prepared, extracted and analysed immediately after sampling.

3. Analytical procedures

Surface water was separated from the sediment by decantation and directly analysed by liquid scintillation counting (LSC).

Sediments were extracted with 1 M NH_3 up to 6 times (laboratory shaker: 350 rpm/min for 12 h maximum at room temperature). The ratio of the extraction solvent and sediment was 1:1 (volume:dry weight, corresponding to 200 mL 1M NH_3 for system Bickenbach and 130 mL 1M NH_3 for system Unter Widdersheim) maximum. Before the addition of fresh solvent the slurry was centrifuged (up to 4500 rpm/10 min) and the supernatant decanted. The sequential extractability of radioactivity was checked by analysis of each individual sediment extract using LSC.

Radioactive components in water and sediment extracts were analysed by two TLC/radiodetection systems with a limit of detection 0.3% AR. Recoveries for the analytical procedure were in the range from 94.2 to 103.7 % AR for both systems.

After sediment extraction, the remaining bound residues were assayed by combustion/LSC. In addition, extracted sediment of 100 DAT was further characterized for radioactivity bound to the humic and fulvic acids and the humin fraction.

Aliquots from the volatile traps were radioassayed at each sampling point (excluding zero-time) or approximately in 14-day intervals, whichever came first. The traps were assayed by adding aliquots of the trapping solutions directly into the liquid scintillation cocktail and counting by LSC. For the sodium hydroxide traps, the identification of $^{14}CO_2$ (trapping solution containing ≥2 % AR) was performed by precipitation of $Ba^{14}CO_3$, using a saturated aqueous solution of barium chloride.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 7.9 and 8.3 in system Bickenbach and between 7.6 and 8.2 for system Unter Widdersheim. The redox potential of the water at study end was 495 mV for system Bickenbach and 450 mV for system Unter Widdersheim. The redox potential of the sediment at study end was -175 mV in system Bickenbach and -233 mV for system Unter Widdersheim.

The results of analysis with two TLC solvent systems were found to be very similar at each sampling interval. Therefore, further discussion refers to average values of the two TLC solvent systems.

Table 8.2.2.3-53: Distribution of radioactivity in water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water total	A	101.8	94.7	86.1	73.3	54.7	39.2	27.1	13.2	8.4
	B	100.5	93.8	80.5	78.5	57.6	40.8	34.1	14.6	12.0
	Mean	101.2	94.3	83.3	75.9	56.2	40.0	30.6	13.9	10.2
Sediment extractables	A	1.5	8.3	15.9	24.5	39.7	46.1	46.8	51.5	30.4
	B	2.5	8.4	19.8	21.1	39.4	50.0	46.8	50.1	29.4
	Mean	2.0	8.4	17.9	22.8	39.6	48.1	46.8	50.8	29.9
Non-extractable residues	A	0.3	1.1	3.0	4.7	7.4	10.4	20.6	18.9	21.8
	B	0.2	1.6	4.0	3.8	6.6	11.6	16.8	17.9	16.3
	Mean	0.3	1.4	3.5	4.3	7.0	11.0	18.7	18.4	19.1
Sediment total	Mean	2.3	9.8	21.4	27.1	46.6	59.1	65.5	69.2	49.0
Carbon Dioxide	A	n.p.	<0.1	<0.1	0.1	1.5	8.2	12.9	20.8	36.9
	B	n.p.	<0.1	<0.1	0.2	1.4	4.5	7.9	20.4	39.1
	Mean	n.p.	<0.1	<0.1	0.2	1.5	6.4	10.4	20.6	38.0
Other Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
Mass balance	Mean	103.5	104.1	104.7	103.2	104.3	105.5	106.5	103.7	97.4

DAT: days after treatment
n.p.: not performed

Table 8.2.2.3-54: Distribution of radioactivity in water/sediment system Unter Widdersheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water total	A	100.7	86.4	57.5	66.7	39.0	30.1	2.3	3.1	3.5
	B	100.1	83.5	49.7	65.1	27.2	8.6	4.3	6.5	2.3
	Mean	100.4	85.0	53.6	65.9	33.1	19.4	3.3	4.8	2.9
Sediment extractables	A	1.3	14.4	40.2	30.9	51.3	54.6	57.9	46.1	38.2
	B	1.5	16.5	49.2	29.9	59.7	74.3	51.4	41.0	45.9
	Mean	1.4	15.5	44.7	30.4	55.5	64.5	54.7	43.6	42.1
Non-extractable residues	A	0.2	2.6	5.5	6.5	10.2	16.4	24.8	23.0	23.8
	B	0.5	3.3	6.0	7.0	12.5	13.8	22.6	24.7	25.9
	Mean	0.4	3.0	5.8	6.8	11.4	15.1	23.7	23.9	24.9
Sediment total	Mean	1.8	18.5	50.5	37.2	66.9	79.6	78.4	67.5	67.0
Carbon Dioxide	A	n.p.	<0.1	<0.1	0.3	2.7	4.1	15.4	23.4	32.6
	B	n.p.	<0.1	<0.1	0.2	4.6	7.8	20.9	27.3	25.5
	Mean	n.p.	<0.1	<0.1	0.3	3.7	6.0	18.2	25.4	29.1
Other Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mass balance	Mean	102.2	103.5	104.1	103.4	103.7	105.0	99.9	97.7	99.0

DAT: days after treatment
n.p.: not performed

Table 8.2.2.3-55: Degradation of [^{14}C]AMPA in water of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	101.8	90.5	86.1	68.4	54.7	32.0	18.5	9.1	6.1
	B	100.5	89.1	80.5	72.9	57.6	35.5	26.9	10.6	3.6
	Mean	101.2	89.8	83.3	70.7	56.2	33.8	22.7	9.9	4.9
About zero	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	n.d.	0.3
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7
	Mean	-	-	-	-	-	0.2	-	-	0.5
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5	n.d.	n.d.
	Mean	-	-	-	-	-	-	1.8	-	-
About 0.9	A	n.d.	4.2	n.d.	5.0	n.d.	6.8	6.6	4.1	2.0
	B	n.d.	4.8	n.d.	5.7	n.d.	5.3	5.8	4.0	7.7
	Mean	-	4.5	-	5.4	-	6.1	6.2	4.1	4.9
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	101.8	92.7	83.0	67.9	50.8	31.2	16.9	5.8	3.6
	B	100.5	91.3	78.2	74.3	46.3	34.4	25.0	8.1	3.0
	Mean	101.2	92.0	80.6	71.1	48.6	32.8	21.0	7.0	3.3
About zero	A	n.d.	2.0	3.2	5.4	2.2	3.6	4.3	2.1	2.4
	B	n.d.	2.6	2.3	4.2	4.4	4.4	4.6	2.4	1.7
	Mean	-	2.3	2.8	4.8	3.3	4.0	4.5	2.3	2.1
About 0.2	A	n.d.	n.d.	n.d.	n.d.	1.7	4.4	5.9	5.3	2.5
	B	n.d.	n.d.	n.d.	n.d.	6.9	1.9	4.5	4.1	7.3
	Mean	-	-	-	-	4.3	3.2	5.2	4.7	4.9

DAT: days after treatment
n.d.: not detectable (calculated detection limit of 0.3 % AR)

Table 8.2.2.3-56: Degradation of [^{14}C]AMPA in sediment extracts of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	1.5	8.3	15.9	21.2	30.6	33.2	34.3	42.0	21.3
	B	2.5	8.4	19.8	17.6	30.9	38.2	35.7	42.0	20.7
	Mean	2.0	8.4	17.9	19.4	30.8	35.7	35.0	42.0	21.0
About zero	A	n.d.	n.d.	n.d.	n.d.	2.3	2.0	3.8	n.d.	1.7
	B	n.d.	n.d.	n.d.	0.7	1.8	1.6	3.4	n.d.	1.9
	Mean	-	-	-	0.4	2.1	1.8	3.6	-	1.8
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.8	n.d.	1.9
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.2	n.d.	0.9
	Mean	-	-	-	-	-	-	2.5	-	1.4
About 0.9	A	n.d.	n.d.	n.d.	3.3	6.8	10.9	6.1	9.6	5.5
	B	n.d.	n.d.	n.d.	2.9	6.7	10.4	5.7	8.1	6.0
	Mean	-	-	-	3.1	6.8	10.7	5.9	8.9	5.8
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	n.a.	8.3	15.4	23.4	31.8	36.5	32.3	39.9	23.0
	B	n.a.	8.3	19.0	18.7	32.0	39.6	35.2	39.2	16.6
	Mean	-	8.3	17.2	21.1	31.9	38.1	33.8	39.6	19.8
About zero	A	n.a.	n.d.	0.5	1.2	4.2	6.2	6.2	6.8	7.4
	B	n.a.	0.1[1]	0.8	2.4	3.7	6.8	8.3	6.9	5.9
	Mean	-	0.1	0.7	1.8	4.0	6.5	7.3	6.9	6.7
About 0.2	A	n.a.	n.d.	n.d.	n.d.	3.7	3.5	3.8	4.8	n.d.
	B	n.a.	n.d.	n.d.	n.d.	3.8	3.6	3.3	4.1	6.9
	Mean	-	-	-	-	3.8	3.6	3.6	4.5	3.5

DAT: days after treatment
n.d.: not detectable (calculated detection limit of 0.3 % AR)

n.a.: not analysed (below 5 % AR in the extract to be analysed)
[1] reduced detection limit of 0.1 % AR

Table 8.2.2.3-57: Degradation of [^{14}C]AMPA in the total system of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	103.3	98.8	102.0	89.6	85.3	65.2	52.8	51.1	27.4
	B	103.0	97.5	100.3	90.5	88.5	73.7	62.6	52.6	24.3
	Mean	103.2	98.2	101.2	90.1	86.9	69.5	57.7	51.9	25.9
About zero	A	n.d.	n.d.	n.d.	n.d.	2.3	2.4	3.8	n.d.	2.0
	B	n.d.	n.d.	n.d.	0.7	1.8	1.6	3.4	n.d.	2.6
	Mean	-	-	-	0.4	2.1	2.0	3.6	-	2.3
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.8	n.d.	1.9
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.7	n.d.	0.9
	Mean	-	-	-	-	-	-	4.3	-	1.4
About 0.9	A	n.d.	4.2	n.d.	8.3	6.8	17.7	12.7	13.7	7.5
	B	n.d.	4.8	n.d.	8.6	6.7	15.7	11.5	12.1	13.7
	Mean	-	4.5	-	8.5	6.8	16.7	12.1	12.9	10.6
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	101.8	101.0	98.4	91.3	82.6	67.7	49.2	45.7	26.6
	B	100.5	99.6	97.2	93.0	78.3	74.0	60.2	47.3	19.6
	Mean	101.2	100.3	97.8	92.2	80.5	70.9	54.7	46.5	23.1
About zero	A	n.d.	2.0	3.7	6.6	6.4	9.8	10.5	8.9	9.8
	B	n.d.	2.7	3.1	6.6	8.1	11.2	12.9	9.3	7.6
	Mean	-	2.4	3.4	6.6	7.3	10.5	11.7	9.1	8.7
About 0.2	A	n.d.	n.d.	n.d.	n.d.	5.4	7.9	9.7	10.1	2.5
	B	n.d.	n.d.	n.d.	n.d.	10.7	5.5	7.8	8.2	14..2
	Mean	-	-	-	-	8.1	6.7	8.8	9.2	8.4

DAT: days after treatment
n.d.: not detectable (calculated detection limit of 0.3 % AR)
n.a.: not analysed (below 5 % AR in the extract to be analysed)
[1] reduced detection limit of 0.1 % AR

Table 8.2.2.3-58: Amounts of [^{14}C]AMPA in water, sediment extracts and total system of test system Bickenbach (mean of both TLC systems, expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water	A	*101.8*	*91.6*	*84.6*	*68.2*	*52.8*	*31.6*	*17.7*	*7.5*	*4.9*
	B	*100.5*	*90.2*	*79.4*	*73.6*	*52.0*	*35.0*	*26.0*	*9.4*	*3.3*
	Mean	*101.2*	*90.9*	*82.0*	*70.9*	*52.4*	*33.3*	*21.9*	*8.5*	*4.1*
Sediment	A	*1.5*	*8.3*	*15.7*	*22.3*	*31.2*	*34.9*	*33.3*	*41.0*	*22.2*
	B	*2.5*	*8.4*	*19.4*	*18.2*	*31.5*	*38.9*	*35.5*	*40.6*	*18.7*
	Mean	*2.0*	*8.4*	*17.6*	*20.3*	*31.4*	*36.9*	*34.4*	*40.8*	*20.5*
Total system	A	*102.6*	*99.9*	*100.2*	*90.5*	*84.0*	*66.5*	*51.0*	*48.4*	*27.0*
	B	*101.8*	*98.6*	*98.8*	*91.8*	*83.4*	*73.9*	*61.4*	*50.0*	*22.0*
	Mean	*102.2*	*99.25*	*99.5*	*91.15*	*83.7*	*70.2*	*56.2*	*49.2*	*24.5*

DAT: days after treatment
Values calculated during dossier preparation are given in *italics*

Table 8.2.2.3-59: Degradation of [^{14}C]AMPA in water of test system Unter Widdersheim quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	100.7	81.6	53.8	58.4	33.0	20.5	n.a.	n.a.	n.a.
	B	100.1	79.1	49.7	58.8	27.2	6.1	n.a.	2.6	n.a.
	Mean	100.4	80.4	51.8	58.6	30.1	13.3	-	1.3	-
About zero	A	n.d.	n.d.	3.7	1.0	n.d.	n.d.	n.a.	n.a.	n.a.

	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.a.
	Mean	-	-	1.9	0.5	-	-	-	-	-
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.a.	n.d.	n.a.
	Mean	-	-	-	-	-	0.2	-	-	-
About 0.9	A	n.d.	4.8	n.d.	7.3	6.1	9.6	n.a.	n.a.	n.a.
	B	n.d.	4.5	n.d.	6.3	n.d.	2.2	n.a.	3.9	n.a.
	Mean	-	4.7	-	6.8	3.1	5.9	-	2.0	-
Rf-value “SS2”										
AMPA (Parent) about 0.3	A	100.7	86.4	52.9	58.5	35.1	20.2	n.a.	n.a.	n.a.
	B	100.1	83.5	47.0	57.5	24.4	6.0	n.a.	2.0	n.a.
	Mean	100.4	85.0	50.0	58.0	29.8	13.1	-	1.0	-
About zero	A	n.d.	n.d.	2.2	8.3	2.5	4.6	n.a.	n.a.	n.a.
	B	n.d.	n.d.	2.7	7.7	1.7	1.2	n.a.	1.8	n.a.
	Mean	-	-	2.5	8.0	2.1	2.9	-	0.9	-
About 0.2	A	n.d.	n.d.	2.5	n.d.	1.4	5.3	n.a.	n.a.	n.a.
	B	n.d.	n.d.	n.d.	n.d.	1.1	1.4	n.a.	2.6	n.a.
	Mean	-	-	1.3	-	1.3	3.4	-	1.3	-

DAT: days after treatment
n.d.: not detectable (calculated detection limit of 0.3 % AR)
n.a.: not analysed (below 5 % AR in the extract to be analysed)

Table 8.2.2.3-60: Degradation of [^{14}C]AMPA in sediment extracts of test system Unter Widdersheim quantified by two different TLC systems “SSl” and “SS2” (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value “SS1”										
AMPA (Parent) about 0.8	A	1.3	14.4	40.2	24.1	38.5	39.1	40.0	33.6	28.3
	B	1.5	16.5	49.2	24.8	46.4	53.5	33.5	30.7	35.8
	Mean	1.4	15.5	44.7	24.5	42.5	46.3	36.8	32.2	32.1
About zero	A	n.d.	n.d.	n.d.	1.5	1.8	2.5	7.5	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	1.9	3.5	7.6	n.d.	n.d.
	Mean	-	-	-	0.8	1.9	3.0	7.6	-	-
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
About 0.8	A	n.d.	n.d.	n.d.	n.d.	5.1	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	6.5	n.d.	n.d.	n.d.	n.d.
	Mean	-	-	-	-	5.8	-	-	-	-
About 0.9	A	n.d.	n.d.	n.d.	5.3	5.9	13.0	10.5	12.5	10.0
	B	n.d.	n.d.	n.d.	5.1	5.0	17.3	10.4	10.4	10.1
	Mean	-	-	-	5.2	5.5	15.2	10.5	11.5	10.1
Rf-value “SS2”										
AMPA (Parent) about 0.3	A	n.a.	14.0	38.8	30.2	40.0	37.2	38.2	34.2	24.9
	B	n.a.	15.9	47.0	29.1	44.5	54.7	33.6	28.7	34.1
	Mean	-	15.0	42.9	29.7	42.3	46.0	35.9	31.5	29.5
About zero	A	n.a.	0.4	1.4	0.7	6.1	9.0	13.4	6.7	8.7
	B	n.a.	0.6	2.2	0.9	6.3	7.7	11.4	5.9	8.4
	Mean	-	0.5	1.8	0.8	6.2	8.4	12.4	6.3	8.6
About 0.2	A	n.a.	n.d.	n.d.	n.d.	5.3	8.5	6.3	5.3	4.7
	B	n.a.	n.d.	n.d.	n.d.	9.0	12.0	6.4	6.5	3.4
	Mean	-	-	-	-	7.2	10.3	6.4	5.9	4.1

DAT: days after treatment
n.d.: not detectable (calculated detection limit of 0.3 % AR)
n.a.: not analysed (below 5 % AR in the extract to be analysed)
[1] reduced detection limit of 0.1 % AR

Table 8.2.2.3-61: Degradation of [^{14}C]AMPA in the total system of test system Unter Widdersheim quantified by two different TLC systems “SSl” and “SS2” (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100

Rf-value "SS1"										
AMPA (Parent) about 0.8	A	102.0	96.0	94.0	82.5	71.5	59.6	40.0	33.6	28.3
	B	101.6	95.6	98.9	83.6	73.6	59.6	33.5	33.3	35.8
	Mean	101.8	95.8	96.5	83.1	72.6	59.6	36.8	33.5	32.1
About zero	A	n.d.	n.d.	3.7	2.5	1.8	2.5	7.5	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	1.9	3.5	7.6	n.d.	n.d.
	Mean	-	-	1.9	1.3	1.9	3.0	7.6	-	-
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
	Mean	-	-	-	-	-	0.2	-	-	-
About 0.8	A	n.d.	n.d.	n.d.	n.d.	5.1	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	6.5	n.d.	n.d.	n.d.	n.d.
	Mean	-	-	-	-	5.8	-	-	-	-
About 0.9	A	n.d.	4.8	n.d.	12.6	12.0	22.6	10.5	12.5	10.0
	B	n.d.	4.5	n.d.	11.4	5.0	19.5	10.4	14.3	10.1
	Mean	-	4.7	-	12.0	8.5	21.1	10.5	13.4	10.1
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	100.7	100.4	91.7	88.7	75.1	57.4	38.2	34.2	24.9
	B	100.1	99.4	94.0	86.6	68.9	60.7	33.6	30.7	34.1
	Mean	100.4	99.9	92.9	87.7	72.0	59.1	35.9	32.5	29.5
About zero	A	n.d.	0.4	3.6	9.0	8.6	13.6	13.4	6.7	8.7
	B	n.d.	0.6	4.9	8.6	8.0	8.9	11.4	7.7	8.4
	Mean	-	0.5	4.3	8.8	8.3	11.3	12.4	7.2	8.6
About 0.2	A	n.d.	n.d.	2.5	n.d.	6.7	13.8	6.3	5.3	4.7
	B	n.d.	n.d.	n.d.	n.d.	10.1	13.4	6.4	9.1	3.4
	Mean	-	-	-	-	8.4	13.6	6.4	7.2	4.1

DAT: days after treatment
n.d.: not detectable (calculated detection limit of 0.3 % AR)

Table 8.2.2.3-62: Amounts of [^{14}C]AMPA in water, sediment extracts and total system of test system Unter Widderheim (mean of both TLC systems, expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water	A	*100.7*	*84.0*	*53.4*	*58.5*	*34.1*	*20.4*	*n.a.*	*n.a.*	*1.2*
	B	*100.1*	*81.3*	*48.4*	*58.2*	*25.8*	*6.1*	*n.a.*	*2.3*	*n.a.*
	Mean	*100.4*	*82.65*	*50.9*	*58.35*	*29.95*	*13.25*	*n.a.*	*2.3*	*1.2*
Sediment	A	*1.3*	*14.2*	*39.5*	*27.2*	*39.3*	*38.2*	*39.1*	*33.9*	*26.6*
	B	*1.5*	*16.2*	*48.1*	*27.0*	*45.5*	*54.1*	*33.6*	*29.7*	*35.0*
	Mean	*1.4*	*15.2*	*43.8*	*27.1*	*42.4*	*46.2*	*36.4*	*31.8*	*30.8*
Total system	A	*101.4*	*98.2*	*92.9*	*85.6*	*73.3*	*58.5*	*39.1*	*33.9*	*27.8*
	B	*100.9*	*97.5*	*96.5*	*85.1*	*71.3*	*60.2*	*33.6*	*32.0*	*35.0*
	Mean	*101.2*	*97.9*	*94.7*	*85.35*	*72.3*	*59.4*	*36.4*	*33.0*	*31.4*

DAT: days after treatment
n.a.: not analysed (below 5 % AR in the extract to be analysed)
Values calculated during dossier preparation are given in *italics*

Table 8.2.2.3-63:Fractionation of day 100 post extracted sediment (in % AR)

	Bickenbach Rep 1	Bickenbach Rep 2	Unter Widdersheim Rep 1	Unter Widdersheim Rep 2
Combustion (residual radioactivity)	21.8	16.3	23.8	25.9
Pool [1]	8.5	6.9	7.7	8.5
fulvic acids	7.1	5.8	4.3	5.3
humic acids	0.7	0.5	3.0	2.5
∑ fulvic acids and humic acids	7.8	6.3	7.3	7.8
Humin [2]	13.3	9.4	16.1	17.4

∑ fulvic acids, humic acids and humin in % of residual radioactivity [3]	96.8	96.3	98.3	97.3

[1] Previous measurement of summed % AR of fulvic acids and humic acids (pool)
[2] Calculated: % AR combustion - % AR pool
[3] ((fulvic acids + humic acids + humin [in % AR])/residual radioactivity [% AR]) x 100%

B. MASS BALANCE

The mean recoveries of applied radioactivity (AR) were 103.7 % (97.4 to 106.5 % AR) for the Bickenbach system and 102.1 % (97.7 to 105 % AR) for the Unter Widdersheim system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

In water at 20°C, the level of applied radioactivity declined very rapidly from 101.2 % at 0 DAT, 40.0 % at 14 DAT and to 10.2 % at 100 DAT in the Bickenbach water and from 100.4 % to 19.4 % and to 2.9 % in Unter Widdersheim water at the same time points.

This decline was associated with an increasing concentration in sediment extracts to 49.0 % AR (Bickenbach) and to 67.0 % AR (Unter Widdersheim) by 100 DAT.

Non-extractable sediment residues represented 19.1 % (Bickenbach) and 24.9 % (Unter Widdersheim) of applied radioactivity at 100 DAT. When the non extractable radioactivity at 100 DAT was further fractionated into humin, humic and fulvic acid fractions, the residual radioactivity was mainly associated with the humin fraction, accounting for, 13.3 % AR (Bickenbach) and 17.4 % AR (Unter Widdersheim). Radioactivity associated with the fulvic acid fraction amounted to 7.1 % AR (Bickenbach) and 5.3 % AR (Unter Widdersheim), and with the humic acid fraction it amounted to 0.7 % AR (Bickenbach) and 3.0 % AR (Unter Widdersheim).

D. VOLATILE RADIOACTIVITY

Significant mineralisation was observed with volatile radioactivity (identified as CO_2) representing 38.0 % AR (Bickenbach) and 29.1 % AR (Unter Widdersheim) at 100 DAT. Organic volatiles determined were ≤0.3 % AR for both systems at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The two TLC systems (SS1 and SS2) separated the water samples and sediment extracts into AMPA and either three (SS1) or two (SS2) radioactive metabolite zones. The AMPA results for the two TLC systems were in good agreement, indicating that AMPA was well separated in both systems. There was not a simple correlation between the radioactivity in the metabolite zones in the two systems, suggesting that there were at least four compounds present, some of which co-eluted.

The amounts of AMPA in the water (mean of both TLC systems) decreased from 0 DAT to 100 DAT from 101.2 to 4.1 % AR in system Bickenbach and from 100.4 to 1.2 % AR in system Unter Widdersheim.

The amounts of AMPA in the sediment of system Bickenbach (mean of both TLC systems) increased from 2.0% AR at 0 DAT to 40.8 % AR at 59 DAT and decreased to 20.5 % AR at 100 DAT. The amounts of AMPA in the sediment of system Unter Widdersheim (mean of both TLC systems) increased from 1.4% AR at 0 DAT to 46.2 % AR at 14 DAT and decreased to 30.8 % AR at 100 DAT.

The amounts of AMPA in the total system (mean of both TLC systems) decreased from 0 DAT to 100 DAT from 102.2 to 24.5 % AR in system Bickenbach and from 101.2 to 31.4 % AR in system Unter Widdersheim.

For both test systems, the unidentified radioactivity in the water phase remained below 10 % AR at all time points for both TLC systems. For the sediment extracts, the TLC system with the best separation ("SS2") showed radioactive zones containing up to 12 % AR. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, no attempts were made to identify these breakdown products.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted], 2020, CA 7.2.2.3/001.

III. CONCLUSIONS

The major route of dissipation of AMPA is through partitioning to the sediment. Once in the sediment, AMPA degrades through degradation to metabolites, mineralisation, and formation of non-extractable residues.

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing minor deviations. Samples were collected from top 15 cm instead of recommended 5-10 cm. Furthermore, the air used for purging the systems was CO_2 free. The deviations are considered to not influence the overall outcome of the study.

The study is considered valid to evaluate the degradation of AMPA in water/sediment systems.

Assessment and conclusion by RMS:

The following deviations to OECD 308 are identified.

Sediment history is not reported, and sediment was sampled from the top 15 cm instead of 5-10 cm. These deviations are not expected to impact the reliability of the results.

According to OECD 308, CO2-free air should not be used as this can result in increases in the pH of the water. For both systems, RMS considers that there is no unusual increase of pH; then this deviation should not significantly impact the results of the study.

A number of unidentified breakdown products exceeding 10% AR or 5% AR at 2 consecutive times were observed. It is stated that no further attempts were made to identify the breakdown products since the study was a metabolite study, not a parent glyphosate study. It would have been more suitable to characterize all the breakdown products exceeding 5 % AR. In this case, considering that metabolite AMPA is formed at a maximal level of 27% AR from parent in total water-sediment systems (based on studies with glyphosate applied), a rough estimation of the maximal occurrence level of these fractions indicate that they are not expected to be formed above 10% or above 5% of glyphosate at 2 consecutive timepoints. Therefore, there is no need to consider these fractions for risk assessment.

The study is considered acceptable.

Frozen storage stability

Table 8.2.2.3-64: studies on frozen storage stability

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Acceptability in RAR (2021)
CA 7.2.2.3/022	[redacted], 1989	Not mentionned in RAR (2015) but reported as additional data in DAR (2001)	Acceptable

[redacted], 1989

Data point:	CA 7.2.2.3/022
Report author	[redacted]
Report year	1989
Report title	Storage Stability of Glyphosate in Environmental Water
Report No	MSL-8626
Document No	R.D. No. 1005
Guidelines followed in study	None

Deviations from current test guideline	No guideline available
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, not mentionned in RAR (2015) but reported as additional data in DAR (1998)
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test item 1:

Identification: Glyphosate (batch no and purity not reported)

Test item 2:

Identification: Aminomethylphosphonic acid (AMPA, batch no and purity not reported)

2. Water:

Water samples were collected from a lake in the Busch Wildlife Area, St. Charles County, Missouri, USA. Samples were filtered through glass wool or filter paper and kept in refrigerated storage until fortification.

B. STUDY DESIGN

1. Experimental conditions

Water samples in Nalgene plastic bottles were fortified with both glyphosate and AMPA at 0.5 ppm and kept in frozen storage at < -18 °C until analysis.

2. Sampling

Duplicate samples were removed from frozen storage after 0, 186, 313, 368, 551, and 734 days (0, 6, 10, 12, 18, and 24 months). Stability and control samples were also analysed after 96 days (3 months) of frozen storage but as no fortified samples were measured, no results were reported.

3. Analytical procedures

To each sample 5 mL of concentrated hydrochloric acid were added and the solvent was evaporated to dryness under reduced pressure. The remainder is reconstituted in 2.9 mL of 5 mM KH_2PO_4 in 4 % methanol/deionized water at pH 2.1. 0.1 mL of 0.03 M disodium EDTA solution were added and the sample was filtered through a 0.45 µm filter. Samples were separated by HPLC using a cation-exchange column. Fluorescence detection was performed after a post-column reaction. Therefore, a calcium hypochlorite solution was introduced into the stream to oxidize glyphosate to a primary amine prior to fluorogenic derivatisation with o-phthaladehyde (OPA). OPA also reacts with AMPA and the two derivatised compounds were quantitated via a fluorometer at 455 nm after excitation at 340 nm.

II. RESULTS AND DISCUSSION

Recoveries for the fortified samples ranged from 81.5 % to 100.9 % for glyphosate and from 73.3 % to 96.1 % for AMPA. The average glyphosate residues, corrected for recoveries in fortified control samples, ranged from 95.8 to 110.7 %. Average AMPA residues, corrected for recovery in fortified control samples, ranged from 96.2 to 108.8 %. Detailed values can be found in the table below.

Table 8.2.2.3-65: Storage stability of glyphosate and AMPA at <-18 °C (mean values of two replicates)

Compound	Corrected recovery (%)					
	Days in storage					
	0	186	313	368	551	734
Glyphosate	*97.1*	101.1	95.8	110.5	101.9	110.7

AMPA	*99.2*	106.1	96.2	110.2	108.1	108.8

Values calculated during dossier preparation are given in *italics*

III. CONCLUSIONS

The data indicate that both glyphosate and AMPA residues are stable in environmental water after two years in frozen storage. At all analysis points, average glyphosate and AMPA residues were greater than 95 % of their original levels.

Assessment and conclusion by applicant:

The study confirmed glyphosate and AMPA to be stable in natural water for a period of 24 months.

The study is considered as supportive information.

Assessment and conclusion by RMS:

The study indicates that both glyphosate and AMPA can be considered as stable in water after frozen storage.

The study is considered acceptable.

Kinetic assessment

The results of water/sediment studies were evaluated according to the current FOCUS kinetic guidance ([redacted], 2020, CA 7.2.2.3/001).

Table 8.2.2.3-66: kinetic assessment

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR (2021)
CA 7.2.2.3/001	[redacted], 2020	-	Acceptable

[redacted], 2020

Data point:	CA 7.2.2.3/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolites AMPA and HMPA from laboratory water-sediment studies
Report No	112148-002
Guidelines followed in study	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
GLP/Officially recognised testing facilities	Not relevant
Previous evaluation	Not previously submitted
Acceptability/Reliability:	Yes

Please note that for easier reading, RMS comments on the kinetic evaluation provided by the applicant are reported system by system in the study summary.

I. MATERIAL AND METHODS

Data pre-processing

Datasets were prepared for the kinetic analysis at different evaluation levels. According to the FOCUS kinetic guidance (2006, 2014), the kinetic analyses for water-sediment study were conducted at Level P-I and Level P-II for the parent and Level M-I for the metabolites. At Level P-I, the kinetic analyses were conducted using the dataset in a single compartment to determine the degradation $DegT_{50}$ in the total system and dissipation DT_{50} in water and sediment. At Level P-II, kinetic analyses were conducted as a two-compartmental approach to estimate degradation in the water column and sediment compartments. At Level M-I, pathway and decline fits were conducted to determine the degradation $DegT_{50}$ in total system and dissipation DT_{50} in total system, water and sediment, where applicable.

The standard procedures recommended by FOCUS (2006, 2014) were followed for all residues to adjust the experimental data for the kinetic modelling. Replicate samples were available for all studies except [redacted] (2004, CA 7.2.2.3/018).

The initial amount of the test substance in total system and water was set to the value of the material balance at day 0. Accordingly, the initial amount was set to zero for the test substance in sediment for evaluation at Level P-II and for the metabolites AMPA and HMPA for evaluation at Level M-I degradation. The assessment of dissipation in sediment at Level P-I and Level M-I dissipation (total system, water and sediment) only requires kinetics to be fitted to the corresponding decline data, starting from the maximum observed concentration in the compartment. The dissipation of the respective compound was thus evaluated starting at the day of maximum occurrence that was defined as 0 days after maximum concentration. All later time points were adjusted accordingly as days after maximum concentrations.

It is recommended that values below the LOD should be replaced by half the LOD (FOCUS; 2006, 2014). Where LOD values were not available, the values were set to half the lowest measured value.

Kinetic models

Four kinetic degradation models were considered to describe the degradation behaviour of the compounds in the water-sediment systems: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model), double-first-order-in-parallel (DFOP) and Hockey-stick (HS) (FOCUS; 2006, 2014). At Level P-I and M-I dissipation, all of the four models were considered, where applicable, based on the recommended procedure to derive endpoints in FOCUS (2014) and the number of available data points. At Level P-II, the SFO model was applied for both water and sediment compartments. At Level M-I degradation, the best-fit model derived from Level P-I was applied for parent, and the SFO model was used for the metabolites.

The best-fit model was accepted for deriving trigger endpoints, while the DT_{50} calculated from SFO model was preferably selected as modelling endpoint.

Optimisation

The kinetic analyses were conducted using the software KinGUI v2.1. The data were directly fitted with the complete dataset and unconstrained initial concentration (M_0) for glyphosate and AMPA (when applied as test substance). Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in KinGUI. Optimisations were carried out for the initial residue (M_0), degradation model parameters k, α, β, g or t_b, depending on the respective kinetic model. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. By default, the initial amount of the parent substance in sediment and the metabolite in total system were fixed to 0 at Level P-II and Level M-I degradation, respectively. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused

on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line.

A statistical measure of the quality of a fit is given by the χ^2-test which considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, it is recommended that if the χ^2 error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. Depending on the complexity of the curve fitting for multiple components and the scattering of the experimental data, also fits with higher χ^2 error values may be acceptable if overall the measured data are well described by the fitted curve.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised degradation rate constants (k) of the SFO, DFOP and HS kinetic models were significantly different from zero at a chosen significance level of 10 % for water-sediment kinetics. For the FOMC kinetic model, only the confidence interval of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The KinGUI software also reports a 95 % confidence interval on the estimated parameters. It should be relatively tight and not contain 0 to be considered statistically robust.

At level P-II, no further evaluation was conducted if the visual fits are poor or the degradation rates of the water or sediment phase failed the t-test.

II. RESULTS AND DISCUSSION

[redacted] *(1999, CA 7.2.2.3/002)*

The sediment residue values were not reported in the study report. Therefore, the sediment values were obtained by subtracting the water phase residues values from the total system residues values.

At Level P-I for glyphosate, no evaluation could be conducted for the sediment phase for system Putah due to the limited number of data points available after the peak concentration. For the same reason, no evaluation could be conducted at Level M-I dissipation for AMPA in sediment in system Cache as well as in water and sediment in system Putah.

Table 8.2.2.3-67: Experimental data for system Cache of study [redacted] (1999, CA 7.2.2.3/002) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% AR)		
	Total system	Water	Sediment[1]	Total system	Water	Sediment[2]
0	99.0[3]	99.0[3]	0.00[4]	0.00[5]	0.24	0.00
0	101.7[3]	101.7[3]	0.00[4]	0.00[5]	0.66	0.00
0.25	94.09	87.38	6.71	0.91	0.33	0.58
0.25	94.65	87.17	7.48	1.08	0.46	0.62
1	84.94	74.26	10.68	2.02	1.30	0.72
1	85.92	71.54	14.38	2.28	1.30	0.98

2	79.04	66.24	12.80	3.47	1.62	1.85
2	82.44	67.13	15.31	4.03	2.17	1.86
3	76.88	59.90	16.98	5.18	2.19	2.99
3	76.04	61.27	14.77	5.12	2.45	2.67
7	53.61	39.51	14.10	12.32	5.24	7.08
7	54.18	39.82	14.36	11.56	5.30	6.26
14	33.25	21.98	11.27	17.93	8.07	9.86
14	34.57	22.22	12.35	18.92	8.93	9.99
30	16.79	7.34	9.45	26.97	10.52	16.45
30	18.60	8.30	10.30	27.18	10.10	17.08
58	4.56	1.53	3.03	27.26	8.07	19.19
58	5.37	1.61	3.76	26.28	8.08	18.20
100	4.85	0.79	4.06	20.71	3.69	17.02
100	4.17	0.87	3.30	22.89	3.97	18.92

[1] Since the sediment phase residues were not reported in the study report, they were obtained by subtracting the water phase residues values from the total system residues values
[2] No evaluation was conducted at Level M-I dissipation since no decline was observed in the sediment phase
[3] Values at day 0 were set to material balance according to FOCUS (2014)
[4] Set to zero for evaluation at Level P-II
[5] Set to zero for evaluation at Level M-I degradation

Table 8.2.2.3-68: Experimental data for system Putah of study [redacted] (1999) used for kinetic evaluation

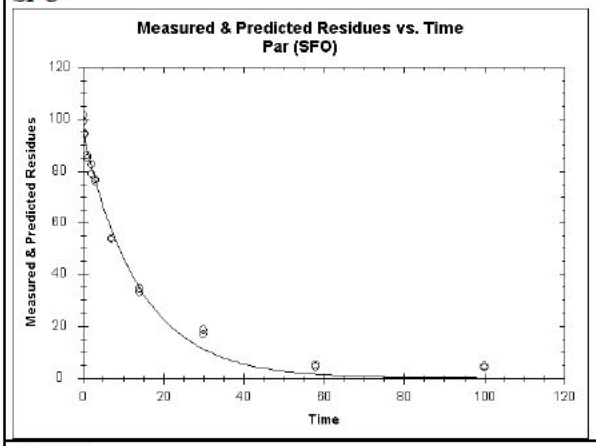
Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% AR)		
	Total system	Water	Sediment[1]	Total system[2]	Water[2]	Sediment[2]
0	103.9[3]	103.9[3]	0.0[4]	0.0[5]	0.41	0.00
0	101.6[3]	101.6[3]	0.0[4]	0.0[5]	0.37	0.00
0.25	96.11	90.68	5.43	0.80	0.80	0.00
0.25	97.67	91.77	5.90	0.69	0.69	0.00
1	86.13	74.05	12.08	1.71	0.96	0.75
1	79.04	65.68	13.36	1.79	0.89	0.90
2	89.22	76.63	12.59	0.81	0.81	0.00
2	89.17	75.39	13.78	1.39	0.90	0.49
3	82.40	63.52	18.88	1.53	0.64	0.89
3	83.45	63.28	20.17	1.84	0.86	0.98
7	81.42	60.24	21.18	2.01	1.10	0.91
7	81.43	60.74	20.69	1.43	0.82	0.61
14	70.31	34.02	36.29	2.68	1.08	1.60
14	67.03	32.47	34.56	2.15	1.11	1.04
30	70.82	18.64	52.18	5.33	1.32	4.01
30	79.88	22.11	57.77	3.02	0.58	2.44
58	65.73	11.45	54.28	6.15	1.78	4.37
58	69.19	9.04	60.15	4.37	1.12	3.25
100	61.77	5.26	56.51	3.67	0.54	3.13
100	64.90	4.97	59.93	3.46	0.50	2.96

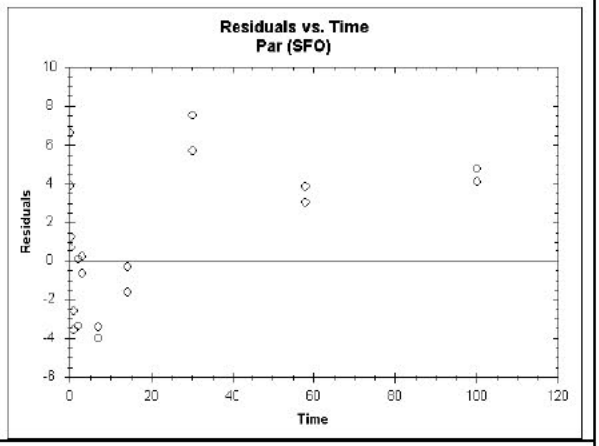
[1] Since no decline was observed in the sediment phase, no evaluation could be conducted at Level P-I for the sediment phase
[2] No evaluation was conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration
[3] Values at day 0 were set to material balance according to FOCUS (2014)
[4] Set to zero for evaluation at Level P-II
[5] Set to zero for evaluation at Level M-I degradation

Table 8.2.2.3-69: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

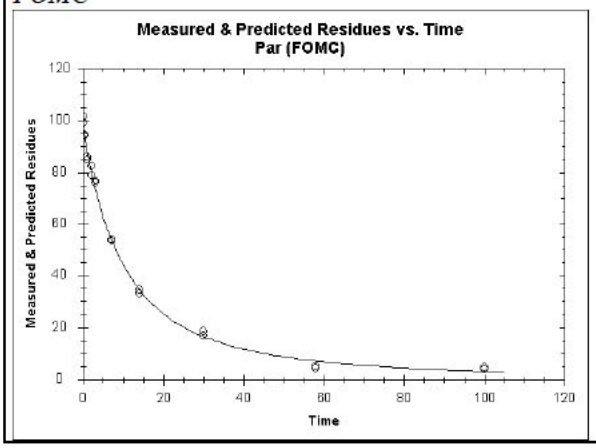
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	95.1	k: 0.0716	5.3	k: <0.001	k: 0.0634	k: 0.0800	9.7	32.1
FOMC	Good	97.8	α: 1.8669 β: 18.7550	2.7	-[1]	β: 11.2434	β: 26.2670	8.4	45.6
DFOP	Good	97.5	k_1: 0.1386 k_2: 0.0298 g: 0.5982	3.1	k_1: <0.001 k_2: <0.001	k_1: 0.0845 k_2: 0.0148	k_1: 0.1930 k_2: 0.0450	8.4	47.0
HS	Good	97.0	k_1: 0.0853 k_2: 0.0394 tb: 11	3.2	k_1: <0.001 k_2: <0.001	k_1: 0.0762 k_2: 0.0301	k_1: 0.0940 k_2: 0.0490	8.1	45.6
Conclusion from notifier	Although the visual and statistical fits of the SFO model are acceptable, the degradation of glyphosate is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually good results but the least χ^2 error is provided by the FOMC model. FOMC to be used for trigger endpoints and for further evaluation at Level M-I degradation. SFO to be used for modelling endpoints								
Conclusion from RMS	RMS agrees with notifier conclusion.								

SFO





FOMC



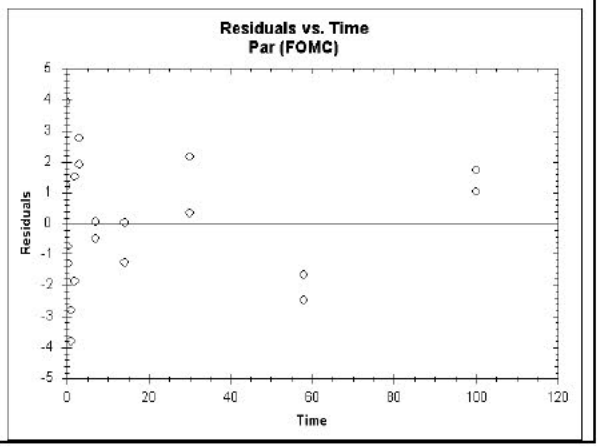
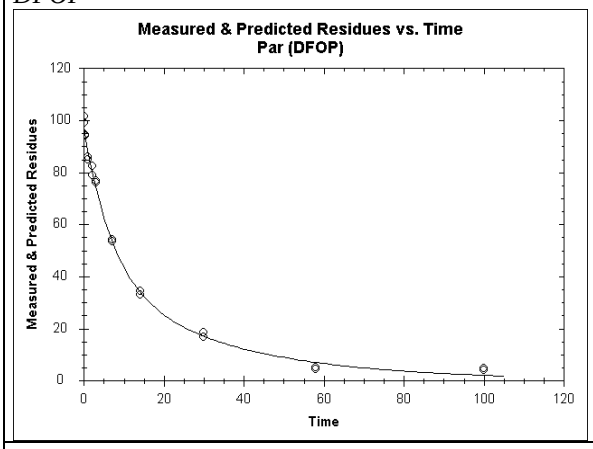
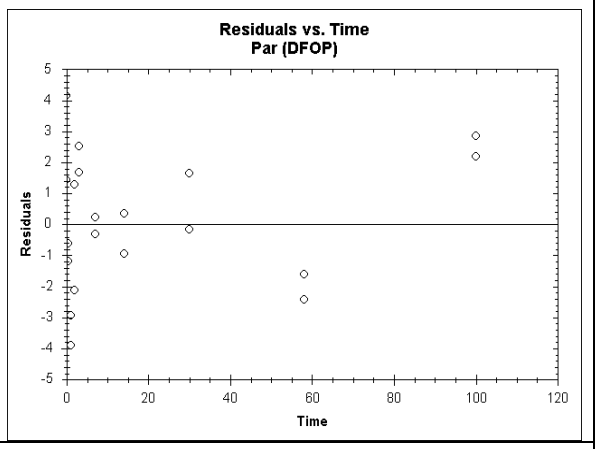


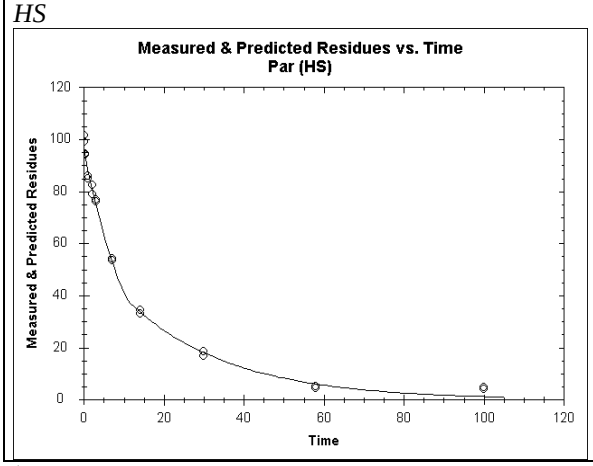
Table 8.2.2.3-69: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

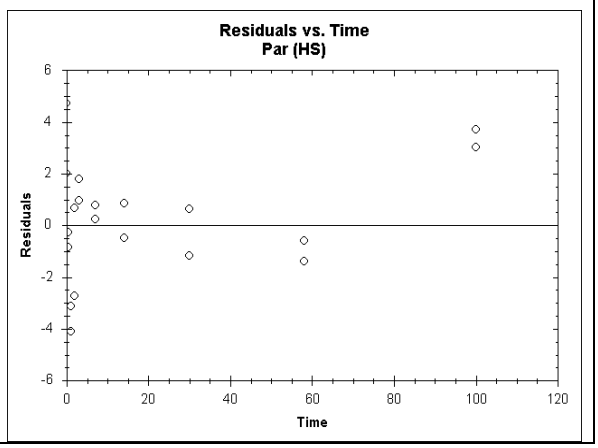
DFOP





HS





[1] t-test not relevant for kinetic parameter β

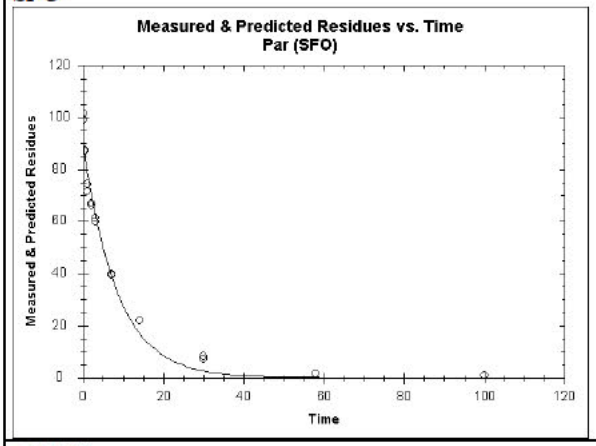
Table 8.2.2.3-70: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

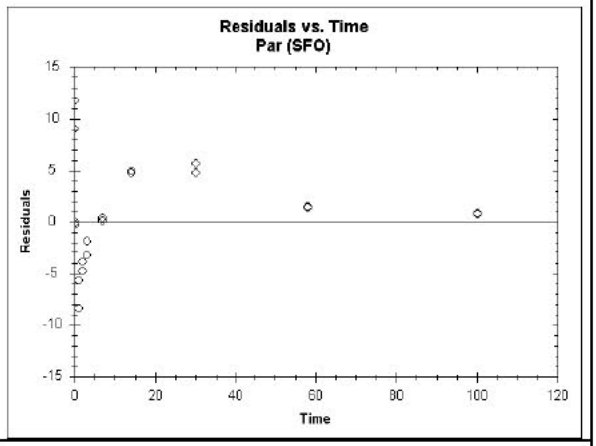
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-70: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

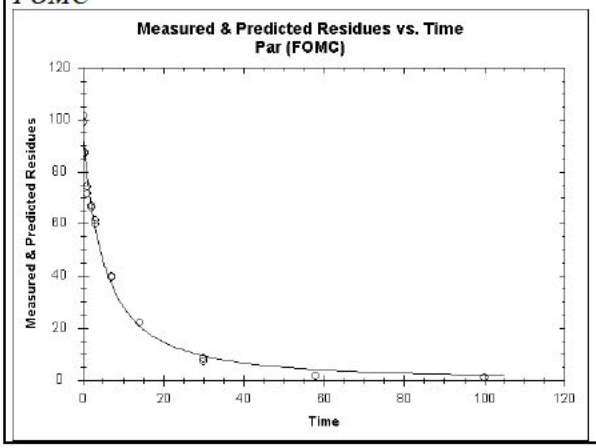
SFO	Acceptable	90.0	k: 0.1181	8.5	k: <0.001	k: 0.0989	k: 0.1370	5.9	19.5
FOMC	Acceptable	94.2	α: 1.4851 β: 7.9603	6.0	-[1]	β: 3.2128	β: 12.7080	4.7	29.6
DFOP	Good	100.4	k_1: 3.0100 k_2: 0.0908 g: 0.2141	2.3	k_1: <0.001. k_2: <0.001	k_1: 1.9370 k_2: 0.0845	k_1: 4.0830 k_2: 0.0970	5.0	22.7
HS	Good	94.0	k_1: 0.1682 k_2: 0.0752 tb: 3.7	6.4	k_1: <0.001 k_2: <0.001.	k_1: 0.1381 k_2: 0.0498	k_1: 0.1980 k_2: 0.1010	4.6	26.0
Conclusion from notifier	Although the visual and statistical fits of the SFO model are acceptable, the dissipation of glyphosate in the water phase is best described by bi-phasic models. The FOMC model provides acceptable fits while the DFOP and HS models provide equally reliable and visually good results. However, the least χ^2 error is provided by the DFOP model. DFOP to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



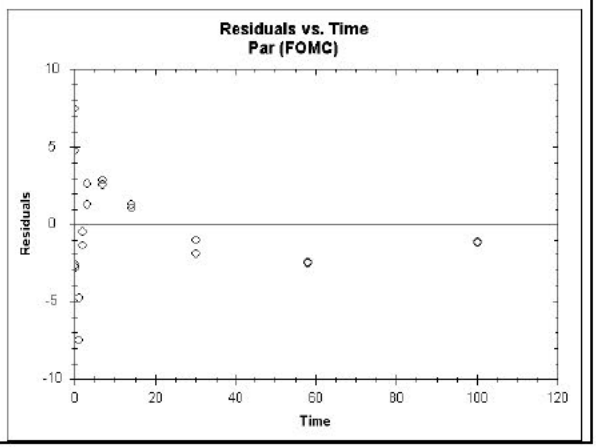
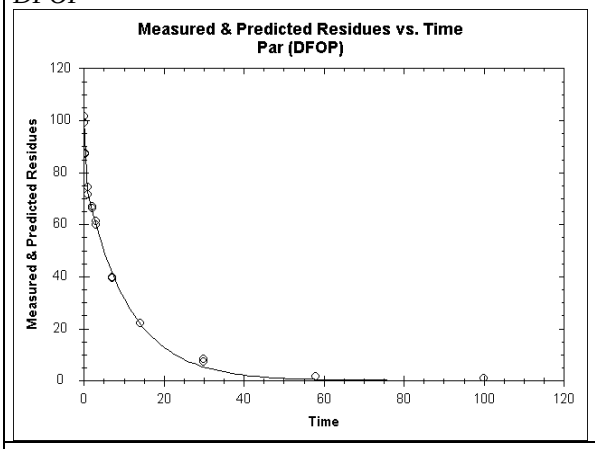
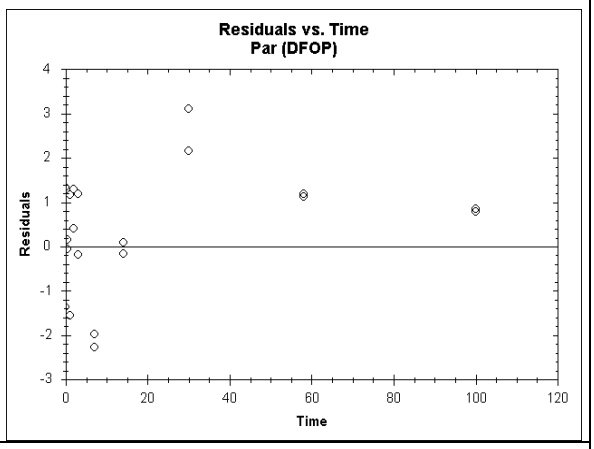


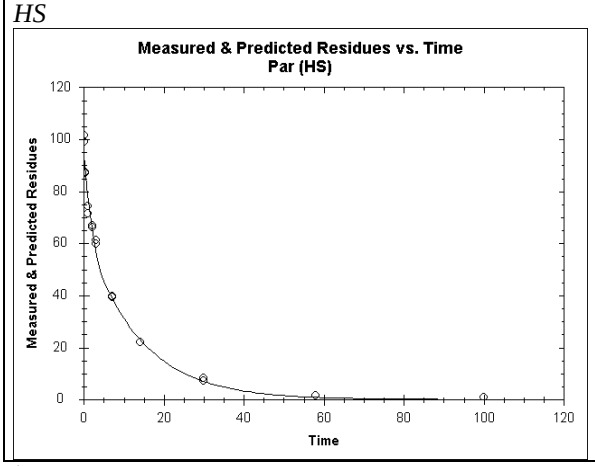
Table 8.2.2.3-70: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

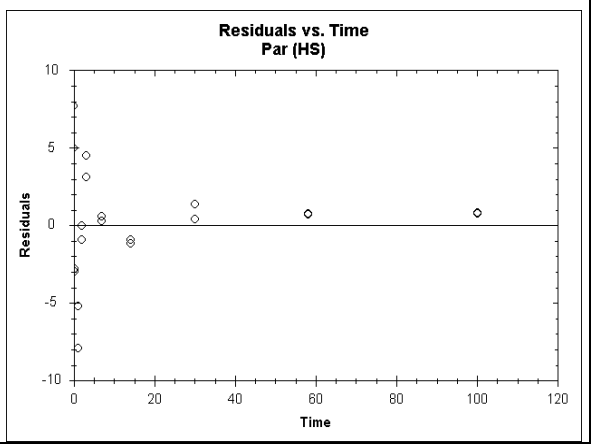
DFOP





HS





[1] t-test not relevant for kinetic parameter β

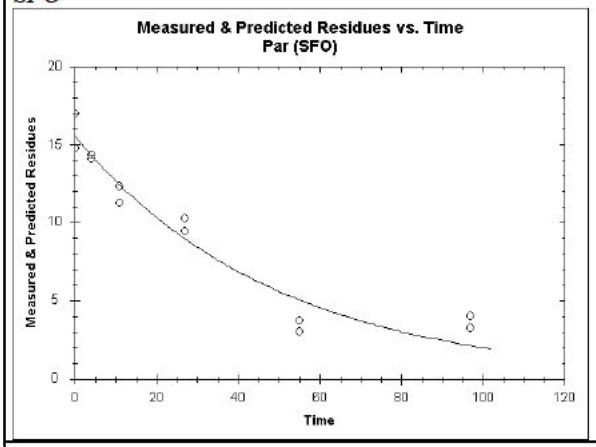
Table 8.2.2.3-71: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, sediment phase

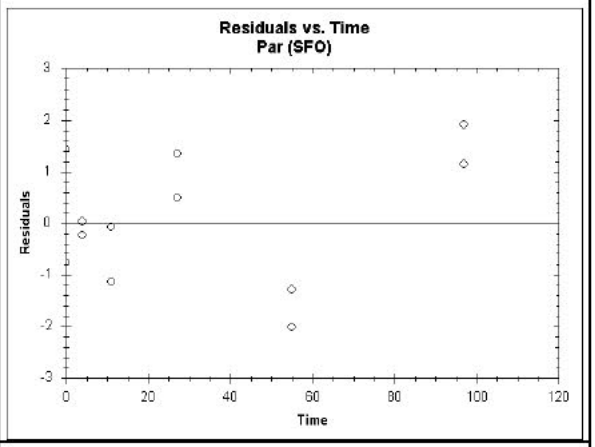
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-71: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, sediment phase

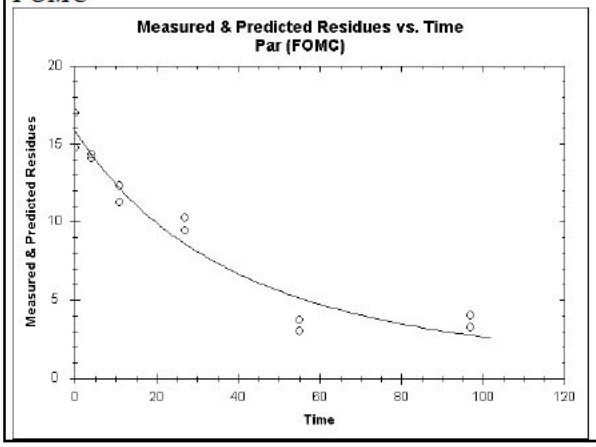
SFO	Acceptable	15.5	k: 0.0204	8.4	k: <0.001	k: 0.0156	k: 0.0250	33.9	112.6
FOMC	Acceptable	15.8	α: 2.6471 β: 103.695	8.7	-[1]	β: -191.77	β: 399.16	31.0	143.8
DFOP	Acceptable	15.9	k_1: 0.0287 k_2: 2.34×10^{-14} g: 0.8631	9.4	k_1: 0.220 k_2: >0.500	k_1: -0.0406 k_2: -0.1295	k_1: 0.0980 k_2: 0.1300	30.2	>1000
HS	Acceptable	15.9	k_1: 0.0467 k_2: 0.0197 tb: 1.6	10.4	k_1: 0.21 k_2: <0.001	k_1: -0.0609 k_2: 0.0138	k_1: 0.1540 k_2: 0.0260	33.0	114.6
Conclusion from notifier	Dissipation of glyphosate in sediment is best described by the SFO model. Thus, the SFO model is selected as the best-fit model as well as for deriving modelling endpoints. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in sediment (please refer to RMS comments).								

SFO





FOMC



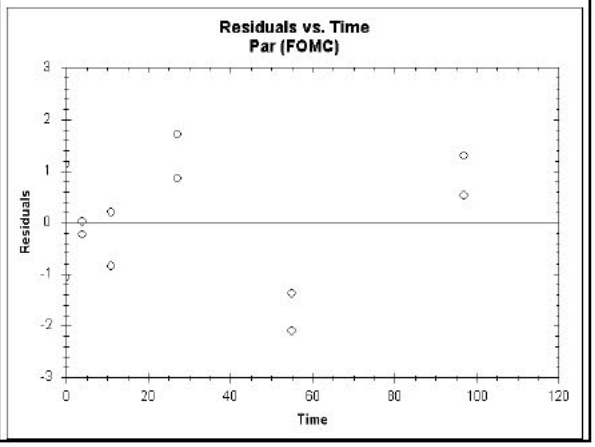
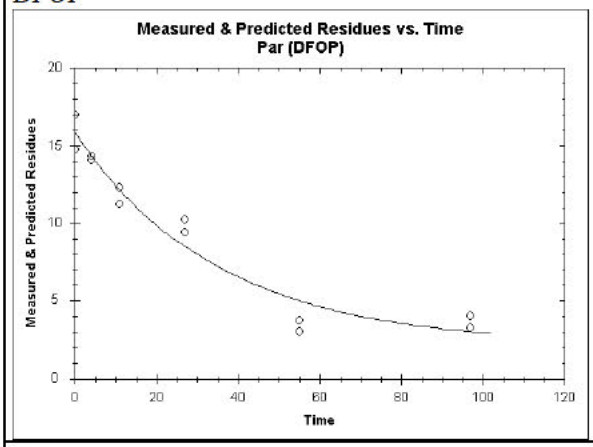
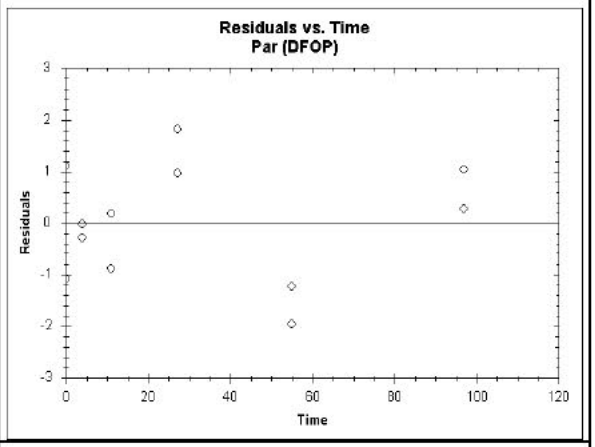


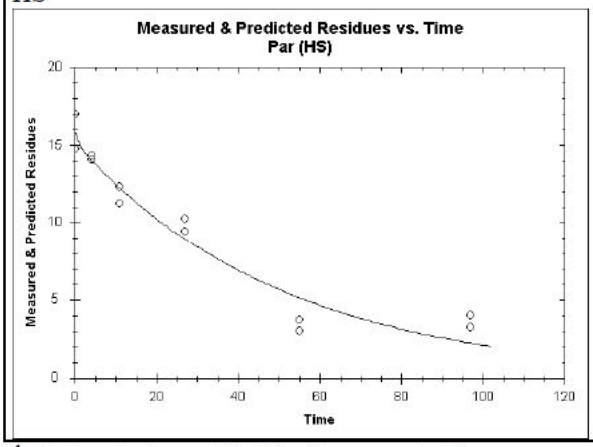
Table 8.2.2.3-71: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, sediment phase

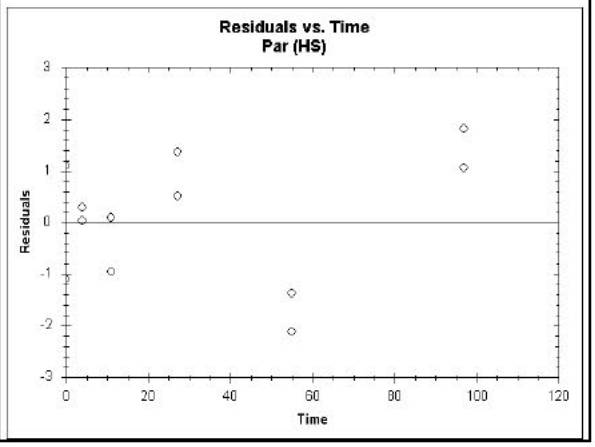
DFOP





HS





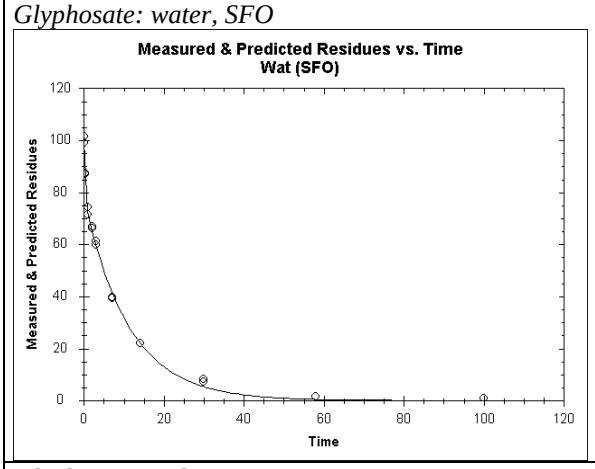
[1] t-test not relevant for kinetic parameter β

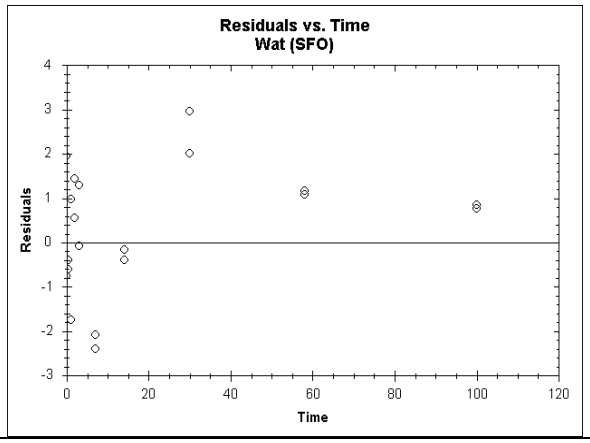
Table 8.2.2.3-72: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Good	99.7	k_{wat}: 0.1129 $k_{wat\ sed}$: 0.5175	2.3	k_{wat}: 0.0442 $k_{wat\ sed}$: <0.001	k_{wat}: -0.0133 $k_{wat\ sed}$: 0.3302	k_{wat}: 0.239 k_{wat_sed}: 0.705	6.1	20.4
Sediment: SFO	Poor	0.0	k_{sed}: 2.34×10^{-14} $k_{sed\ wat}$: 2.082	34.6	k_{sed}: 0.5 $k_{sed\ wat}$: <0.001	k_{sed}: -0.5082 $k_{sed\ wat}$: -1.177	k_{sed}: 0.508 k_{sed_wat}: 2.987	>1000	>1000
Conclusion from notifier	The visual and statistical fits obtained for the water phase are reliable but the visual fit obtained for the sediment phase is poor. No further evaluation was conducted. No reliable endpoints could be derived.								
Conclusion from RMS	Agrees with notifier conclusion.								

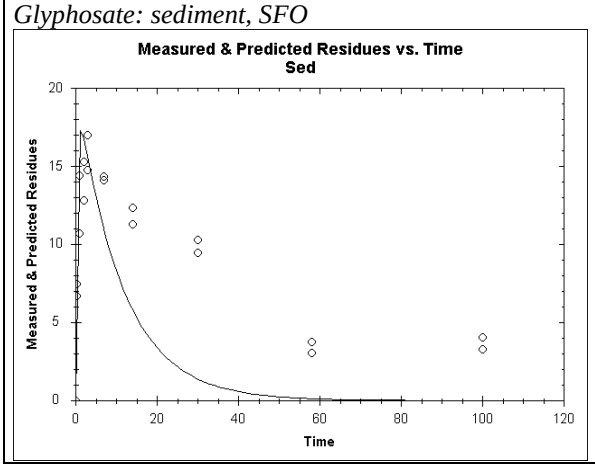
Table 8.2.2.3-72: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

Glyphosate: water, SFO





Glyphosate: sediment, SFO



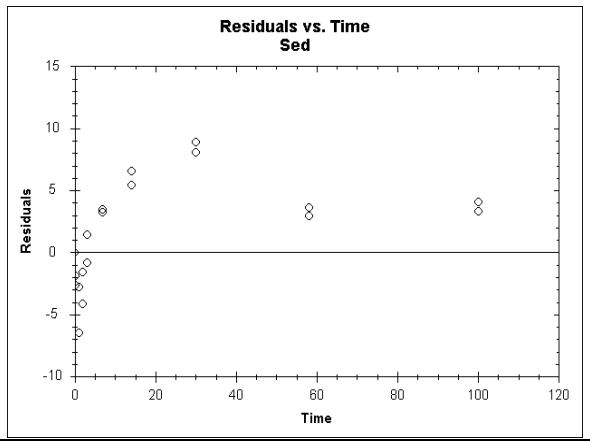


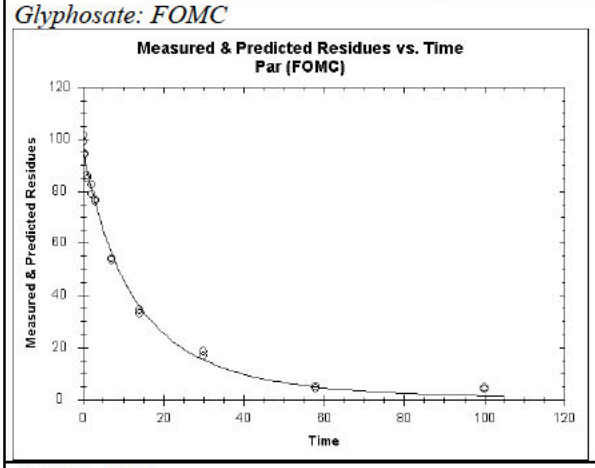
Table 8.2.2.3-73: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I degradation

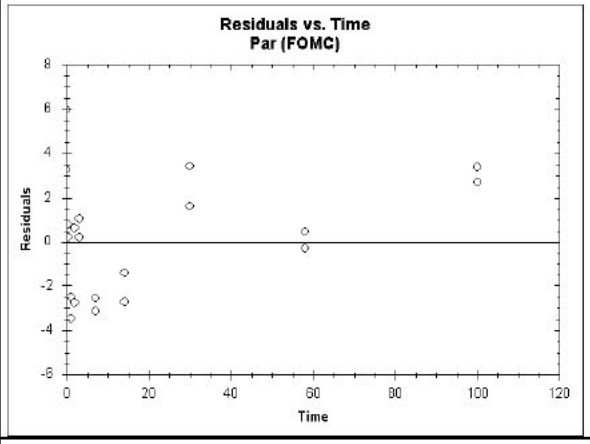
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)

Table 8.2.2.3-73: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I degradation

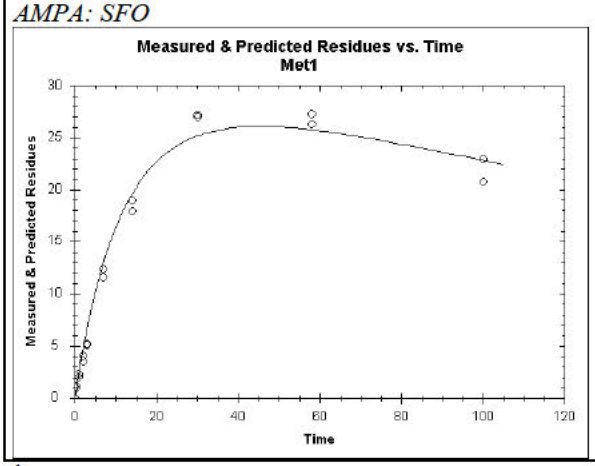
Glyphosate: FOMC	Good	95.7	α: 3.544 β: 44.02	3.7	-[1]	β: 13.510	β: 74.518	9.5	40.3	-
AMPA: SFO	Acceptable	0.0	k: 0.004	7.0	k: <0.001	k: 0.0026	k: 0.0050	172.8	573.9	0.339 (±0.014)
Conclusion from notifier	The fit of glyphosate at Level M-I degradation is comparable to that at Level P-I total system degradation. For AMPA, both the visual fit and the statistical parameters from the SFO model are acceptable. FOMC-SFO to be used for trigger endpoints for AMPA FOMC-SFO to be used for modelling endpoints for AMPA									
Conclusion from RMS	Agrees with notifier conclusion. The notifier approach is in line with FOCUS kinetic guidance (2014), figure 10-9, which recommends that parent best-fit is used to determine modelling endpoint for metabolite at level M-I degradation.									

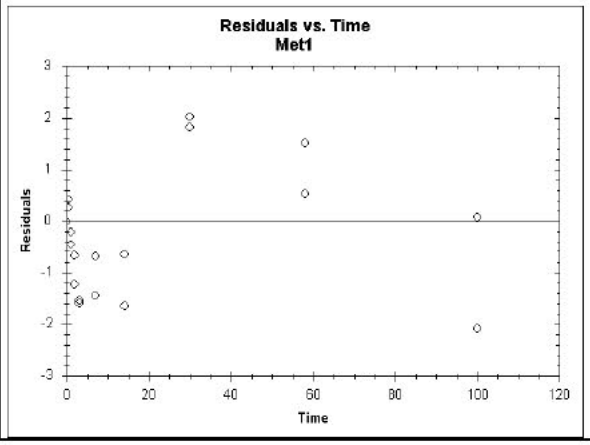
Glyphosate: FOMC





AMPA: SFO





[1] t-test not relevant for kinetic parameter β

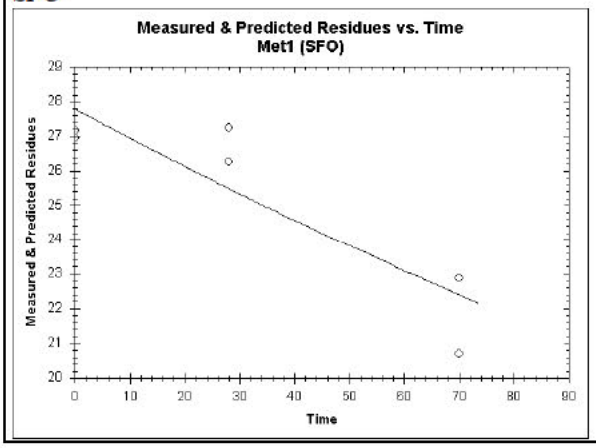
Table 8.2.2.3-74: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I dissipation, total system

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-74: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I dissipation, total system

SFO	Acceptable	27.8	k: 0.0031	3.2	k: 0.010	k: 0.0015	k: 0.0050	224.6	746.2
Conclusion from notifier	Only the SFO model was used for evaluation due to the limited number of data points. The visual and statistical fit from the SFO model is acceptable. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	The number of data points is too restricted (only 3 points available) to determine a DT_{50} from the maximal occurrence for metabolite AMPA. Since reliable endpoints can be derived for AMPA at level M-I degradation, no further kinetic assessment at level M-I dissipation is required for AMPA. .								

SFO



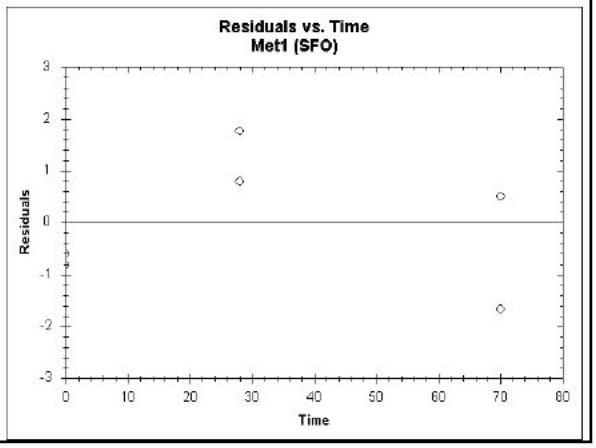


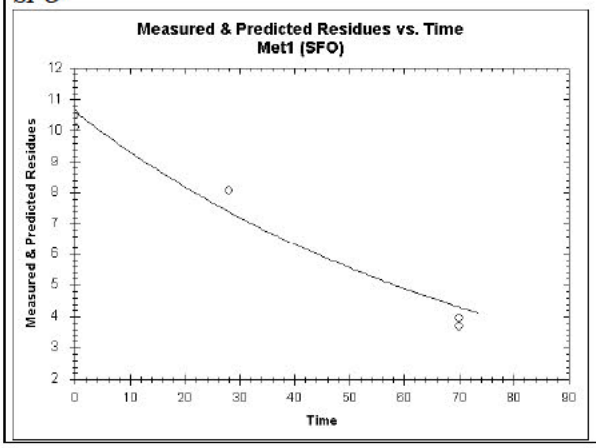
Table 8.2.2.3-75: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I dissipation, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-75: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I dissipation, water phase

SFO	Acceptable	10.6	k: 0.0129	6.1	k: <0.001	k: 0.0099	k: 0.0160	53.8	178.8
Conclusion from notifier	Only the SFO model was used for evaluation due to the limited number of data points. The visual and statistical fits from the SFO model are acceptable. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	The number of data points is too restricted (only 3 points available) to determine a DT_{50} from the maximal occurrence for metabolite AMPA. Since reliable endpoints can be derived for AMPA at level M-I degradation, no further kinetic assessment at level M-I dissipation is required for AMPA.								

SFO



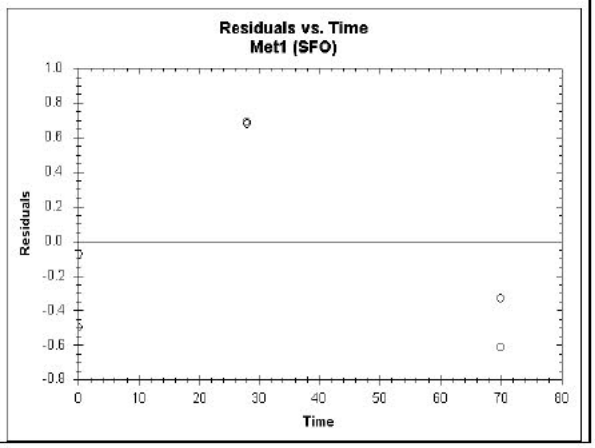
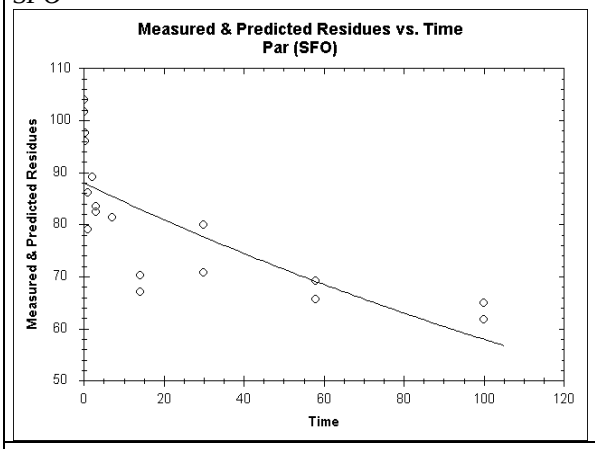


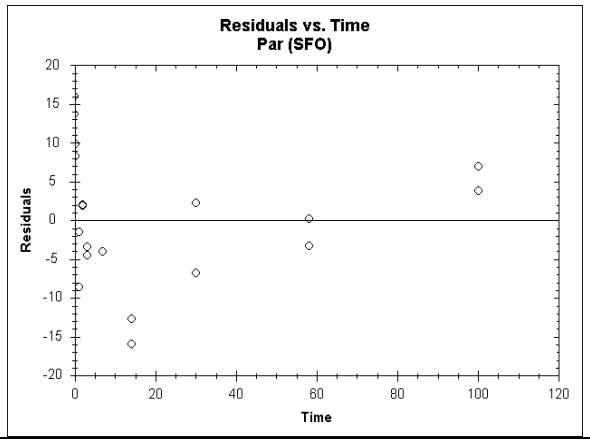
Table 8.2.2.3-76: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.9	k: 0.0042	7.7	k: <0.001	k: 0.0024	k: 0.0060	166.0	551.5
FOMC	Acceptable	102.8	α: 0.0686 β: 0.1287	3.7	-[1]	β: -0.0829	β: 0.3400	>1000	>1000
DFOP	Acceptable	100.4	k_1: 0.6409 k_2: 0.0023 g: 0.2189	4.4	k_1: 0.016 k_2: 0.003	k_1: 0.1071 k_2: 0.0009	k_1: 1.1750 k_2: 0.0040	195.8	902.3
HS	Acceptable	98.0	k_1: 0.0623 k_2: 0.0021 tb: 3.9	4.9	k_1: 0.001 k_2: 0.006	k_1: 0.0275 k_2: 0.0007	k_1: 0.0970 k_2: 0.0040	215.3	966.2
Conclusion from notifier	Degradation of glyphosate is best described by bi-phasic models. The statistical fit of the FOMC model is not reliable as the confidence interval of parameter β includes zero. Both DFOP and HS models provide equally reliable and visually acceptable results but the DFOP model provides a smaller χ^2 error and is selected as the best-fit model as well as for deriving modelling endpoints. DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion. It is additionally noted that according to FOCUS recommendations, FOMC is not a reliable option in this case since more than 10% AR is still remaining as glyphosate at the end of the study.								

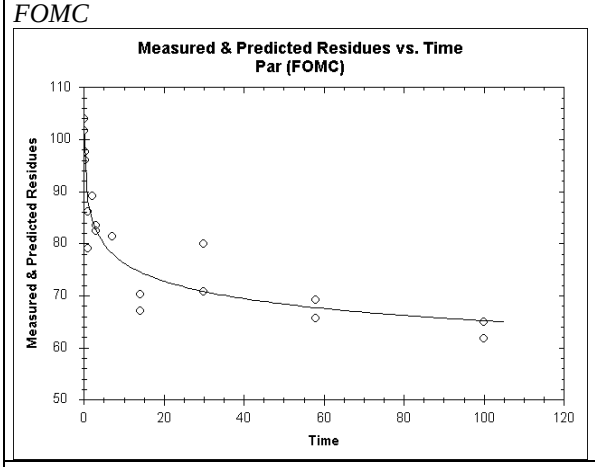
Table 8.2.2.3-76: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

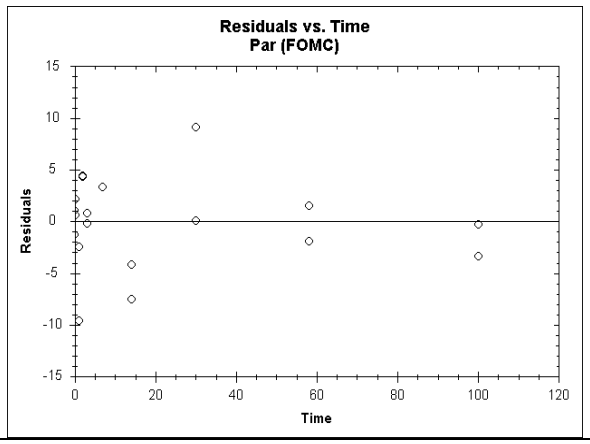
SFO



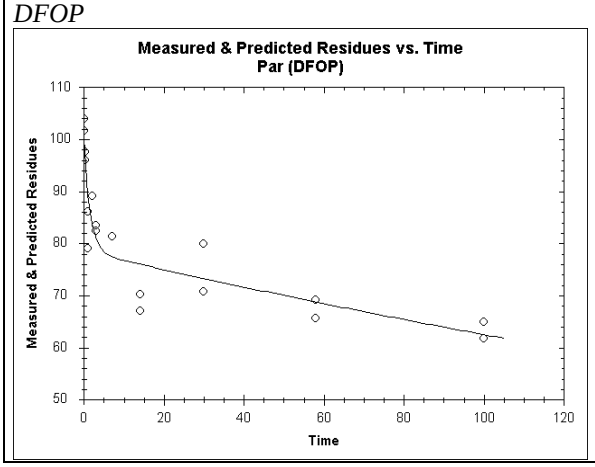


FOMC





DFOP



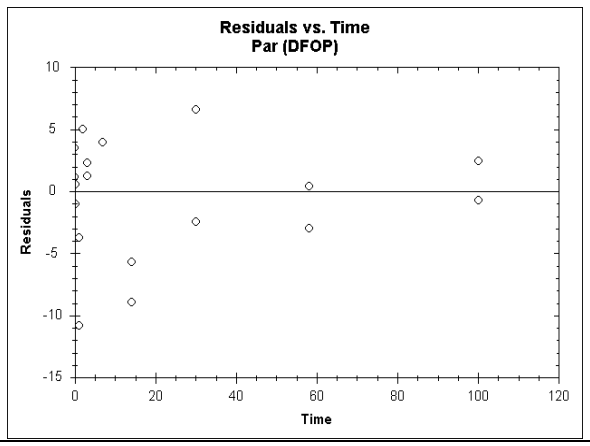
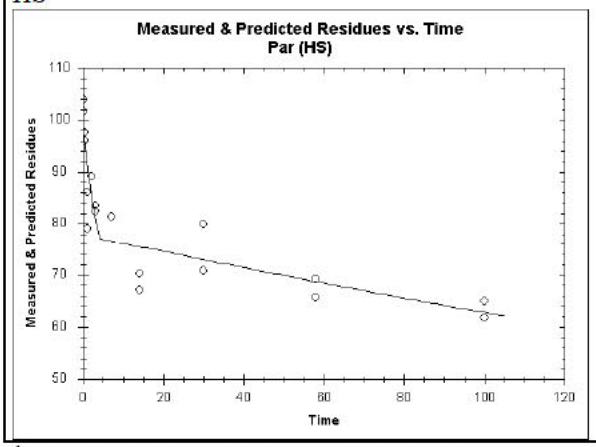
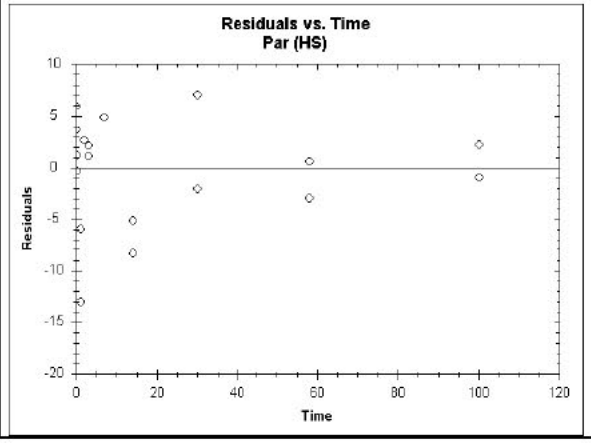


Table 8.2.2.3-76: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

HS



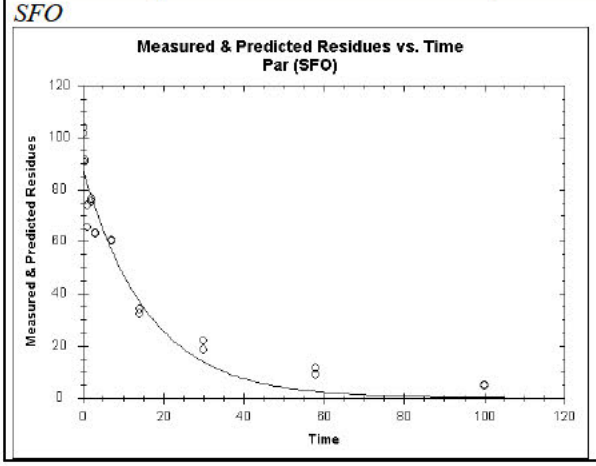


[1] t-test not relevant for kinetic parameter β

Table 8.2.2.3-77: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.9	k: 0.0617	12.2	k: <0.001	k: 0.0448	k: 0.0780	11.2	37.4
FOMC	Good	93.9	α: 0.9292 β: 7.158	10.0	-[1]	β: 0.0281	β: 14.288	7.9	78.2
DFOP	Acceptable	103.5	k_1: 2.724 k_2: 0.0451 g: 0.2639	7.4	k_1: 0.023 k_2: <0.001	k_1: 0.2677 k_2: 0.0354	k_1: 5.1800 k_2: 0.0550	8.6	44.3
HS	Acceptable	96.6	k_1: 0.1696 k_2: 0.0428 tb: 2.2	9.9	k_1: <0.001 k_2: <0.001	k_1: 0.0915 k_2: 0.0289	k_1: 0.2480 k_2: 0.0570	9.8	47.4
Conclusion from notifier	Dissipation of glyphosate in the water phase is best described by bi-phasic models. The FOMC model provides the best visual fit and is statistically reliable. Since 10 % of the initially measured substance concentration was reached within the experimental period, FOMC is selected also for deriving modelling endpoints. FOMC to be used for trigger endpoints FOMC to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO



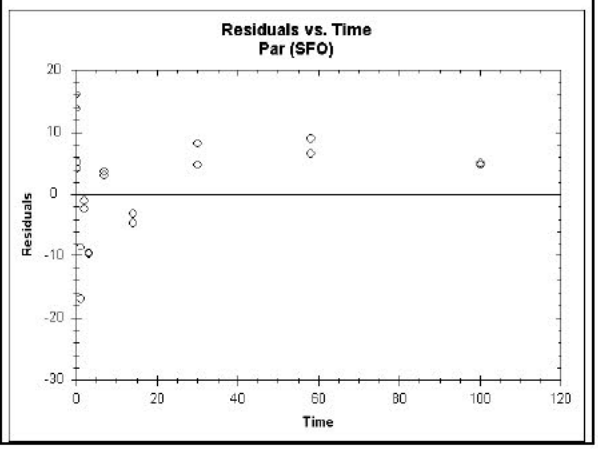
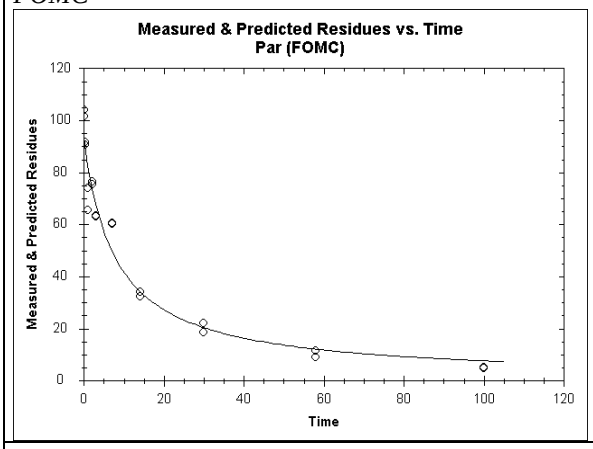
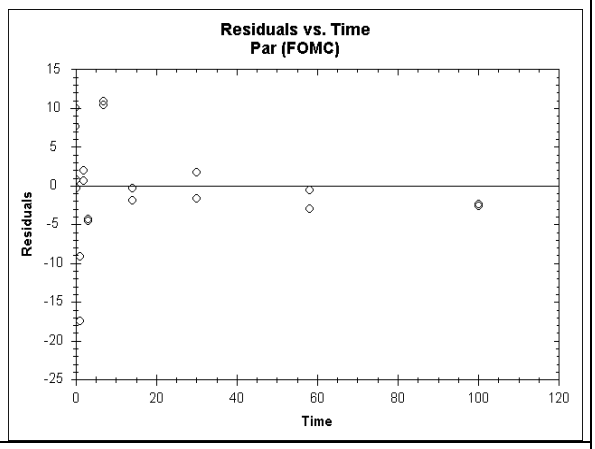


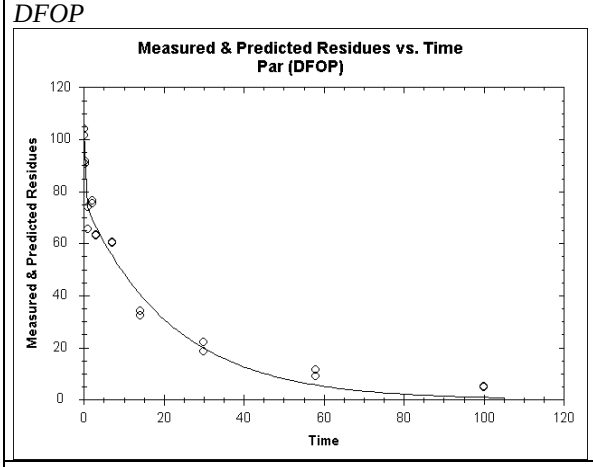
Table 8.2.2.3-77: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

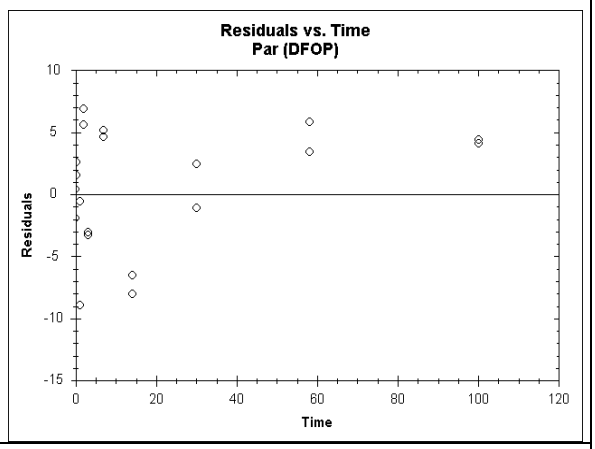
FOMC



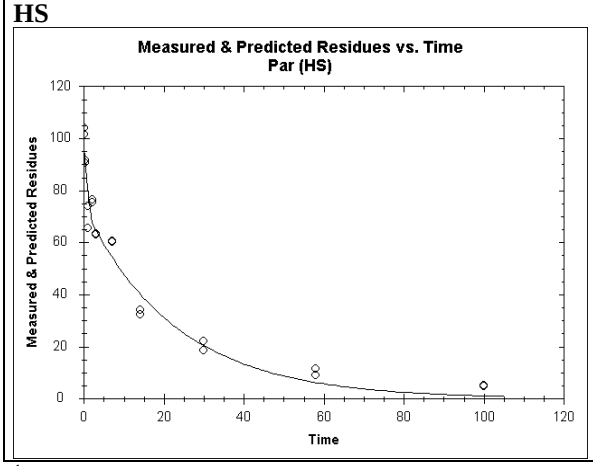


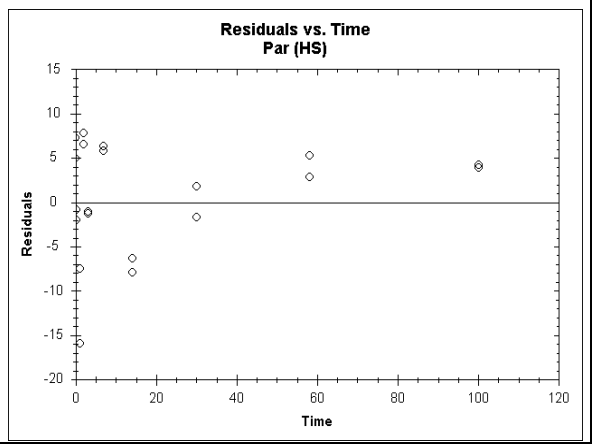
DFOP





HS





[1] t-test not relevant for kinetic parameter β

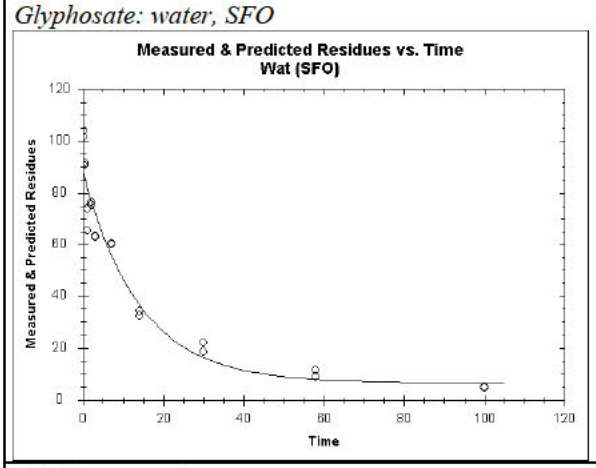
Table 8.2.2.3-78: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

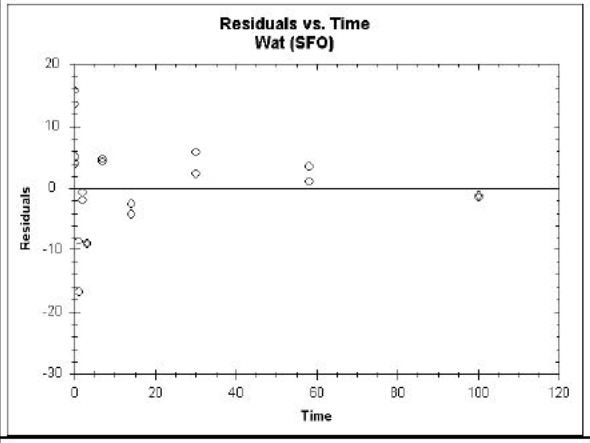
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-78: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

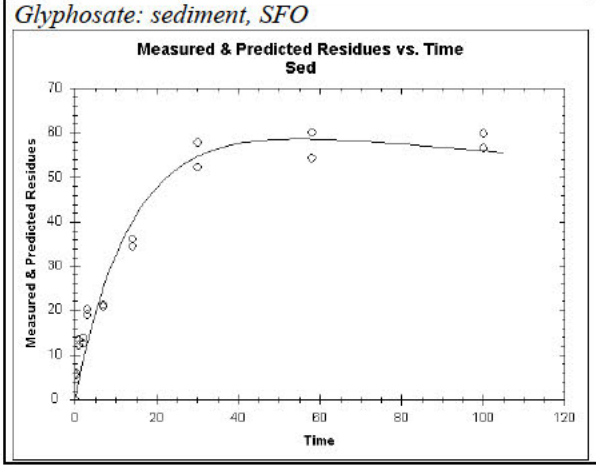
Water: SFO	Good	88.1	k_{wat}: 0.0144 $k_{wat\ sed}$: 0.0521	11.5	k_{wat}: 0.0226 $k_{wat\ sed}$: <0.001	k_{wat}: 0.0008 $k_{wat\ sed}$: 0.0434	k_{wat}: 0.028 $k_{wat\ sed}$: 0.061	48.3	160.4
Sediment: SFO	Good	0.0	k_{sed}: 2.34×10^{-14} $k_{sed\ wat}$: 0.0073	12.4	k_{sed}: 0.5 k_{sed_wat}: 0.0676	k_{sed}: -0.0052 $k_{sed\ wat}$: -0.0021	k_{sed}: 0.005 $k_{sed\ wat}$: 0.017	>1000	>1000
Conclusion from notifier	Although the visual fits obtained for the water and sediment phases are good, the degradation rate in sediment is not significantly different from zero. Therefore, the statistical fits obtained for the sediment phase are not reliable. No further evaluation was conducted. No reliable endpoints could be derived								
Conclusion from RMS	Agrees with notifier conclusion.								

Glyphosate: water, SFO





Glyphosate: sediment, SFO



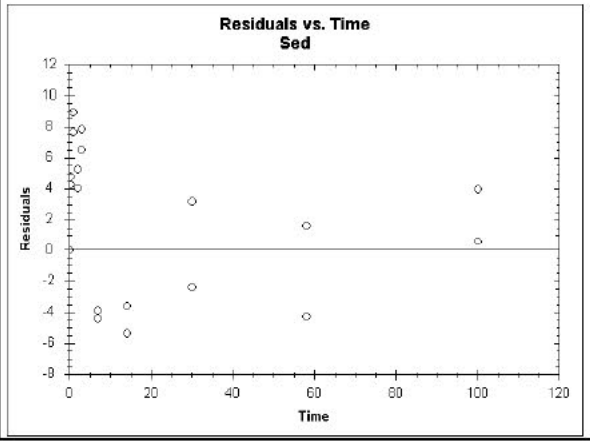


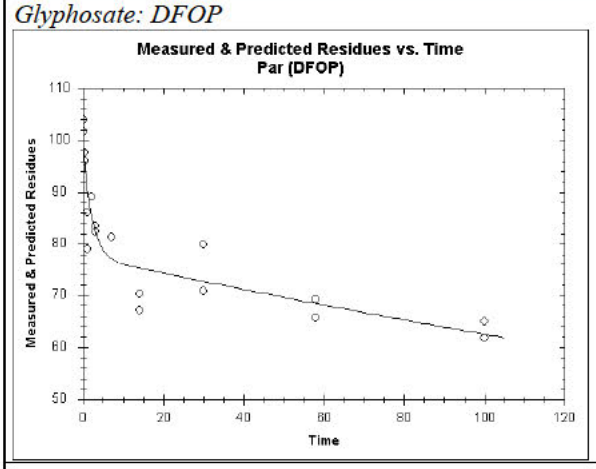
Table 8.2.2.3-79: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level M-I, degradation

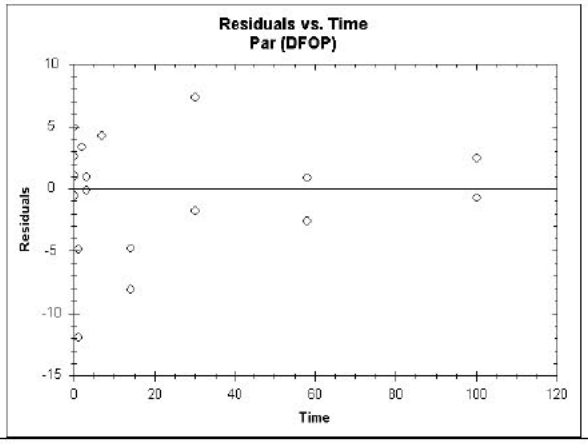
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)

Table 8.2.2.3-79: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level M-I, degradation

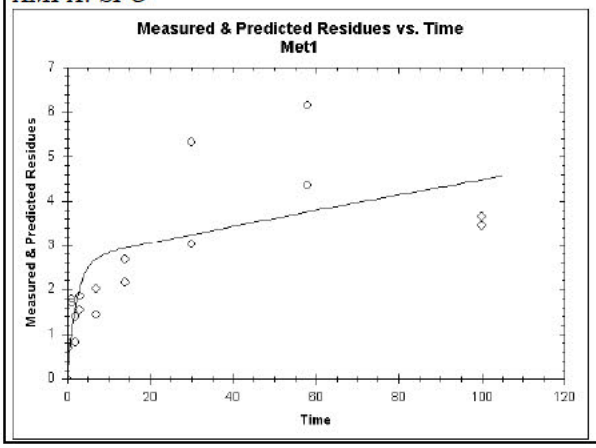
Glyphosate: DFOP	Accep-table	99.0	k_1: 0.4485 k_2: 0.0021 g: 0.2183	4.5	k_1: 0.003 k_2: 0.003 g: <0.001	k_1: 0.1427 k_2: 0.0007	k_1: 0.754 k_2: 0.004	208.8	960.7	-
AMPA: SFO	Poor	0.0	k: 2.34×10^{-14}	26.9	k: >0.1	k: -0.0069	k: 0.007	>1000	>1000	0.123 (±0.027)
Conclusion from notifier	No reliable endpoints could be derived for AMPA.									
Conclusion from RMS	Agrees with notifier conclusion.									

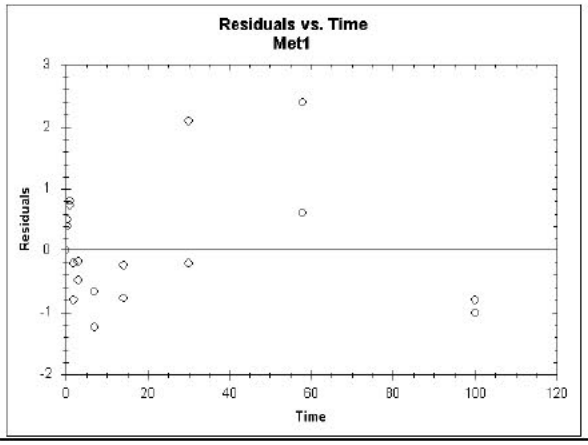
Glyphosate: DFOP





AMPA: SFO





[redacted] *(1993, CA 7.2.2.3/005)*

Since the metabolites AMPA and HMPA were not detected in the sediment phase, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system. However, no evaluation could be conducted at Level M-I dissipation for HMPA for system Bickenbach due to the limited number of data points available after the peak concentration.

The metabolite AMPA was not detected in the system Bickenbach on days 0 and 0.25. Therefore, the residue values on day 0.25 were set to half of the lowest reported value across the experimental study (0.2 % AR for glyphosate on day 100) as no LOD/LOQ values were available.

The same was done for the metabolite HMPA in the system Bickenbach where HMPA was first detected on day 14 of the study period. The residual values on day 7 were set to half of the lowest measured value as described above.

Table 8.2.2.3-80: Experimental data for system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% TAR)[1]		HMPA residues (% AR)[1]	
	Total system	Water	Sediment	Total system	Water	Total system	Water[2]
0	96.93[3]	96.93[3]	0.0[4]	0.0[5]	-[6]	0.00[5]	-[6]
0	98.76[3]	98.76[3]	0.0[4]	0.0[5]	-[6]	0.00[5]	-[6]
0.25	95.79	81.04	14.75	0.10[g]	-[6]	NaN[8]	-[6]
0.25	96.33	80.29	16.04	0.10[g]	-[6]	NaN[8]	-[6]
1	96.41	63.18	33.23	2.86	2.86	NaN[8]	-[6]
1	94.78	64.22	30.56	2.08	2.08	NaN[8]	-[6]
2	86.84	47.68	39.16	4.21	4.21	NaN[8]	-[6]
2	91.30	51.68	39.62	5.65	5.65	NaN[8]	-[6]
7	74.35	21.52	52.83	12.45	12.45	0.10[7]	0.10[7]
7	77.69	24.37	53.32	8.98	8.98	0.10[7]	0.10[7]
14	53.50	14.92	38.60	15.39	15.39	3.75	3.75
14	48.24	12.10	36.14	16.10	16.10	3.01	3.01
30	40.32	5.86	34.46	11.41	11.41	2.67	2.67
30	42.25	9.39	32.86	11.61	11.61	4.78	4.78
61	36.75	1.10	35.65	4.83	4.83	11.37	11.37
61	34.67	0.62	34.05	5.23	5.23	8.58	8.58
100	29.93	0.20	29.73	0.39	0.39	7.63	7.63
100	29.07	0.33	28.74	0.56	0.56	7.41	7.41

[1] Since the metabolites were not detected in sediment, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system
[2] No evaluation was conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration
[3] Values at day 0 were set to material balance according to FOCUS (2014)
[4] Set to zero for evaluation at Level P-II.
[5] Set to zero for evaluation at Level M-I degradation
[6] The metabolites were not detected at the beginning of the experiment
[7] Since no LOD/LOQ values are available in the study report, the value was set to half of the lowest measured value in the study (lowest measured value: 0.2 % AR, system Bickenbach, glyphosate on day 100, water phase)
[8] HMPA not detected; values omitted according to FOCUS (2014), NaN (= not a number) was used as input for KinGUI

Table 8.2.2.3-81: Experimental data for system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% AR)[1]		HMPA residues (% AR)[1]	
	Total system	Water	Sediment	Total system	Water	Total system	Water
0	93.48[2]	93.48[2]	0.0[3]	0.0[4]	4.37	0.0[4]	-[5]
0	95.92[2]	95.92[2]	0.0[3]	0.0[4]	3.65	0.0[4]	-[5]
0.25	99.32	78.03	21.29	2.35	2.35	NaN[6]	-[5]
0.25	96.32	73.24	23.08	1.64	1.64	NaN[6]	-[5]
1	86.69	47.17	39.52	2.95	2.95	0.10[7]	-[5]
1	95.02	50.74	44.28	1.18	1.18	0.10[7]	-[5]
2	82.86	34.41	52.83	2.77	2.77	0.24	0.24
2	82.05	31.06	57.31	2.11	2.11	0.15	0.15
7	82.86	16.77	66.09	3.91	3.91	0.63	0.63

7	76.30	25.43	56.62	4.88	4.88	0.51	0.51
14	61.16	14.78	46.38	5.41	5.41	0.81	0.81
14	59.91	17.07	42.84	6.14	6.14	0.77	0.77
30	51.67	8.30	43.37	3.22	3.22	1.76	1.76
30	52.47	8.25	44.22	2.45	2.45	2.09	2.09
61	58.07	3.31	54.76	0.47	0.47	0.11	0.11
61	58.68	3.66	55.02	0.51	0.51	0.21	0.21
100	45.97	1.83	44.14	0.39	0.39	0.12	0.12
100	47.17	3.02	44.15	0.39	0.39	0.10	0.10

[1] since the metabolites were not detected in sediment, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system
[2] Values at day 0 were set to material balance according to FOCUS (2014)
[3] Set to zero for evaluation at Level P-II
[4] Set to zero for evaluation at Level M-I degradation
[5] The metabolite HMPA was not detected at the beginning of the experiment
[6] HMPA not detected; values omitted according to FOCUS (2014), NaN (= not a number) was used as input for KinGUI
[7] Since no LOD/LOQ values are available in the study report, the value was set to half of the lowest measured value in the study (lowest measured value: 0.2 %, system Sandy Sediment, glyphosate on day 100, water phase)

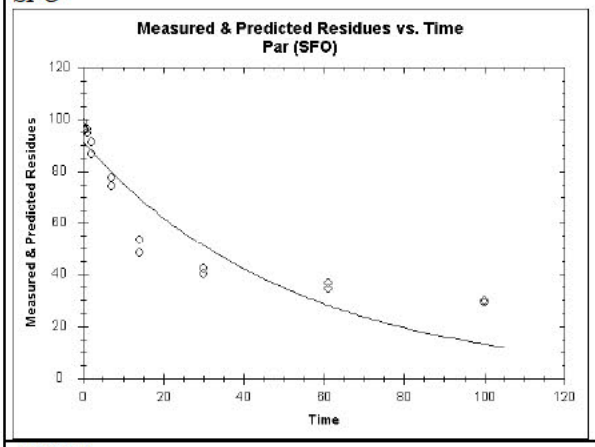
Table 8.2.2.3-82: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

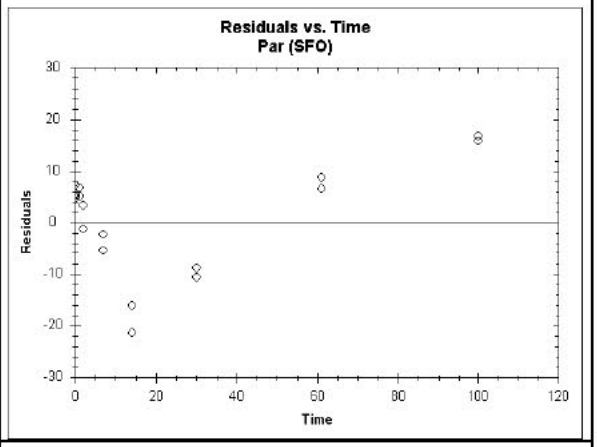
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-82: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

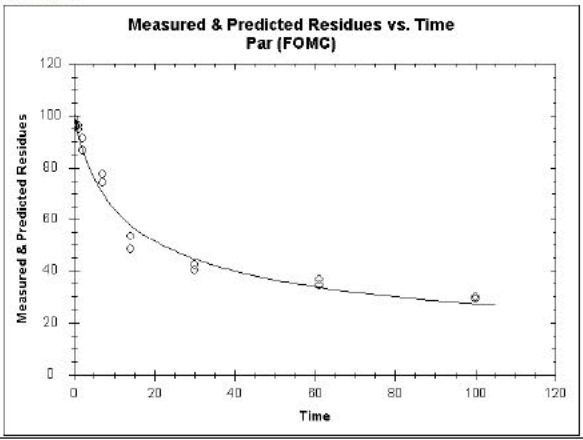
SFO	Poor	91.4	k: 0.0194	11.7	k: <0.001	k: 0.0136	k: 0.0250	35.8	118.8
FOMC	Good	99.7	α: 0.4556 β: 6.1123	4.5	-[1]	β: 1.9556	β: 10.2690	21.9	951.8
DFOP	Good	98.9	k_1: 0.0863 k_2: 0.0026 g: 0.6014	3.4	k_1: <0.001 k_2: 0.12	k_1: 0.0556 k_2: -0.0016	k_1: 0.1170 k_2: 0.0070	18.7	531.2
HS	Good	98.4	k_1: 0.0439 k_2: 0.0048 tb: 18.5	2.2	k_1: <0.001 k_2: <0.001	k_1: 0.0395 k_2: 0.0028	k_1: 0.0480 k_2: 0.0070	15.8	329.4
Conclusion from notifier	Degradation of glyphosate is best described by bi-phasic models. All bi-phasic models provide visually good fits. The HS model provided the least χ^2 error and its degradation parameters are significantly different from zero. Thus, the HS model is selected as the best-fit model as well as for deriving modelling endpoints. HS to be used for trigger endpoints HS to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion.								

SFO





FOMC



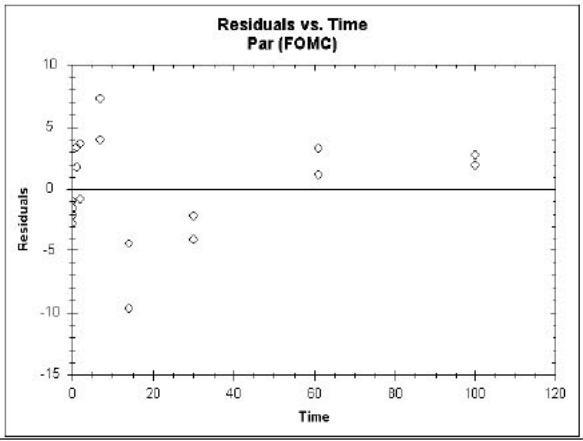
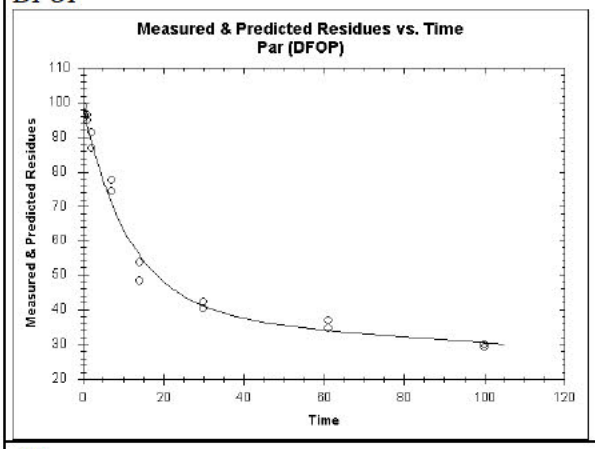
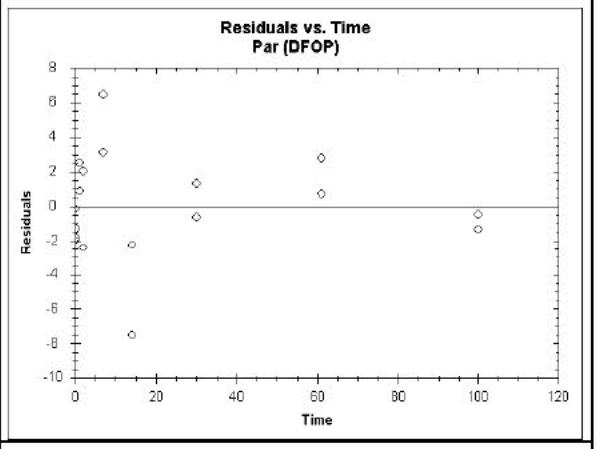


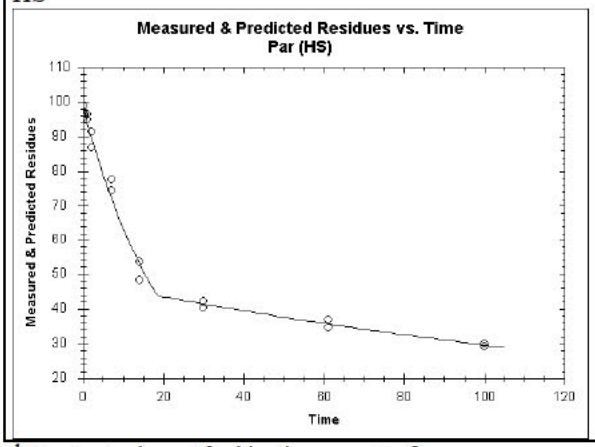
Table 8.2.2.3-82: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

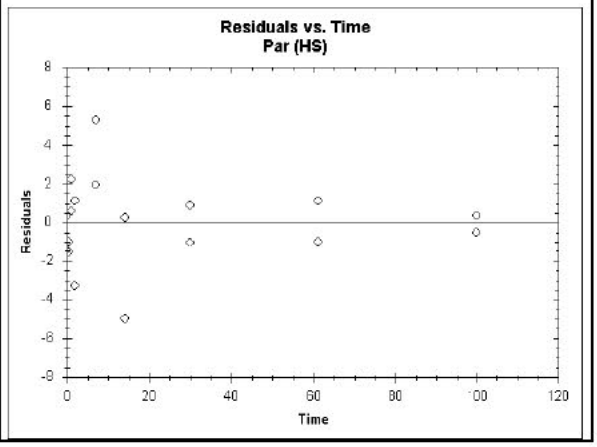
DFOP





HS





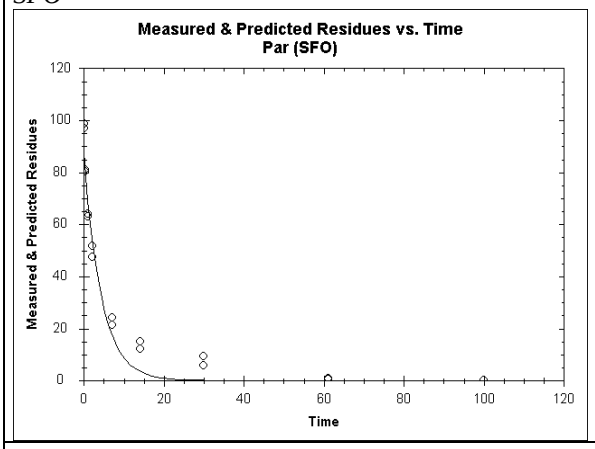
[1] t-test not relevant for kinetic parameter β

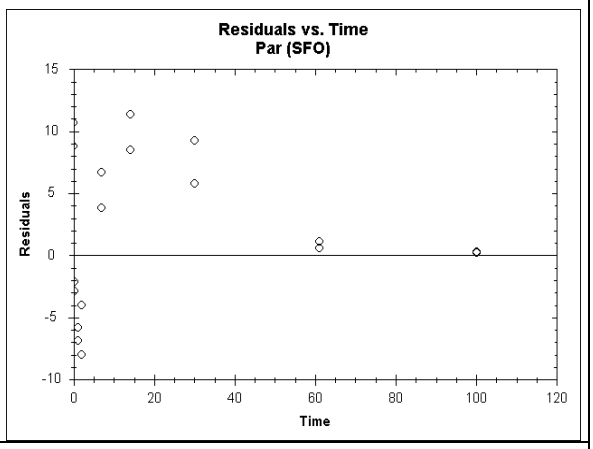
Table 8.2.2.3-83: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	88.1	k: 0.2293	13.5	k: <0.001	k: 0.1699	k: 0.2980	3.0	10.0
FOMC	Good	95.1	α: 0.9278 β: 1.8600	4.6	-[1]	β: 1.1856	β: 2.5340	2.1	20.4
DFOP	Good	94.3	k_1: 0.6169 k_2: 0.0565 g: 0.6488	5.2	k_1: <0.001 k_2: <0.001	k_1: 0.4463 k_2: 0.0306	k_1: 0.7880 k_2: 0.0820	2.0	22.2
HS	Good	93.1	k_1: 0.34 k_2: 0.0546 tb: 3.6	6.4	k_1: <0.001 k_2: <0.001	k_1: 0.2938 k_2: 0.0271	k_1: 0.3920 k_2: 0.0820	2.0	22.9
Conclusion from notifier	Dissipation of glyphosate is best described by bi-phasic models. All bi-phasic models provide visually good fits. However, the DFOP model provides the best visual fit and is selected as the best-fit model as well as for deriving modelling endpoints. DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoints. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

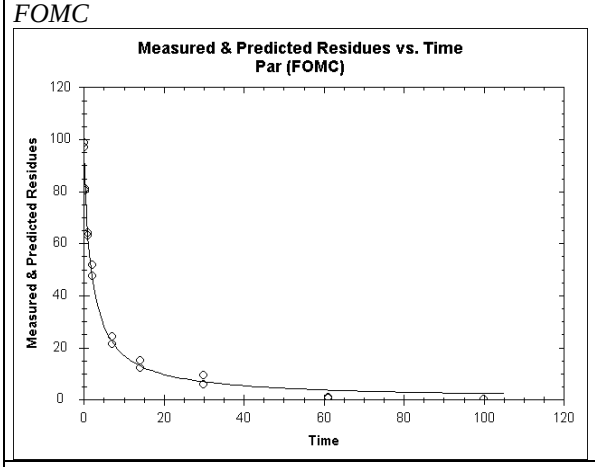
Table 8.2.2.3-82: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

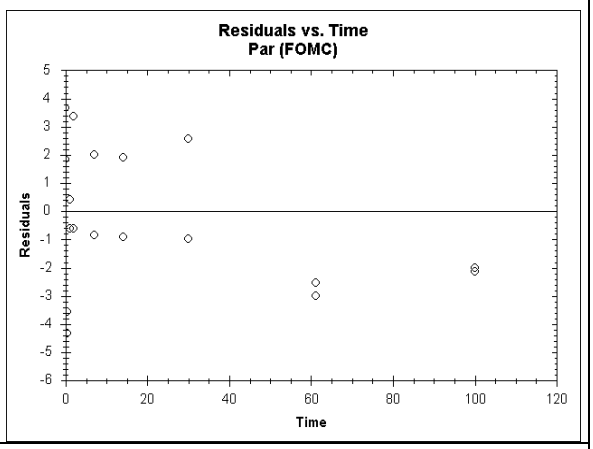
SFO



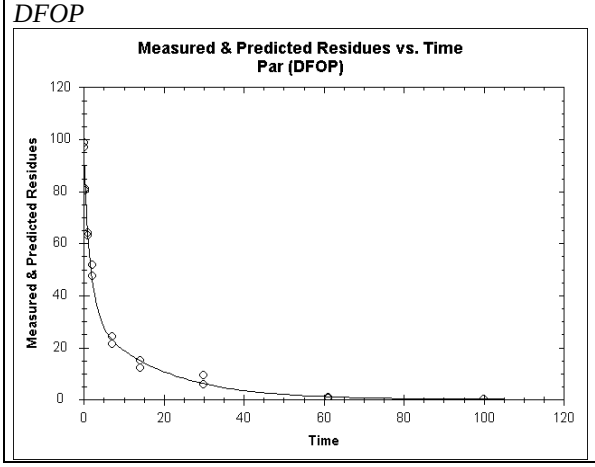


FOMC





DFOP



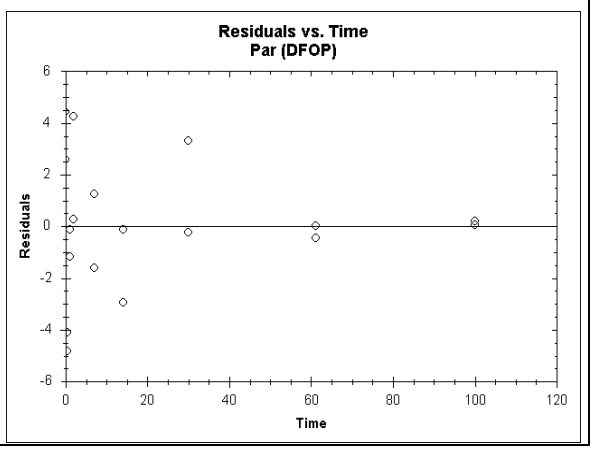
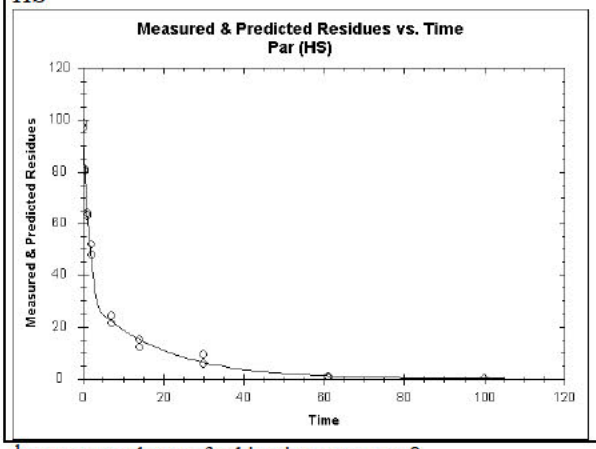
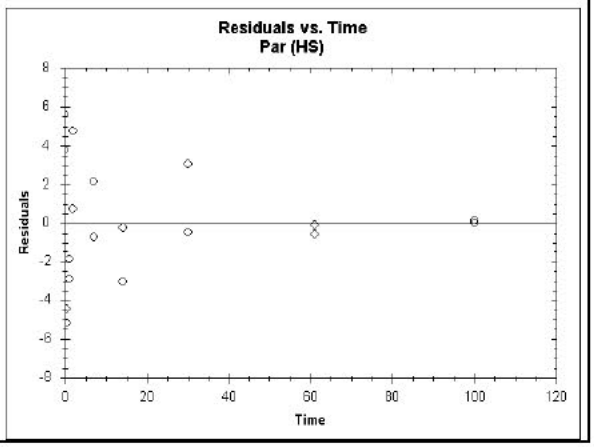


Table 8.2.2.3-82: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

HS





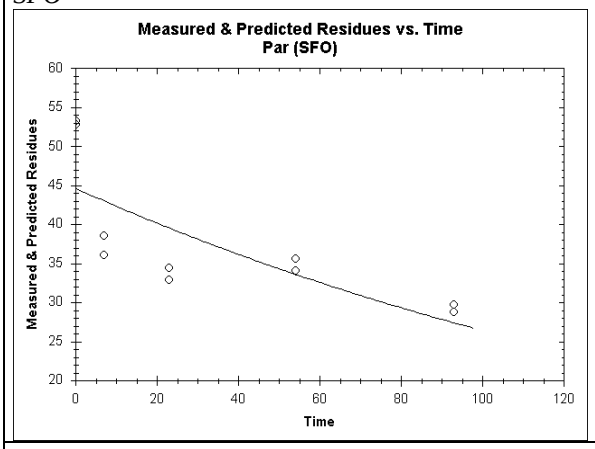
[1] t-test not relevant for kinetic parameter β

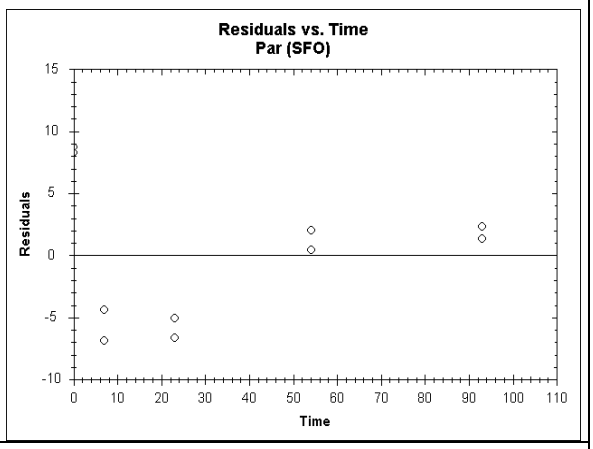
Table 8.2.2.3-84: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	44.6	k: 0.0052	11.4	k: 0.008	k: 0.0019	k: 0.0090	132.3	439.4
FOMC	Acceptable	53.1	α: 0.0738 β: 0.0637	3.5	-[1]	β: -0.1582	β: 0.2860	764.6	>1000
DFOP	Good	53.1	k_1: 0.3503 k_2: 0.0020 g: 0.3137	3.6	k_1: 0.061 k_2: 0.025	k_1: -0.0310 k_2: 0.0004	k_1: 0.7320 k_2: 0.0040	158.7	965.3
HS	Good	53.1	k_1: 0.1242 k_2: 0.0024 tb: 2.9	3.8	k_1: <0.001 k_2: 0.005	k_1: 0.0973 k_2: 0.0011	k_1: 0.1510 k_2: 0.0040	145.0	825.5
Conclusion from notifier	Dissipation of glyphosate in sediment is best described by bi-phasic models. All bi-phasic models provide visually acceptable or good fits. The statistical fit of the FOMC model is not reliable as the confidence interval of parameter β includes zero. The DFOP model provides the best visual fit and is selected as the best-fit model as well as for deriving modelling endpoints. DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoints. Both t-test for k_1 and k_2 are acceptable when considering a significance level of 10% (as recommended in FOCUS Guidance for water/sediment studies). 95% confidence interval for k_1 includes 0, but it is expected that 90% confidence interval would not include 0. Indeed, as indicated in the FOCUS guidance, "Both, the t-test and the confidence interval give the same answer, provided the underlying assumptions are identical (distribution of the parameter and level of probability)". According to FOCUS guidance, no modelling endpoint should be derived from P-I level in sediment (please refer to RMS comments).								

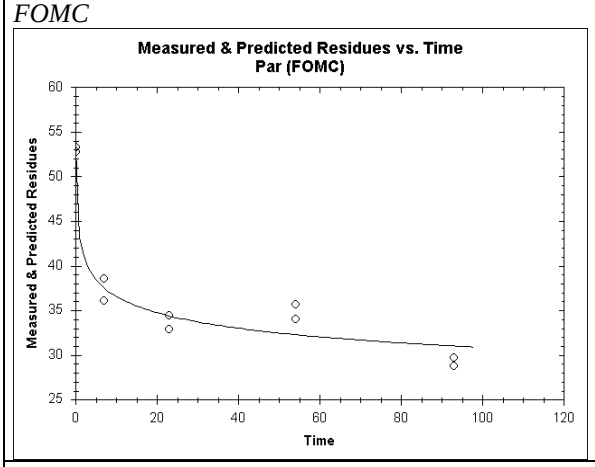
Table 8.2.2.3-84: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

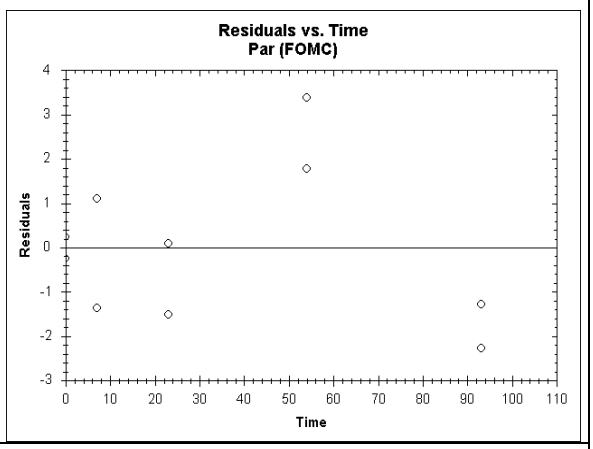
SFO



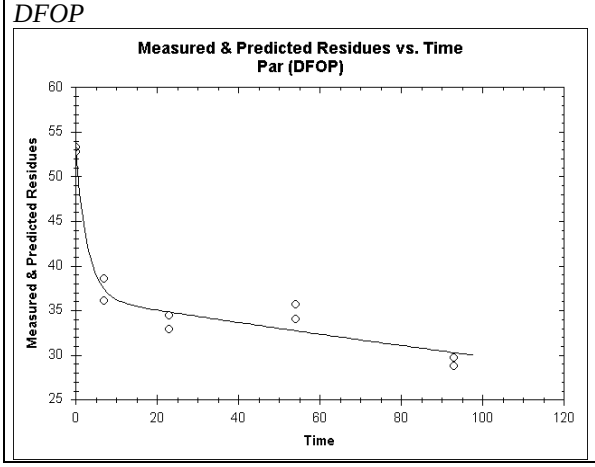


FOMC





DFOP



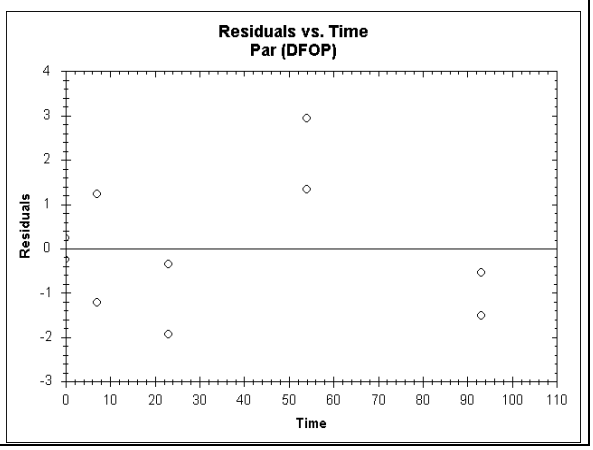
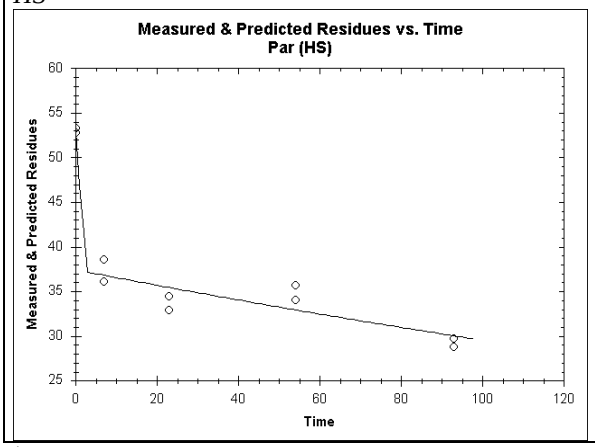
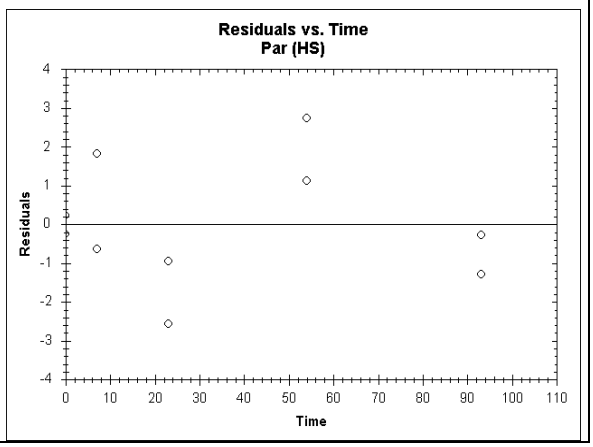


Table 8.2.2.3-84: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

HS





[1] t-test not relevant for kinetic parameter β

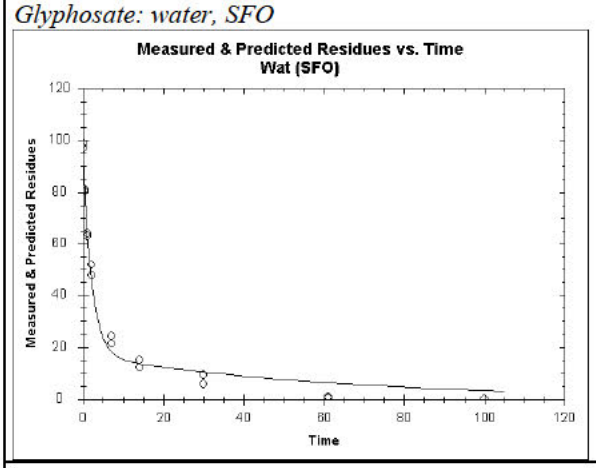
Table 8.2.2.3-85: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

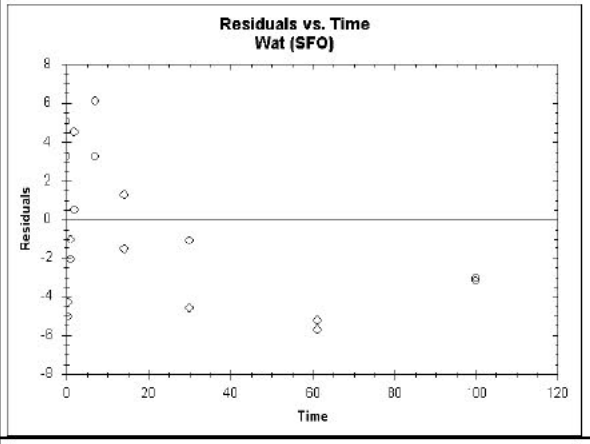
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-85: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

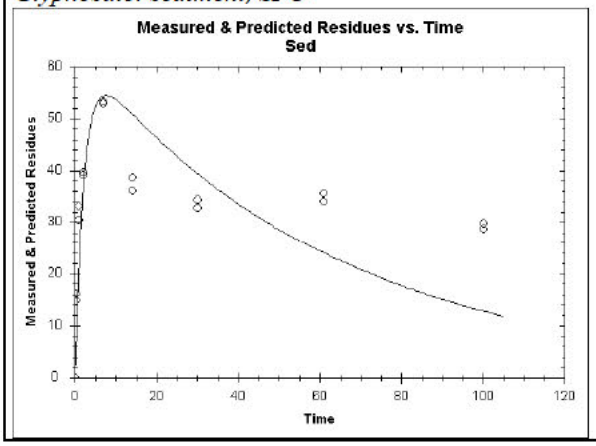
Water: SFO	Accep-table	93.7	k_{wat}: 0.0768 k_{wat_sed}: 0.3007	8.2	k_{wat}: 0.0144 $k_{wat\ sed}$: <0.001	k_{wat}: 0.0111 k_{wat_sed}:0.2301	k_{wat}: 0.143 k_{wat_sed}: 0.371	9.0	30.0
Sediment: SFO	Poor	0.0	k_{sed}: 2.34×10^{-14} $k_{sed\ wat}$: 0.0958	23.0	k_{sed}: 0.5 $k_{sed\ wat}$: <0.001	k_{sed}: -0.0243 k_{sed_wat}:0.0551	k_{sed}: 0.024 k_{sed_wat}: 0.136	>1000	>1000
Conclusion from notifier	The visual and statistical fits obtained for the water phase are reliable but the visual fit obtained for the sediment phase is poor. No further evaluation was conducted. No reliable endpoints could be derived								
Conclusion from RMS	Agrees with notifier conclusion.								

Glyphosate: water, SFO





Glyphosate: sediment, SFO



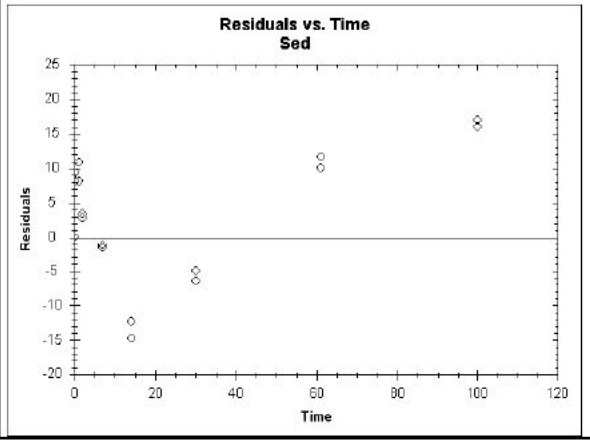


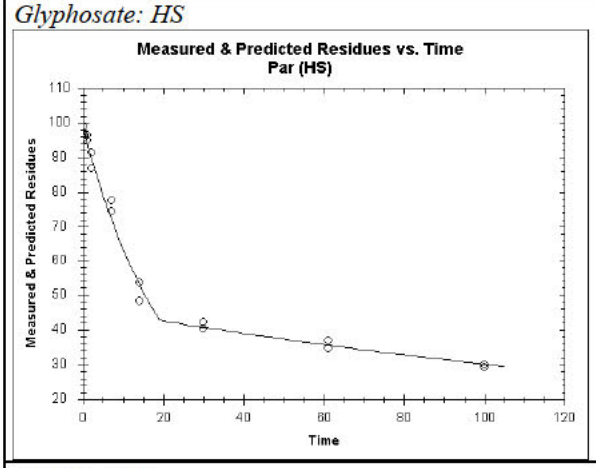
Table 8.2.2.3-86: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

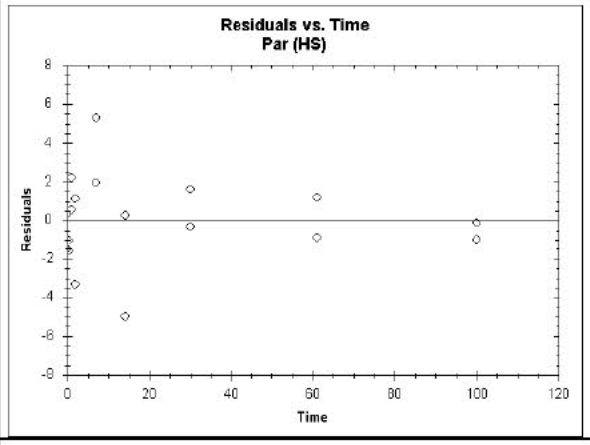
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)

Table 8.2.2.3-86: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

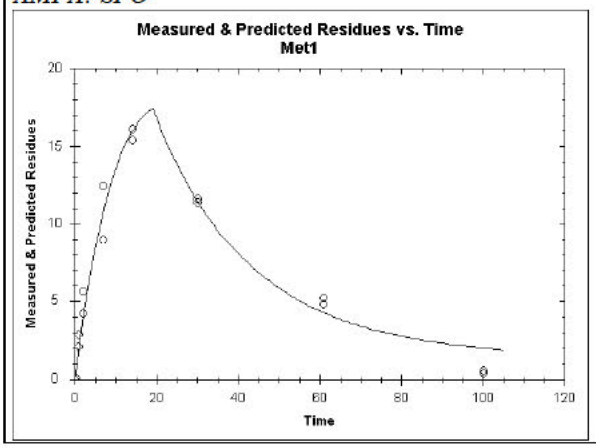
Glyphosate: HS	Good	98.4	k_1: 0.0439 k_2: 0.0043 tb: 19.1	2.2	k_1: <0.001 k_2: <0.001	k_1: 0.0398 k_2: 0.0026	k_1: 0.0480 k_2: 0.0060	15.8	358.4	-
AMPA: SFO	Accep-table	0.0	k: 0.0442	9.4	k: <0.001	k: 0.0351	k: 0.0530	15.7	52.2	0.489 (±0.035) (from parent)
HMPA: SFO	Accep-table	0.0	k: 0.0052	22.6	k: 0.130	k: -0.0037	k: 0.0140	133.6	443.9	0.366 (±0.085) (from AMPA)
Conclusion from notifier	The fit of glyphosate at Level M-I degradation is comparable to that at Level P-I total system. For AMPA, both the visual and statistical fits from the SFO model are acceptable. For HMPA, the visual fit is acceptable but the degradation parameter k is not significantly different from zero. As the formation of HMPA is correctly described by the model and the standard deviation of the estimated formation fraction is low, the derived formation fraction is considered reliable. A second fitting step with fixed formation fraction from AMPA to HMPA was conducted.									
Conclusion from RMS	Agrees with notifier conclusion, please refer to next fit with fixed ffm.									

Glyphosate: HS





AMPA: SFO



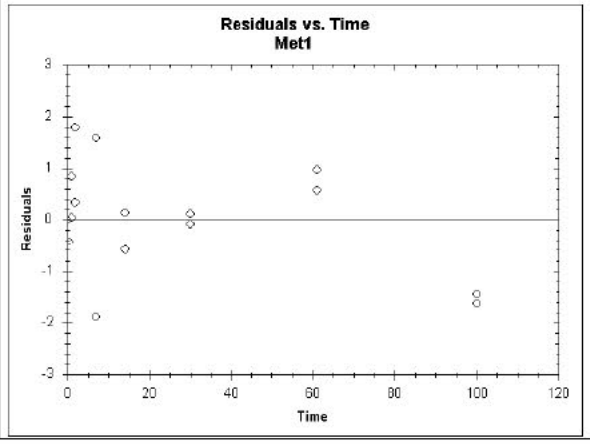
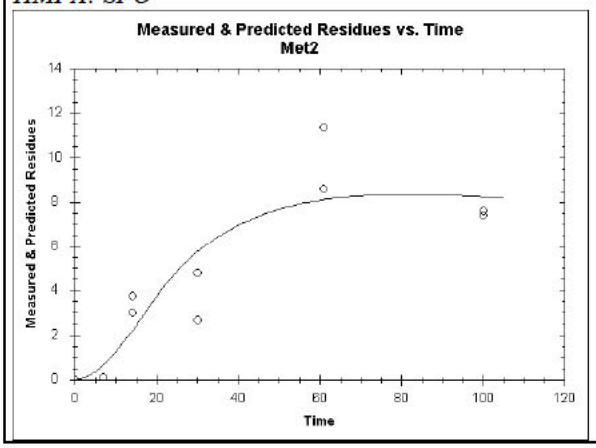


Table 8.2.2.3-86: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

HMPA: SFO



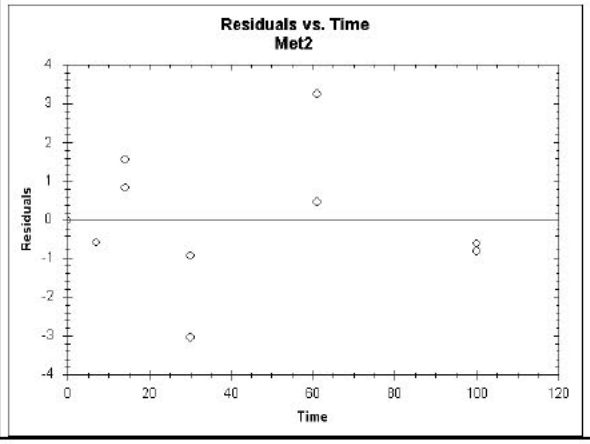
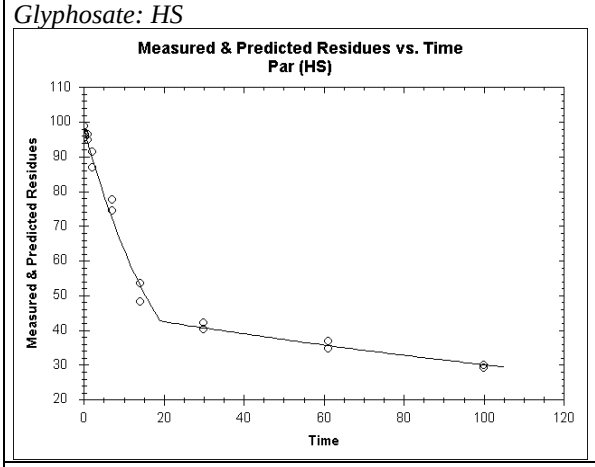


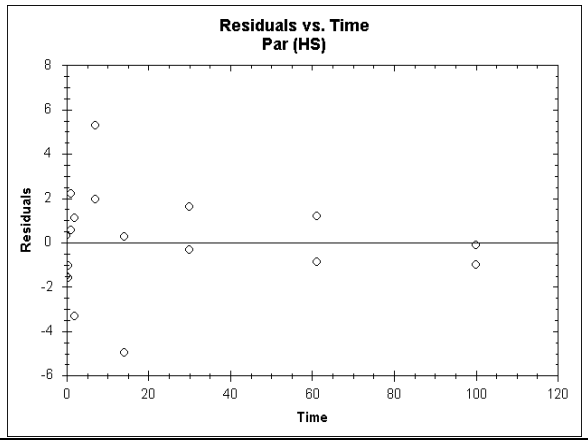
Table 8.2.2.3-87: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation (formation fraction from AMPA to HMPA fixed)

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: HS	Good	98.4	k_1: 0.0440 k_2: 0.0043 tb: 19.1	2.2	k_1: <0.001 k_2: <0.001	k_1: 0.0401 k_2: 0.0026	k_1: 0.0480 k_2: 0.0060	15.8	358.9	-
AMPA: SFO	Accep-table	0.0	k: 0.0440	9.4	k: <0.001	k: 0.0364	k: 0.0520	15.7	52.3	0.488 (±0.032) (from parent)
HMPA: SFO	Accep-table	0.0	k: 0.0054	20.5	k: 0.0157	k: 0.0006	k: 0.0100	128.8	427.8	0.366[1] (from AMPA)
Conclusion from notifier	The fit of glyphosate at Level M-I degradation is comparable to that at Level P-I total system degradation. For AMPA, both the visual and statistical fits from the SFO model are acceptable. For HMPA, both the visual and statistical fits from the SFO model are acceptable. HS-SFO-SFO to be used for trigger endpoints of AMPA and HMPA HS-SFO-SFO to be used for modelling endpoints of AMPA and HMPA									
Conclusion from RMS	Agrees with notifier conclusion.									

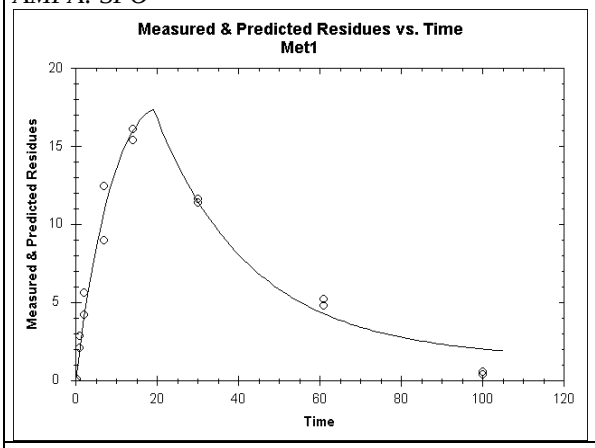
Table 8.2.2.3-87: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation (formation fraction from AMPA to HMPA fixed)

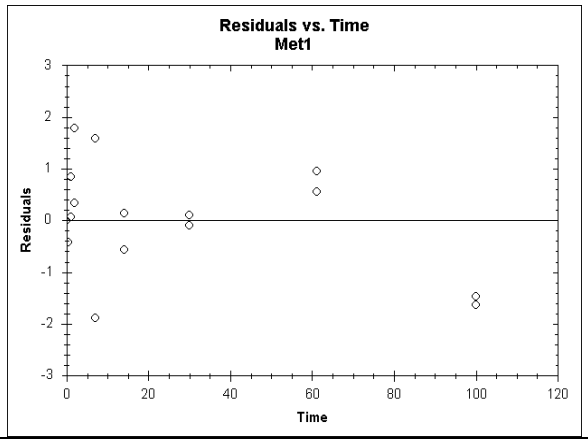
Glyphosate: HS



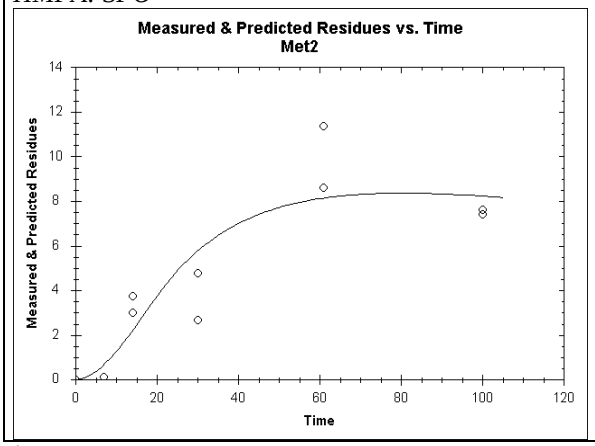


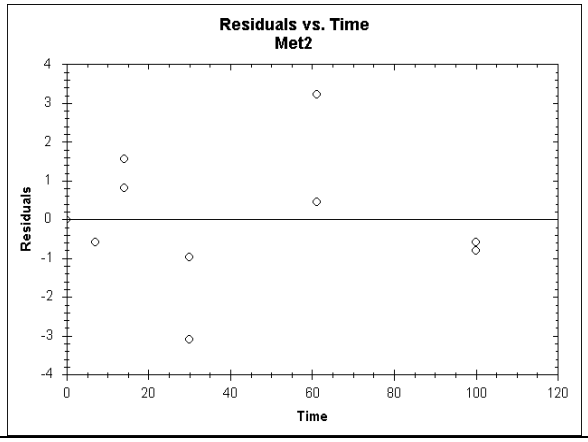
AMPA: SFO





HMPA: SFO





[1] Formation fraction from AMPA to HMPA was fixed to the estimated value obtained from an initial fitting step

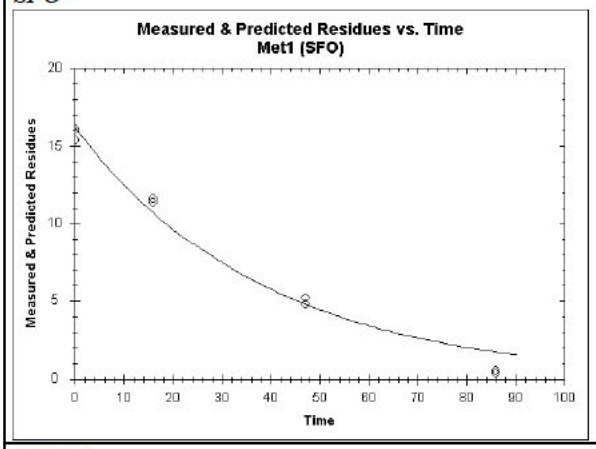
Table 8.2.2.3-88: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

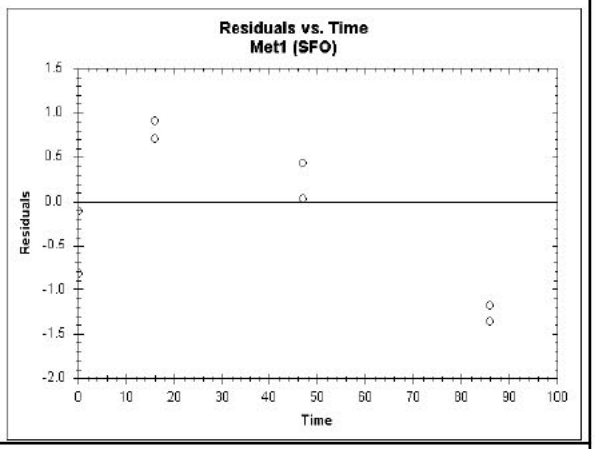
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	16.2	k: 0.0259	7.9	k: <0.001	k: 0.0212	k: 0.0310	26.8	88.9

Table 8.2.2.3-88: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

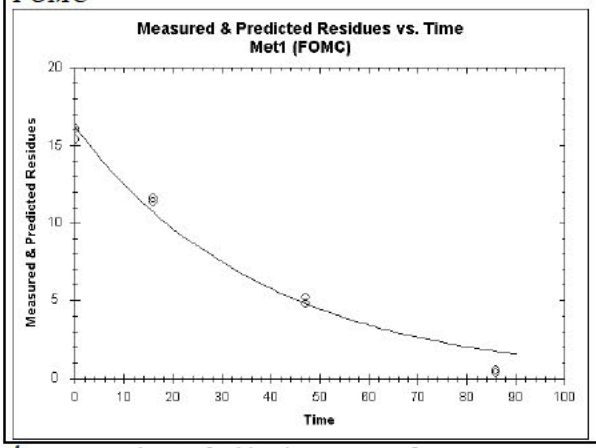
FOMC	Acceptable	16.2	α: 7234 β: 279100	9.9	-[1]	β: 279100	β: 279167	26.8	88.9
Conclusion from notifier	Only the SFO and FOMC model was used for evaluation due to the limited number of data points. The visual and statistical fits from both models are acceptable but the χ^2 error resulting from the SFO model is smaller. Thus, the SFO model is selected as the best-fit model as well as for modelling endpoints. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Since reliable endpoints can be derived for glyphosate and its metabolites at level M-I degradation, no further kinetic assessment is required for AMPA. In addition, the number of available data from maximum occurrence is limited (4).								

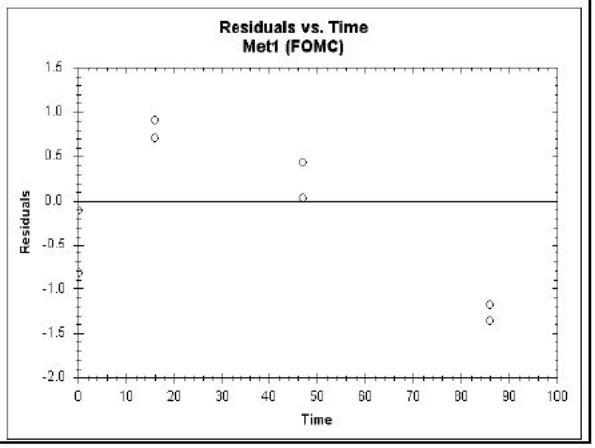
SFO





FOMC





[1] t-test not relevant for kinetic parameter β

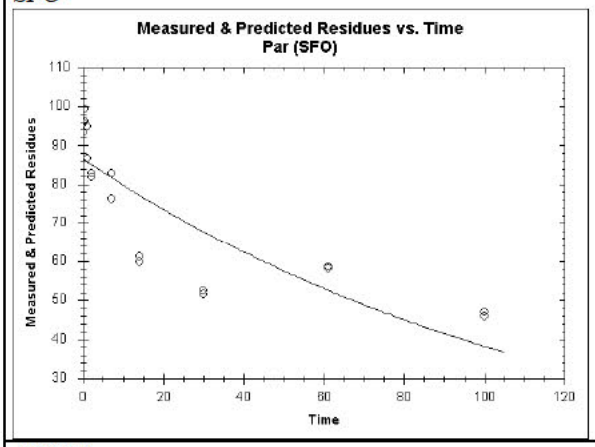
Table 8.2.2.3-89: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

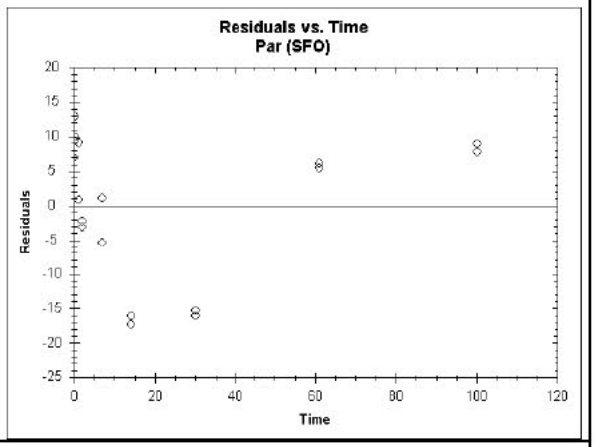
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-89: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

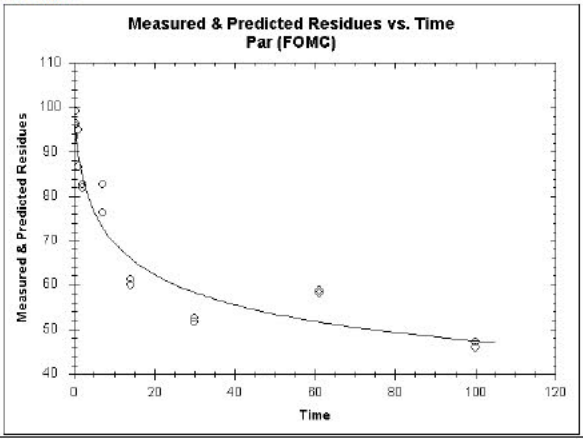
SFO	Poor	86.6	k: 0.0082	10.6	k: <0.001	k: 0.0052	k: 0.0110	84.7	281.3
FOMC	Acceptable	97.3	α: 0.1766 β: 1.7272	5.1	-[1]	β: -0.4493	β: 3.9040	85.7	>1000
DFOP	Acceptable	95.6	k_1: 0.1152 k_2: 0.0014 g: 0.4052	4.8	k_1: 0.007 k_2: 0.199	k_1: 0.0340 k_2:-0.0018	k_1: 0.1960 k_2: 0.0050	121.6	>1000
HS	Poor	97.2	k_1: 0.0774 k_2: 0.0044 tb: 4.3	6.9	k_1: 0.022 k_2: 0.001	k_1: 0.0091 k_2: 0.0021	k_1: 0.1460 k_2: 0.0070	85.0	447.6
Conclusion from notifier	Degradation of glyphosate is best described by bi-phasic models. The FOMC and DFOP models provide visually acceptable fits but the resulting parameters are not statistically reliable. Nevertheless, the DFOP model provides more reliable estimates of the DT_{50} and DT_{90} values as well as a smaller χ^2 error. Thus, the DFOP model is selected as the best-fit model. DFOP to be used for trigger endpoints 1000 d to be used for modelling as conservative approach								
Conclusion from RMS	T-test for HS is acceptable, however visually it is not as good as DFOP. Agrees with notifier conclusion.								

SFO





FOMC



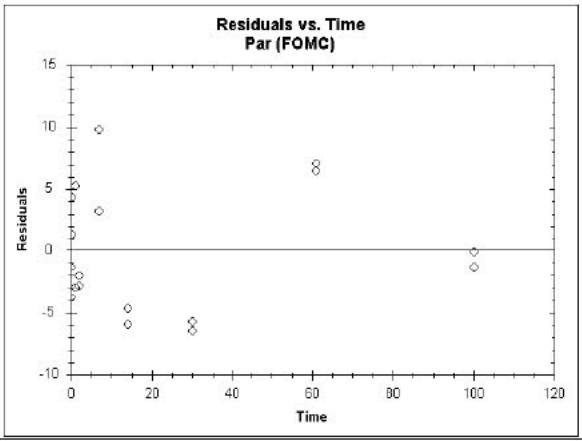
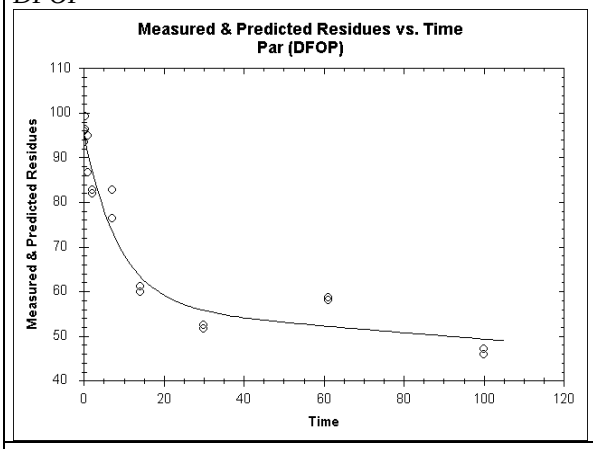
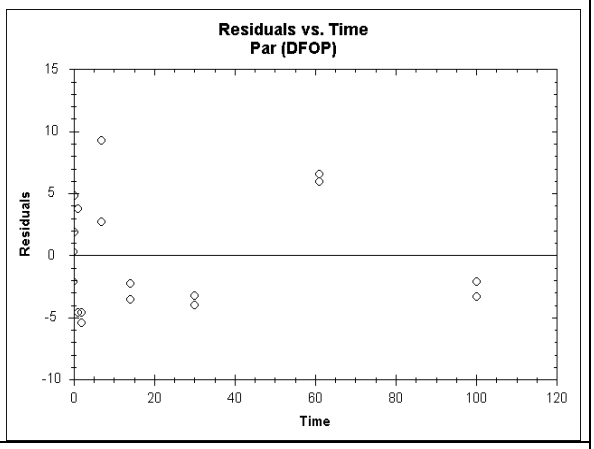


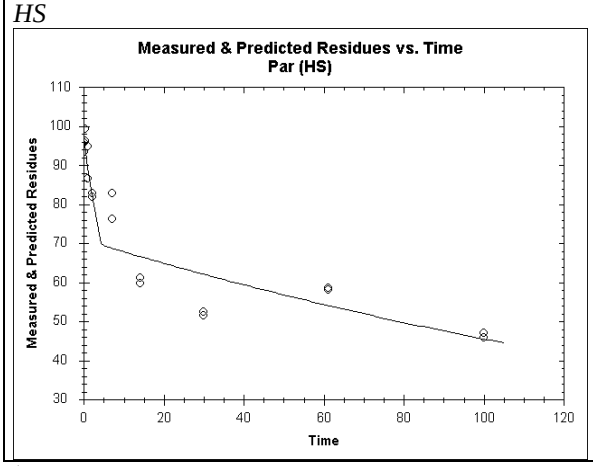
Table 8.2.2.3-89: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

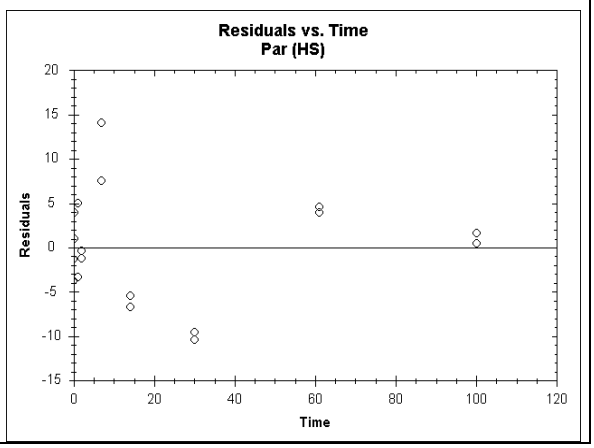
DFOP





HS





[1] t-test not relevant for kinetic parameter β

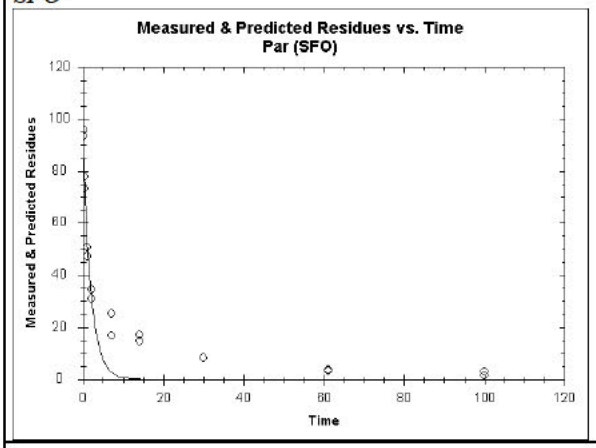
Table 8.2.2.3-90: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

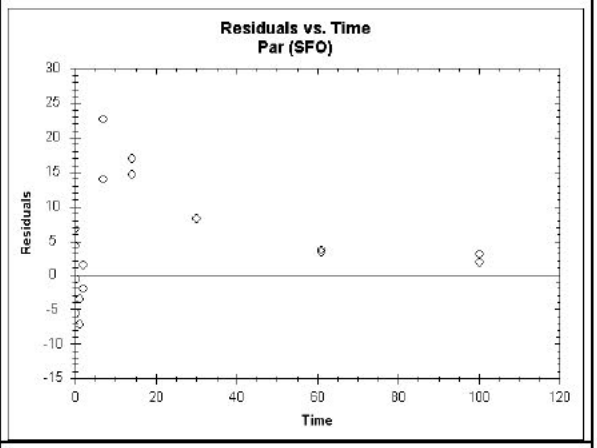
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-90: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

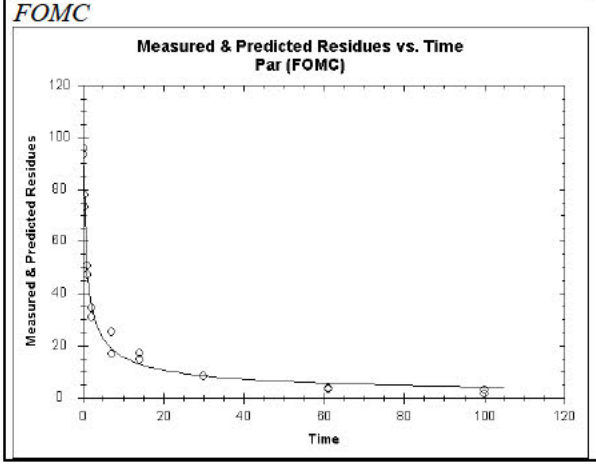
SFO	Poor	89.1	k: 0.4955	21.6	k: <0.001	k: 0.3130	k: 0.6780	1.4	4.6
FOMC	Good	95.3	α: 0.5818 β: 0.4649	4.9	-[1]	β: 0.2784	β: 0.6510	1.1	23.9
DFOP	Good	93.7	k_1: 1.1159 k_2: 0.0373 g: 0.7078	2.6	k_1: <0.001 k_2: <0.001	k_1:0.9006 k_2:0.0243	k_1: 1.3310 k_2: 0.0500	1.1	28.7
HS	Good	92.8	k_1: 0.665 k_2: 0.0518 tb: 1.6	5.3	k_1: <0.001 k_2: <0.001	k_1:0.5627 k_2:0.0353	k_1: 0.7670 k_2: 0.0680	1.0	25.1
Conclusion from notifier	Dissipation of glyphosate is best described by bi-phasic models. All the bi-phasic models provide equally reliable and visually good results but the least χ^2 error is provided by the the DFOP model. DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoints. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



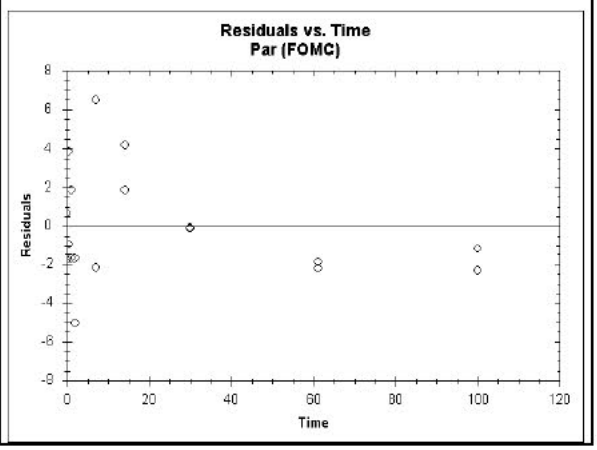
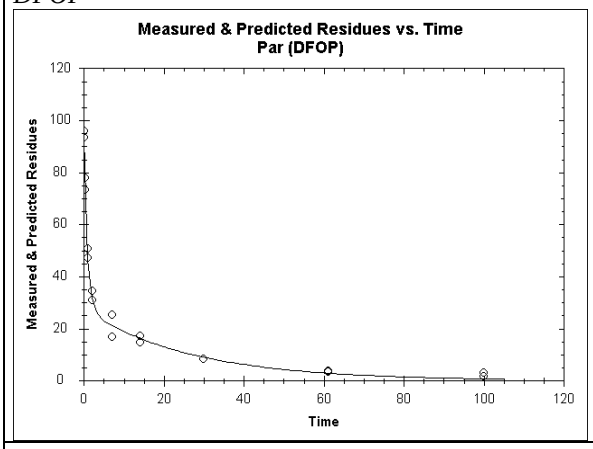
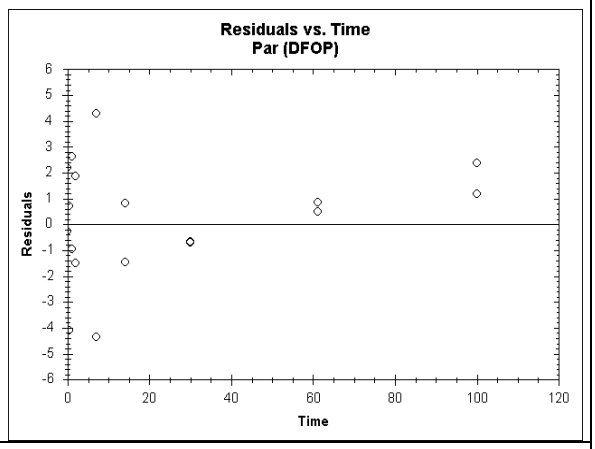


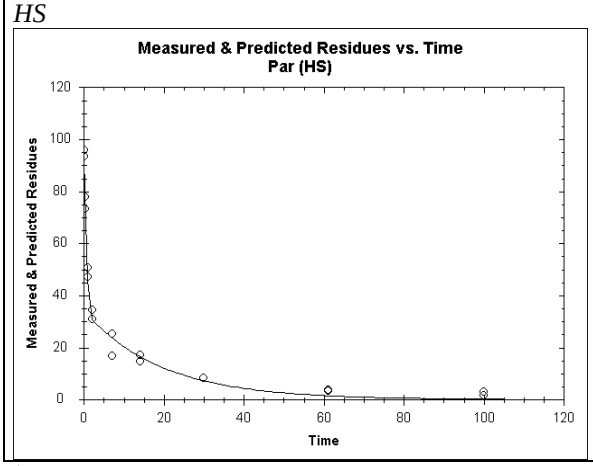
Table 8.2.2.3-90: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

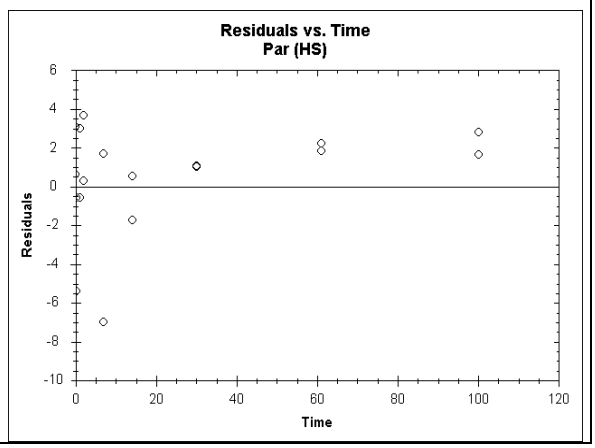
DFOP





HS





[1] t-test not relevant for kinetic parameter β

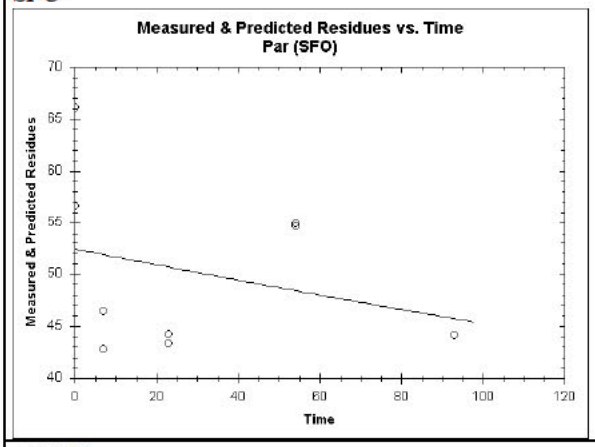
Table 8.2.2.3-91:Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

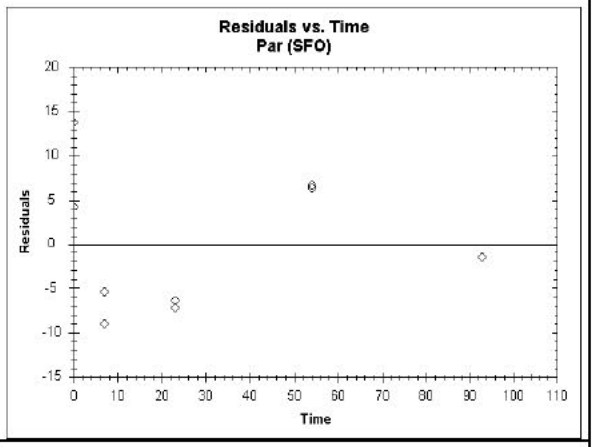
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-91:Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

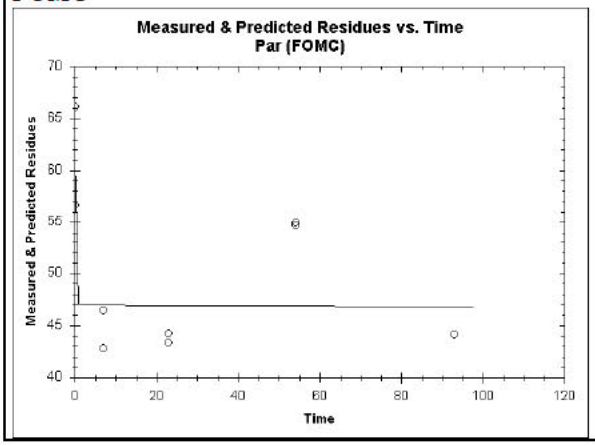
SFO	Poor	52.3	k: 0.0015	10.8	k: 0.181	k: -0.0015	k: 0.0040	473.4	>1000
FOMC	Poor	61.4	α: 0.0012 β: 0	Inf	-[1]	β: NA[2]	β: NA[b]	>1000	>1000
DFOP	Poor	61.4	k_1: 11.3 k_2: 2.22×10^{-14} g: 0.2362	9.5	k_1: 0.5 k_2: 0.5	k_1: NA[2] k_2:-0.0028	k_1: NA[2] k_2: 0.0030	>1000	>1000
HS	Poor	52.3	k_1: 0.0015 k_2: 2.22×10^{-14} tb: 1078	15.4	k_1: 0.218 k_2: <0.001	k_1:-0.0020 k_2: 0.0000	k_1: 0.0050 k_2: 0.0000	473.4	>1000
Conclusion from notifier	No reliable endpoints could be derived.								
Conclusion from RMS	No reliable endpoint can be derived due the scatter of the data. Agrees with notifier conclusion.								

SFO





FOMC



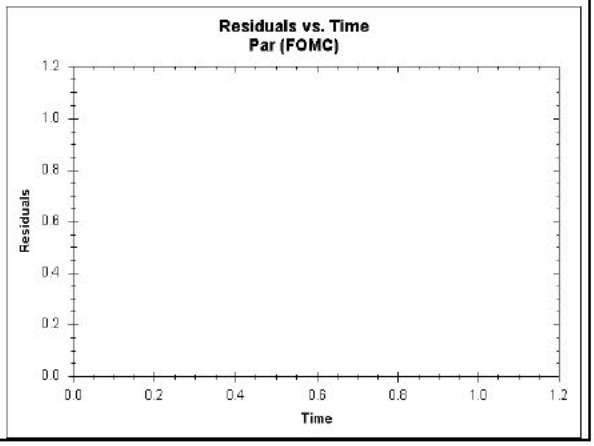
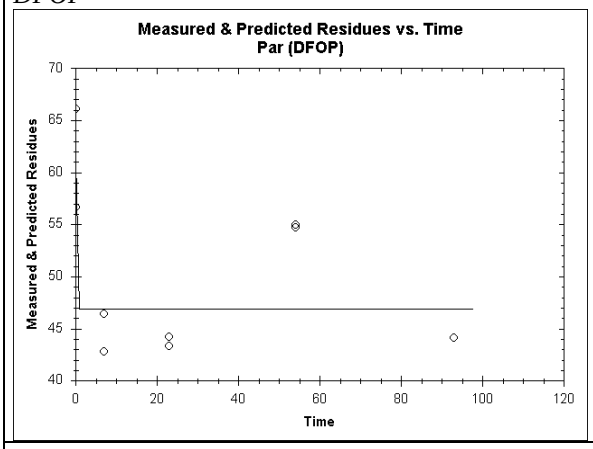
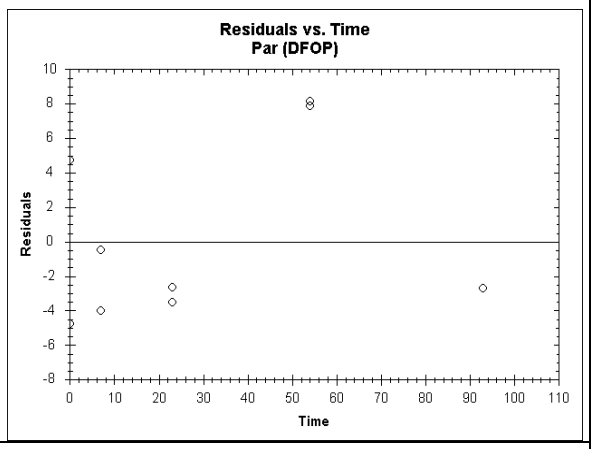


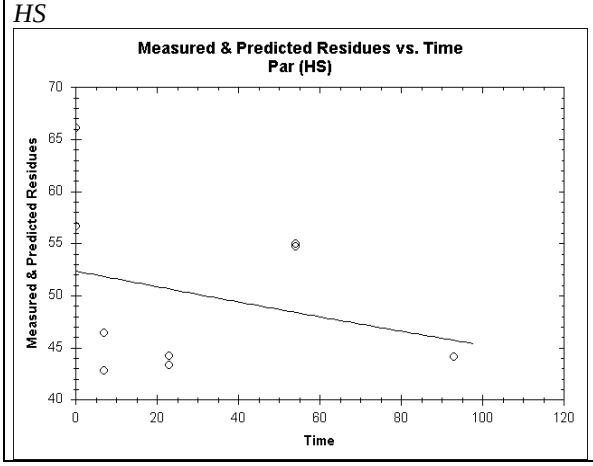
Table 8.2.2.3-91:Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

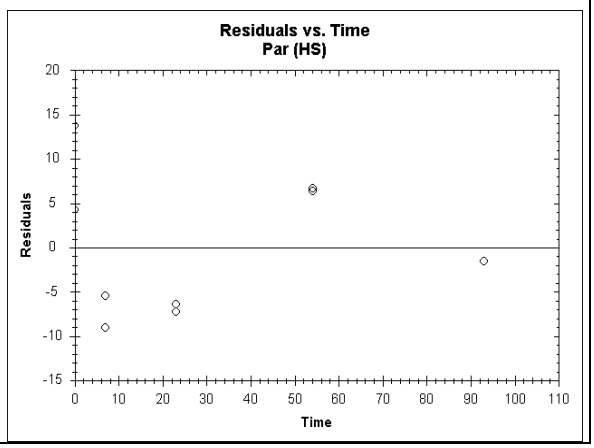
DFOP





HS





Inf = infinite; χ^2 error cannot be calculated

[1] t-test not relevant for kinetic parameter β

[2] Information from the KinGUI output: 'Hessian not invertible – NA was calculated for standard deviation, confidence interval and t-test'

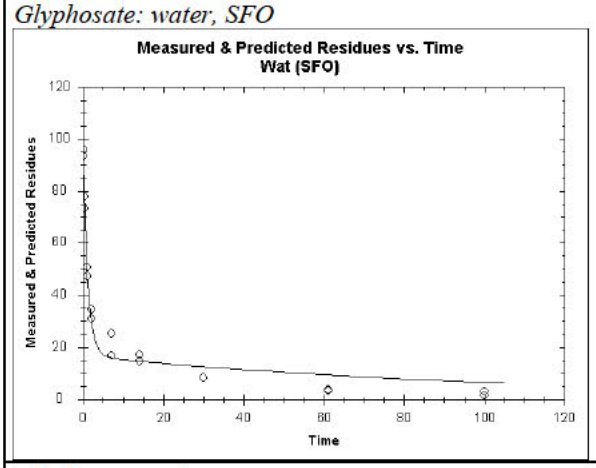
Table 8.2.2.3-92: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

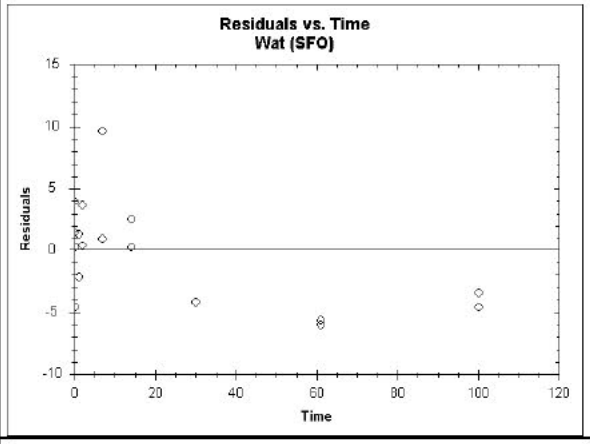
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-92: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

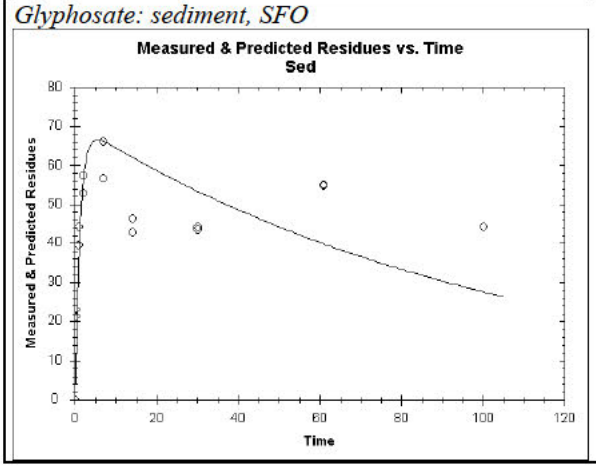
Water: SFO	Acceptable	91.9	k_{wat}: 0.0498 $k_{wat\ sed}$: 0.6299	9.0	k_{wat}: 0.203 $k_{wat\ sed}$: <0.001	k_{wat}: -0.0658 $k_{wat\ sed}$: -0.4942	k_{wat}: 0.165 k_{wat_sed}: 0.766	13.9	46.3
Sediment: SFO	Poor	0.0	k_{sed}: 2.77×10^{-14} $k_{sed\ wat}$: 0.1571	19.5	k_{sed}: 0.5 $k_{sed\ wat}$: <0.001	k_{sed}: -0.0309 $k_{sed\ wat}$: 0.1002	k_{sed}: 0.031 k_{sed_wat}: 0.214	>1000	>1000
Conclusion from notifier	The visual fit obtained for the water phase is acceptable, but the visual fit obtained for the sediment phase is poor. No further evaluation was conducted. No reliable endpoints could be derived								
Conclusion from RMS	Agrees with notifier conclusion.								

Glyphosate: water, SFO





Glyphosate: sediment, SFO



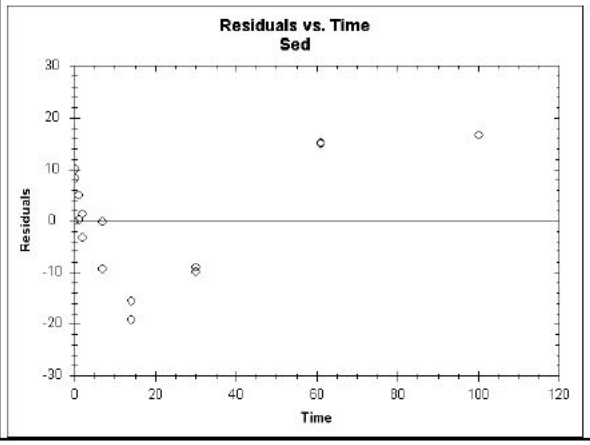
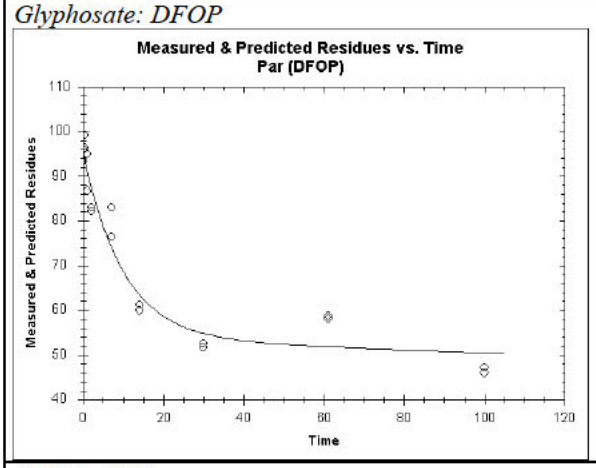


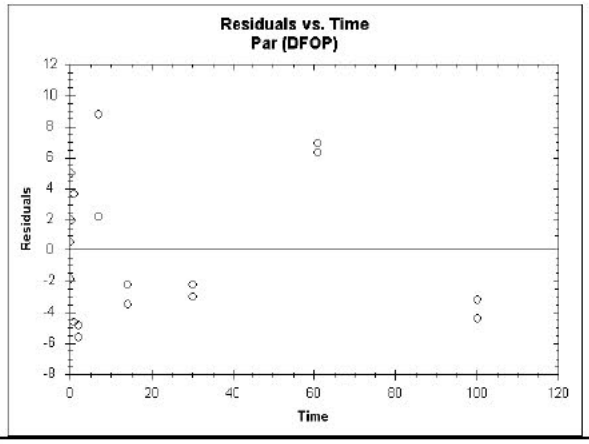
Table 8.2.2.3-93: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)

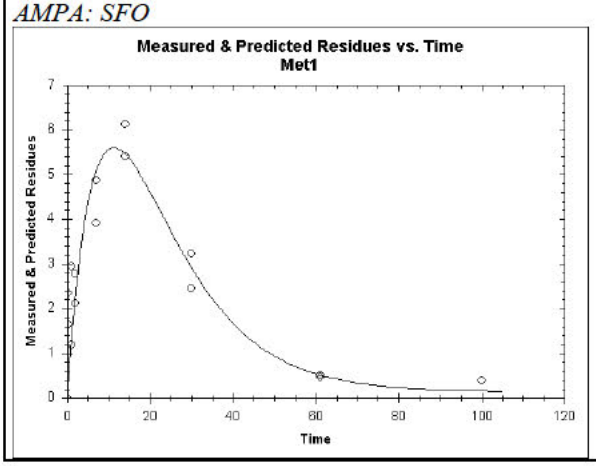
Glyphosate: DFOP	Acceptable	95.4	k_1: 0.1004 k_2: 0.0006 g: 0.4370	4.9	k_1: <0.001 k_2: 0.2986	k_1: 0.0479 k_2: -0.0017	k_1: 0.1530 k_2: 0.0030	187.9	>1000	-
AMPA: SFO	Acceptable	0.0	k: 0.0788	22.4	k: <0.001	k: 0.0329	k: 0.1250	8.8	29.2	0.321 (±0.076) (from parent)
HMPA: SFO	Acceptable	0.0	k: 0.0690	39.3	k: 0.0032	k: 0.0218	k: 0.1160	10.0	33.4	0.359 (±0.159) (from AMPA)
Conclusion from notifier	The fit of glyphosate at level M-I degradation is comparable to that at level P-I total system. For AMPA and HMPA, both the visual and statistical fits from the SFO model are acceptable The χ^2 values above 15 % are acceptable as measured data are overall well represented by the fit. DFOP-SFO to be used for trigger endpoints of AMPA and HMPA DFOP-SFO to be used for modelling endpoints of AMPA and HMPA									
Conclusion from RMS	Agrees with notifier conclusion.									

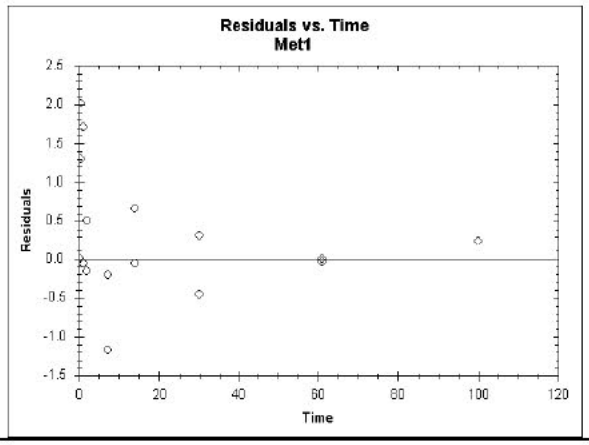
Glyphosate: DFOP



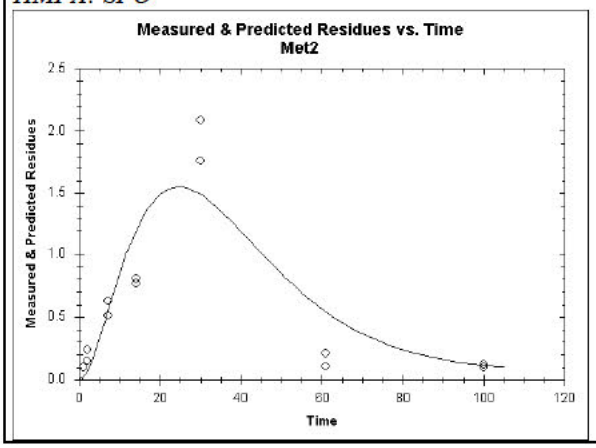


AMPA: SFO





HMPA: SFO



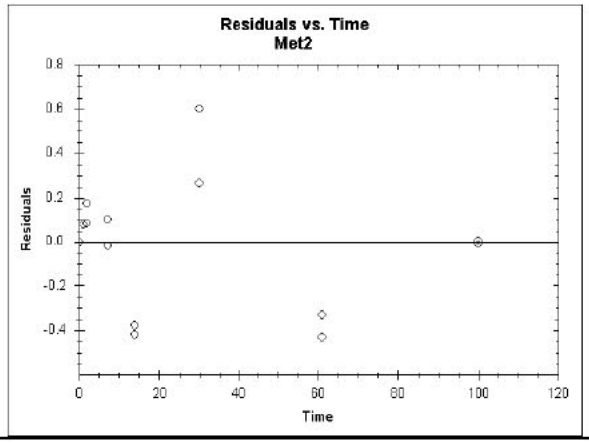
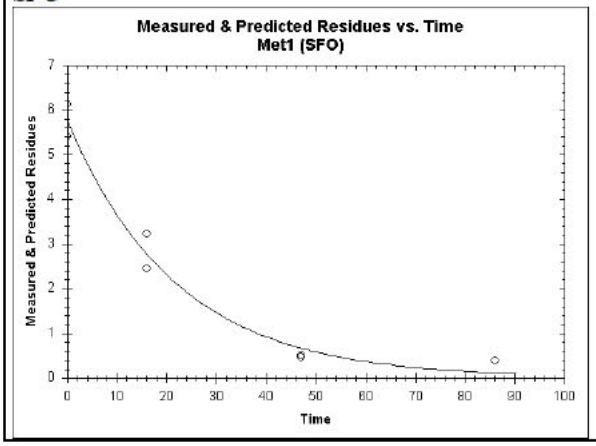


Table 8.2.2.3-94: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	5.8	k: 0.0461	5.8	k: <0.001	k: 0.0361	k: 0.056	15.1	50.0
FOMC	Acceptable	5.8	α: 43.940 β: 940.9	7.2	-[1]	β: -1013	β: 23915	15.0	50.6
Conclusion from notifier	Only the SFO and FOMC model were used for evaluation due to the limited number of data points. The visual and statistical fits from the SFO model are acceptable while the statistical fit of the FOMC model is not reliable as the confidence interval of parameter β includes zero. Thus, the SFO model is selected as the best-fit model as well as for modelling endpoints. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Since reliable endpoints can be derived for AMPA at level M-I degradation, no further kinetic assessment at level M-I dissipation is required for AMPA. In addition, the number of available data points after maximum occurrence (4) is limited.								

SFO



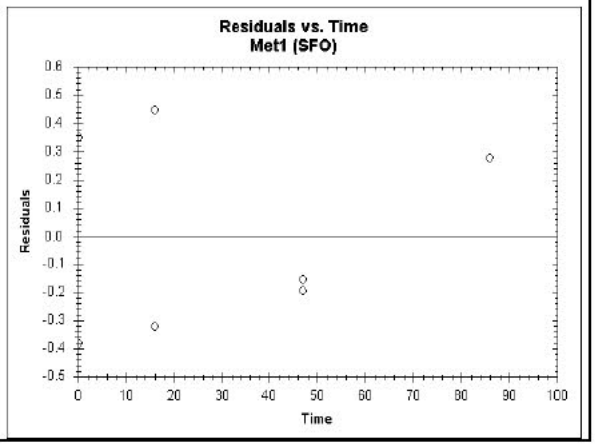
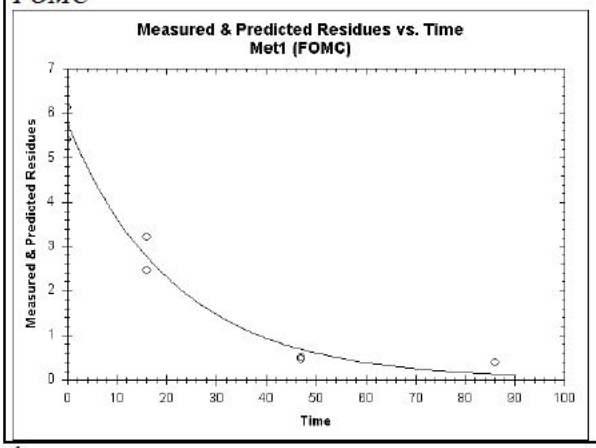
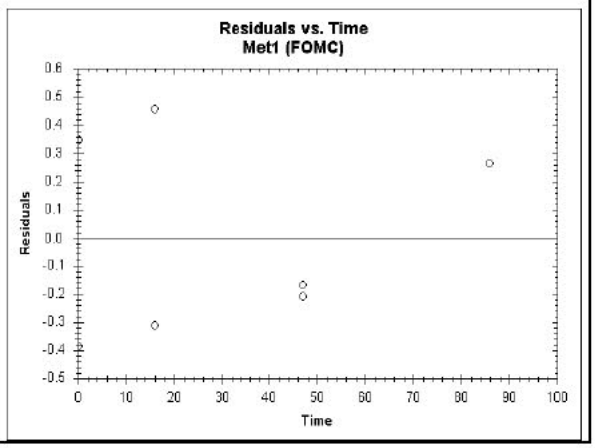


Table 8.2.2.3-94: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

FOMC



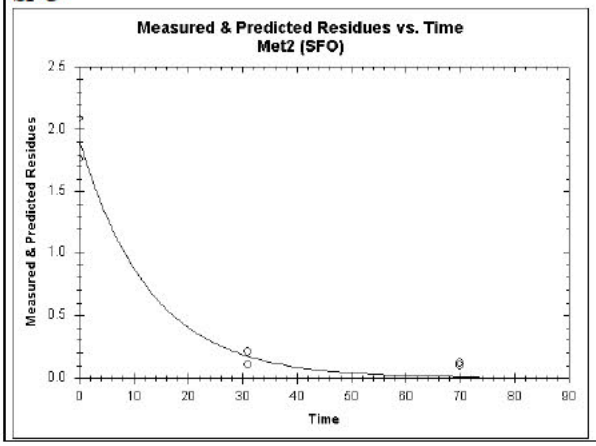


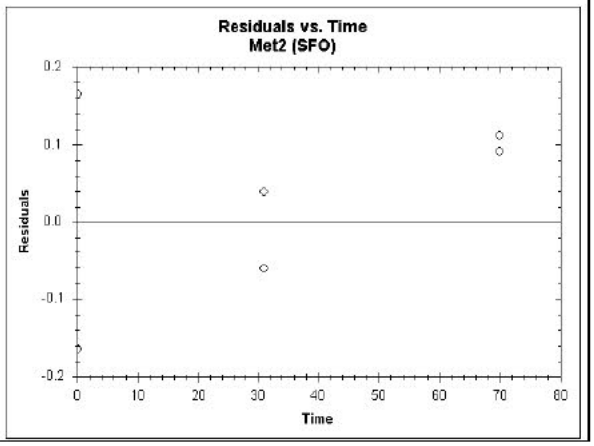
[1] t-test not relevant for kinetic parameter β

Table 8.2.2.3-95:Kinetic models and goodness-of-fit statistics of metabolite HMPA dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	1.9	k: 0.0781	7.1	k: 0.007	k: 0.0410	k: 0.1150	8.9	29.5
Conclusion from notifier	Only the SFO model was used for the evaluation due to the limited number of data points. The visual and statistical fit from the SFO model is acceptable. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Since reliable endpoints can be derived for HMPA at level M-I degradation, no further kinetic assessment at level M-I dissipation is required for HMPA. In addition, the number of available data points after maximum occurrence (3) is limited.								

SFO





[redacted] *(2002, CA 7.2.2.3/020)*

In the water phase of the system Schäphysen, AMPA was not detected in one of the replicates on day 60 while in one replicate each on days 90 and 119 amounts of AMPA were below the LOQ. The residue values were set to half of the individual reported LOQ values on the respective sampling dates (0.48 % AR on day 60, 0.42 % AR on day 90 and 0.39 % AR on day 119).

Table 8.2.2.3-96: Experimental data of AMPA for system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment
0	100.2[1]	100.2[1]	0.0[2]
0	100.4[1]	100.4[1]	0.0[2]
3	68.08	39.27	28.82
3	72.94	46.05	26.90
7	59.42	24.96	34.46
7	59.59	25.39	34.19
14	51.90	19.17	32.73
14	48.41	11.94	36.47
31	39.40	7.36	32.04
31	37.43	8.37	29.05
60	30.67	4.04	26.62
60	31.21	3.27	27.94
89	28.78	0.88	27.91
89	24.38	3.18	21.21
119	19.86	2.06	17.80
119	27.86	0.75	27.10

[1] Values at day 0 were set to material balance according to FOCUS (2014)
[2] Set to zero for evaluation at Level P-II

Table 8.2.2.3-97: Experimental data of AMPA for system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020) used for kinetic evaluation

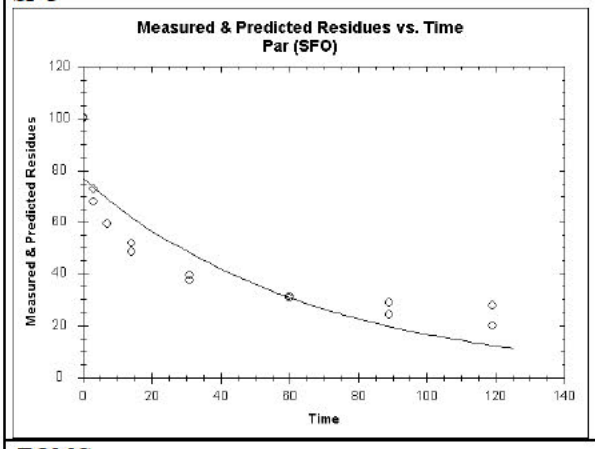
Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment
0	97.4[1]	97.4[1]	0.0[2]
0	99.7[1]	99.7[1]	0.0[2]
3	38.49	18.91	19.58
3	44.67	24.94	19.73
7	35.28	15.80	19.48
7	33.38	13.95	19.43
14	25.16	3.69	21.47
14	26.95	3.46	23.49
31	19.87	0.82	19.04
31	18.65	0.30	18.35
60	27.52	0.24[3]	27.28
60	24.36	0.88	23.48
89	20.75	0.21[4]	20.54
89	19.54	0.36	19.18
119	23.18	0.24	22.94
119	23.75	0.20[5]	23.55

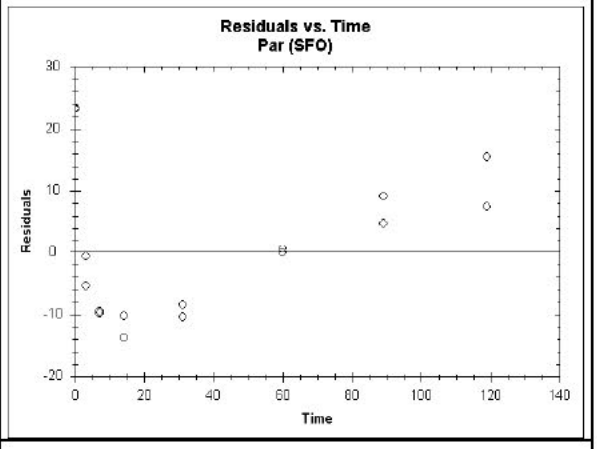
[1] Values at day 0 were set to material balance according to FOCUS (2014)
[2] Set to zero for evaluation at Level P-II
[3] Value was set to ½ LOQ (LOQ at sampling day 60: 0.48 %)
[4] Value was set to ½ LOQ (LOQ at sampling day 89: 0.42 %)
[5] Value was set to ½ LOQ (LOQ at sampling day 119: 0.39 %)

Table 8.2.2.3-98: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

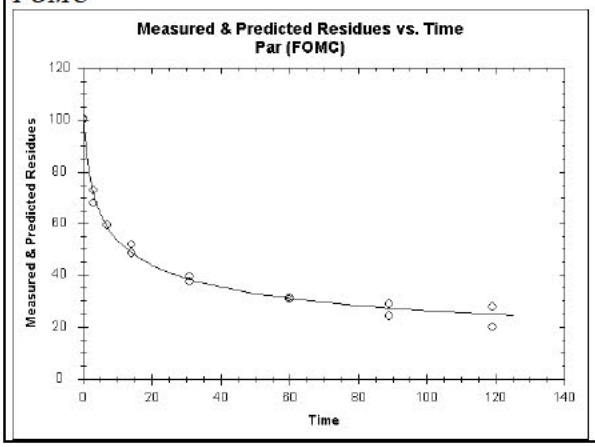
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	77.0	k: 0.0154	18.3	k: <0.001	k: 0.0094	k: 0.0210	45.1	149.8
FOMC	Good	100.0	α: 0.3317 β: 1.7846	1.6	-[1]	β: 1.1339	β: 2.4350	12.6	>1000
DFOP	Good	99.5	k_1: 0.2458 k_2: 0.0073 g: 0.4858	3.8	k_1: <0.001 k_2: <0.001	k_1: 0.1645 k_2: 0.0053	k_1: 0.3270 k_2: 0.0090	11.7	225.3
HS	Acceptable	100.3	k_1: 0.1175 k_2: 0.0091 tb: 5.0	5.8	k_1: <0.001 k_2: <0.001	k_1: 0.0830 k_2: 0.0071	k_1: 0.1520 k_2: 0.0110	16.1	193.0
Conclusion from notifier	Degradation of AMPA is best described by bi-phasic models. The FOMC and the DFOP models provide better visual and statistical fits than the HS model. The FOMC model provides the best visual fit and is selected as the best-fit model. Since 10 % of the initially measured substance concentration was not reached within the experimental period, the DFOP model is selected for deriving modelling endpoints. FOMC to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion.								

SFO





FOMC



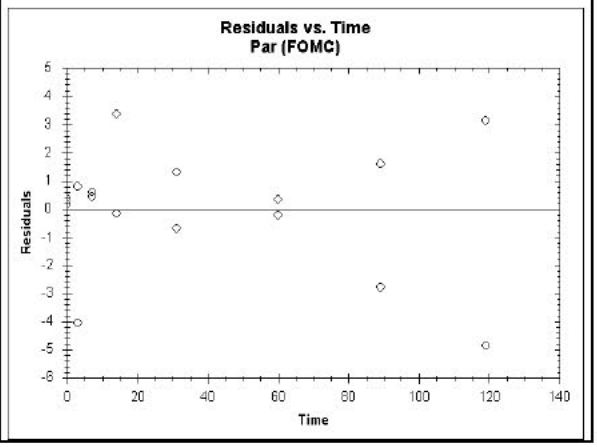
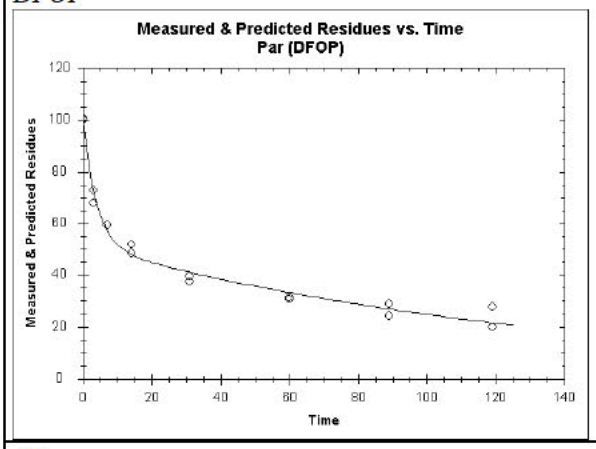
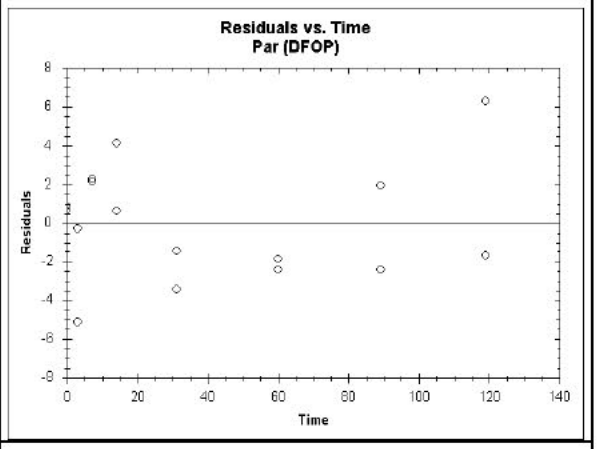


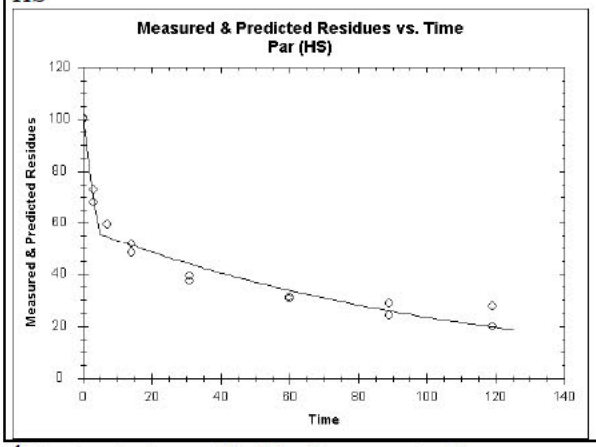
Table 8.2.2.3-98: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

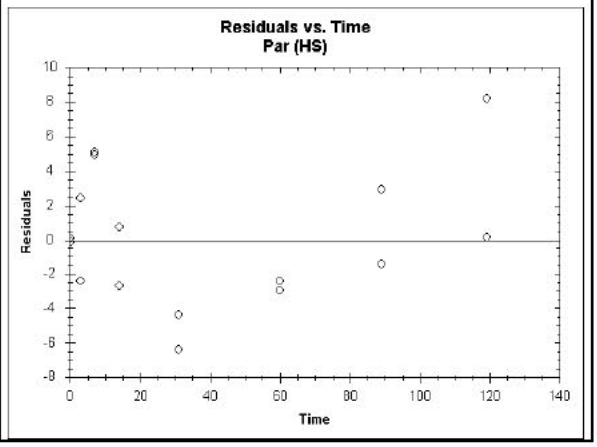
DFOP





HS





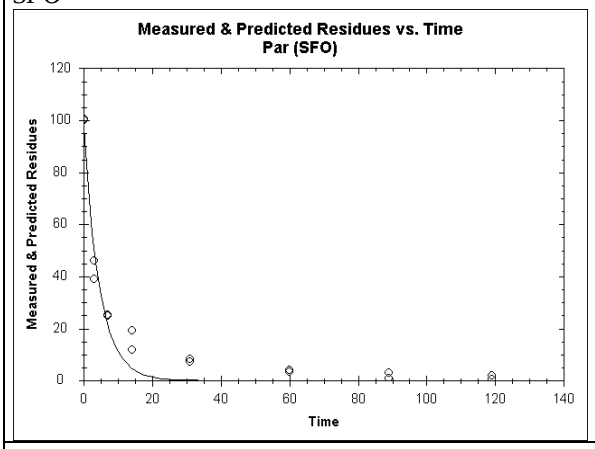
[1] t-test not relevant for kinetic parameter β

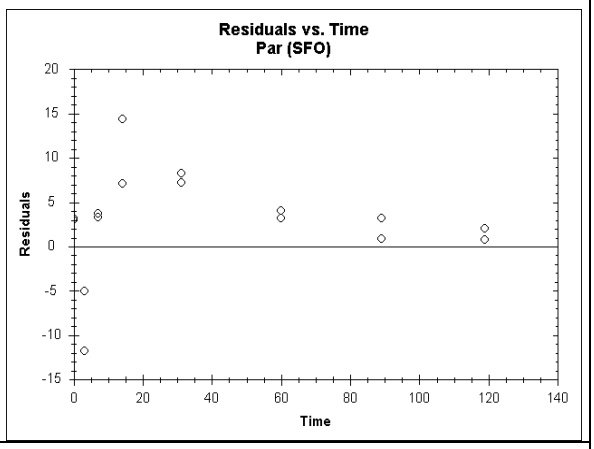
Table 8.2.2.3-99: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	97.2	k: 0.2145	19.2	k: <0.001	k: 0.1685	k: 0.2610	3.2	10.7
FOMC	Good	100.3	α: 0.9301 β: 2.0284	2.1	-[1]	β: 1.2818	β: 2.7750	2.2	22.1
DFOP	Good	100.2	k_1: 0.4512 k_2: 0.0365 g: 0.7311	3.0	k_1: <0.001 k_2: <0.001	k_1: 0.3505 k_2: 0.0221	k_1: 0.5520 k_2: 0.0510	2.4	27.1
HS	Good	100.3	k_1: 0.285 k_2: 0.0455 tb: 4.7	4.3	k_1: <0.001 k_2: <0.001	k_1: 0.2555 k_2: 0.0301	k_1: 0.3140 k_2: 0.0610	2.4	26.1
Conclusion from notifier	Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually acceptable results but the FOMC model provides the least χ^2 error and is selected as best-fit model as well as for modelling endpoints. FOMC to be used for trigger endpoints FOMC to be used for modelling endpoints								
Conclusion from RMS	Agrees with the notifier conclusion for trigger endpoints. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

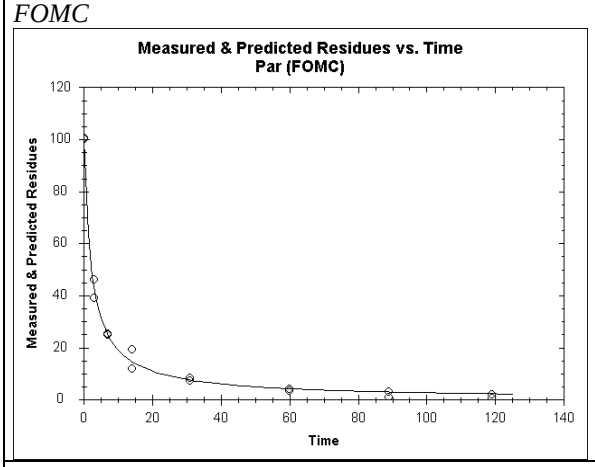
Table 8.2.2.3-99: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

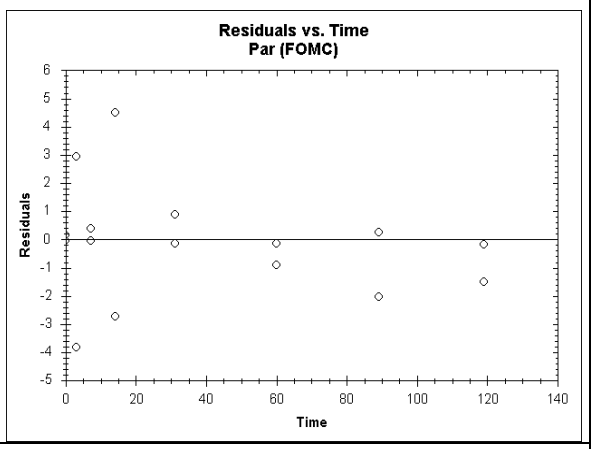
SFO



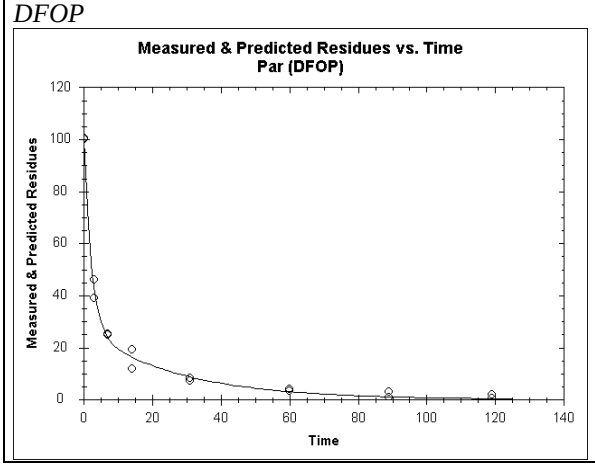


FOMC





DFOP



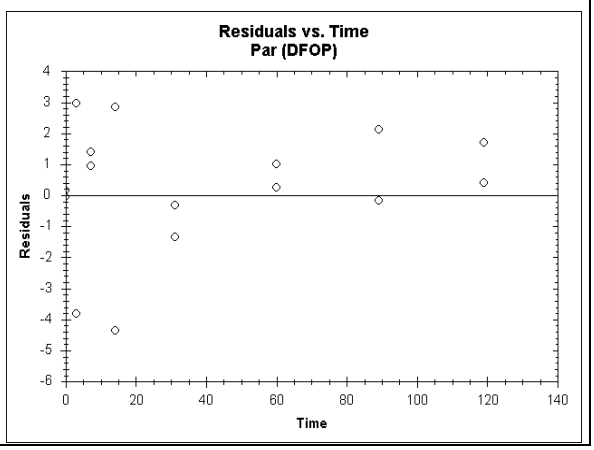
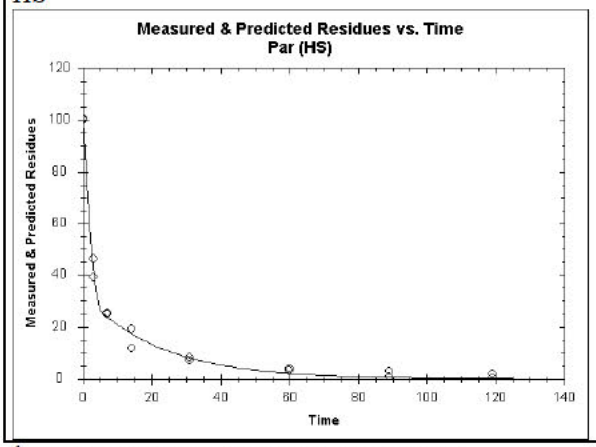
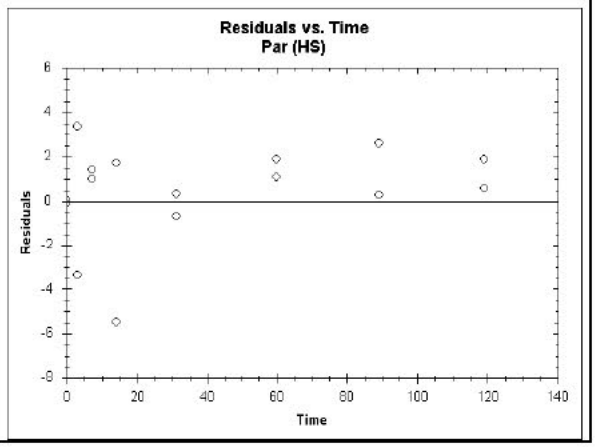


Table 8.2.2.3-99: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

HS



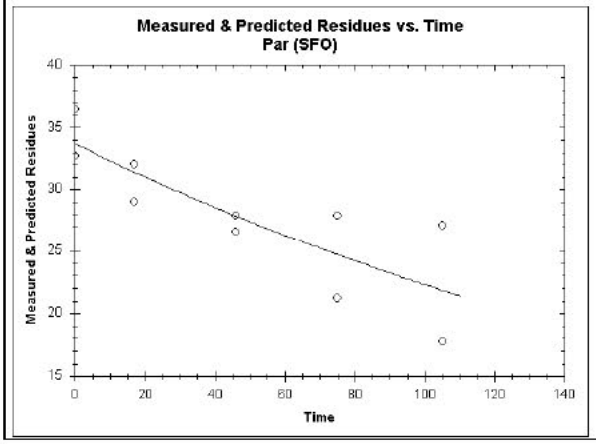


[1] t-test not relevant for kinetic parameter β

Table 8.2.2.3-100: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Good	33.7	k: 0.0041	1.9	k: 0.002	k: 0.0022	k: 0.0060	168.1	558.3
FOMC	Good	34.5	α: 0.3612 β: 47.3107	0.7	-[1]	β: -194.89	β: 289.52	275.1	>1000
DFOP	Good	34.6	k_1: 0.0843 k_2: 0.0033 g: 0.0888	0.3	k_1: 0.427 k_2: 0.174	k_1: -0.7781 k_2: -0.0030	k_1: 0.9470 k_2: 0.0100	184.0	677.5
HS	Good	34.6	k_1: 0.0073 k_2: 0.0033 tb: 21.8	0.3	k_1: 0.153 k_2: 0.115	k_1: -0.0055 k_2: -0.0016	k_1: 0.0200 k_2: 0.0080	182.4	666.9
Conclusion from notifier	All models provide good visual fits but the statistical fits provided by the bi-phasic models are not reliable. Thus, the SFO model is selected as the best-fit model as well as for deriving modelling endpoints. SFO to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoints. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in sediment (please refer to RMS comments).								

SFO



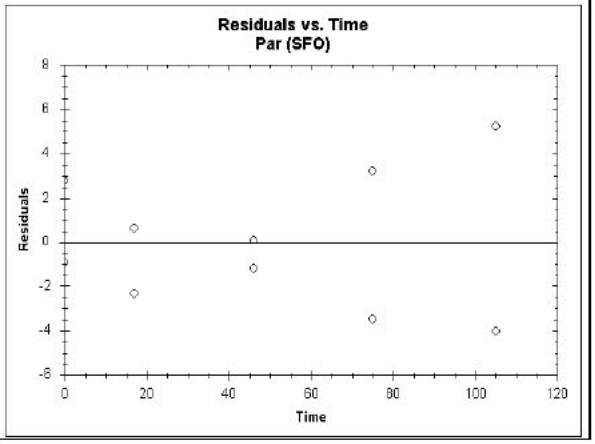
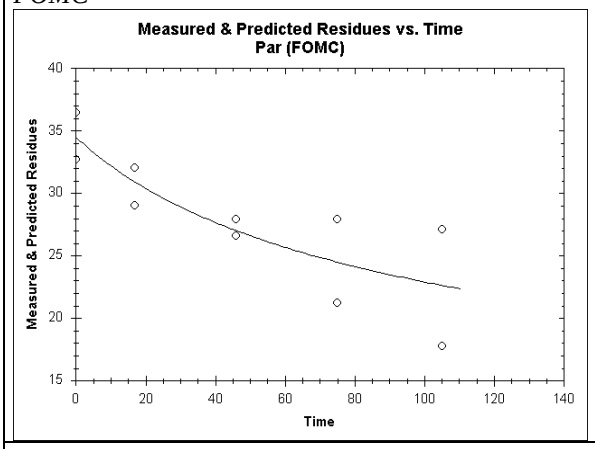
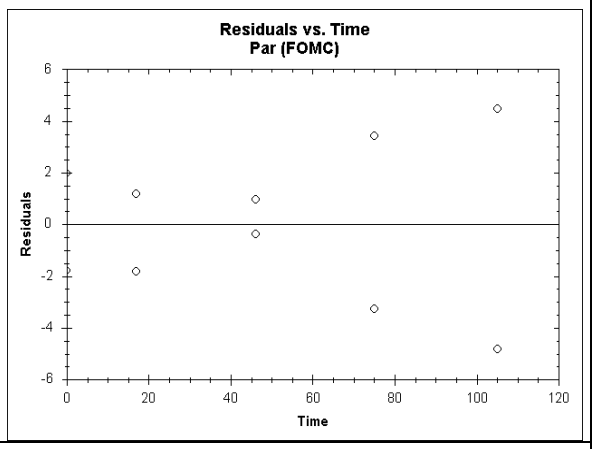


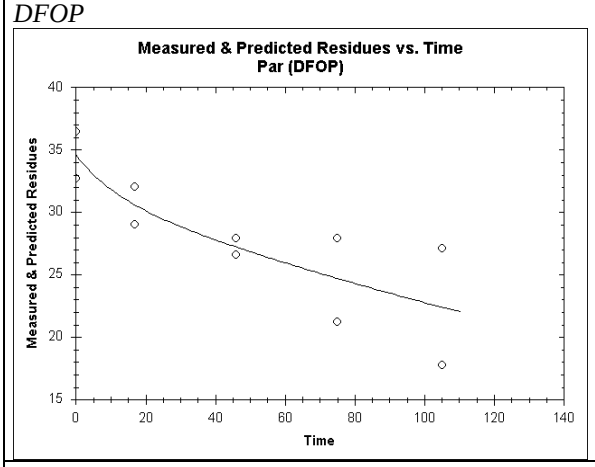
Table 8.2.2.3-100: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

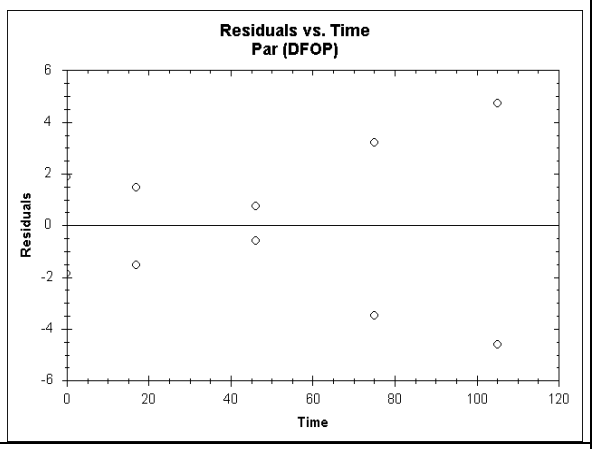
FOMC



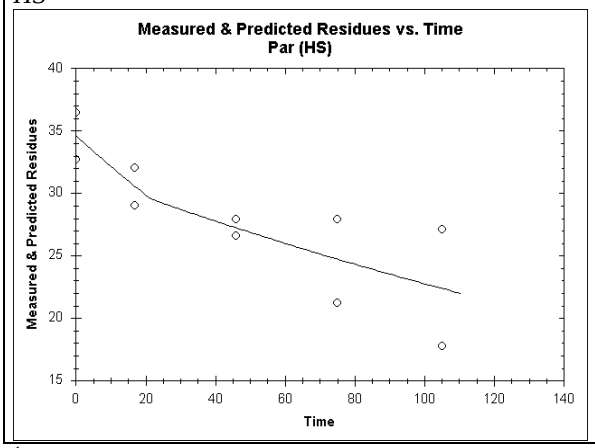


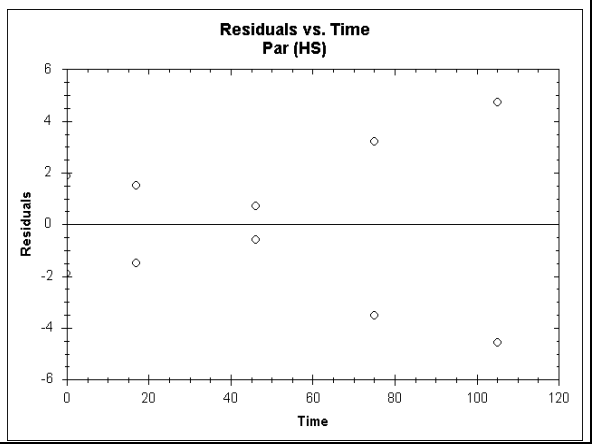
DFOP





HS





[1] t-test not relevant for kinetic parameter β

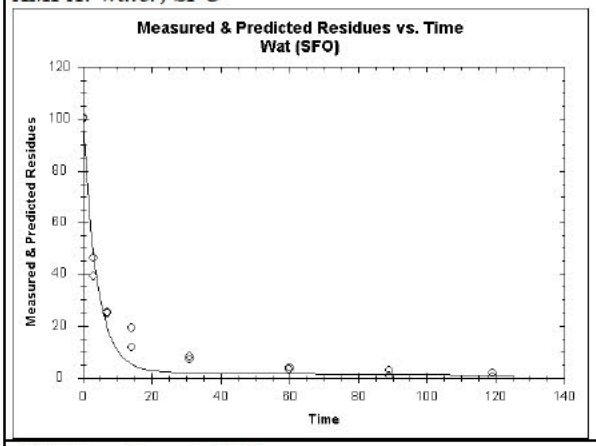
Table 8.2.2.3-101: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-II

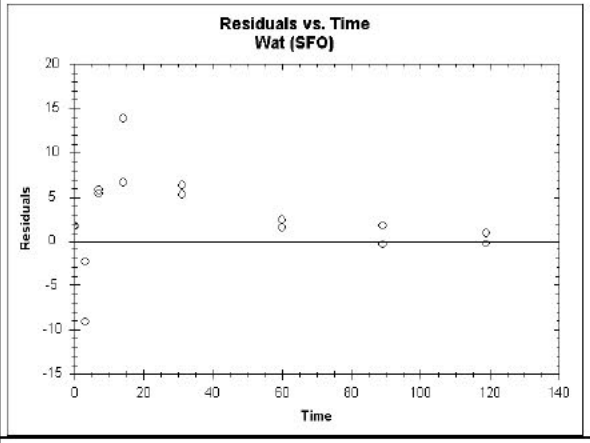
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-101: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-II

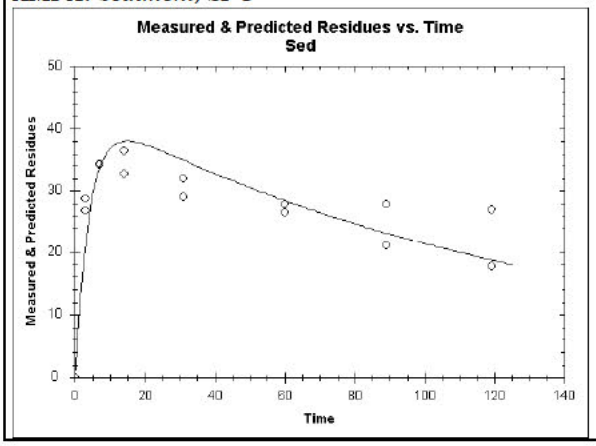
Water: SFO	Poor	98.5	k_{wat}: 0.1343 $k_{wat\ sed}$: 0.1052	17.6	k_{wat}: <0.001 $k_{wat\ sed}$: <0.001	k_{wat}: 0.0996 $k_{wat\ sed}$: -0.0857	k_{wat}: 0.169 k_{wat_sed}: 0.125	5.1	17.2
Sediment: SFO	Acceptable	0.0	k_{sed}: 2.34×10^{-14} $k_{sed\ wat}$: 0.0129	9.6	k_{sed}: 0.5 k_{sed_wat}: 0.199	k_{sed}: -0.0169 $k_{sed\ wat}$: -0.0166	k_{sed}: 0.017 k_{sed_wat}: 0.042	>1000	>1000
Conclusion from notifier	Although the visual fit obtained for the sediment phase is acceptable, the visual fit obtained for the water phase is poor. No further evaluation was conducted, no reliable endpoints could be derived.								
Conclusion from RMS	Agrees with notifier conclusion.								

AMPA: water, SFO





AMPA: sediment, SFO



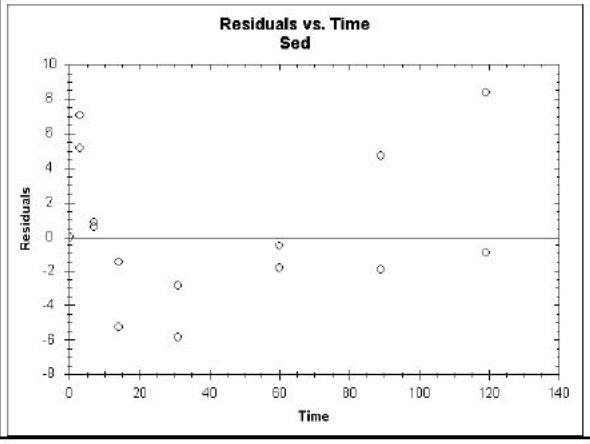


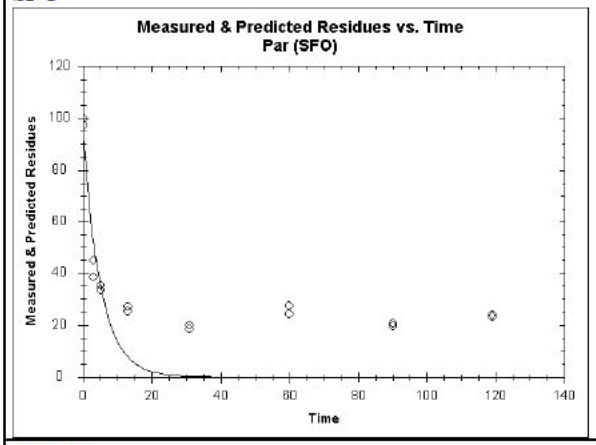
Table 8.2.2.3-102: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

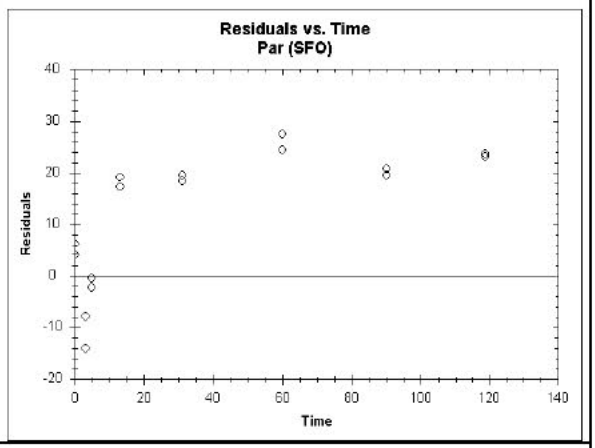
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-102: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

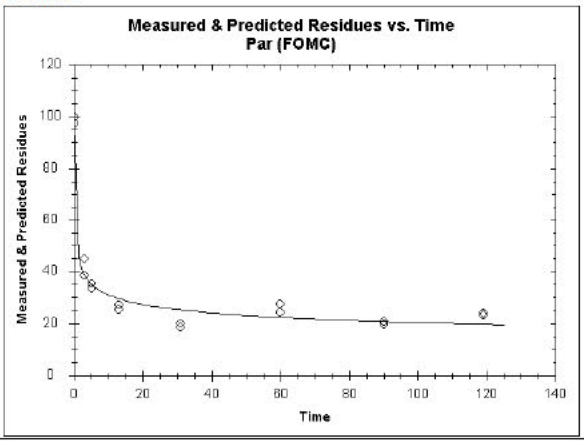
SFO	Poor	93.4	k: 0.1919	38.8	k: 0.003	k: 0.0730	k: 0.3110	3.6	12.0
FOMC	Acceptable	98.6	α: 0.1836 β: 0.0179	7.6	-[1]	β: -0.0196	β: 0.0550	0.8	>1000
DFOP	Good	98.4	k_1: 0.4522 k_2: 0.0008 g: 0.7532	6.2	k_1: <0.001 k_2: 0.253	k_1: 0.3606 k_2: -0.0015	k_1: 0.5440 k_2: 0.0030	2.4	>1000
HS	Acceptable	97.5	k_1: 0.2427 k_2: 0.0006 tb: 5.8	8.9	k_1: <0.001 k_2: 0.364	k_1: 0.2085 k_2: -0.0025	k_1: 0.2770 k_2: 0.0040	2.9	>1000
Conclusion from notifier	Degradation of AMPA is best described by bi-phasic models. The FOMC and HS models provide visually acceptable fits while the DFOP model provides a good fit. However, the resulting parameters from the models are not statistically reliable. Nevertheless, the DFOP model provides more realistic estimates of the DT_{50} and DT_{90} values as well as a smaller χ^2 error. Thus, the DFOP model is selected as the best-fit model. DFOP to be used for trigger endpoints 1000 d to be used for modelling as conservative approach								
Conclusion from RMS	Agrees with notifier conclusion.								

SFO





FOMC



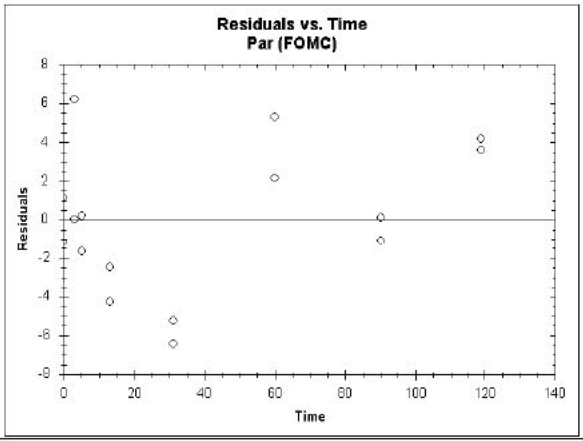
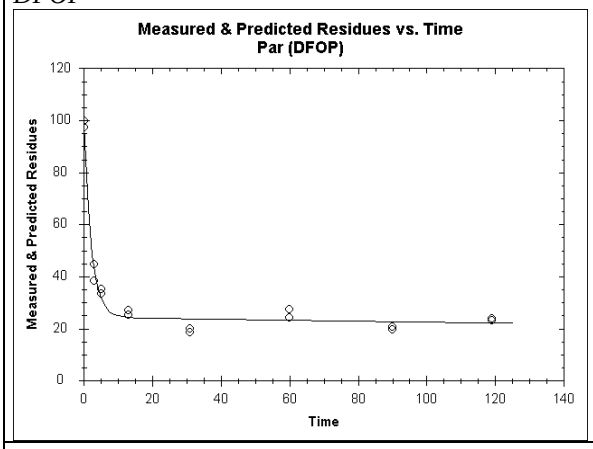
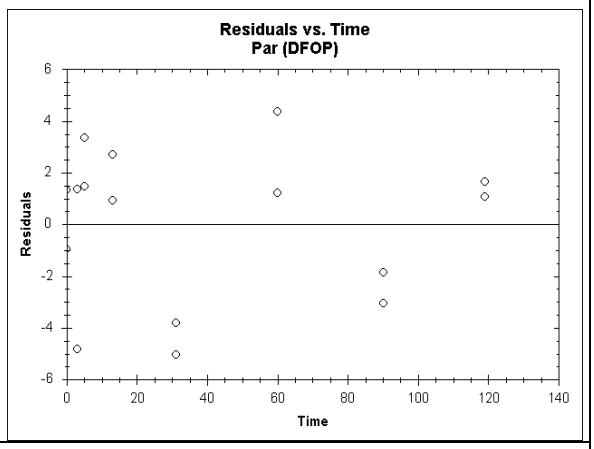


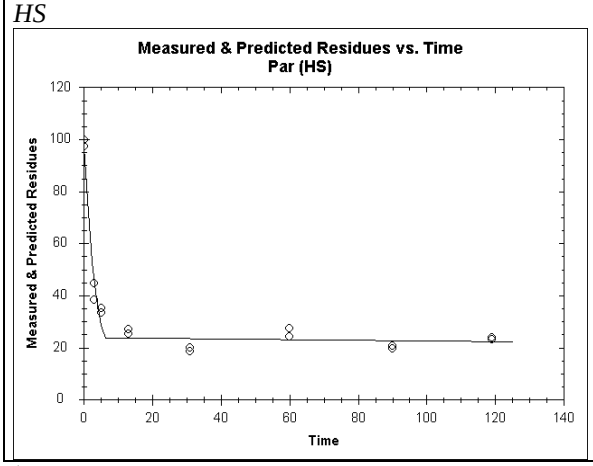
Table 8.2.2.3-102: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

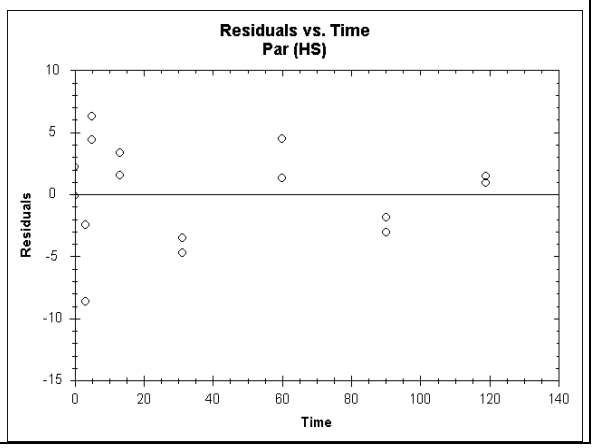
DFOP





HS





[1] t-test not relevant for kinetic parameter β

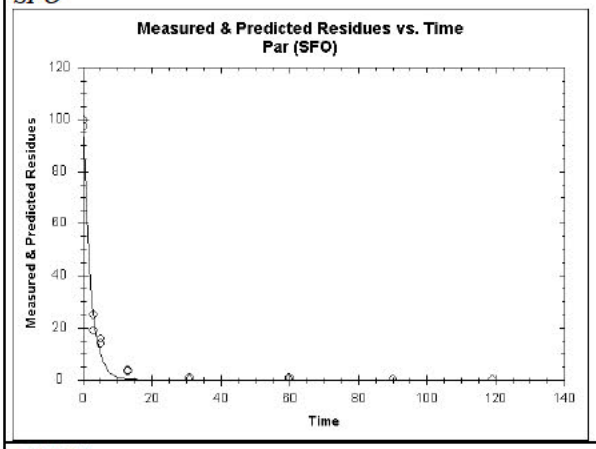
Table 8.2.2.3-103: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

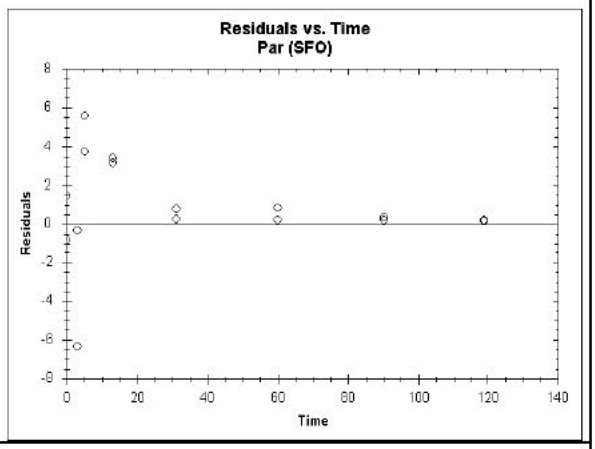
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-103: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

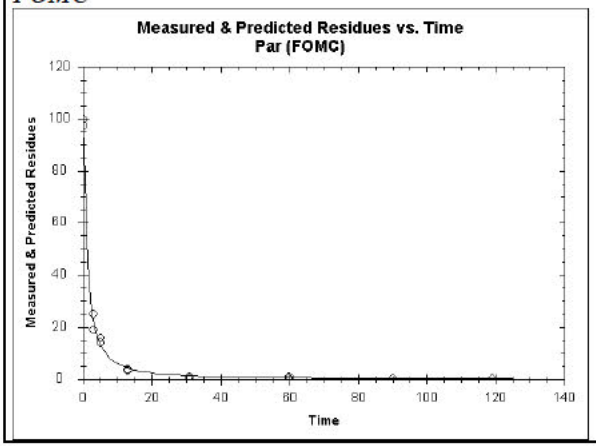
SFO	Acceptable	98.2	k: 0.4530	10.7	k: <0.001	k: 0.4085	k: 0.4970	1.5	5.1
FOMC	Good	98.5	α: 1.4858 β: 1.7767	3.2	-[1]	β: 0.6957	β: 2.8580	1.1	6.6
DFOP	Good	98.5	k_1: 1.4236 k_2: 0.1757 g: 0.6383	1.4	k_1:0.110 k_2: <0.001	k_1: -0.7326 k_2: 0.1033	k_1: 3.5800 k_2: 0.2480	0.9	7.3
HS	Good	98.2	k_1: 0.4546 k_2: 0.0943 tb: 5.8	10.7	k_1: <0.001 k_2: 0.289	k_1: 0.4114 k_2: -0.2286	k_1: 0.4980 k_2: 0.4170	1.5	5.1
Conclusion from notifier	Although the visual fit of the SFO model is acceptable, the dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide equally good visual fits but the statistical parameters resulting from the DFOP and HS models do not indicate reliable fits. Thus, the FOMC model is selected as the best fit model. FOMC to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	For trigger endpoints, RMS notes that most of the degradation is well described by SFO model. In addition, the endpoints between SFO (1.5/5.1 d) and FOMC (1.1/6.6 d) are very close. RMS proposes that SFO is used for trigger endpoints. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



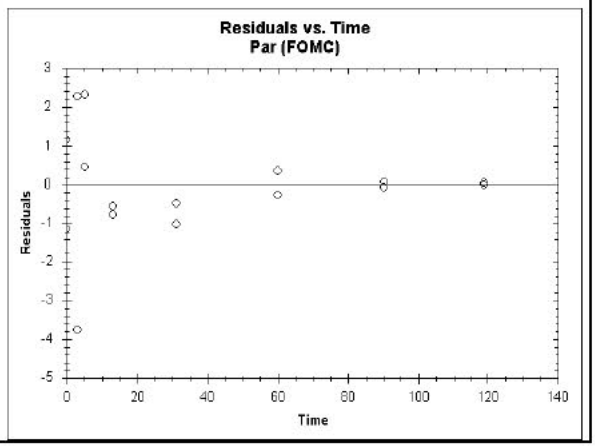
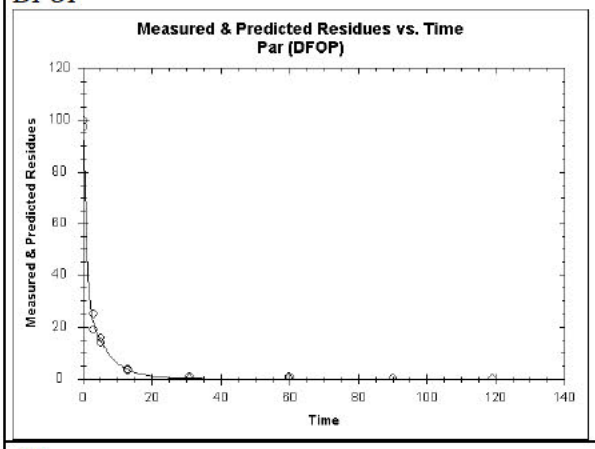
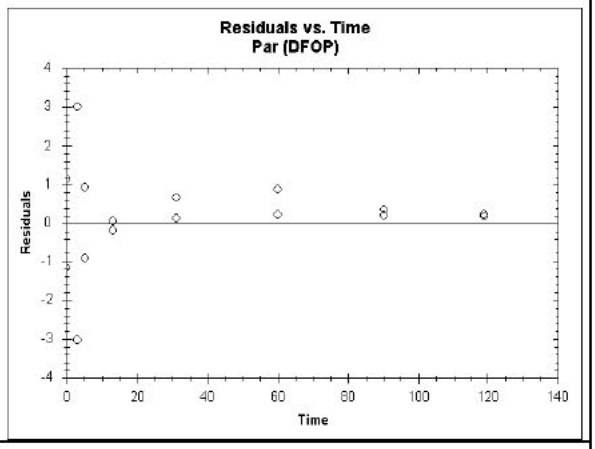


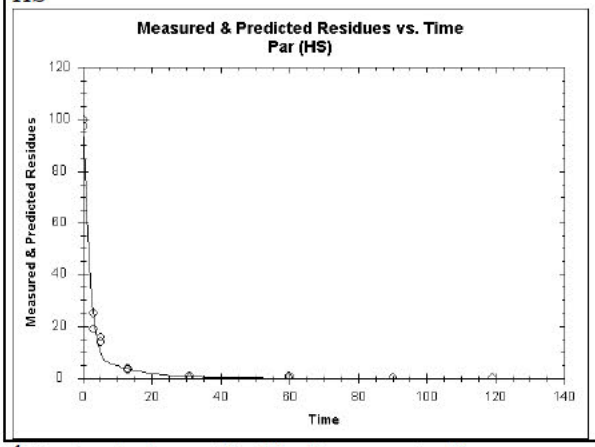
Table 8.2.2.3-103: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

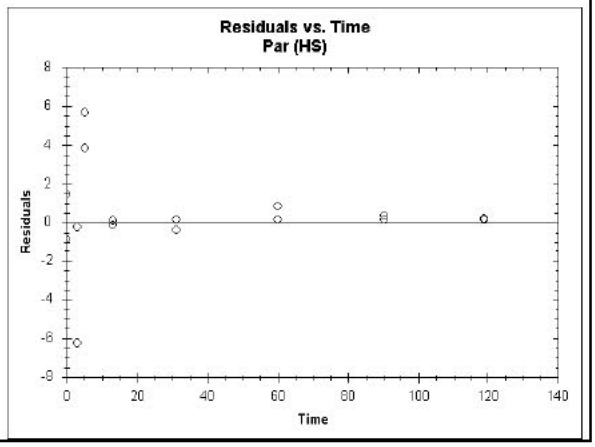
DFOP





HS





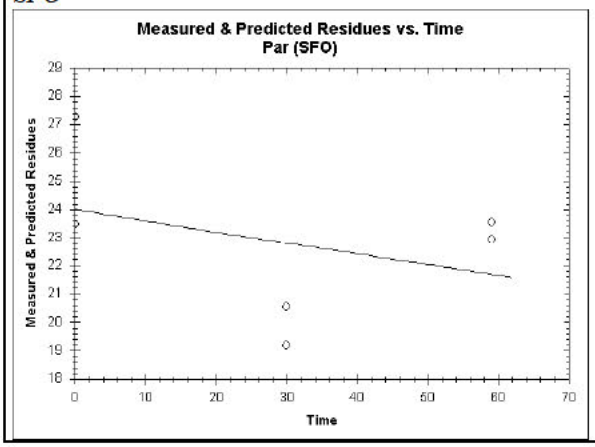
[1] t-test not relevant for kinetic parameter β

Table 8.2.2.3-104: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	24.0	k: 0.0017	8.0	k: 0.235	k: -0.0025	k: 0.0060	399.8	>1000
Conclusion from notifer	Only the SFO model was used for the evaluation due to the number of available data points. The model did not provide an acceptable fit. No reliable endpoints could be derived.								
Conclusion from RMS	Agrees with notifier conclusion.								

Table 8.2.2.3-104: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

SFO



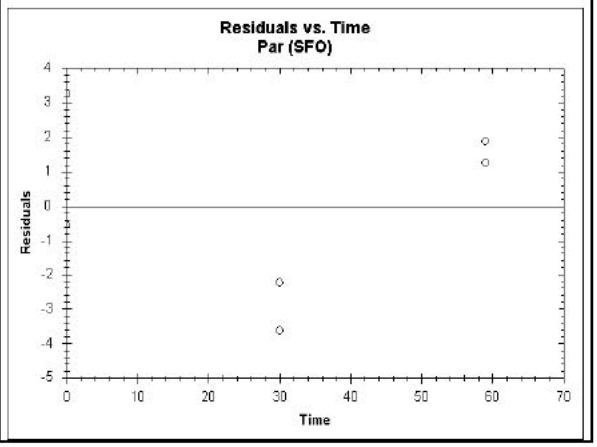
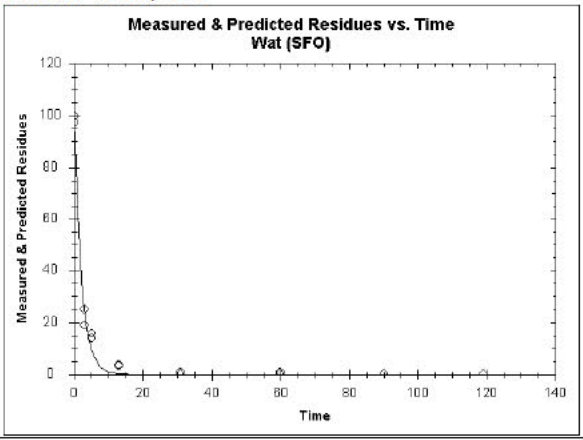


Table 8.2.2.3-105: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-II

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Acceptable	98.3	k_{wat}: 0.3546 $k_{wat\ sed}$: 0.1050	11.4	k_{wat}: <0.001 $k_{wat\ sed}$: <0.001	k_{wat}: 0.3149 $k_{wat\ sed}$: -0.0910	k_{wat}: 0.394 k_{wat_sed}: 0.119	2.0	6.5
Sediment: SFO	Acceptable	0.0	k_{sed}: 0.0000 $k_{sed\ wat}$: 0.0002	8.8	k_{sed}: 0.5 $k_{sed\ wat}$: 0.495	k_{sed}: -0.0276 $k_{sed\ wat}$: -0.0354	k_{sed}: 0.028 $k_{sed\ wat}$: 0.036	>1000	>1000
Conclusion from notifier	The visual and statistical fits obtained for the water phase are reliable but the visual fit obtained for the sediment phase is poor. No reliable endpoints could be derived.								
Conclusion from RMS	Agrres with notifier conclusion.								

AMPA: water, SFO



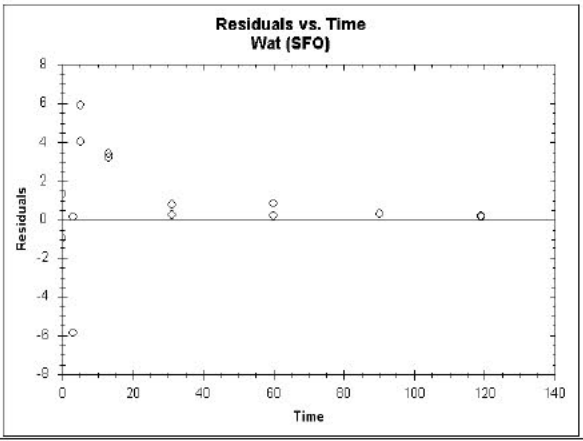
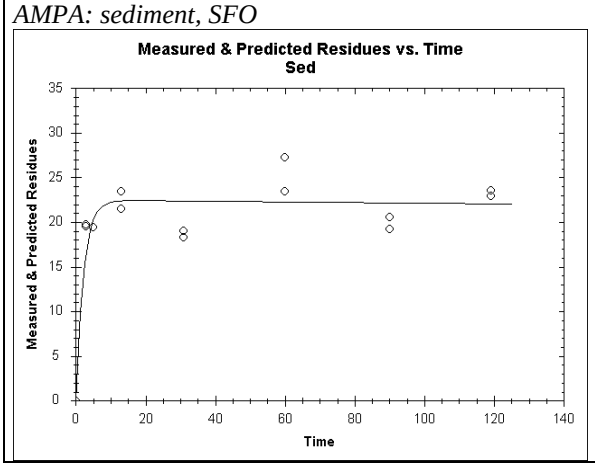
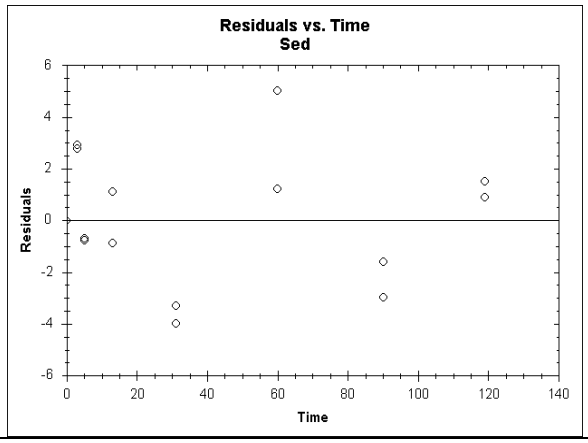


Table 8.2.2.3-105: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-II

AMPA: sediment, SFO





[redacted] *(2003, CA 7.2.2.3/019)*

In the report document available for the evaluation, the individual results of HPLC analysis for water and sediment phase were missing. Thus, the evaluation could only be based on results of TLC analysis. The missing data led to inconsistencies in the reporting of the amounts of AMPA in sediment extracts in the text of the study report compared to tabulated results from TLC analysis. Therefore, no kinetic evaluation was performed for the sediment phase as well as the total system of both systems and only a kinetic evaluation for the water phase is included in the current assessment.

A complete report document including the results of HPLC analysis was received after completion of the kinetic evaluation. The complete data may be used to update the evaluation at a later time point.

Table 8.2.2.3-106: Experimental data of AMPA for system Bickenbach of study [redacted] (2003, CA 7.2.2.3/019) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)[1]
	Water
0	97.3[2]
0	101.7[2]
0.25	87.8
0.25	87.4
1	63.9
1	62.5
2	58.5
2	59.5
7	27.9
7	28.6
14	16.7
14	15.4
30	9.7
30	13.8
62	7.6
62	7.6
104	4.7
104	5.8

[1] The data of the sediment phase and the total system were not considered in the kinetic evaluation

[2] Values at day 0 were set to material balance according to FOCUS (2014)

Table 8.2.2.3-107: Experimental data of AMPA for system Unter Widdersheim of study [redacted] (2003, CA 7.2.2.3/019) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)[1]
	Water
0	100.7[2]
0	102.4[2]
0.25	80.6
0.25	82.1
1	60.2
1	63.1
2	52.7
2	54.4
7	28.9
7	32.0
14	12.2
14	14.4
30	7.2
30	6.8
62	1.3
62	1.5
104	1.8
104	0.8

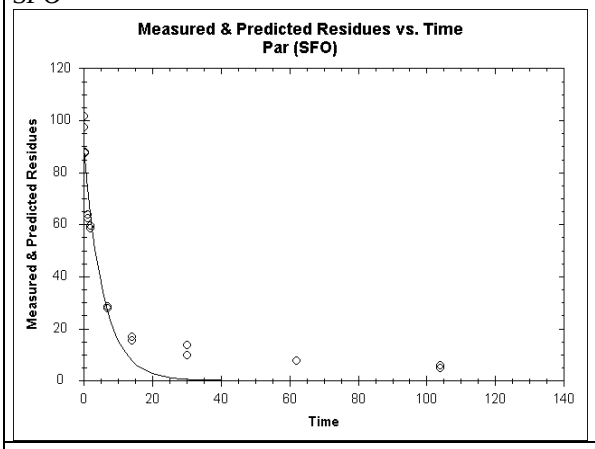
[1] Due to inconsistencies of the reported residues in sediment phase in the study report, the data of the sediment phase and the total system were not considered in the kinetic evaluation
[2] Values at day 0 were set to material balance according to FOCUS (2014)

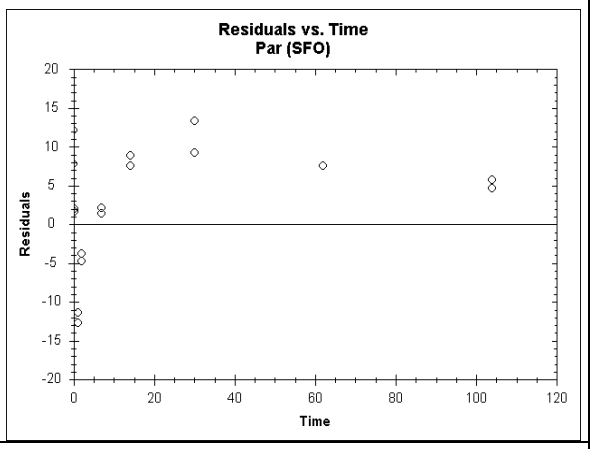
Table 8.2.2.3-108: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	89.5	k: 0.1741	14.9	k: <0.001	k: 0.1229	k: 0.2250	4.0	13.2
FOMC	Good	98.5	α: 0.6972 β: 1.418	5.3	-[1]	β: 0.8552	β: 1.9810	2.4	37.1
DFOP	Good	95.9	k_1: 0.4238 k_2: 0.02 g: 0.7434	7.8	k_1: <0.001 k_2: 0.005	k_1: 0.2998 k_2: 0.0069	k_1: 0.5480 k_2: 0.0330	2.5	47.1
HS	Good	91.5	k_1: 0.2066 k_2: 0.0137 tb: 8.2	11.1	k_1: <0.001 k_2: 0.068	k_1: 0.1594 k_2: -0.0032	k_1: 0.2540 k_2: 0.0310	3.4	52.4
Conclusion from notifier	Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually acceptable results but the FOMC model provides the least χ^2 error and is selected as best-fit model as well as for modelling endpoints. FOMC to be used for trigger endpoints FOMC to be used for modelling endpoints								
Conclusion from RMS	Waiting for the assessment based on HPLC results, RMS agrees with the applicant for trigger endpoints. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

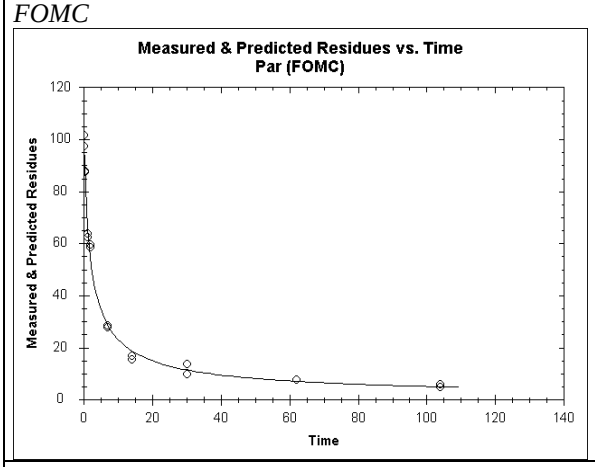
Table 8.2.2.3-108: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

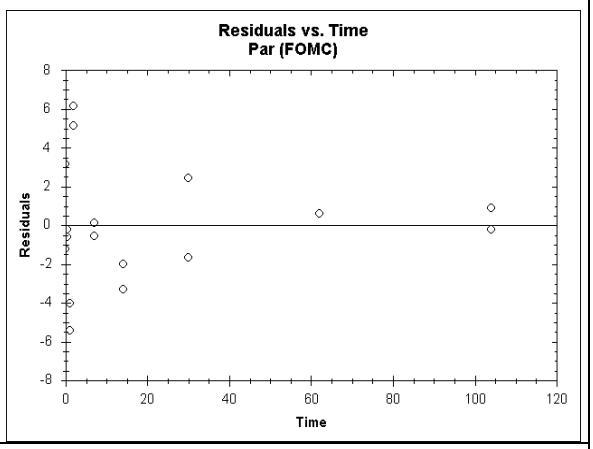
SFO



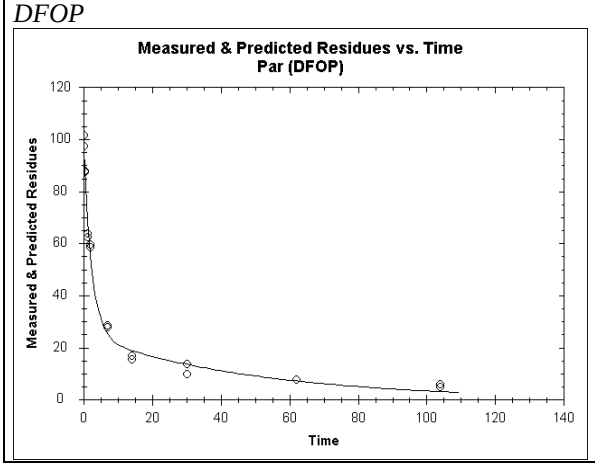


FOMC





DFOP



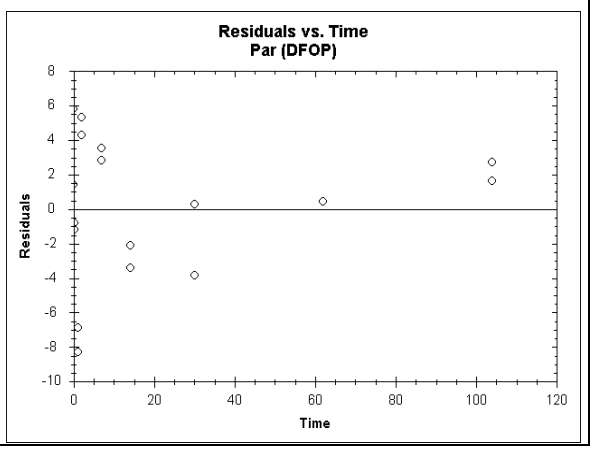
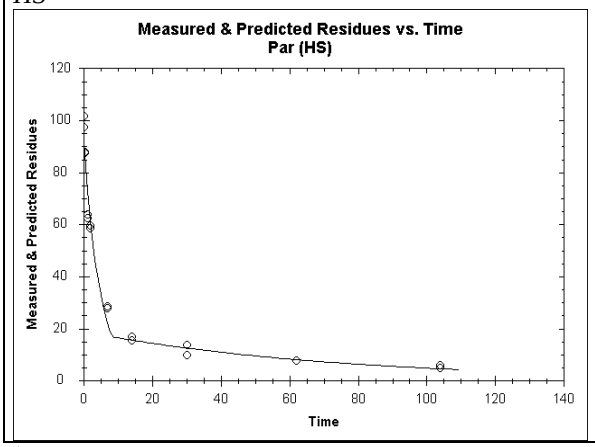
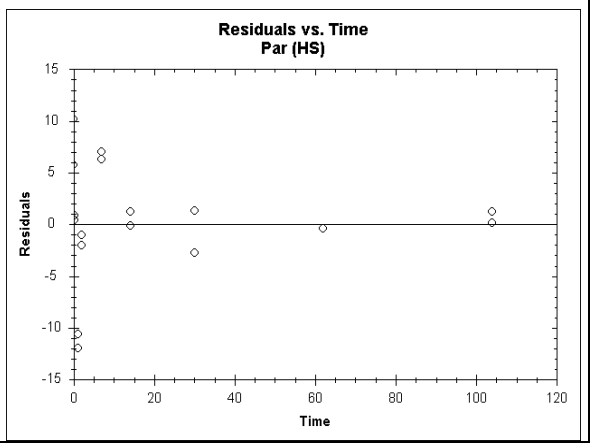


Table 8.2.2.3-108: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

HS





[1] t-test not relevant for kinetic parameter β

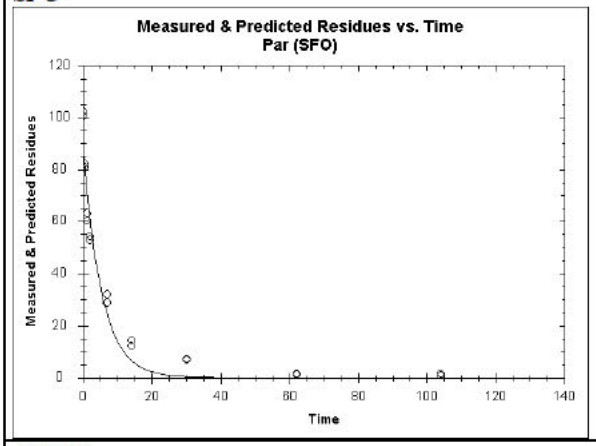
Table 8.2.2.3-109: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

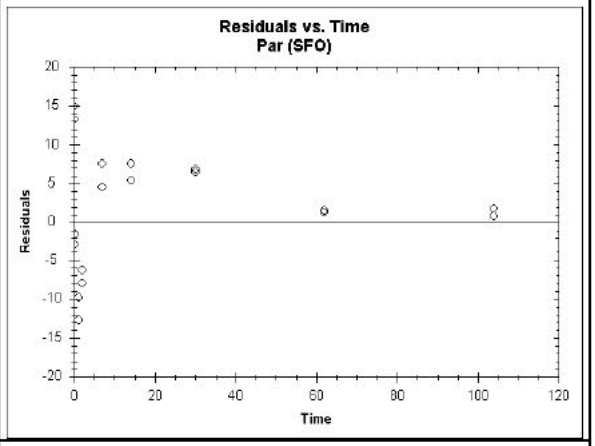
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-109: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

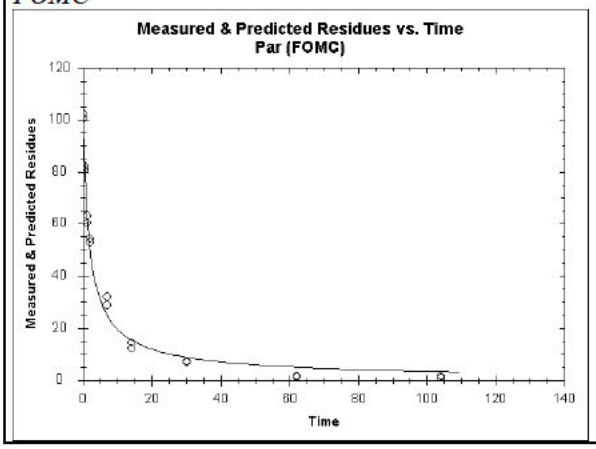
SFO	Poor	87.4	k: 0.1824	15.4	k: <0.001	k: 0.1291	k: 0.2360	3.8	12.6
FOMC	Good	97.4	α: 0.7981 β: 1.5317	8.0	-[1]	β: 0.6887	β: 2.3750	2.1	25.9
DFOP	Good	101.5	k_1: 2.883 k_2: 0.107 g: 0.3491	3.9	k_1: <0.001 k_2: <0.001	k_1: 1.9260 k_2: 0.0947	k_1: 3.8400 k_2: 0.1210	2.5	17.4
HS	Good	89.0	k_1: 0.2089 k_2: 0.0411 tb: 7.9	15.4	k_1: <0.001 k_2: 0.169	k_1: 0.1460 k_2: -0.0400	k_1: 0.2720 k_2: 0.1220	3.3	23.8
Conclusion from notifier	Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide good visual fits but the statistical parameters of the HS model do not indicate a reliable fit. Since FOMC provides a slightly better visual fit than the DFOP model and 10 % of the initially measured substance concentration was reached within the experimental period, the FOMC is selected as the best-fit model for as well as for deriving modelling endpoints. FOMC to be used for trigger endpoints FOMC to be used for modelling endpoints								
Conclusion from RMS	DFOP coud have been selected based on lowest chi2, however the latest sampling points are more underestimated than with FOMC. Waiting for the assessment based on HPLC results, RMS agrees with the notifier for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



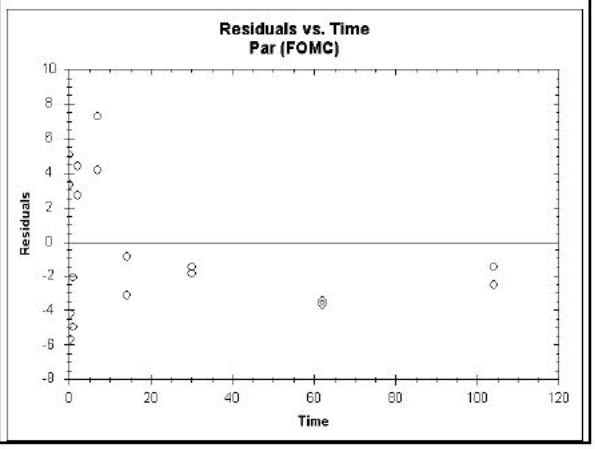
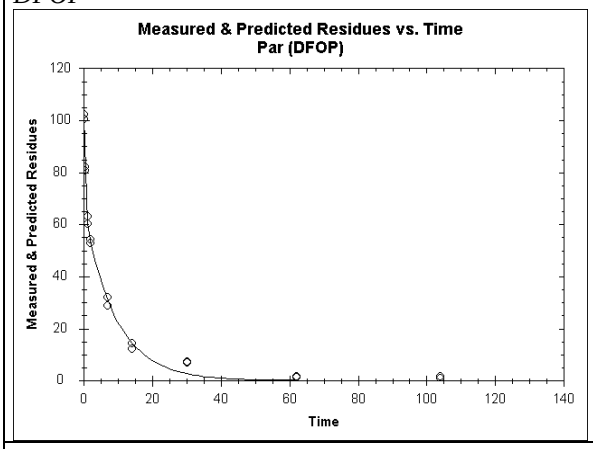
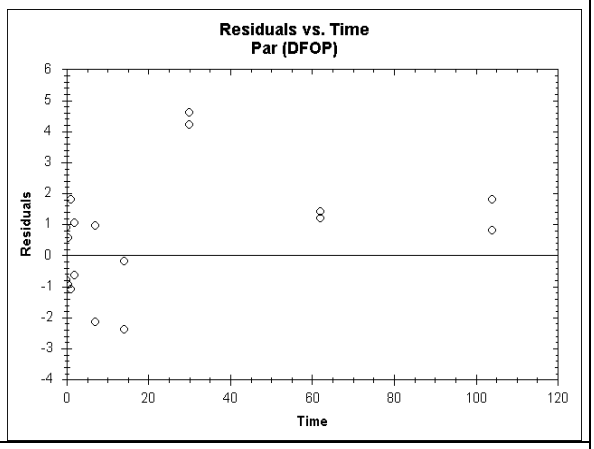


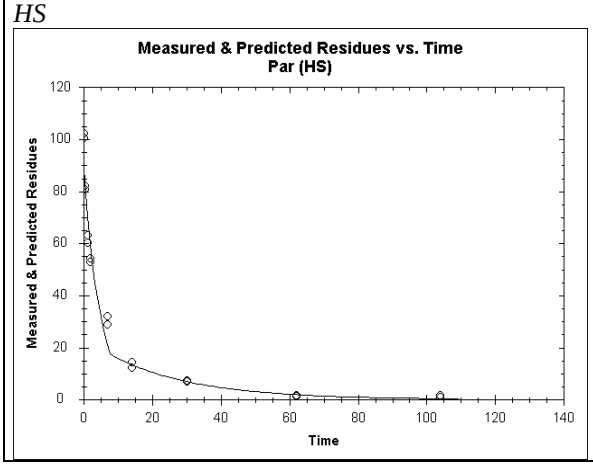
Table 8.2.2.3-109: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

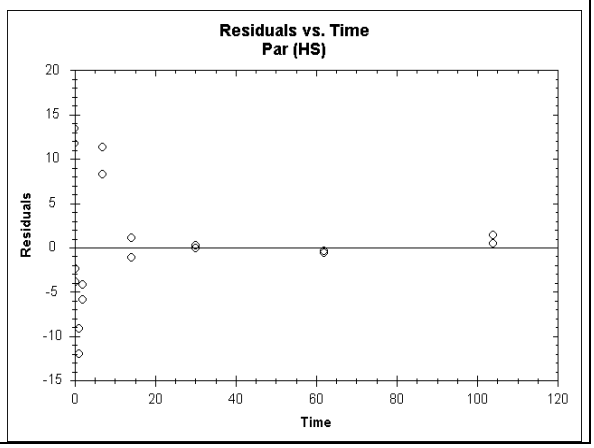
DFOP





HS





[1] t-test not relevant for kinetic parameter β

[redacted] *(1999, CA 7.2.2.3/021)*

For each water-sediment system, the extracts were analysed at each sampling time, with two different TLC systems (SS 1 and SS 2). The values resulting from the TLC systems were considered to be analytical replicates and were therefore averaged prior to kinetic evaluation for each sampling time.

For the system Unter Widdersheim some water samples with overall less than 5 % AR were not analysed by TLC on days 30, 59 (one of two replicates) and 100 (both replicates). These data points were not considered in the kinetic evaluation.

At Level P-I, no evaluation could be conducted for the sediment phase for system Bickenbach due to the limited number of data points available after the peak concentration.

Table 8.2.2.3-110: Experimental data of AMPA for system Bickenbach of study [redacted] (1999, CA 7.2.2.3/021) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment[1]
0	103.6[2]	103.6[2]	0.0[3]
0	103.2[2]	103.2[2]	0.0[3]
0.25	99.9	91.6	8.3
0.25	98.6	90.2	8.4
1	100.2	84.6	15.7
1	98.8	79.4	19.4
2	90.5	68.2	22.3

2	91.8	73.6	18.2
7	84.0	52.8	31.2
7	83.4	52.0	31.5
14	66.5	31.6	34.9
14	73.9	35.0	38.9
30	51.0	17.7	33.3
30	61.4	26.0	35.5
59	48.4	7.5	41.0
59	50.0	9.4	40.6
100	27.0	4.9	22.2
100	22.0	3.3	18.7

[1] No evaluation was conducted at Level P-I for the sediment phase due to the limited number of data points available after the peak concentration
[2] Values at day 0 were set to material balance according to FOCUS (2014)
[3] Set to zero for evaluation at Level P-II

Table 8.2.2.3-111: Experimental data of AMPA for system Unter Widdersheim of study [redacted] (1999, CA 7.2.2.3/021) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment
0	102.2[1]	102.2[1]	0.0[2]
0	102.1[1]	102.1[1]	0.0[2]
0.25	98.2	84.0	14.2
0.25	97.5	81.3	16.2
1	92.9	53.4	39.5
1	96.5	48.4	48.1
2	85.6	58.5	27.2
2	85.1	58.2	27.0
7	73.3	34.1	39.3
7	71.3	25.8	45.5
14	58.5	20.4	38.2
14	60.2	6.1	54.1
30	39.1	-[3]	39.1
30	33.6	-[3]	33.6
59	33.9	-[3]	33.9
59	32.0	2.3	29.7
100	26.6	-[3]	26.6
100	35.0	-[3]	35.0

[1] Values at day 0 were set to material balance according to FOCUS (2014)
[2] Set to zero for evaluation at Level P-II
[3] Sediment extracts containing less than 5 % AR were not analysed further. These data points were not considered in the kinetic evaluation

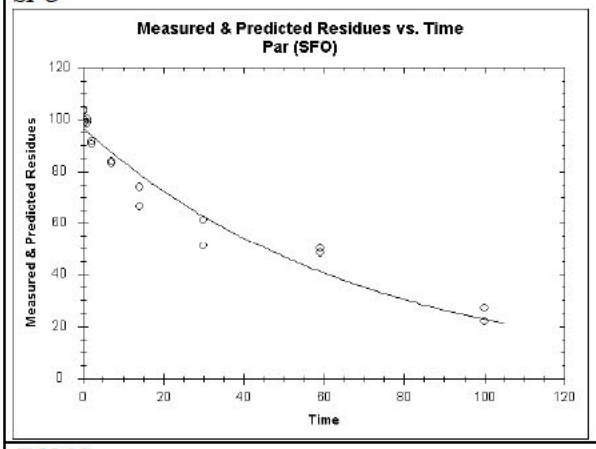
Table 8.2.2.3-112: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

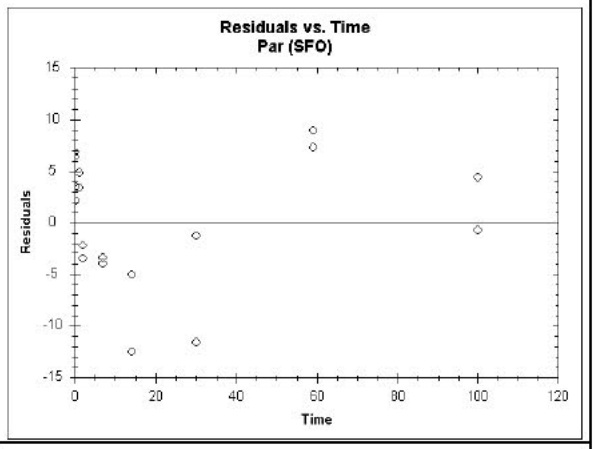
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-112: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

SFO	Acceptable	96.8	k: 0.0145	5.9	k: <0.001	k: 0.0121	k: 0.0170	47.7	158.4
FOMC	Good	100.6	α: 0.7336 β: 24.7523	4.4	-[1]	β: 1.2073	β: 48.297	38.9	546.5
DFOP	Good	101.9	k_1: 0.1684 k_2: 0.0105 g: 0.2114	3.5	k_1: 0.058 k_2: <0.001	k_1: -0.0286 k_2: 0.0075	k_1: 0.3650 k_2: 0.0140	43.5	196.8
HS	Good	102.7	k_1: 0.0547 k_2: 0.0118 tb: 4.0	4.0	k_1: 0.012 k_2: <0.001	k_1: 0.0121 k_2: 0.0096	k_1: 0.0970 k_2: 0.0140	44.4	181.0
Conclusion from notifier	Although the visual and statistical fits of the SFO model are acceptable, the degradation of AMPA is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually good results but the least χ^2 error is provided by the DFOP model. DFOP to be used for trigger endpoints SFO to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion. For DFOP, both t-test for k_1 and k_2 are acceptable when considering a significance level of 10% (as recommended in FOCUS Guidance for water/sediment studies). 95% confidence interval for k_1 includes 0, but it is expected that 90% confidence interval would not include 0. Indeed, as indicated in the FOCUS guidance, "Both, the t-test and the confidence interval give the same answer, provided the underlying assumptions are identical (distribution of the parameter and level of probability)".								

SFO





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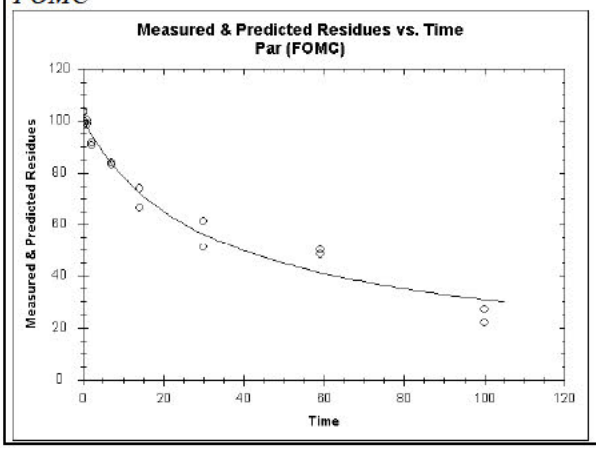


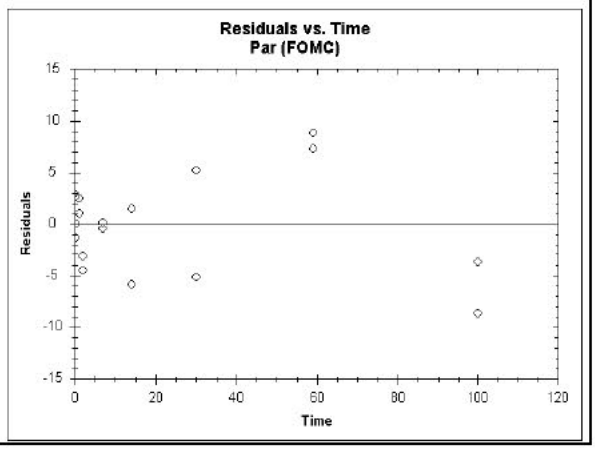
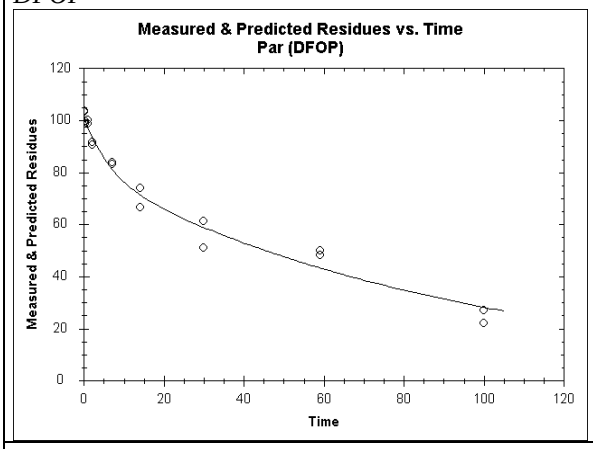
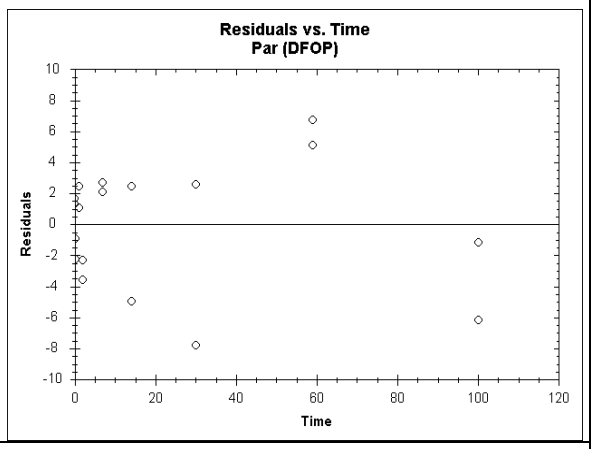


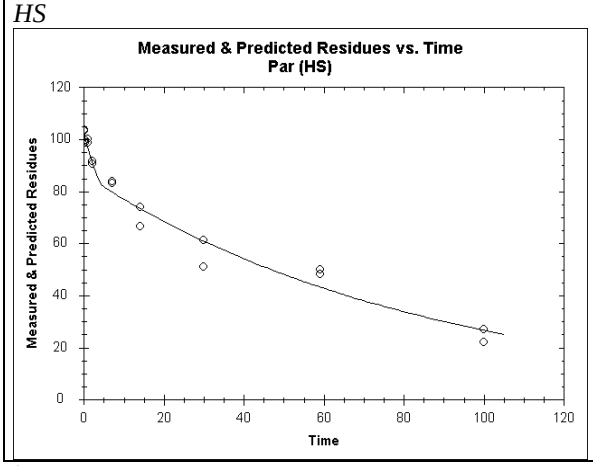
Table 8.2.2.3-112: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

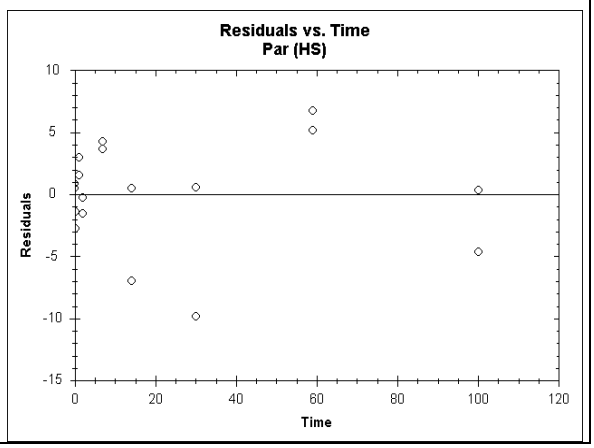
DFOP





HS





[1] t-test not relevant for kinetic parameter β

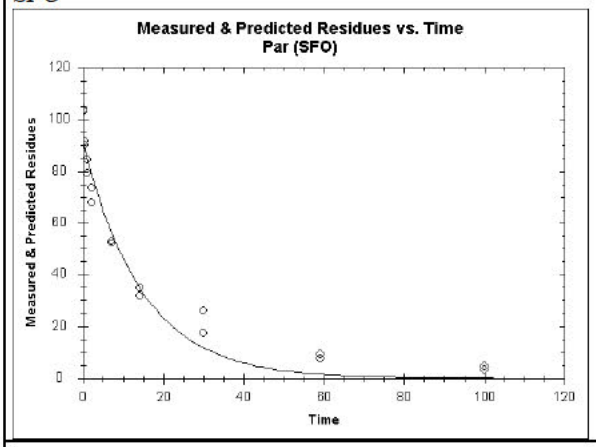
Table 8.2.2.3-113: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

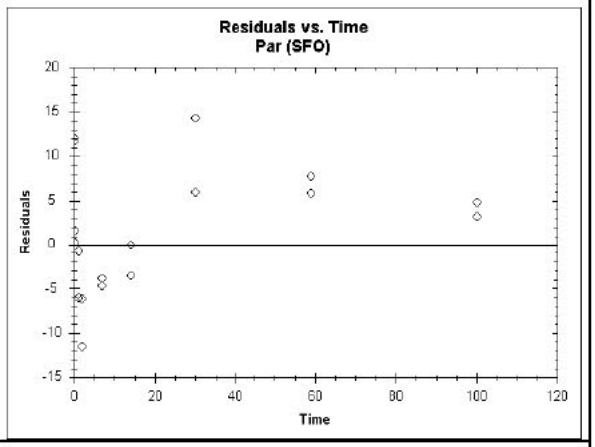
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-113: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

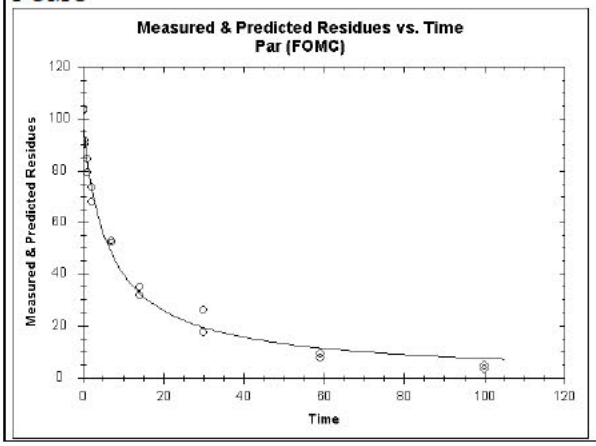
SFO	Poor	91.5	k: 0.0685	10.5	k: <0.001	k: 0.0527	k: 0.0840	10.1	33.6
FOMC	Good	97.6	α: 0.8827 β: 5.6835	5.7	-[1]	β: 2.0266	β: 9.3400	6.8	71.5
DFOP	Good	100.4	k_1: 0.567 k_2: 0.0361 g: 0.3765	4.5	k_1: <0.001 k_2: <0.001	k_1: 0.2831 k_2: 0.0268	k_1: 0.8510 k_2: 0.0450	6.6	50.7
HS	Good	99.4	k_1: 0.179 k_2: 0.0372 tb: 3.1	5.1	k_1: <0.001 k_2: <0.001	k_1: 0.1338 k_2: 0.0276	k_1: 0.2240 k_2: 0.0470	6.8	50.1
Conclusion from notifier	The dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide reliable and visually good results but the best visual fit with the least χ^2 error is provided by the DFOP model. DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



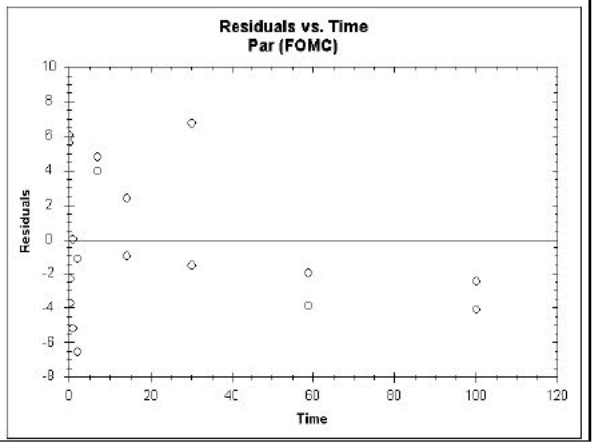
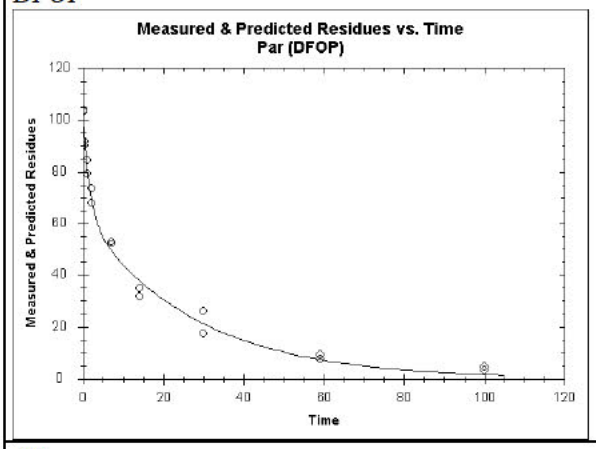
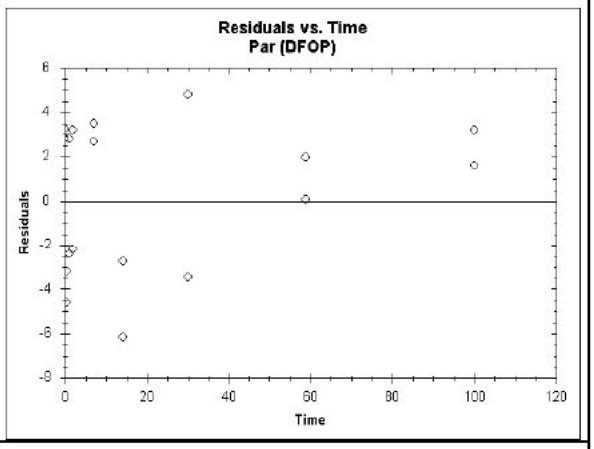


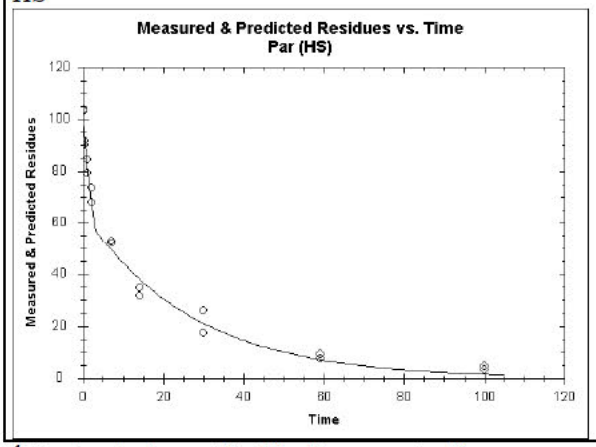
Table 8.2.2.3-113: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

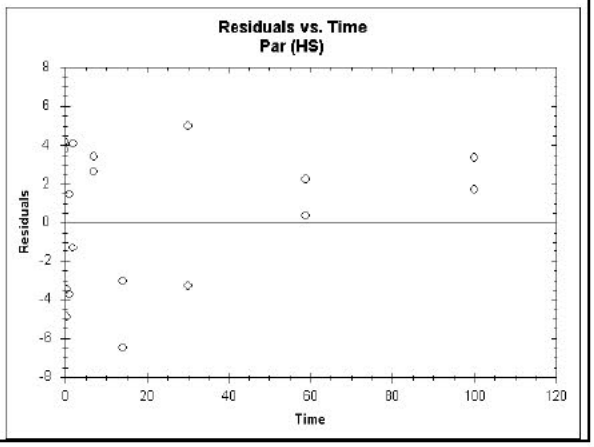
DFOP





HS





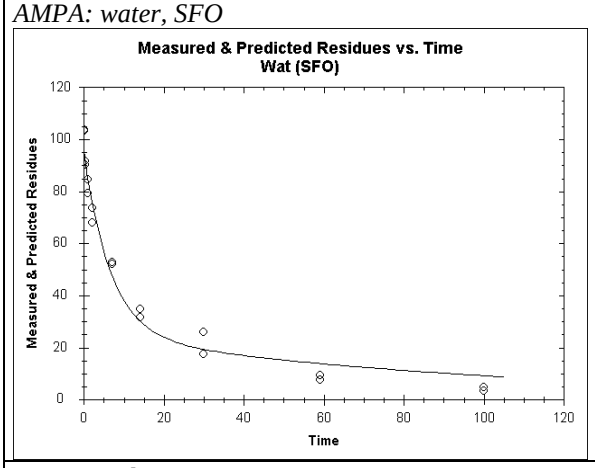
[1] t-test not relevant for kinetic parameter β

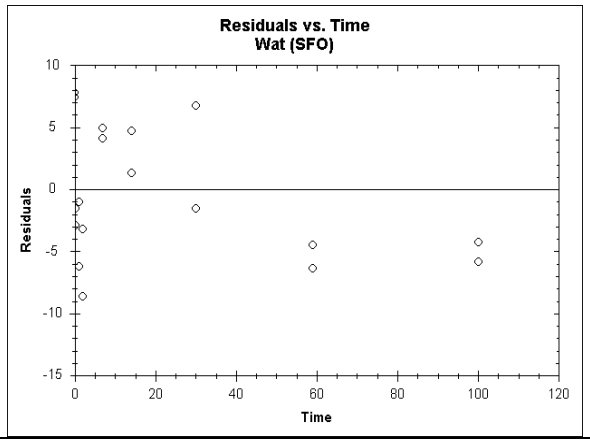
Table 8.2.2.3-114: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Acceptable	95.8	k_{wat}: 0.0324 $k_{wat\ sed}$: 0.0820	7.7	k_{wat}: 0.0041 k_{wat_sed}: <0.001	k_{wat}: 0.0100 $k_{wat\ sed}$: -0.0611	k_{wat}: 0.055 k_{wat_sed}: 0.103	21.4	71.1
Sediment: SFO	Acceptable	0.0	k_{sed}: 2.34×10^{-14} k_{sed_wat}: 0.0469	20.3	k_{sed}: 0.5 $k_{sed\ wat}$: 0.006	k_{sed}: -0.0159 k_{sed_wat}: -0.0213	k_{sed}: 0.016 k_{sed_wat}: 0.073	>1000	>1000
Conclusion from notifier	Although the visual fits obtained for the water and sediment phases are acceptable, the degradation rate in sediment is not significantly different from zero. Therefore, the statistical fit obtained for the sediment phase is not reliable. No further evaluation was conducted. No reliable endpoints could be derived.								
Conclusion from RMS	Agrees with notifier conclusion.								

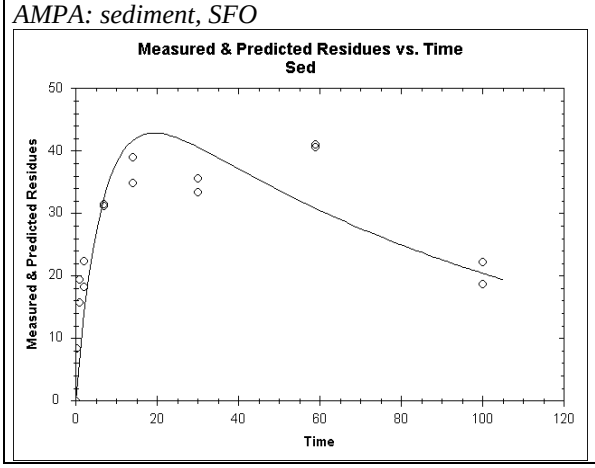
Table 8.2.2.3-114: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

AMPA: water, SFO





AMPA: sediment, SFO



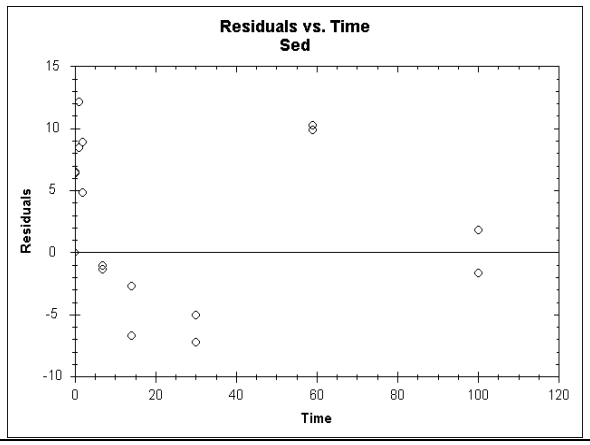


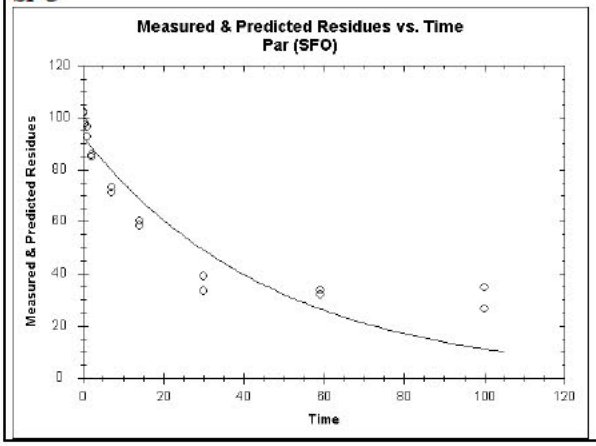
Table 8.2.2.3-115: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-115: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

SFO	Poor	92.6	k: 0.0212	11.7	k: <0.001	k: 0.0150	k: 0.0270	32.7	108.5
FOMC	Good	101.2	α: 0.4410 β: 5.1794	3.9	-[1]	β: 1.9157	β: 8.4430	19.8	954.3
DFOP	Good	99.1	k_1: 0.071 k_2: 2.33 × 10^{-14} g: 0.6886	3.1	k_1: <0.001 k_2: 0.5	k_1: 0.0475 k_2: -0.0053	k_1: 0.0940 k_2: 0.0050	18.2	>1000
HS	Good	98.1	k_1: 0.0391 k_2: 0.0024 tb: 25.3	3.4	k_1: <0.001 k_2: 0.075	k_1: 0.0331 k_2: -0.0007	k_1: 0.0450 k_2: 0.0050	17.7	579.8
Conclusion from notifier	The degradation of AMPA is best described by bi-phasic models. The DFOP and HS models provide the best visual fits. However, the slow-phase degradation parameter (k_2) resulting from the DFOP model is not significantly different from zero. Thus, the HS model is selected as the best-fit model as well as for deriving modelling endpoints. HS to be used for trigger endpoints HS to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion. The visual fit obtained with SFO is not considered visually acceptable and significantly underestimates the DT_{90}. Biphasic models significantly improve the visual fits. For trigger endpoints, DFOP and HS models provide a slightly better visual fit than FOMC. For modelling endpoint, FOMC is not a reliable option since more than 10% AR remain as glyphosate at the end of the study. T-test for k_2 DFOP is not acceptable. T-test for k_2 HS is considered acceptable based on the significance level of 10% as recommended in FOCUS guidance for water/sediment studies. 95% confidence interval for HS k_1 includes 0, but it is expected that 90% confidence interval would not include 0. Indeed, as indicated in the FOCUS guidance, "Both, the t-test and the confidence interval give the same answer, provided the underlying assumptions are identical (distribution of the parameter and level of probability)". It is therefore agreed that HS is the best option for trigger and modelling endpoints.								

SFO



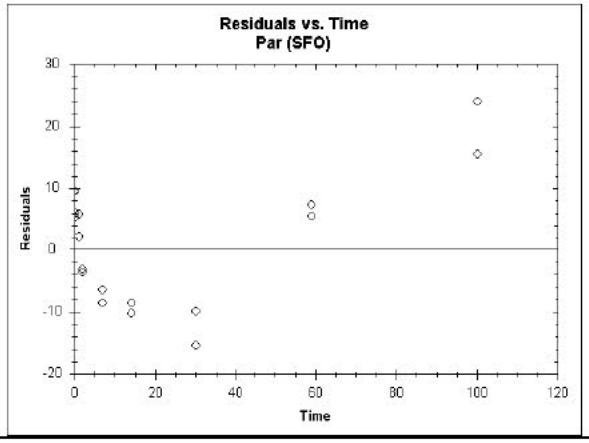
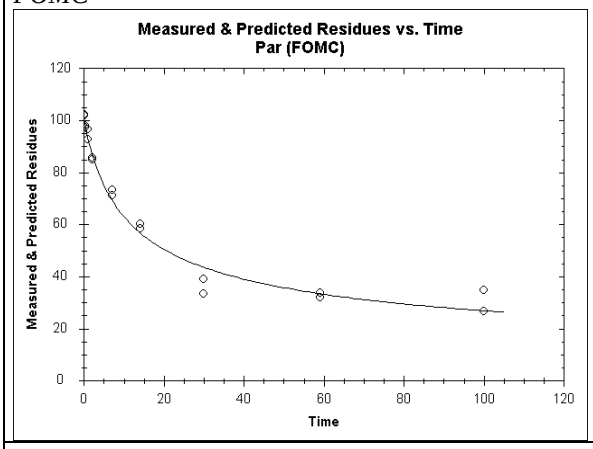
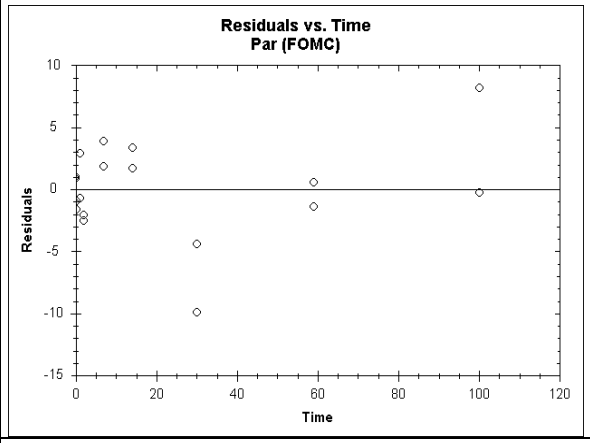


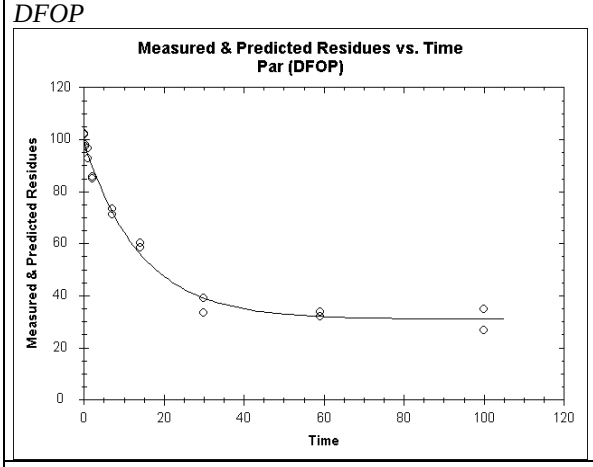
Table 8.2.2.3-115: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

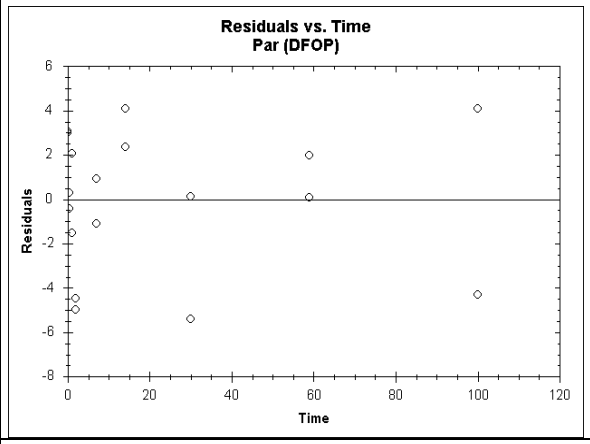
FOMC



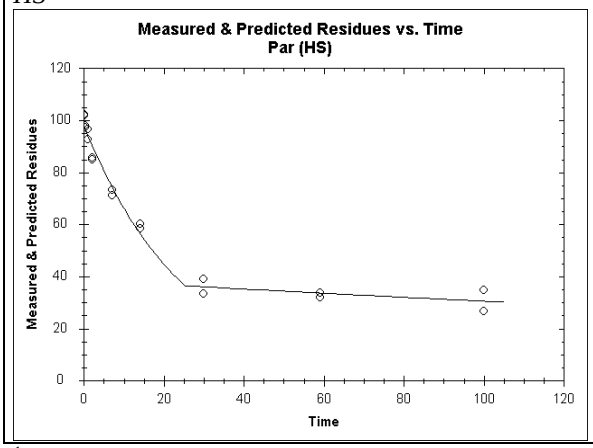


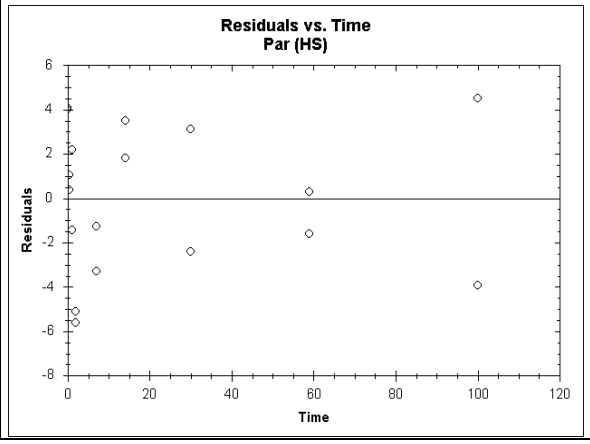
DFOP





HS





[1] t-test not relevant for kinetic parameter β

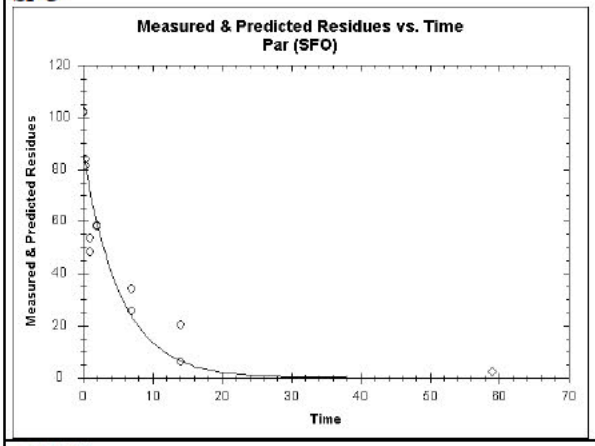
Table 8.2.2.3-116: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

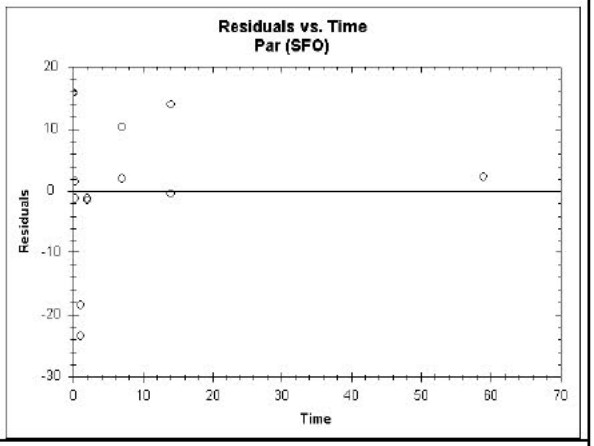
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-116: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

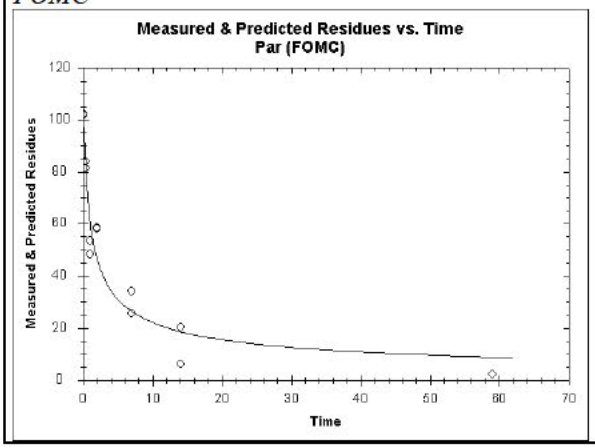
SFO	Poor	86.3	k: 0.1846	17.3	k: <0.001	k: 0.0996	k: 0.2700	3.8	12.5
FOMC	Poor	100.5	α: 0.5353 β: 0.6254	11.8	-[1]	β: -0.1641	β: 1.4150	1.7	45.5
DFOP	Acceptable	103.1	k_1: 3.1154 k_2: 0.1051 g: 0.3867	8.2	k_1: 0.027 k_2: <0.001	k_1: 0.3677 k_2: 0.0625	k_1: 5.8630 k_2: 0.1480	2.0	17.3
HS	Poor	87.8	k_1: 0.2099 k_2: 0.0389 tb: 7.9	19.7	k_1: 0.002 k_2: 0.385	k_1: 0.0991 k_2: -0.2138	k_1: 0.3210 k_2: 0.2920	3.3	24.6
Conclusion from notifier	The dissipation of AMPA in the water phase is best described by bi-phasic models. The visual fit and the statistical parameters resulting from the FOMC and HS models do not indicate an acceptable fit. The DFOP model provides acceptable visual and statistical fit. Thus, the DFOP model is selected as the best fit model as well as for deriving modelling endpoints. DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



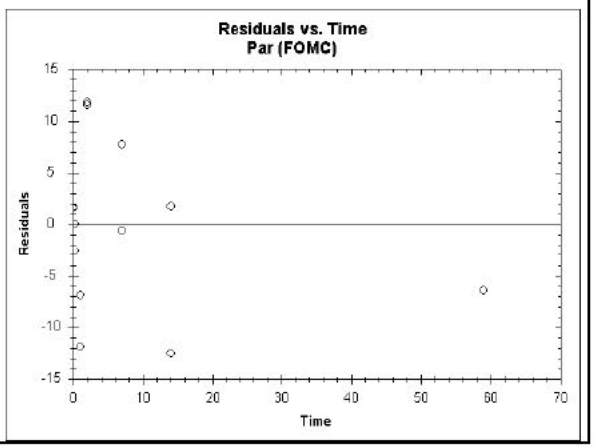
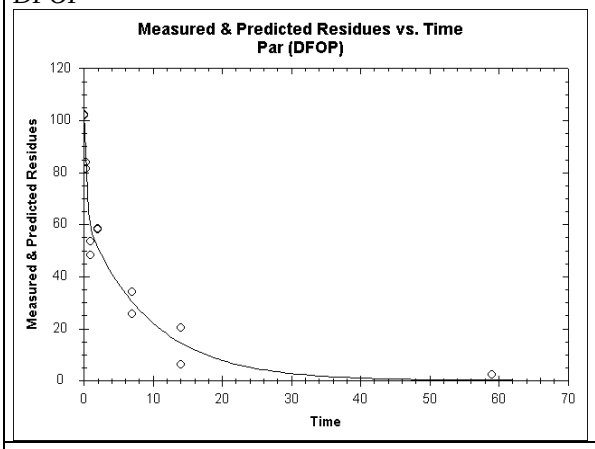
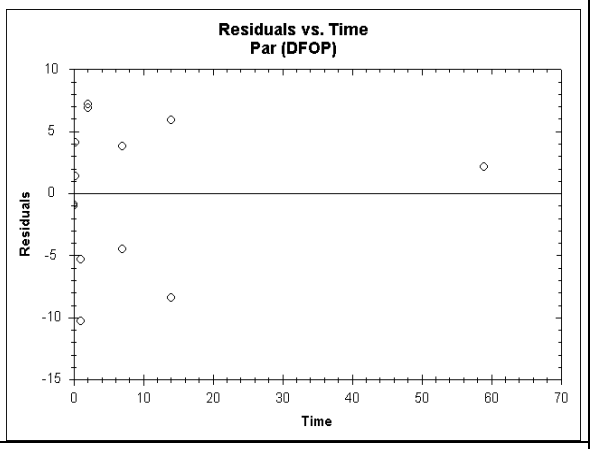


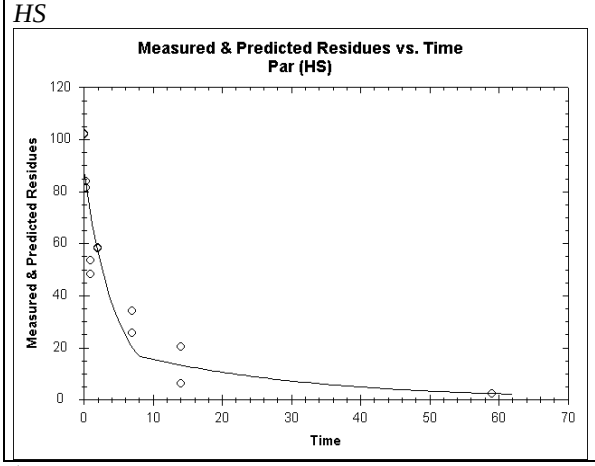
Table 8.2.2.3-116: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

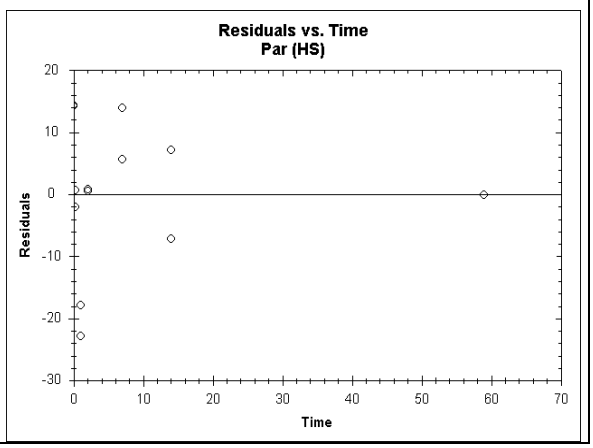
DFOP





HS





[1] t-test not relevant for kinetic parameter β

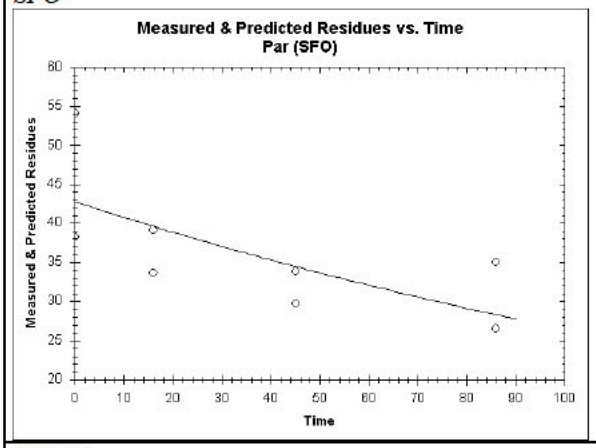
Table 8.2.2.3-117: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, sediment phase

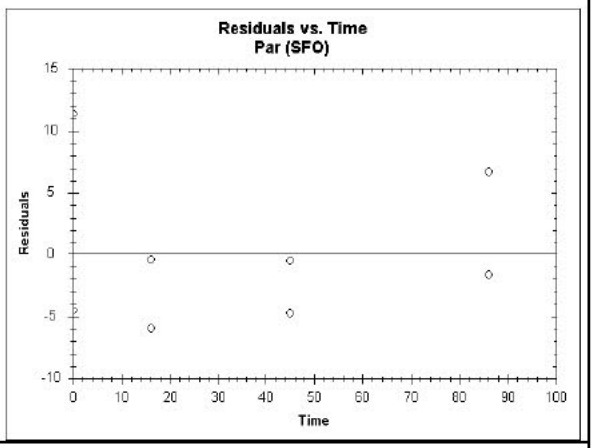
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-117: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, sediment phase

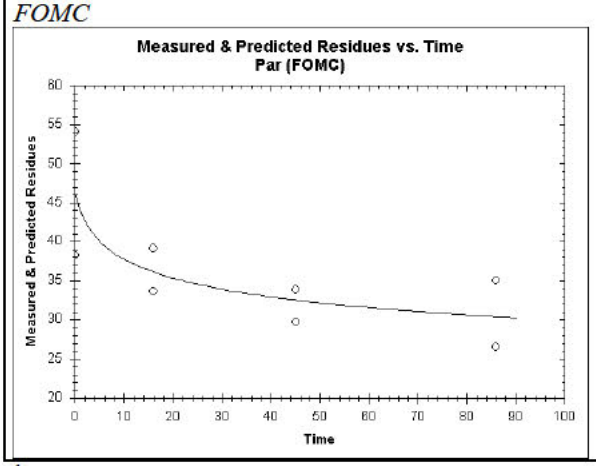
SFO	Poor	42.7	k: 0.0048	6.7	k: 0.033	k: 0.0006	k: 0.0090	144.6	480.5
FOMC	Acceptable	46.2	α: 0.108 β: 1.8179	1.2	-[1]	β: -13.706	β: 17.341	>1000	>1000
Conclusion from notifier	Only the SFO and FOMC models were used for the evaluation due to the limited number of available data points. The SFO model did not properly describe the dissipation. Although the FOMC model provides an acceptable visual fit, the confidence interval of the parameter β is wide and includes zero. No reliable endpoints could be derived								
Conclusion from RMS	Agrees with notifier conclusion.								

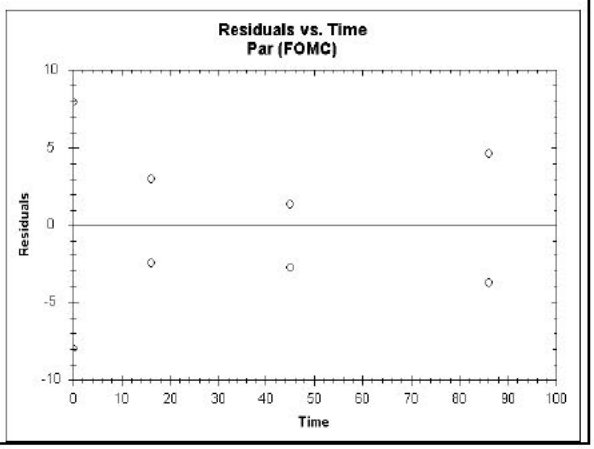
SFO





FOMC





[1] t-test not relevant for kinetic parameter β

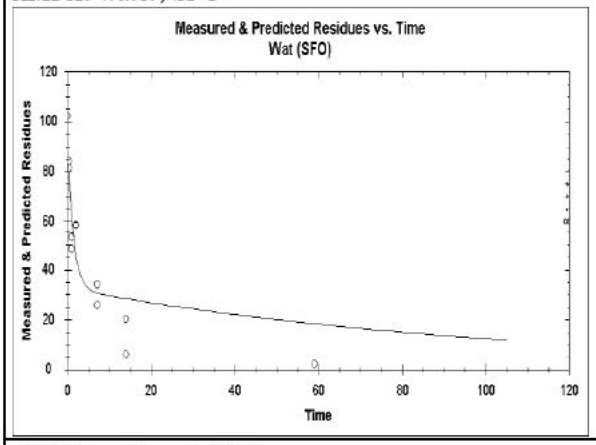
Table 8.2.2.3-118: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

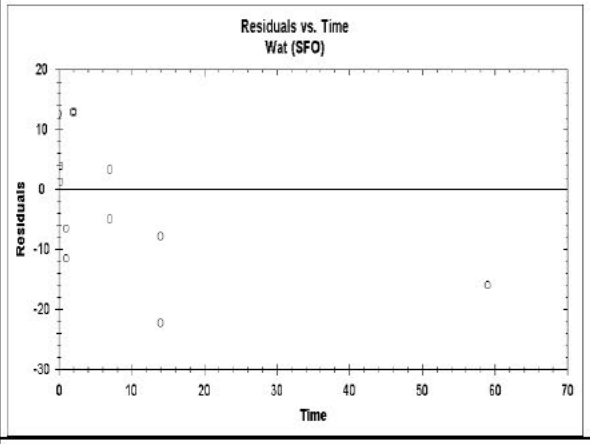
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-118: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

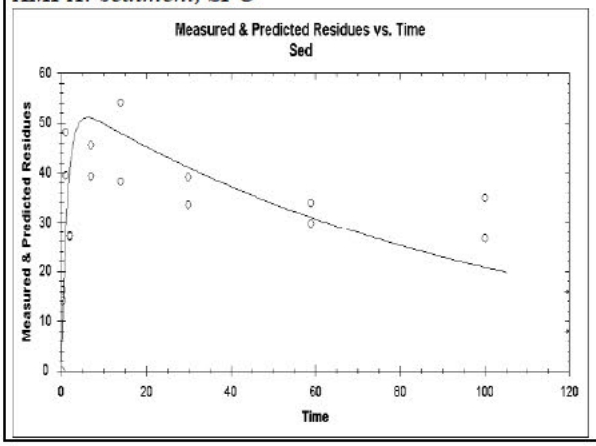
Water: SFO	Poor	89.5	k_{wat}: 0.0263 $k_{wat\ sed}$: 0.4356	20.1	k_{wat}: 0.3885 $k_{wat\ sed}$: <0.001	k_{wat}: -0.1536 $k_{wat\ sed}$: -0.2501	k_{wat}: 0.206 k_{wat_sed}: 0.621	26.4	87.7
Sediment: SFO	Acceptable	0.0	k_{sed}: $2.34 \times 10^{-14}0$ $k_{sed\ wat}$: 0.2671	21.7	k_{sed}: 0.5 k_{sed_wat}: 0.0062	k_{sed}: -0.1100 $k_{sed\ wat}$: -0.0722	k_{sed}: 0.110 k_{sed_wat}: 0.462	>1000	>1000
Conclusion from notifier	Although the visual fit obtained for the sediment phase is acceptable, the statistical fit is not and the visual fit obtained for the water phase is poor. No further evaluation was conducted. No reliable endpoints could be derived								
Conclusion from RMS	Agrees with notifier conclusion.								

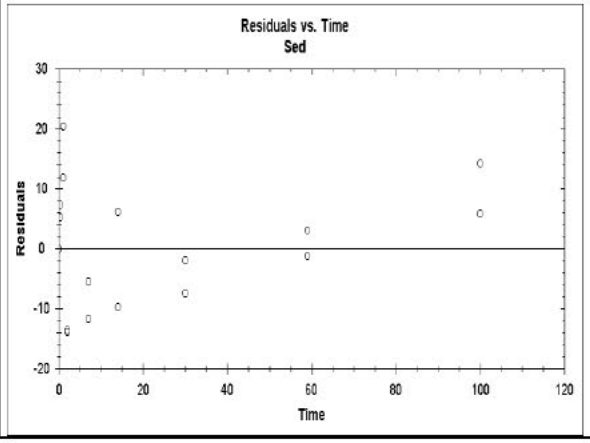
AMPA: water, SFO





AMPA: sediment, SFO





[redacted] *(2004, CA 7.2.2.3/018)*

At Level P-I, no evaluation could be conducted for the sediment phase for system Manningtree A due to the limited number of data points available after the peak concentration.

Due to problems analysing extracts obtained from system Manningtree B, explained to be caused by endogenous co-extracted material disrupting the ion-exchange chromatography, the total system and sediment phase of the system Manningtree B were not considered in the kinetic evaluation. Therefore, Level P-II evaluation could not be conducted for the system Manningtree B.

Table 8.2.2.3-119: Experimental data of AMPA for system Manningtree A of study [redacted] (2004, CA 7.2.2.3/018) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	**Water**	**Sediment[1]**
0	96.6[2]	96.6[2]	0.0[3]
1	53.1	37.7	15.4
7	27.1	10.7	16.4
14	9.4	5.9	3.5
29	34.3	4.7	29.6
61	32.5	2.3	30.2
103	13.1	0.8	12.3

Number in **bold** represent peak concentration considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] No evaluation was conducted at Level P-I for the sediment phase due to the limited number of data points available after the peak concentration

[2] Values at day 0 were set to material balance according to FOCUS (2014)

[3] Set to zero for evaluation at Level P-II

Table 8.2.2.3-120: Experimental data of AMPA for system Manningtree B of study [redacted] (2004, CA 7.2.2.3/018) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)[1]
	Water
0	97.2[2]
1	52.7
7	8.60
14	6.20
29	1.90
61	0.10
103	0.20

[1] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation

[2] Values at day 0 were set to material balance according to FOCUS (2014)

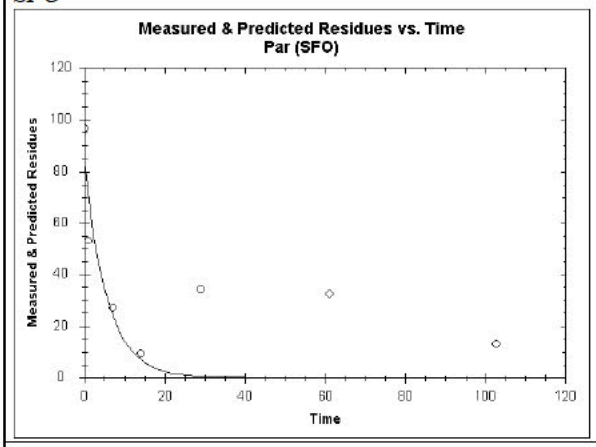
Table 8.2.2.3-121: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, total system

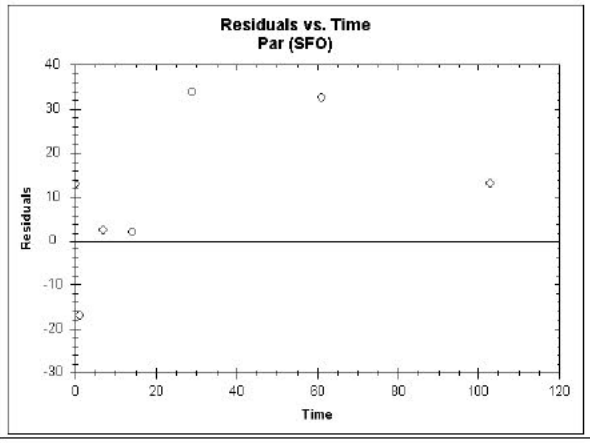
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-121: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, total system

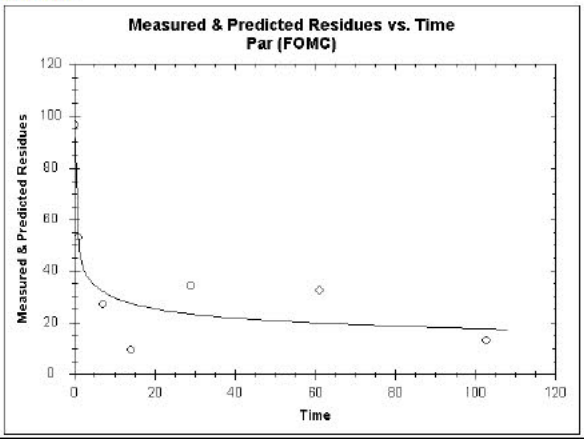
SFO	Poor	83.5	k: 0.1757	42.1	k: 0.109	k: -0.0683	k: 0.4200	3.9	13.1
FOMC	Poor	96.7	α: 0.2234 β: 0.0488	22.1	-[1]	β: -0.2142	β: 0.3120	1.0	>1000
DFOP	Poor	96.6	k_1: 0.9354 k_2: 0.0019 g: 0.7393	21.1	k_1: 0.090 k_2: 0.407	k_1: -0.1201 k_2: -0.0126	k_1: 1.9910 k_2: 0.0160	1.2	503.7
HS	Poor	96.6	k_1: 0.5984 k_2: 0.0019 tb: 2.3	21.1	k_1: 0.060 k_2: 0.405	k_1: 0.0532 k_2: -0.0126	k_1: 1.1440 k_2: 0.0160	1.2	497.0
Conclusion from notifier	No reliable endpoints could be derived.								
Conclusion from RMS	Agrees with notifier conclusion.								

SFO





FOMC



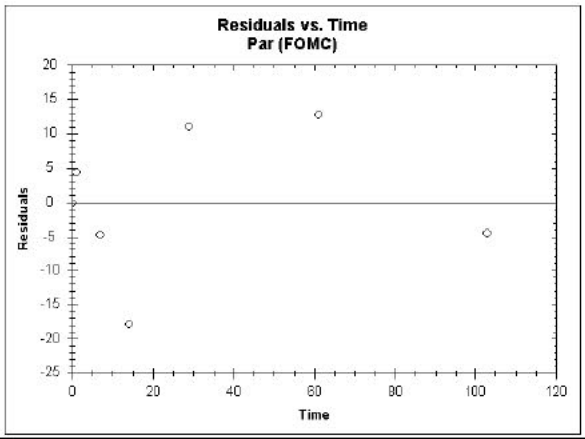
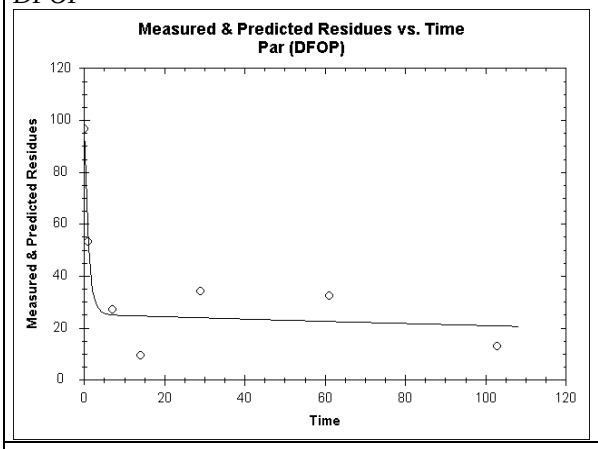
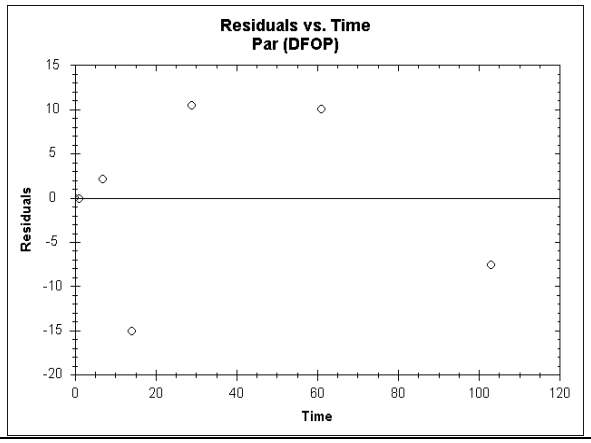


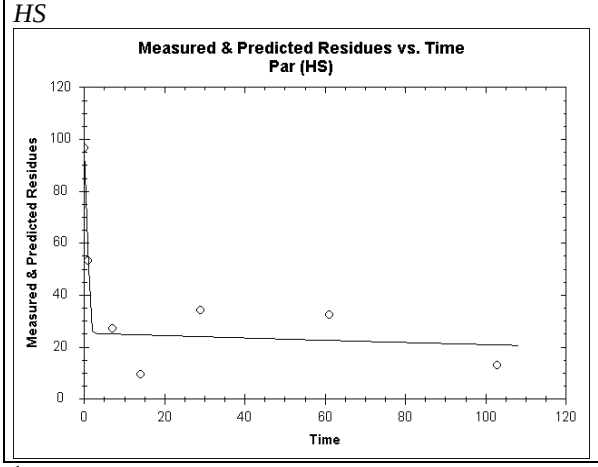
Table 8.2.2.3-121: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, total system

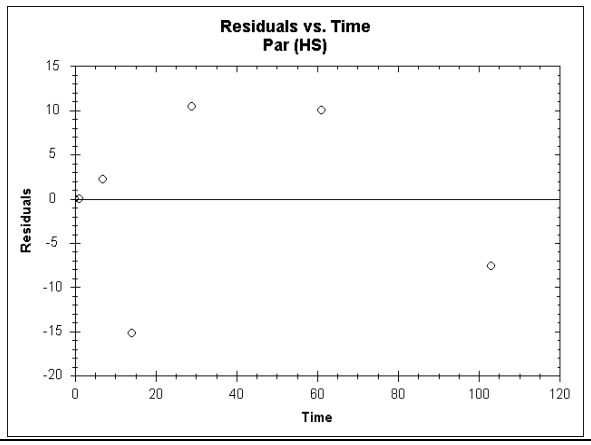
DFOP





HS





[1] t-test not relevant for kinetic parameter β

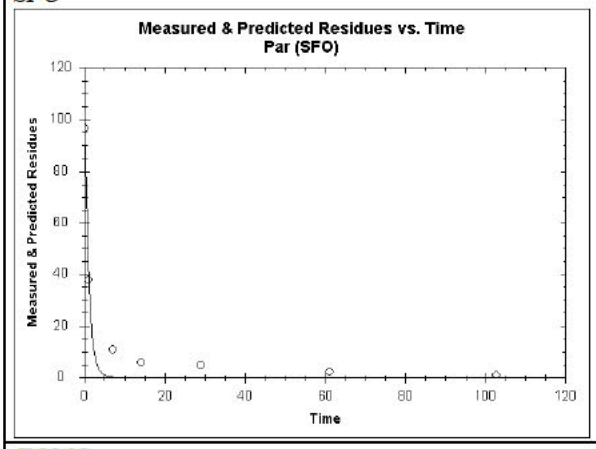
Table 8.2.2.3-122: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

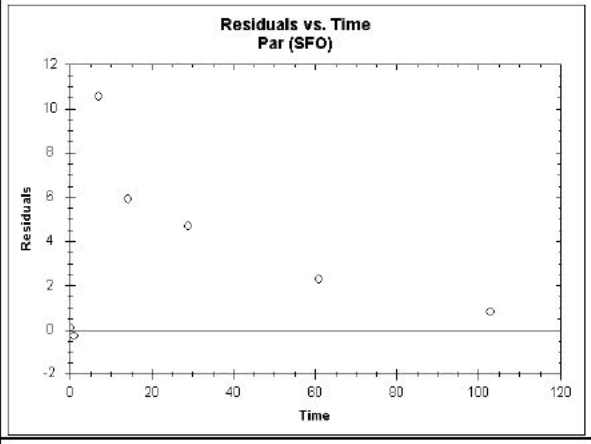
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)

Table 8.2.2.3-122: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

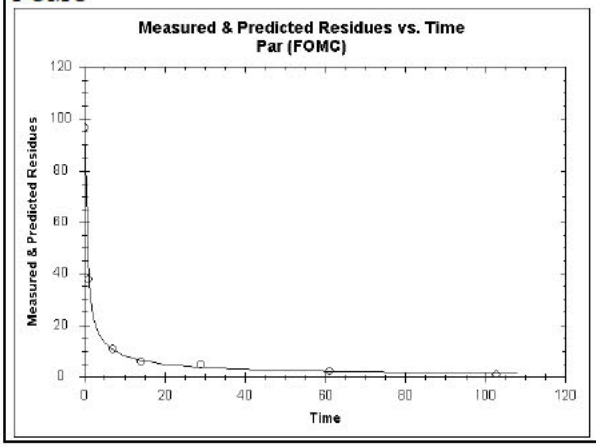
SFO	Poor	96.5	k: 0.9327	17.5	k: 0.001	k: 0.6052	k: 1.2600	0.7	2.5
FOMC	Good	96.6	α: 0.7584 β: 0.4064	1.8	-[1]	β: 0.3340	β: 0.4790	0.6	8.1
DFOP	Good	96.6	k_1: 1.1772 k_2: 0.0333 g: 0.8753	3.4	k_1: <0.001 k_2: 0.023	k_1: 1.0434 k_2: 0.0136	k_1: 1.3110 k_2: 0.0530	0.7	6.7
HS	Good	96.6	k_1: 0.9409 k_2: 0.0334 tb: 2.3	3.5	k_1: <0.001 k_2: >0.023	k_1: 0.8704 k_2: 0.0136	k_1: 1.0110 k_2: 0.0530	0.7	6.7
Conclusion from notifier	Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide good visual fits and reliable statistical parameters. Since FOMC provides the least χ^2 error and 10 % of the initially measured substance concentration was reached within the experimental period, the FOMC model is selected as the best-fit model as well as for deriving modelling endpoints. FOMC to be used for trigger endpoints FOMC to be used for modelling endpoints								
Conclusion from RMS	Agrees with notifier conclusion for trigger endpoint. According to FOCUS guidance, no modelling endpoint should be derived from P-I level in water (please refer to RMS comments).								

SFO





FOMC



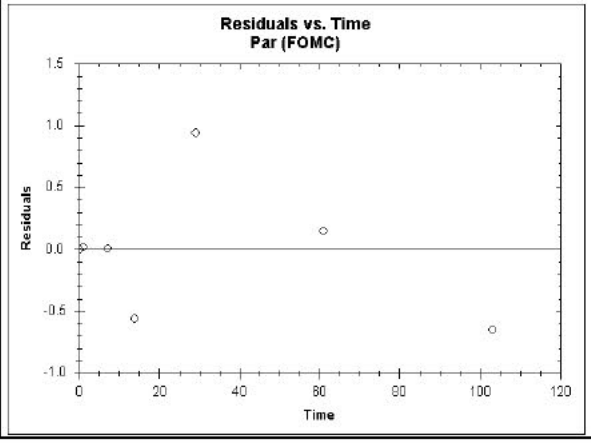
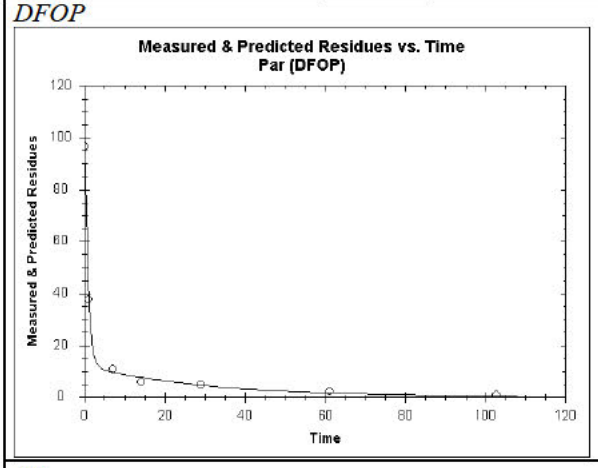
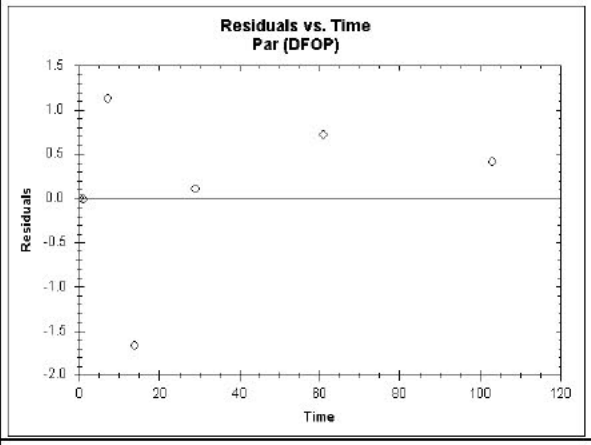


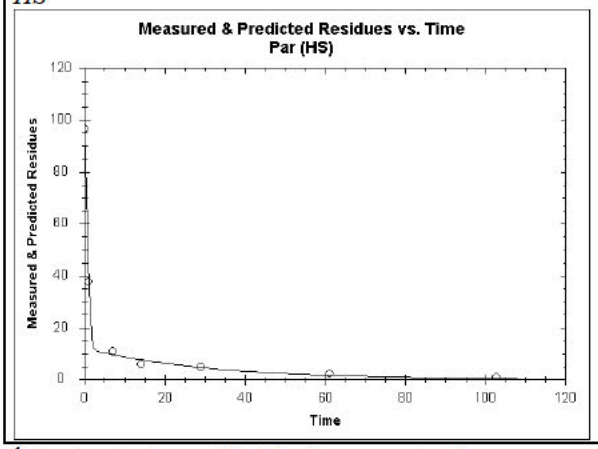
Table 8.2.2.3-122: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

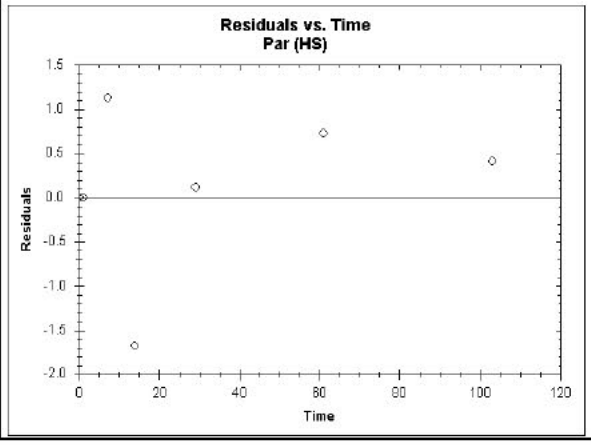
DFOP





HS





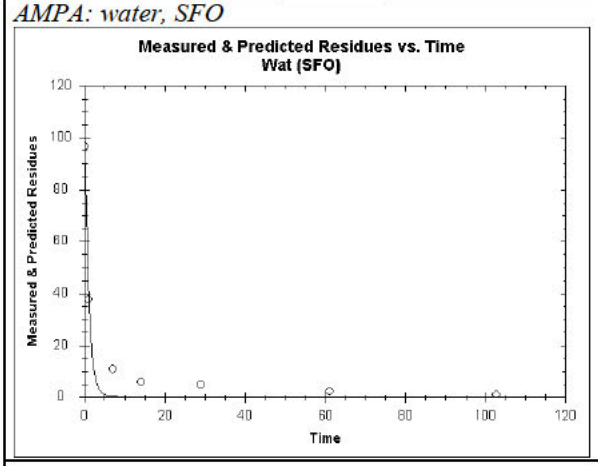
[1] t-test not relevant for kinetic parameter β

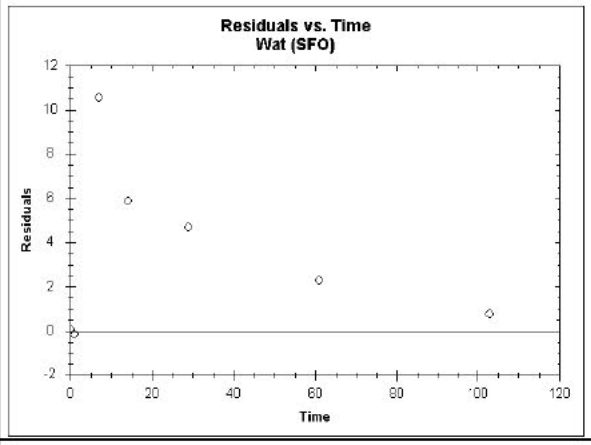
Table 8.2.2.3-123: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of study [redacted] (2004, CA 7.2.2.3/018), Level P-II

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Poor	96.6	k_{wat}: 0.7478 $k_{wat\ sed}$: 0.1884	18.9	k_{wat}: <0.001 $k_{wat\ sed}$: 0.0303	k_{wat}: 0.4450 $k_{wat\ sed}$: -0.0162	k_{wat}: 1.051 k_{wat_sed}: 0.361	0.9	3.1
Sediment: SFO	Poor	0.0	k_{sed}: 2.24×10^{-14} $k_{sed\ wat}$: 0.0009	42.5	k_{sed}: 0.5 k_{sed_wat}: 0.4970	k_{sed}: -0.1898 $k_{sed\ wat}$: -0.2356	k_{sed}: 0.190 k_{sed_wat}: 0.237	>1000	>1000
Conclusion from notifier	The visual and statistical fits obtained for the water phase and sediment phase are not acceptable. No further evaluation was conducted. No reliable endpoints could be derived								
Conclusion from RMS	Agrees with notifier conclusion.								

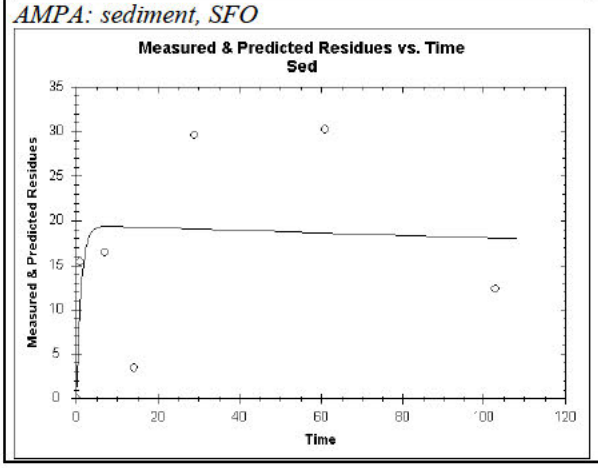
Table 8.2.2.3-123: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of study [redacted] (2004, CA 7.2.2.3/018), Level P-II

AMPA: water, SFO





AMPA: sediment, SFO



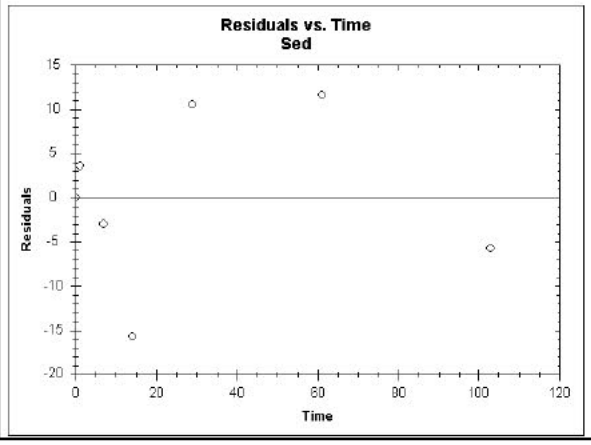
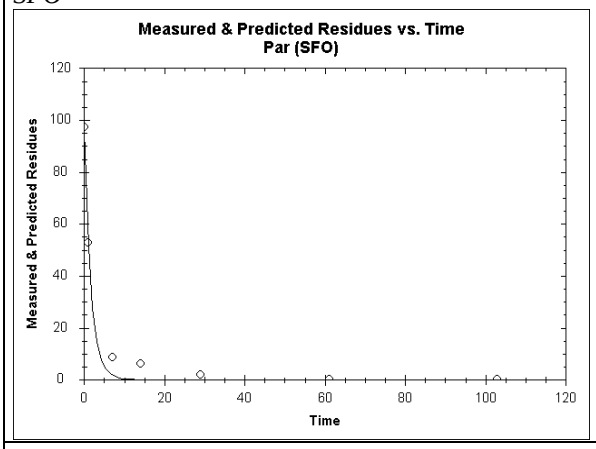


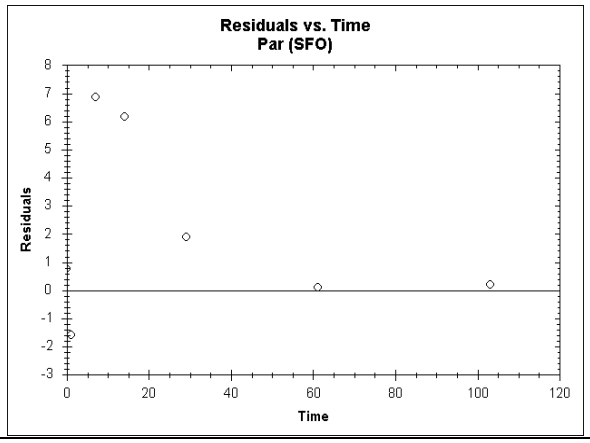
Table 8.2.2.3-124: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree B of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.4	k: 0.5747	12.1	k: <0.001	k: 0.4021	k: 0.7470	1.2	4.0
FOMC	Good	97.3	α: 1.4791 β: 1.93	3.4	-[1]	β: 1.2645	β: 2.5960	1.2	7.2
DFOP	Good	97.2	k_1: 0.7311 k_2: 0.0592 g: 0.8689	1.3	k_1: <0.001 k_2: 0.004	k_1: 0.6921 k_2: 0.0415	k_1: 0.7700 k_2: 0.0770	1.1	6.2
HS	Good	97.2	k_1: 0.6122 k_2: 0.0623 tb: 3.6	1.0	k_1: <0.001 k_2: 0.002	k_1: 0.5951 k_2: 0.0486	k_1: 0.6290 k_2: 0.0760	1.1	5.5
Conclusion from notifier	Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide good visual fits and reliable statistical parameters but the HS model provies the least χ^2 error. Thus, the HS model is selected as the best-fit model as well as for deriving modelling endpoints. HS to be used for trigger endpoints HS to be used for modelling endpoints								
Conclusion from RMS	Results from this system were not considered as reliable by RMS, therefore no endpoints should be derived.								

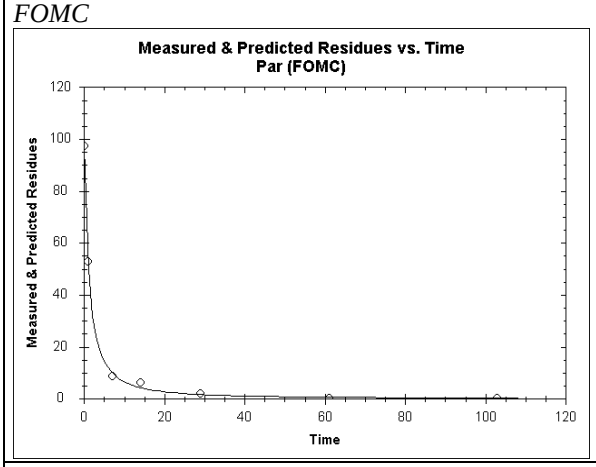
Table 8.2.2.3-124: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree B of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

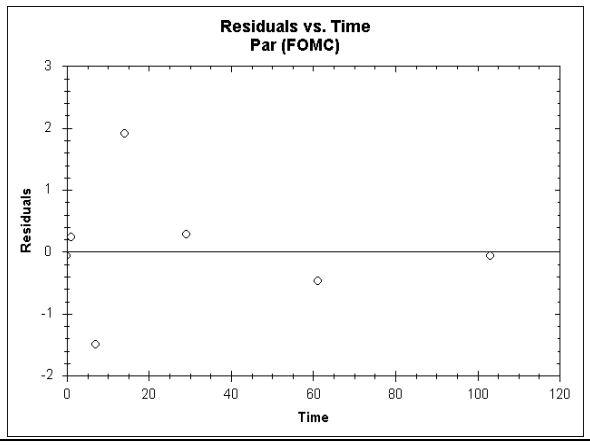
SFO



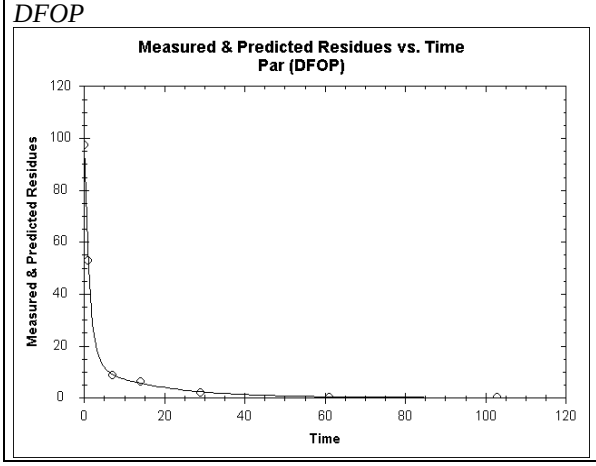


FOMC





DFOP



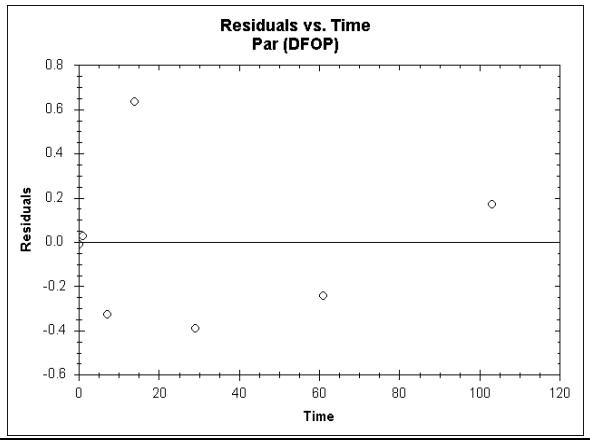
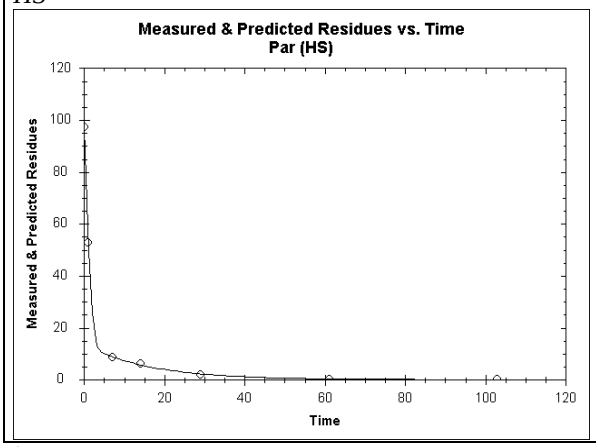
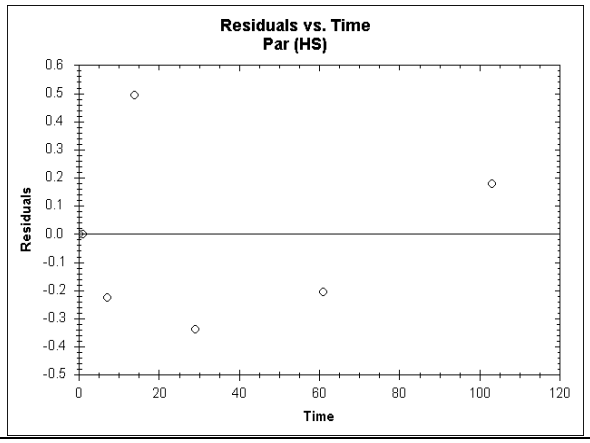


Table 8.2.2.3-124: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree B of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

HS





[1] t-test not relevant for kinetic parameter β

Overview of trigger and modelling endpoints

No reliable endpoints could be derived at Level P-II. A summary of trigger and modelling endpoints for glyphosate and its metabolites AMPA and HMPA is given in the tables below:

Table 8.2.2.3-125: Degradation and dissipation in water / sediment systems: trigger endpoints of glyphosate, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	χ^2 error (%)	Kinetic model
Total system								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	8.4	45.6	2.7	FOMC
	Putah	8.4	7.5	20	195.8	902.3	4.4	DFOP
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	15.8	329.4	2.2	HS
	Unter Widdersheim	8.6	7.68	20	121.6	>1000	4.8	DFOP
Water phase								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	5.0	22.7	2.3	DFOP
	Putah	8.4	7.5	20	7.9	78.2	10.0	FOMC
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	2.0	22.2	5.2	DFOP
	Unter Widdersheim	8.6	7.68	20	1.1	28.7	2.6	DFOP
Sediment phase								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	33.9	112.6	8.4	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	158.7	965.3	3.6	DFOP
	Unter Widdersheim	8.6	7.68	20	-[3]	-[3]	-[3]	-[3]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[3] No acceptable fit obtained and no endpoints could be derived

Table 8.2.2.3-126: Degradation and dissipation in water / sediment systems: modelling endpoints of glyphosate, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	SFO DT_{50} (d)[1]	χ^2 error (%)	Kinetic model
Total system							
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	9.7	5.3	SFO
	Putah	8.4	7.5	20	301.4[2]	4.4	DFOP
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	144.4[2]	2.2	HS
	Unter Widdersheim	8.6	7.68	20	1000[3]	4.8	DFOP
Water phase							
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	5.9	8.5	SFO
	Putah	8.4	7.5	20	23.6[4]	10.0	FOMC
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	6.7[4]	5.2	DFOP
	Unter Widdersheim	8.6	7.68	20	8.6[4]	2.6	DFOP
Sediment phase							
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	33.9	8.4	SFO
	Putah	8.4	7.5	20	-[5]	-[5]	-[5]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	346.6[2]	3.6	DFOP
	Unter Widdersheim	8.6	7.68	20	-[6]	-[6]	-[6]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow-phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used
[4] Back-calculated from DT_{90}/3.32 as 10 % of the initial amount was reached within experimental period
[5] No evaluation could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[6] No acceptable fit obtained and no endpoints could be derived

Table 8.2.2.3-127: Degradation and dissipation in water / sediment systems: trigger endpoints of AMPA, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	DT_{50} (d)[1]	DT_{90} (d)[1]	χ^2 error (%)	Kinetic model

Total system								
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	12.6	>1000	1.6	FOMC
	Schäphysen	8.0	7.34	20	2.4	>1000	6.2	DFOP
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	7.3	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	43.5	196.8	3.5	DFOP
	Unter Widdersheim	8.2	7.6	20	17.7	579.8	3.4	HS
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-[3]	-[3]	-[3]	-[3]
	Manningtree B	7.1	6.3	20	-[4]	-[4]	-[4]	-[4]
Water phase								
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	2.2	22.1	2.1	FOMC
	Schäphysen	8.0	7.34	20	1.1	6.6	3.2	FOMC
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	2.4	37.1	5.3	FOMC
	Unter Widdersheim	8.5	7.3	20	2.1	25.9	8.0	FOMC
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	6.6	50.7	4.5	DFOP
	Unter Widdersheim	8.2	7.6	20	2.0	17.3	8.2	DFOP
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	0.6	8.1	1.8	FOMC
	Manningtree B	7.1	6.3	20	1.1	5.5	1.0	HS
Sediment phase								
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	168.1	558.3	1.9	SFO
	Schäphysen	8.0	7.34	20	-[3]	-[3]	-[3]	-[3]
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	7.3	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	-[5]	-[5]	-[5]	-[5]
	Unter Widdersheim	8.2	7.6	20	-[3]	-[3]	-[3]	-[3]
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-[5]	-[5]	-[5]	-[5]
	Manningtree B	7.1	6.3	20	-[4]	-[4]	-[4]	-[4]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[3] No acceptable fit obtained and no endpoints could be derived
[4] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation
[5] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration

Table 8.2.2.3-128: Degradation and dissipation in water / sediment systems: modelling endpoints of AMPA, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	SFO DT_{50} (d)[1]	χ^2 error (%)	Kinetic model

Total system							
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	95.0[2]	3.8	DFOP
	Schäphysen	8.0	7.34	20	1000[3]	6.2	DFOP
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-[4]	-[4]	-[4]
	Unter Widdersheim	8.5	7.3	20	-[4]	-[4]	-[4]
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	47.7	5.9	SFO
	Unter Widdersheim	8.2	7.6	20	288.8[2]	3.4	HS
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-[5]	-[5]	-[5]
	Manningtree B	7.1	6.3	20	-[6]	-[6]	-[6]
Water phase							
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	6.7[7]	2.1	FOMC
	Schäphysen	8.0	7.34	20	1.5	10.7	SFO
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	11.2[7]	5.3	FOMC
	Unter Widdersheim	8.5	7.3	20	7.8[7]	8.0	FOMC
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	15.3[7]	4.5	DFOP
	Unter Widdersheim	8.2	7.6	20	5.2[7]	8.2	DFOP
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	2.4[7]	1.8	FOMC
	Manningtree B	7.1	6.3	20	1.7[7]	1.0	HS
Sediment phase							
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	168.1	1.9	SFO
	Schäphysen	8.0	7.34	20	-[5]	-[5]	-[5]
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-[4]	-[4]	-[4]
	Unter Widdersheim	8.5	7.3	20	-[4]	-[4]	-[4]
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	-[8]	-[8]	-[8]
	Unter Widdersheim	8.2	7.6	20	-[5]	-[5]	-[5]
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-[8]	-[8]	-[8]
	Manningtree B	7.1	6.3	20	-[6]	-[6]	-[6]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow-phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used
[4] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[5] No acceptable fit obtained and no endpoints could be derived
[6] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation
[7] Back-calculated from DT_{90}/3.32 as 10 % of the initial amount was reached within experimental period
[8] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration

Table 8.2.2.3-129: Degradation in water / sediment systems: trigger and modelling endpoints of AMPA, Level M-I, degradation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction	χ^2 error (%)	Kinetic model

[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	172.8	573.9	0.339 (from parent)	7.0	FOMC-SFO
	Putah	8.4	7.5	20	-[1]	-[1]	-[1]	-[1]	-[1]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	15.7	52.3	0.488 (from parent)	9.4	HS-SFO
	Unter Widdersheim	8.6	7.68	20	8.8	29.2	0.321 (from parent)	22.4	DFOP-SFO

[1] No acceptable fit obtained and no endpoints could be derived

Table 8.2.2.3-130: Dissipation in water / sediment systems: trigger and modelling endpoints of AMPA, Level M-I dissipation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DisT_{50}$ (d)	$DisT_{90}$ (d)	χ^2 error (%)	Kinetic model
Total system								
[redacted] (1999, CA 7.2.2.3/002)	Cache[1]	8.2	8.1	20	224.6	746.2	3.2	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	26.8[3]	88.9[3]	7.9	SFO
	Unter Widdersheim	8.6	7.68	20	15.1[3]	50.0[3]	5.8	SFO
Water phase								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	53.8	178.8	6.1	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	26.8	88.9	7.9	SFO
	Unter Widdersheim	8.6	7.68	20	15.1	50.0	5.8	SFO

[1] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration

[2] No evaluations could be conducted for any compartment at Level M-I dissipation due to the limited number of data points available after the peak concentration

[3] Since AMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system

Table 8.2.2.3-131: Degradation in water / sediment systems: trigger and modelling endpoints of HMPA, Level M-I, degradation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction	χ^2 error (%)	Kinetic model
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	128.8	427.8	0.366 (from AMPA)	20.5	HS-SFO
	Unter Widdersheim	8.6	7.68	20	10.0	33.4	0.359 (from AMPA)	39.3	DFOP-SFO

Table 8.2.2.3-132: Dissipation in water / sediment systems: trigger and modelling endpoints of HMPA, Level M-I dissipation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DisT_{50}$ (d)	$DisT_{90}$ (d)	χ^2 error (%)	Kinetic model
Total system & water phase[1]								
[redacted]	Bickenbach	8.6	7.8	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Unter Widdersheim	8.6	7.68	20	8.9	29.5	7.1	SFO

[1] Since HMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system

[2] No evaluations could be conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration

Assessment and conclusion by applicant:

The kinetic evaluation was performed according to the current guidances without any deviation. Thus, the provided endpoints can be used for risk assessment.

Assessment and conclusion by RMS:

The study is overall well performed.

RMS highlights that concentration at t0 were set to material balance, but were not corrected for radiochemical purity as recommended in FOCUS guidance. Considering that all radiochemical purities from the concerned studies are above 97.8% AR, RMS considers that no significant impact on the derived endpoints is expected.

RMS highlights that according to FOCUS guidance, only level P-I for total system is appropriate to derive modelling endpoints. Level P-1 for water and for sediment give dissipation DT_{50} and are therefore not suitable to derive modelling endpoints.

Finally, it is also noted that FOCUS kinetic guidance recommends to use a significance level of 10% for the t-test for water sediment studies. This was followed by the applicant. For consistency, the 90% confidence interval could have been reported instead of the 95% confidence interval.

The study is considered acceptable. It is noted that an updated kinetic assessment of data from [redacted], 2003 based on HPLC analysis should be provided by the applicant. This is identified as a data gap.

Relevant articles from literature search

Within the actual review of scientific literature for glyphosate (2010-2020), only one article was identified to potentially provide relevant information to the data point.

Table 8.2.2.3-133: Water/Sediment – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status
CA 7.2.2.3/023	Wang *et al.*, 2016	(Bio)degradation	Glyphosate	Reliable with retrictions

Wang et al., 2016

Data point:	CA 7.2.2.3/023
Report author	Wang, S. *et al.*
Report year	2016
Report title	(Bio)degradation of glyphosate in water-sediment microcosms - A stable isotope co-labeling approach
Document No	DOI 10.1016/j.watres.2016.04.041 E-ISSN 1879-2448
Guidelines followed in study	OECD guideline 308

Deviations from current test guideline	From OECD 308_ - Exremely high application rate. - Water/sediment systems may have received inputs of glyphosate or AMPA within the previous 4 years.
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Materials and Methods

Chemicals

All the chemicals used were analytical or reagent grade and were obtained from the Carl Roth Company (Karlsruhe, Germany) if not specified otherwise. Resin for amino acid purification (Dowex 50W-X8, 50-100 mesh) was purchased from VWR/Merck (Darmstadt, Germany). Methanol and ammonium acetate for ultraperformance liquid chromatography-mass spectrometry (UPLC/MS) measurements were provided by Biosolve (Valkenswaard, Netherlands). Labeled $^{13}C_3{}^{15}N$-glyphosate was purchased from IsoSciences Company (Trevose, PA, USA). The isotopical enrichment of glyphosate was 99% for ^{13}C and 98% for ^{15}N; the chemical purity was 98 %.

Sediments and water

The sediments and associated water were collected from the Getel creek, Harz Mountains in Saxony-Anhalt, Germany. The catchment of this creek comprises agricultural lowlands with continuous crop rotation and pesticide application. It is thus a high risk area for exposure to pesticides. The sediments contained 38 % (±0.7 %) sand (>0.05 mm), 62 % (±0.7 %) silt + clay (<0.05 mm), 85 mg/g (±2 mg/g) total organic carbon and 15 mg/g (±1 mg/g) total nitrogen. The pH of the sediments and creek water was 7.1 and 8.8, respectively. The content of total organic carbon of the suspended matter in the creek water was 8 mg/L (±1 mg/L), and the content of total nitrogen was 3 mg/L (±0.6 mg/L). Neither glyphosate nor AMPA were detected in the sediments or creek water. Sediments and associated water were taken from the upper layer (up to 5 cm) of the Getel creek sediment. The sediments were separated from the water by filtration, wet sieved and gently homogenized.

Incubation experiment

Degradation experiments were conducted according to the OECD guideline 308 in biometer flasks to address the transformation in aquatic sediment systems. Six incubations were performed:

1) water-sediment without glyphosate (non-amended control),

2) water-sediment with unlabeled glyphosate (unlabeled control),

3) water-sediment with labeled glyphosate (biotic system),

4) water with unlabeled glyphosate (unlabeled control),

5) water with labeled glyphosate and

6) sterilized water sediment with labeled glyphosate (abiotic system).

The two controls without glyphosate and unlabeled glyphosate were used to correct for the natural abundances of ^{13}C (~1.1 at %) and ^{15}N (~0.37 at %) in the sediment, and water systems without sediment were prepared to test the effect of sediment on the microbial degradation of glyphosate. Abiotic controls were incubated to distinguish between abiotic and biotic degradation of $^{13}C_3{}^{15}N$-glyphosate. In these controls, sediment and water were sterilized by autoclaving three times at 120°C for 20 min prior to incubation.

50 g (dw) of sediment and 90 mL of creek water containing either unlabeled or labeled glyphosate were added to glass bottles. The initial concentration of glyphosate was 50 mg/L in water and water-sediment systems, except in the blanks containing no glyphosate. This concentration is well above environmentally relevant levels, but it was required to obtain reliable isotopic enrichment results in the water-sediment systems given the limited sensitivity of $^{13}C/^{15}N$ isotope analytical methods and the high background due to natural abundance of the heavy isotopes in the controls. To assess the overall fate and turnover at lower

concentrations that were closer to environmentally relevant concentrations, additional water-sediment experiments at 3 mg/L (minimum ^{13}C and ^{15}N label detection limit) were prepared.

Incubation experiments were conducted in the dark and at constant temperature (20°C) for 80 days. The bottles were sampled after 0, 5, 10, 20, 40 and 80 days (abiotic, blank, water and 3 mg/L systems only after 80 days). At each sampling time, the respective systems were destructively sampled, and the water and sediments were separated by filtration and subjected to further analyses. The CO_2 evolved from the mineralised glyphosate was trapped in 2 M NaOH; the NaOH solution was exchanged at regular intervals. Because the pH of the water was >7.0, a certain amount of CO_2 originating from the mineralisation of glyphosate may partition into the water phase, which therefore has been analyzed in addition to the NaOH traps. Mineralisation in biotic and abiotic systems includes $^{13}CO_2$ in both the sodium hydroxide and water phases.

Chemical analyses

A general mass balance of the ^{13}C and ^{15}N labels in the systems was set up based on the contents and isotopic compositions of CO_2, the extractable glyphosate and its metabolites and either ^{13}C or ^{15}N in the total NER. Proteins were hydrolyzed, and the amino acids (AA) were extracted and analyzed for their concentration and isotopic composition to estimate the extent that C and N from $^{13}C_3{}^{15}N$-glyphosate were incorporated into microbial biomass and ultimately into biogenic residues. Proteins are the main constituents of microbial biomass (50 % of cells); therefore, the quantification of biogenic residues formation was based on a factor of 2 for both ^{13}C and ^{15}N-amino acids (AA).

CO_2 measurements

The ^{13}C labeled CO_2 was quantified by measuring the total inorganic C in a 2 M NaOH solution on a total organic carbon analyzer. The isotopic composition of CO_2 (at % ^{13}C) was measured by GC-combustion-isotope ratio mass spectrometry (GC-C-irMS; Finnigan MAT252 Thermo Electron coupled to a Hewlett Packard 6890 GC) with a Porabond Q-HT Plot FS column (50 m - 0.32 m - 5 µm).

Extractable glyphosate and AMPA

Glyphosate and AMPA were extracted with borate buffer (40 mM, pH 9.2) from sediments and derivatized with 0.5 mL of fluorenylmethyloxycarbonyl (Fmoc). The water samples were directly derivatized with Fmoc in borate buffer. The concentrations of glyphosate and AMPA were determined by UPLC-MS i-Class system (Waters, Manchester, UK) with an Acquity UPLC HSS T3 column (1.7 µm, 2.1 x 100 mm; Waters, Milford, MA, USA). The temperatures of the column and the autosampler were set at 60°C and 4°C, respectively. The injection volume was 10 µL. The eluents were 5 mM NH_4 acetate (pH 8) in water (eluent A) and methanol (eluent B). The flow rate was set to 0.6 mL/min. The gradient program was as follows: 0-3 min 5 % B, 7-8 min 95 % B, 8.1-10 min 5 % B. The MS analysis was performed using a Xevo TQ-S mass spectrometer (Waters, Manchester, UK) equipped with an ESI source in negative ion mode working in multiple reaction monitoring (MRM) mode. A capillary voltage of 2 kV and a desolvation temperature of 600°C were used. The flow of the desolvation gas was set at 1000 L/h. Unlabeled glyphosate and AMPA were used for calibration and as internal standards for correction of possible matrix effects which may occur during the measurement of glyphosate and AMPA concentrations. Transitions, cone voltages, and collision energies were automatically tuned for the compounds: $^{13}C_3{}^{15}N$-glyphosate (m/z 172 / m/z 154, cone: 58 V, collision energy: 10 V; m/z 172 / m/z 63, cone: 58 V, collision energy: 16 V) and $^{13}C_3{}^{15}N$-AMPA (m/z 112 / m/z 63, cone: 58 V, collision energy: 16 V; m/z 112 / m/z 79, cone: 58 V, collision energy: 10 V). The detection limit (LOD) of glyphosate was determined at 20 µg/L, and the LOD for AMPA was 30 µg/L based on the signal-to-noise method (signal >3 S/N). For the entire procedure, including the extraction of the sediment samples, the detection limits were 0.608 mg/kg (glyphosate) and 0.912 mg/kg (AMPA). The recovery of glyphosate and AMPA was >98 %. The values of the coefficient of determination (R^2) for all calibration curves were greater than 0.99. The relative error of UPLC-MS measurements was <10 %.

Non-extractable residues (NER)

After the extraction of glyphosate and AMPA, the sediment sample containing unextracted ^{13}C and ^{15}N label as NER was airdried. An aliquot of 4-5 mg was weighed and combusted using an elemental analyzer-combustion-isotope ratio mass spectrometer combination (EA-C-irMS; Euro EA 3000, Eurovector, Milano, Italy + Finnigan MAT 253, Thermo Electron, Bremen, Germany). Glyphosate-derived

C and N were calculated as the excess ^{13}C and ^{15}N over the controls. The values of the coefficient of determination (R^2) for all calibration curves were greater than 0.99.

Amino acids (AA)

Amino acids were analyzed in the living microbial biomass AA fraction of sediment (bioAA) and in the total AA pool of the sediment fraction (tAA). Microbial biomass was extracted from the sediment with ion exchanger and sodium deoxycholate/polyethylenglycol solution. The sediment and microbial biomass pellets containing accordingly tAA and bioAA were hydrolyzed using 6 M HCl. Thereafter, the hydrolysate was purified over a cation exchange resin. The detailed extraction, purification and derivatization methods for bioAA and tAA were described previously. The identity and quantity of AA were measured using GC-MS, HP 6890 with a BPX-5 column. The isotopic composition of the respective AA (at % ^{13}C and at % ^{15}N) was determined by GC-C-irMS, Finnigan MAT 253 coupled to a Trace GC, with a BPX-5 column. The details on the analytical conditions for AA separation by GC-MS and GC-C-irMS are reported in Nowak *et al.* (2013). For quantification and identification of respective AA in samples, an external standard containing all detectable AA in the samples (alanine, glycine, threonine, valine, leucine, isoleucine, proline, aspartate, glutamate, phenylalanine and lysine) was used. The internal standard L-norleucine was added to each sample before hydrolysis to estimate the losses in AA analyses. The recovery of all measured AA was >90 %, except from threonine (>80 %). The measured isotopic compositions were corrected for shifts due to derivatization.

Data analyses and mass balance

All incubation experiments and chemical analyses were conducted in triplicate, and the data are presented as averages of three replicates. Mineralisation, extractable and non-extractable $^{13}C_3$ ^{15}N-glyphosate residues were quantified for each sampling date in order to set up the full carbon and nitrogen mass balance, and to determine the compound degradation kinetics. The contents of the $^{13}CO_2$, ^{13}C and ^{15}N-NER, ^{13}C and ^{15}N-AA (bioAA + tAA) were based on quantitation of the total concentration of the respective carbon or nitrogen pool and on analyzing the excess of ^{13}C (^{15}N) over the controls (non-amended without glyphosate and unlabeled containing unlabeled glyphosate) as described by Lerch *et al.* (2009). The results were expressed as a percentage of ^{13}C or ^{15}N label relative to the initial $^{13}C_3$-glyphosate equivalents or ^{15}N-glyphosate equivalents. The total uncertainty of the carbon pool in CO_2 and of the carbon and nitrogen pools in NER was <10 %, whereas the total uncertainty of the determination of at % ^{13}C and at % ^{13}N isotope signatures was <0.5 % for unlabeled samples, but <3 % for the labeled ones. The relative average error of the label excess (based on Gaussian error propagation) was <10 % for CO_2 and NER.

The total uncertainty of carbon and nitrogen pool in tAA and bioAA was <15 %. The total uncertainty on the determination of at % ^{13}C and at % ^{13}N isotope analysis was <0.5 % for unlabeled samples, but <1 % for labeled ones. The relative average error of the label excess (based on Gaussian error propagation) was <10 % for tAA and bioAA.

The recovery of the ^{13}C and ^{15}N labels expressed as a percentage of the initially applied isotope label equivalents ranged from 93 to 110 % for C and from 86 to 110 % for N. Incorporation of the ^{13}C and ^{15}N labels into the microbial biomass and thus the total content of biogenic residues formed during degradation of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system were estimated from ^{13}C -tAA and ^{15}N-tAA, considering that AA constituted approximately 50 % of the total C and total N in the biomass. The recovery of microbial biomass extraction is estimated at 40 %. The bioAA results are presented both as the original data and the recalculated values based on 40 % extraction efficiency, but interpretation of bioAA was based on the original data.

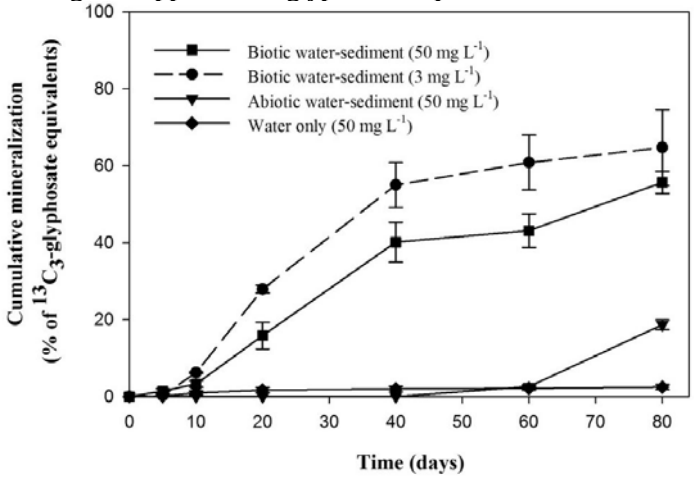
Results and Discussion

Mineralisation of $^{13}C_3$-glyphosate

Mineralisation of $^{13}C_3$-glyphosate in the biotic water-sediment system consisted of three periods (Figure 8.2.2.3-6): an initial short lag-phase from day 0 to day 10 characterized by low mineralisation rates (0.3 %/day), day 10-40 characterized by the highest mineralisation rate (1.2 %/day), and day 40-80 characterized by decreasing mineralisation rates to 0.4 %/day. At the end of incubation, a total of 56 % of the $^{13}C_3$-glyphosate had been mineralised. Abiotic processes played a minor role in the mineralisation of $^{13}C_3$-glyphosate (<20 %). The mineralisation rates of $^{13}C_3$-glyphosate in the water system (without

sediment) were very low and increased slowly during the first ten days (~0.1 %/day). Thereafter, the mineralisation rate decreased and only 2 % of $^{13}C_3$-glyphosate equivalents were mineralised at the end, demonstrating the key role of sediments in the mineralisation of $^{13}C_3$-glyphosate. Mineralisation of $^{13}C_3$-glyphosate at 3 mg/L was slightly higher (65 % of $^{13}C_3$-glyphosate equivalents) than at 50 mg/L. The acclimation period at 50 mg/L was longer (10 days vs. 5 days for 3 mg/L). The mineralisation rate in the initial phase (0 – 10 days) was two-fold higher at 3 mg/L (0.6 %/day) than at 50 mg/L (0.3 %/day) and 1.3-fold higher in the second phase (10 – 40 days; 1.6 %/day compared to 1.2 %/day, respectively). In the third phase (40 – 80 days), the mineralisation rate was 2-fold lower at 3 mg/L (0.2 %/day) than at 50 mg/L (0.4 %/day).To date, there are no reports on the mineralisation of $^{13}C_3$-glyphosate in water-sediment systems. Although glyphosate was below the detection limit in the sediment and associated water used in the present experiments, prior exposure to this herbicide is very likely due to input from the agricultural area in the catchment, with major biodegradation occurring in the sediment phase. In contrast to the high $^{13}CO_2$ evolution from $^{13}C_3$-glyphosate equivalents, no or minimal mineralisation of ^{15}N-glyphosate was found in the present study because the total recovery of the ^{15}N label ranged from 86 to 110 %.

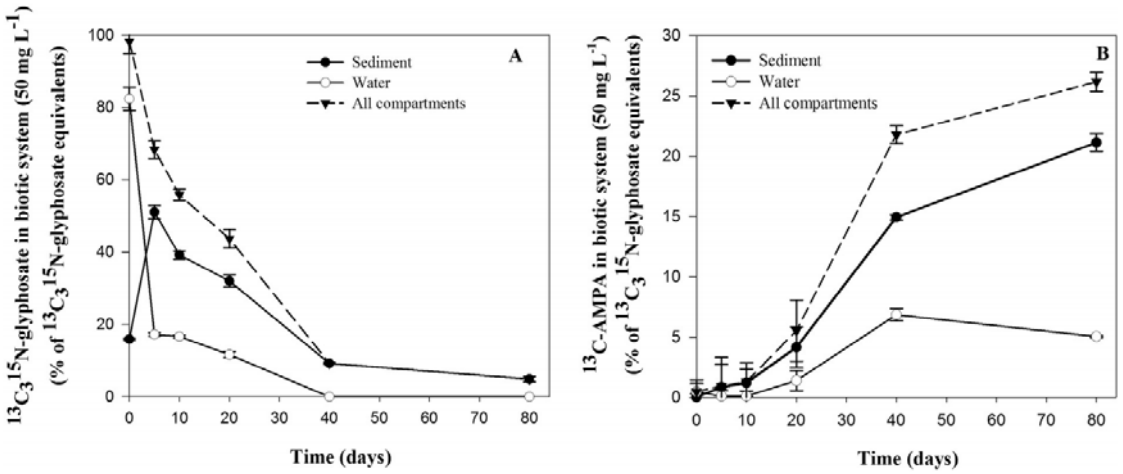
Figure 8.2.2.3-6: Cumulative mineralisation of $^{13}C_3$-glyphosate in water-sediment and water only systems over 80 days given as percentages of applied $^{13}C_3$-glyphosate equivalents



Turnover of $^{13}C_3{}^{15}N$-glyphosate

The content of $^{13}C_3{}^{15}$N-glyphosate in the biotic 50 mg/L water sediment system decreased rapidly until day 40 (Figure 8.2.2.3-7), indicating its low persistence reflected in its half-life (DT_{50}) of 15 days. In the water compartment, glyphosate dissipated rapidly during the first five days. Thereafter, elimination of this herbicide continued slowly until its ultimate removal by day 40. From day 40 onwards, $^{13}C_3{}^{15}$N-glyphosate was detected only in the sediment compartment although it was initially spiked in the water phase. This indicates that elimination from the water compartment was a combined process of sorption onto sediments and microbial transformation. A quick partitioning of glyphosate from the water compartment to the sediments had already been observed on day 0. At the initial sampling, which was performed 3 h after the addition of the glyphosate-spiked water to allow for particle sedimentation, 16 % of the initially added $^{13}C_3{}^{15}$N-glyphosate was already detected in the sediment phase. The high abundance of the silt + clay fraction (62 %), which is typically rich in oxides, of the sediments might explain the rapid elimination of glyphosate from the water by adsorption to the sediments. The turnover kinetics of $^{13}C_3{}^{15}$N-glyphosate in the sediment was much slower than in the water. A maximum amount of $^{13}C_3{}^{15}$N-glyphosate (51 % of the initially added $^{13}C_3{}^{15}$N label) was detected in the sediments on day 5. Therefore, a potential risk by residual glyphosate in the sediment is given. Thereafter, elimination of $^{13}C_3{}^{15}$N-glyphosate from sediments was rapid (days 5-40), followed by a slower disappearance towards the end to ultimately result in 5 % of the initially added $^{13}C_3{}^{15}$N label.

Figure 8.2.2.3-7: Distribution of the extracted $^{13}C_3{}^{15}$N-glyphosate (A) and ^{13}C1-AMPA (B) in biotic water-sediment systems (50 mg/L) over 80 days expressed as the percentage of applied $^{13}C_3{}^{15}$N-glyphosate. (Please note: ^{13}C-AMPA only contains one labeled carbon atom; the second metabolite glyoxylate contains the other two)



The decrease in $^{13}C_3{}^{15}N$-glyphosate in both the water and sediment compartments during days 5-40 parallels the increasing mineralisation of glyphosate. At the same time, a large amount of the recovered $^{13}C_3{}^{15}N$-glyphosate from the water-sediment system was associated with the sediments (~74 %), whereas only ~25 % was dissolved in the water. Finally, when $^{13}C_3{}^{15}N$-glyphosate was detected only in the sediment compartment (40 – 80 days), mineralisation kinetics were slower indicating a limited bioavailability of glyphosate adsorbed onto sediment particles (Katagi, 2013). In the first ten days of the present experiments, only low contents of ^{13}C in AMPA (1 % of $^{13}C_3{}^{15}N$-glyphosate equivalents; Figure 8.2.2.3-7) and ^{15}N in AMPA (4 % of $^{13}C_3{}^{15}N$-glyphosate equivalents) were observed. Thereafter, (10-40 days), a rapid increase in the $^{13}C_3{}^{15}N$-AMPA contents was noted and was accompanied by the rapid degradation of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system with concomitant $^{13}CO_2$ formation. From day 40 onwards, when glyphosate partitioned into the sediment and its mineralisation rate decreased, the increase in the $^{13}C_3{}^{15}N$-AMPA contents slowed down. At the end of the experiment, ^{13}C in AMPA accounted for 26 % of the $^{13}C_3$-glyphosate equivalents. As only one of the three labeled ^{13}C atoms from the glyphosate, but all of the ^{15}N (one atom) is retained in AMPA (Figure 8.2.2.3-9) and the percentages are referred to the initial amount of labeled atoms (not molecules), the percentage of ^{15}N-AMPA was generally 3-fold higher than that of ^{13}C-AMPA and thus amounted to 79 % of the initially added ^{15}N-glyphosate. Similar to $^{13}C_3{}^{15}N$-glyphosate, the recovered $^{13}C_3{}^{15}N$-AMPA from the system was mostly associated to the sediment (70-90 %), whereas the residual (10-30 %) was dissolved in the water phase. In contrast to $^{13}C_3{}^{15}N$-glyphosate, $^{13}C_3{}^{15}N$-AMPA was more persistent; this was indicated by its continuous increase until the end of the experiment, indicating that $^{13}C_3{}^{15}N$-AMPAwas degraded more slowly than it was produced from glyphosate, as reported earlier (Mamy *et al.*,2005). Unfortunately, our data do not allow quantification of microbial AMPA degradation due to the simultaneous formation and degradation. Due to the continuing production of AMPA at a higher rate than degradation, a potential risk may be given by this metabolite. Compared to the biotic systems, the abiotic controls, water without sediment and biotic systems at 3 mg/L showed much lower formation of $^{13}C_3{}^{15}N$-AMPA from glyphosate.

Figure 8.2.2.3-8: Time dependent ^{13}C- (A) and ^{15}N-label (B) incorporation into tAA, bioAA and recalculated bioAA (40 % extraction efficiency) during microbial degradation of $^{13}C_3{}^{15}N$-glyphosate in biotic water-sediment system (50 mg/L) expressed as the percentage of applied $^{13}C_3{}^{15}N$-glyphosate equivalents

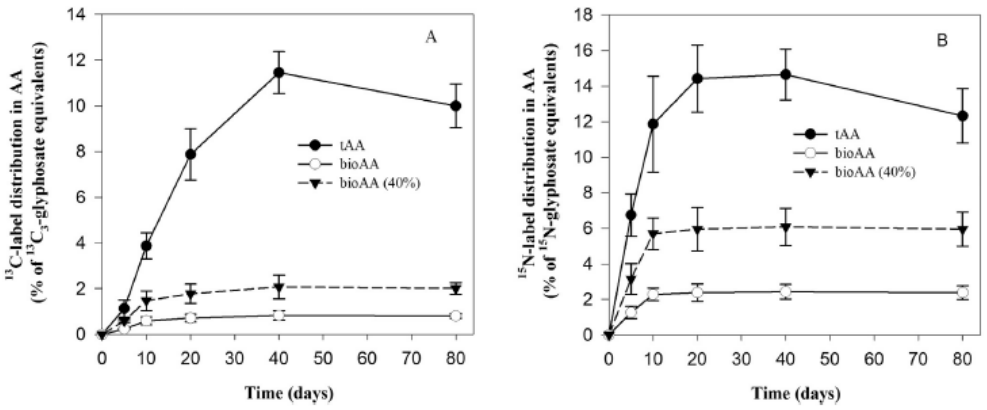
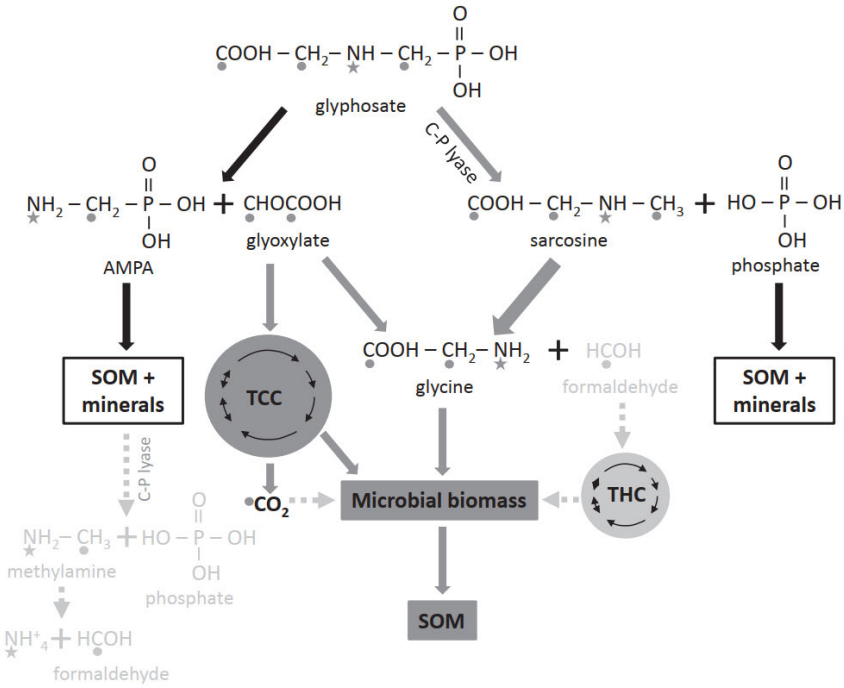


Figure 8.2.2.3-9: Pathways of microbial degradation of glyphosate through sarcosine and AMPA in biotic water-sediment system (50 mg/L). Dark grey arrows: biogenic residue formation; black arrows: xenobiotic NER formation; TCC: tricarboxylic acid cycle; THC: tetrahydrofolate cycle; grey circles = ^{13}C label; grey stars = ^{15}N label; Light grey = presumed further degradation



Incorporation of the ^{13}C and ^{15}N labels into AA and biogenic residues

The total AA pool of sediment (tAA) includes the AA in the living biomass and in the dead and decaying necromass. Neither ^{13}C nor ^{15}N enrichment in AA was detected in the suspended particles in either the water compartment of the abiotic sediment-water systems or the water systems. In the biotic water-sediment systems, ^{13}C label incorporation into the bioAA fraction was observed in the first sampling, and the contents of ^{13}C-bioAA increased rapidly to a maximum on day 40 (0.83 % of $^{13}C_3$-glyphosate equivalents; Figure 8.2.2.3-8). A continuous flux of ^{13}C--labeled AA from the living biomass to the non-living fraction OM was noted from day 5 onwards. ^{13}C-tAA initially increased sharply whereas from day 20 onwards, the ^{13}C-bioAA remained nearly constant, and approximately 92 % of the ^{13}C label in the tAA could be attributed to the non-living OM. In contrast to the ^{13}C bioAA, the ^{13}C-tAA contents slightly decreased after 40 days. At the end of the experiment, the contents of ^{13}C in tAA reached 10 % of the initially added $^{13}C_3$-glyphosate. Considering a protein content of 50 % in bacterial cells (Nowak *et al.*, 2013), we arrive at a total of 20 % ^{13}C-biogenic residues at the end of the experiment. Similar to ^{13}CAA, incorporation of the ^{15}N label into bioAA and tAA was also observed starting from day 5 (Figure 8.2.2.3-8). In contrast to ^{13}C-bioAA, ^{15}N-bioAA contents plateaued on day 10 (2.38 % of ^{15}N-glyphosate equivalents). The incorporation of

^{15}N-bioAA into the non-living OM fraction was also similar to that of ^{13}C-bioAA, starting rapidly (on day 5), and approximately 81 % of the ^{15}N-tAA was stabilized in the non-living OM at the end. The rapid initial increase in ^{15}N-tAA continued until day 20 and then remained stable until day 40. Analogous to ^{13}C-tAA, ^{15}N-tAA decreased slightly towards the end.

^{15}N-tAA amounted to 12 % of the initially added ^{15}N-glyphosate at the end (similar to ^{13}C-tAA), and 24 %was observed for ^{15}N-biogenic residues based on a conversion factor of two for biomass in general.

The dominant incorporation of both the ^{13}C and ^{15}N labels into the glycine was observed throughout the experiment and was most pronounced in the initial incubation period. Various AA were progressively enriched in both isotopes over time. In general, incorporation of ^{15}N proceeded faster than that of ^{13}C. In contrast to ^{13}C, the ^{15}N label disappeared from ^{13}C^{15}N-glycine quickly and was distributed within different AA more rapidly than the ^{13}C label.

The results based on the ^{13}C- and ^{15}N-colabeling technique allowed comprehensive insight into the C and N fluxes from the colabeled $^{13}C_3{}^{15}N$ -glyphosate via microbial biomass to the non-living OM. Microorganisms assimilated the carbon and nitrogen from glyphosate to synthesize biomass compounds, as shown by the ^{13}C and ^{15}N-labeled bioAA. After death and cell lysis, their biomass constituents were progressively incorporated into the non-living OM fraction where they were stabilized and ultimately formed non-toxic biogenic residues.

Table 8.2.2.3-134: ^{13}C label distribution in diverse ^{13}C-bioAA (A) and ^{13}C-AA in the non-living SOM (B) during biodegradation of $^{13}C_3$-glyphosate in biotic water-sediment system (50 mg/L)

(A)

Incubation time (days)	^{13}C-bioAA (% of $^{13}C_3$-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.06 (±0.01)	0.07 (±0.00)	0.07 (±0.02)	0.07 (±0.00)	0.06 (±0.01)
Glycine	n.d.	**0.17** (±0.02)	**0.18** (±0.01)	0.14 (±0.01)	0.15 (±0.03)	**0.11**↓ (±0.01)
Threonine	n.d.	n.d.	n.d.	0.07 (±0.00)	0.06 (±0.01)	0.06 (±0.02)
Valine	n.d.	n.d.	0.10 (±0.02)	0.10 (±0.01)	0.09 (±0.02)	0.09 (±0.00)
Leucine	n.d.	n.d.	n.d.	n.d.	0.14 (±0.00)	0.12 (±0.01)
Isoleucine	n.d.	n.d.	0.11 (±0.00)	0.14 (±0.00)	0.05 (±0.01)	0.04 (±0.02)
Proline	n.d.	n.d.	n.d.	0.05 (±0.03)	0.04 (±0.01)	0.09 (±0.00)
Aspartate[a]	n.d.	n.d.	0.02 (±0.00)	0.03 (±0.00)	0.04 (±0.02)	0.08 (±0.01)
Glutamate[b]	n.d.	n.d.	0.06 (±0.03)	0.07 (±0.03)	0.05 (±0.02)	0.05 (±0.00)
Phenylalanine	n.d.	n.d.	n.d.	n.d.	0.10 (±0.03)	0.07 (±0.01)
Lysine	n.d.	n.d.	0.04 (±0.01)	0.04 (±0.03)	0.04 (±0.06)	0.03 (±0.01)
Total	**n.d.**	**0.23 (± 0.03)**	**0.58 (± 0.07)**	**0.71 (± 0.13)**	**0.83 (± 0.21)**	**0.80 (± 0.10)**

(B)

Incubation time (days)	^{13}C-non-living AA (% of $^{13}C_3$-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.4 (±0.1)	0.6 (±0.1)	1.3 (±0.3)	1.4 (±0.1)	0.7 (±0.1)
Glycine	n.d.	**0.5** (±0.2)	**1.0** (±0.1)	1.0 (±0.1)	0.5 (±0.1)	0.6 (±0.2)
Threonine	n.d.	n.d.	n.d.	0.4 (±0.1)	0.1 (±0.1)	0.5 (±0.1)
Valine	n.d.	n.d.	n.d.	1.0 (±0.6)	1.1 (±0.1)	1.3 (±0.3)
Leucine	n.d.	n.d.	n.d.	n.d.	1.6 (±0.3)	1.2 (±0.1)
Isoleucine	n.d.	n.d.	n.d.	0.7 (±0.1)	1.1 (±0.1)	0.9 (±0.3)
Proline	n.d.	n.d.	0.5 (±0.1)	0.5 (±0.1)	0.5 (±0.1)	0.3 (±0.1)
Aspartate[a]	n.d.	n.d.	0.5 (±0.1)	0.5 (±0.1)	1.1 (±0.2)	0.8 (±0.1)
Glutamate[b]	n.d.	n.d.	0.7 (±0.1)	0.5 (±0.1)	1.1 (±0.1)	1.6 (±0.2)
Phenylalanine	n.d.	n.d.	n.d.	n.d.	1.0 (±0.3)	0.6 (±0.1)
Lysine	n.d.	n.d.	n.d.	1.3 (±0.1)	1.3 (±0.4)	0.9 (±0.3)
Total	**n.d.**	**0.9 (± 0.3)**	**3.3 (± 0.5)**	**7.2 (± 1.6)**	**10.8 (± 2.0)**	**9.4 (± 1.9)**

[a] Incl. Asparagine.
[b] Incl. Glutamine; n.d. - not detectable; values are presented as averages ± standard deviation; values printed in bold show characteristic values; arrows illustrates increases or decreases of the respective AA compared to the preceding sampling event.

Table 8.2.2.3-135: ^{15}N label distribution in diverse ^{15}N-bioAA (A) and ^{15}N-AA in the non-living SOM (B) during biodegradation of ^{15}N-glyphosate in biotic water-sediment system (50 mg/L)

(A)

Incubation time (days)	^{15}N-bioAA (% of ^{15}N-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.01 (±0.08)	0.09 (±0.1)	0.03 (±0.02)	0.15 (±0.02)	0.23 (±0.07)
Glycine	n.d.	**0.70** (±0.07)	**1.20**↑(±0.01)	**1.20** (±0.09)	**0.80**↓(±0.10)	**0.43**↓(±0.07)
Threonine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	n.d.	0.38 (±0.07)	0.28 (±0.03)	0.28 (±0.02)	0.30 (±0.05)	0.28 (±0.02)
Leucine	n.d.	0.17 (±0.02)	0.17 (±0.04)	0.14 (±0.07)	0.40 (±0.09)	0.38 (±0.03)
Isoleucine	n.d.	n.d.	0.30 (±0.01)	0.28 (±0.01)	0.09 (±0.07)	0.13 (±0.06)
Proline	n.d.	n.d.	0.04 (±0.04)	0.04 (±0.13)	0.05 (±0.04)	0.22 (±0.05)
Aspartate[a]	n.d.	n.d.	n.d.	0.13 (±0.06)	0.10 (±0.09)	0.22 (±0.10)
Glutamate[b]	n.d.	n.d.	0.10 (±0.03)	0.19 (±0.07)	0.19 (±0.08)	0.20 (±0.02)
Phenylalanine	n.d.	n.d.	0.06 (±0.03)	0.08 (±0.02)	0.21 (±0.04)	0.19 (±0.02)
Lysine	n.d.	n.d.	0.04 (±0.03)	0.01 (±0.01)	0.14 (±0.05)	0.10 (±0.08)
Total	**n.d.**	**1.26 (±0.24)**	**2.28 (±0.32)**	**2.38 (±0.5)**	**2.43 (±0.63)**	**2.38 (±0.52)**

(B)

Incubation time (days)	^{15}N-non-living AA (% of ^{15}N-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.2 (±0.1)	0.6 (±0.1)	0.4 (±0.1)	0.8 (±0.2)	0.2 (±0.1)
Glycine	n.d.	**1.1** (±0.1)	1.2 (±0.2)	1.2 (±0.1)	**0.8**↓(±0.1)	**0.7**↓ (±0.1)
Threonine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	n.d.	0.8 (±0.1)	1.4 (±0.2)	1.5 (±0.5)	1.3 (±0.2)	1.0 (±0.2)
Leucine	n.d.	0.7 (±0.1)	1.2 (±0.8)	2.8 (±0.4)	1.8 (±0.1)	1.5 (±0.1)
Isoleucine	n.d.	n.d.	1.5 (±0.1)	1.2 (±0.2)	1.6 (±0.1)	1.4 (±0.3)
Proline	n.d.	n.d.	0.6 (±0.1)	0.8 (±0.1)	0.8 (±0.1)	0.4 (±0.1)
Aspartate[a]	n.d.	n.d.	0.1 (±0.2)	0.5 (±0.1)	1.0 (±0.1)	0.6 (±0.2)
Glutamate[b]	n.d.	**1.2** (±0.1)	1.0 (±0.1)	0.7 (±0.1)	1.4 (±0.1)	1.8 (±0.1)
Phenylalanine	n.d.	n.d.	0.7 (±0.1)	1.4 (±0.1)	1.4 (±0.1)	1.2 (±0.1)
Lysine	n.d.	n.d.	0.1 (±0.1)	1.6 (±0.1)	1.7 (±0.1)	0.9 (±0.1)
Total	**n.d.**	**4.0 (±0.5)**	**8.4 (±2.0)**	**12.1 (±1.8)**	**12.6 (±1.2)**	**9.7 (±1.4)**

[a] Incl. Asparagine.
[b] Incl. Glutamine; n.d. - not detectable; values are presented as averages ± standard deviation; values printed in bold show characteristic values; arrows illustrates increases or decreases of the respective AA compared to the preceding sampling event.

Indication of different (bio)degradation pathways of glyphosate in water-sediment systems

Based on the detailed glyphosate turnover mass balance and the patterns of ^{13}C and ^{15}N labeled AA over time, particularly of the dominant glycine, we could distinguish between two degradation pathways of this herbicide in water-sediment system. The dominance of co-labeled $^{13}C^{15}N$-glycine especially in the first sampling event indicates its formation via the sarcosine pathway (Borggaard and Gimsing, 2008; Singh and Walker, 2006; Figure 8.2.2.3-9). However, the occurrence of the sarcosine pathway in soil or sediment has not yet been proven (Borggaard and Gimsing, 2008; Singh andWalker, 2006). We could not detect sarcosine in our experiment, but this compound is rapidly oxidized to glycine and thus does not accumulate. The formed glycine is directly incorporated into microbial biomass, resulting in the observed occurrence of co-labeled $^{13}C^{15}N$-glycine in the living biomass AA. The negligible mineralisation (3 % of ^{13}C equivalents initially applied) with high simultaneous removal of $^{13}C_3{}^{15}N$ -glyphosate and the maximum contents of ^{13}C and ^{15}N glycine on day 10 also support the hypothesis that glyphosate is initially degraded via the sarcosine pathway. Hence, the sarcosine pathway was actually contributing at the beginning of glyphosate degradation, whereas the AMPA pathway dominated in the later degradation phase. A later decrease of co-labeled $^{13}C_3{}^{15}N$-glycine (10-20 days) was accompanied by a rapid increase in AMPA over time.

The risk potential of glyphosate residues in water-sediment systems

To date, there is no detailed information on the metabolic fate of glyphosate residues and their distribution in the water-sediment system. The present results provide detailed insight into the biodegradation processes of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system and into the transformation of this herbicide into AMPA, microbial biomass and NER. Since glyphosate is biodegraded and the NER are dominantly biogenic residues, the highest potential risk is provided by the significant concentrations of AMPA.

Non-extractable $^{13}C_3$-glyphosate residues were formed immediately (6 % of the initially added ^{13}C label). The NER contents increased until day 10 and then remained on a high level. From day 20 onwards, their contents decreased and ultimately reached 23 % of the $^{13}C_3$-glyphosate equivalents. The chemical composition of the NER formed during degradation of glyphosate is not yet known, and their analyses are limited to quantification. In the present study, glyphosate was initially a source of xenobiotic ^{13}CNER formation that was dominant until day 10 (Figure 8.2.2.3-10). However, immobilized glyphosate in the NER was microbially degradable, as shown by the continuous decrease of xenobiotic NER over time, specifically of ^{13}C-xenobiotic NER. Microorganisms used the carbon and nitrogen from $^{13}C_3{}^{15}N$-glyphosate

to synthesize their biomass compounds, as shown by the ^{13}C and ^{15}N incorporation into microbial AA, leading to biogenic residues in OM after cell death and lysis. Based on the ^{13}C-tAA content, 20 % of the ^{13}C-biogenic residues were formed and constituted the major fraction of ^{13}C-NER (87 %, Figure 8.2.2.3-10). These results agree with previous studies on biogenic residue formation during biodegradation of ^{13}C-labeled pesticides or pharmaceuticals.

Table 8.2.2.3-136: Mass balance of $^{13}C_3{}^{15}N$-glyphosate degradation in biotic and abiotic water-sediment systems and in water over 80 days (% of initially applied ^{13}C- and ^{15}N-label equivalents)

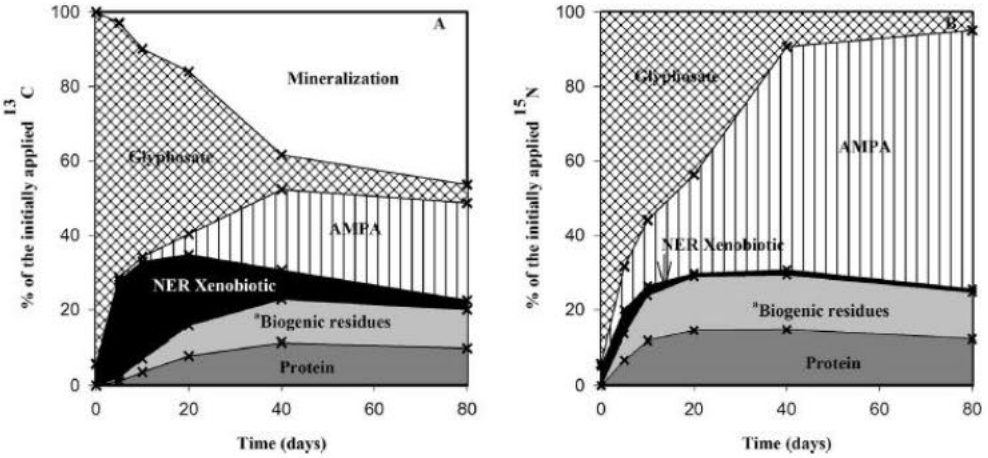
		% of initial ^{13}C				
	Time (days)	Mineralization	Glyphosate	AMPA	NER	Recovery
	0	n.d.	98 (± 1)	0.2 (±0.1)	6 (± 1)	104 (± 2)
Biotic	**5**	1 (± 1)	68 (± 2)	1 (± 1)	28 (± 1)	98 (± 4)
	10	3 (± 1)	56 (± 2)	1 (± 1)	33 (± 5)	93 (± 7)
	20	16 (± 0)	44 (± 2)	6 (± 1)	35 (± 1)	101 (± 5)
	40	40 (± 1)	9 (± 1)	22 (± 1)	31 (± 1)	102 (± 4)
	80	**56 (± 3)**	**5 (± 1)**	**26 (± 1)**	**23 (± 2)**	**110 (± 6)**
Abiotic	**80**	19 (± 1)	41 (± 2)	11 (± 1)	26 (± 0)	97 (± 3)
Only water	**80**	2 (± 1)	90 (± 2)	2 (± 0)	n.d.	94 (± 3)

		% of initial ^{15}N			
	Time (days)	Glyphosate	AMPA	NER	Recovery
	0	98 (± 1)	1 (± 1)	5 (± 0)	104 (± 2)
Biotic	**5**	68 (± 2)	3 (± 1)	20 (± 2)	91 (± 6)
	10	56 (± 2)	4 (± 1)	26 (± 3)	86 (± 6)
	20	44 (± 2)	17 (± 3)	30 (± 1)	91 (± 6)
	40	9 (± 1)	65 (± 4)	31 (± 0)	105 (± 5)
	80	**5 (± 1)**	**79 (± 5)**	**26 (± 0)**	**110 (± 6)**
Abiotic	**80**	41 (± 2)	33 (± 1)	26 (± 3)	100 (± 6)
Only water	**80**	90 (± 2)	6 (± 1)	n.d.	96 (± 2)

n.d. - not detectable; values are shown as averages ± standard deviation

The kinetics of ^{15}N-NER formation showed a similar pattern to that of ^{13}C-NER and reached 26 % of the initially added ^{15}N-glyphosate. Analogous to the ^{13}C-biogenic residues, the ^{15}N-NER were primarily biogenic (Figure 8.2.2.3-10); at the end of the experiment, the ^{15}N-biogenic residues amounted to 24 % of ^{15}N-glyphosate equivalents and constituted 92 % of the ^{15}N-NER. In contrast to the ^{13}C-biogenic residues, the ^{15}N-biogenic residues were formed rapidly, which is in line with the metabolization and mineralisation of $^{13}C_3{}^{15}N$-glyphosate via the sarcosine pathway without N mineralisation in the initial degradation phase. The contents of extractable $^{13}C_3{}^{15}N$-glyphosate residues (31 % of the $^{13}C_3$-glyphosate equivalents and 84 % of the ^{15}N-glyphosate equivalents) comprised a large proportion of the ^{13}C and ^{15}N-isotope mass balance at the end, with AMPA accounting for almost all of these residues. The percentage of ^{15}N-AMPA was 3-fold higher than that of ^{13}C-AMPA because only one out of three ^{13}C atoms, but all ^{15}N atoms from the co-labeled glyphosate are transferred to AMPA during metabolization (see Figure 8.2.2.3-9). In the sediment-water systems nearly all of the NER could be explained by biogenic residues bearing no potential risk. However, high contents of extracted AMPA were detected, which typically biodegrades slower than glyphosate. The detailed fate of AMPA needs to be investigated to assess the potential risks related to this degradation product of glyphosate. In contrast to previous studies in which biogenic residues remained constant, ^{13}C and ^{15}N biogenic residues from glyphosate slightly decreased towards the end of the experiment. Total hydrolysable ^{13}C- and ^{15}N-labeled AA decreased progressively after 69 days in sediments incubated with ^{13}C-glucose and ^{15}N-labeled ammonium, which is in agreement with the present study.

Figure 8.2.2.3-10: Detailed mass balance including biogenic residue formation (A) of $^{13}C_3$-glyphosate and (B) of ^{15}N-glyphosate in biotic water-sediment system (50 mg/L). a: Biogenic residues were calculated based on a conversion factor of 2 for proteins



Conclusions

This is the first detailed glyphosate turnover mass balance including NER speciation in water-sediment systems using stable isotope co-labeled tracers (^{13}C and ^{15}N).
Sediment plays a key role in the microbial degradation of glyphosate via both the sarcosine and AMPA pathway.
At the end, nearly all of the NER can be assigned to non-toxic biogenic residues after degradation of the parent compound.
Accumulation of main metabolite of glyphosate, AMPA, may because for environmental concern; therefore, an additional investigation of the fate of AMPA in water-sediment systems is needed.

Assessment and conclusion by applicant:

The article reports the results from a water-sediment dissipation experiment with ^{13}C-^{15}N-labelled-glyphosate, conducted according to OECD guideline 308. The methods and results are generally well described and conclusive. However, the water and associated sediment were taken from a German small water body located in agricultural lowlands with continuous crop rotation and pesticide application, which is considered a high risk area for exposure to pesticides. Thus, it cannot be excluded that the water/sediment system received inputs of glyphosate or AMPA within the previous 4 years, as is required in OECD 308 guideline.

In addition, the use of stable-isotope enriched glyphosate does not allow to differentiate between applied substance and existing background. This is also documented by the fact that no metabolites were detected other than AMPA which is known for its potential stability under such test conditions.

In the main experiment, the application rate was extremely high (50 mg/L, equivalent to an application rate of 150 kg/ha when assuming overspray of a 30 cm deep water body) while in OECD 308 guideline it is recommended that the test concentration should be close to application rate or environmental concentrations. Hence the exaggerated test concentration may affect the route and rate of degradation.

The article is seen as reliable with restrictions and therefore it is considered as supportive information.

Assessment and conclusion by RMS:

The study is overall well performed, however RMS agrees with the deviations identified above.

The article provides supportive information on the route of degradation of glyphosate in water/sediment system (with emphasis on the nature of NER formation) but no quantitative endpoints regarding degradation rate are derived.

The article concludes that "additional investigation of the fate of AMPA in water-sediment systems is needed". RMS notes that such studies are available in the current dossier.

B.8.2.2.4. Irradiated water/sediment study

An irradiated water-sediment study was not submitted but it is not formally required.

B.8.2.2.5. Summary of route and rate of degradation in water

Ready biodegradability of glyphosate was investigated in 3 studies and showed that it is not readily biodegradable under the conditions of the tests. In addition, results from hydrolysis and water/sediment studies show that glyphosate is not degraded in the aquatic environment to a level > 70 % within a 28-day period. As a consequence, glyphosate is considered not rapidly degradable.

In the mineralization study, glyphosate was found to be well degraded in natural surface water under aerobic conditions at 20°C in the dark with half-lives of 12.3 and 21.8 days, for low and high dose, respectively. Maximum mineralisation of glyphosate was 26.5 and 23.1 % AR, while non extractable radioactivity accounted for 14.0 and 8.8 % AR at the end of the study, in the low and high dose, respectively. AMPA was the only major metabolite identified and was almost exclusively detected in the water phase. The maximum amounts of AMPA, detected in the water phase, were 42.7 and 39.8 % AR, in the low and high dose, respectively.

Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method showed that this peak was comprised of three individual peaks. Further attempts to characterize this radioactivity are currently made and will be reported in an amendment to this study report. A data gap is identified for the notifier to provide the amended report when available.

In water/sediment systems, glyphosate degraded in the water phase and also partitioned to the sediment where it was further degraded. Mineralisation reached a maximum amount of 48 % AR after 100 days. The formation of non-extractable residues reached a maximum amount of 22.0 % AR after 100 days. The major degradation products observed in water/sediment systems were AMPA and hydroxymethylphosphonic acid (HMPA). AMPA was determined in water, sediment and total system with maximum occurrences of 15.7 % AR after 14 days, 18.7 % AR after 58 days and 27.1 % AR after 30 days, respectively. HMPA was not observed in sediment extracts but in the water phase with a maximum occurrence of 10.0 % AR after 61 days.

The proposed degradation pathway for glyphosate in water/sediment system is presented below.

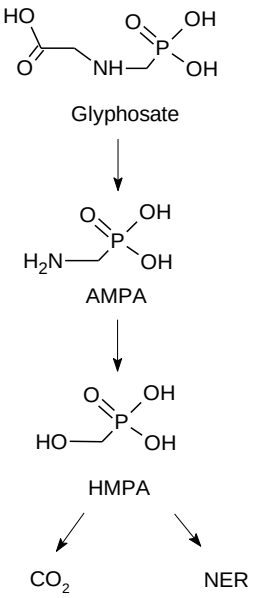


Figure 8.2.2.5-1: Proposed degradation pathway of glyphosate in water/sediment systems

In addition, 4 water/sediment studies with the metabolite AMPA applied provide further information on the behaviour of AMPA in aquatic systems. Reliable results were obtained on 7 water/sediment systems. The results of these studies showed rapid dissipation of AMPA from the water phase by adsorption to the sediment (maximum 63.8% AR after 30 days) followed by microbial degradation to CO_2. The results demonstrated the degradation of AMPA to carbon dioxide and non-extractable residues. Mineralisation reached a maximum of 40.1 % AR after 104 days. The formation of non-extractable residues reached a maximum amount of 40.7 % AR after 29 days. In addition, formation of 1-oxo-AMPA was observed. It should be considered in more details whether this metabolite 1-oxo-AMPA exceeds the trigger for further assessment. A data gap is identified for the applicant to further address this metabolite, quantitatively or qualitatively.

The reliable results for glyphosate, AMPA and HMPA were evaluated according to the current FOCUS kinetic guidance.

The degradation/dissipation of glyphosate in water / sediment systems was mainly described by biphasic kinetics. The persistence DT_{50} and DT_{90} of glyphosate for the total system range from 8.4 to 196 days and from 45.6 to >1000 days, respectively. In addition, the persistence $DissT_{50}$ and $DissT_{90}$ for the water phase range from 1.1 to 7.9 days and from 22.2 to 78.2 days, respectively. The persistence $DissT_{50}$ and $DissT_{90}$ for the sediment phase range from 33.9 to 158.7 days and from 112.6 to 965.3 days, respectively. For modelling purpose the geometric mean $DegT_{50}$ in the total system is 143.3 days (n = 4).

The degradation/dissipation of AMPA in water / sediment systems is described by both single-first-order and biphasic kinetics. The persistence DT_{50} and DT_{90} of AMPA for the total system ranged from 2.4 to 172.8 days and from 29.2 to >1000 days, respectively. In addition, the persistence $DissT_{50}$ and $DissT_{90}$ for the water phase range from 0.6 to 172.8 days and from 5.1 to 573.9 days, respectively. The persistence $DissT_{50}$ and $DissT_{90}$ for the sediment phase could be derived from one system only and are 168.1 days and 558.3 days, respectively. For modelling purpose the geometric mean $DegT_{50}$ in the total system, derived from evaluation at Level P-I and Level M-I dissipation is 98.7 days (n = 7).

The trigger and modelling DT_{50} and DT_{90} of HMPA for the total system ranged from 10 to 128.8 days and from 33.4 to 427.8 days, respectively.

Table 8.2.2.5-1: Summary of degradation endpoints in water/sediment for glyphosate: trigger endpoints Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	St. (χ^2err) (%)	Kinetic model
Total system								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	8.4	45.6	2.7	FOMC
	Putah	8.4	7.5	20	195.8	902.3	4.4	DFOP
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	15.8	329.4	2.2	HS
	Unter Widdersheim	8.6	7.68	20	121.6	>1000	4.8	DFOP
Water phase								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	5.0	22.7	2.3	DFOP
	Putah	8.4	7.5	20	7.9	78.2	10.0	FOMC
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	2.0	22.2	5.2	DFOP
	Unter Widdersheim	8.6	7.68	20	1.1	28.7	2.6	DFOP
Sediment phase								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	33.9	112.6	8.4	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993)	Bickenbach	8.6	7.8	20	158.7	965.3	3.6	DFOP
	Unter Widdersheim	8.6	7.68	20	-[3]	-[3]	-[3]	-[3]

Table 8.2.2.5-1: Summary of degradation endpoints in water/sediment for glyphosate: trigger endpoints Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	DT_{50} (d)[1]	DT_{90} (d)[1]	St. (χ^2err) (%)	Kinetic model
CA 7.2.2.3/005								

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[3] No acceptable fit obtained and no endpoints could be derived

Table 8.2.2.5-2:Summary of degradation endpoints in water/sediment for glyphosate: modelling endpoints Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	Model	SFO DT_{50} (d)[1]	St. (χ^2err) (%)
Total system							
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	SFO	9.7	5.3
	Putah	8.4	7.5	20	DFOP	301.4[2]	4.4
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	HS	144.4[2]	2.2
	Unter Widdersheim	8.6	7.68	20	DFOP	1000[3]	4.8
Geometric mean (total system) (n = 4)						**143.3**	

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used

Table 8.2.2.5-3: Summary of degradation endpoints in water/sediment for AMPA: trigger endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	Ffm (-)	DT_{50} (d)[1]	DT_{90} (d)[1]	St. (χ^2err) (%)	Kinetic model
Total system, Level P-I									
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	-	12.6	>1000	1.6	FOMC
	Schäphysen	8.0	7.34	20	-	2.4	>1000	6.2	DFOP
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	-	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	8.5	20	-	-[2]	-[2]	-[2]	-[2]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	-	43.5	196.8	3.5	DFOP
	Unter Widdersheim	8.2	7.5	20	-	17.7	579.8	3.4	HS
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-	-[3]	-[3]	-[3]	-[3]
Total system, Level M-I dissipation									
[redacted] (1999) CA 7.2.2.3/002	Cache[5]	8.2	8.1	20	0.339	172.8	573.9	7.0	SFO
	Putah	8.4	7.5	20	-[6]	-[6]	-[6]	-[6]	-[6]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	0.488	15.7	52.2	9.4	SFO
	Unter Widdersheim	8.6	7.68	20	0.321	8.8	29.2	22.4	SFO
Water phase, Level P-I									
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	-	2.2	22.1	2.1	FOMC
	Schäphysen	8.0	7.34	20	-	1.5	5.1	10.7	SFO
[redacted] (2003)	Bickenbach	8.5	8.5	20	-	2.4	37.1	5.3	FOMC

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	Ffm (-)	DT_{50} (d)[1]	DT_{90} (d)[1]	St. (χ^2err) (%)	Kinetic model
CA 7.2.2.3/019	Unter Widdersheim	8.5	8.5	20	-	2.1	25.9	8.0	FOMC
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	-	6.6	50.7	4.5	DFOP
	Unter Widdersheim	8.2	7.5	20	-	2.0	17.3	8.2	DFOP
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-	0.6	8.1	1.8	FOMC
Water phase, Level M-I dissipation									
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	0.339	172.8	573.9	7.0	SFO
	Putah	8.4	7.5	20	-	-[5]	-[5]	-[5]	-[5]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	0.488	15.7	52.2	9.4	SFO
	Unter Widdersheim	8.6	7.68	20	0.321	8.8	29.2	22.4	SFO
Sediment phase, Level P-I									
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	-	168.1	558.3	1.9	SFO
	Schäphysen	8.0	7.34	20	-	-[3]	-[3]	-[3]	-[3]
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	-	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	8.5	20	-	-[2]	-[2]	-[2]	-[2]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	-	-[4]	-[4]	-[4]	-[4]
	Unter Widdersheim	8.2	7.5	20	-	-[3]	-[3]	-[3]	-[3]
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-	-[4]	-[4]	-[4]	-[4]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[3] No acceptable fits obtained and no endpoints could be derived
[4] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[5] No evaluations could be conducted for any compartment at Level M-I dissipation due to the limited number of data points available after the peak concentration
[6] Since AMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for total system

Table 8.2.2.5-4: Summary of degradation endpoints in water/sediment for AMPA: modelling endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (ºC)	Model	Ffm from parent (-)	SFO DT_{50} (d)[1]	St. (χ^2err) (%)
Total system, Level P-I								
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	DFOP	-	95.0[2]	3.8
	Schäphysen	8.0	7.34	20	DFOP	-	1000[3]	6.2
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	-[4]	-	-[4]	-[4]
	Unter Widdersheim	8.5	8.5	20	-[4]	-	-[4]	-[4]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	SFO	-	47.7	5.9
	Unter Widdersheim	8.2	7.5	20	HS	-	288.8[2]	3.4
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-[5]	-	-[5]	-[5]
Total system, Level M-I dissipation								
	Cache	8.2	8.1	20	SFO	0.339	172.8	7.0

Table 8.2.2.5-4: Summary of degradation endpoints in water/sediment for AMPA: modelling endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	Model	Ffm from parent (-)	SFO DT_{50} (d)[1]	St. (χ^2err) (%)
[redacted] (1999) CA 7.2.2.3/002	Putah	8.4	7.5	20	-[6]	-[6]	-[6]	-[6]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	SFO	0.488	26.8[7]	7.9
	Unter Widdersheim	8.6	7.68	20	SFO	0.321	15.1[7]	5.8
Geometric mean (total system) (n = 7, derived from Level P-I and M-I dissipation)							**98.7**	

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used
[4] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[5] No acceptable fits obtained and no endpoints could be derived
[6] No evaluations could be conducted for any compartment at Level M-I dissipation due to the limited number of data points available after the peak concentration
[7] Since AMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for total system

Table 8.2.2.5-5: Summary of degradation endpoints in total system for HMPA: modelling and trigger endpoints Level M-I degradation (pathway fit)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction (-)	St. (χ^2err) (%)	Model
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	128.8	427.8	0.366 (from AMPA)	20.5	SFO
	Unter Widdersheim	8.6	7.68	20	10	33.4	0.359 (from AMPA)	39.3	SFO

Table 8.2.2.5-6: Summary of maximum occurrence of parent in sediment, mineralisation and non-extractable residues (from glyphosate dosed experiments)

Study	Water / sediment system	pH water phase	pH sed	Parent max x % in sediment after n d.	Mineralisation x % after n d. (end of the study).	Non-extractable residues in sed. max x % after n d
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	15.9 (3 d)	48.0 (100 d)	13.5 (58 d)
	Putah	8.4	7.5	58.2 (100 d)	5.9 (100 d)	20.3 (58 d)
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	53.1 (7 d)	23.5 (100 d)	22.0 (100 d)
	Unter Widdersheim	8.6	7.68	61.4 (7 d)	19.4 (61 d)	13.6 (100 d)

Table 8.2.2.5-7: Summary of maximum occurrence of major metabolites of glyphosate in different compartments of water/sediment systems (from glyphosate dosed experiments)

Study	Water / sediment system	pH water phase	pH sed	AMPA			HMPA		
				Water	Sediment	Total system	Water	Sediment	Total system

[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	10.3 (30 d)	18.7 (58 d)	27.1 (30 d)	-	-	-
	Putah	8.4	7.5	1.5 (58 d)	3.8 (58 d)	5.3 (58 d)	-	-	-
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	15.7 (14 d)	-	15.7 (14 d)	10.0 (61 d)	-	10.0 (61 d)
	Unter Widdersheim	8.6	7.68	5.8 (14 d)	-	5.8 (14 d)	1.9 (30 d)	-	1.9 (30 d)

AMPA: aminomethylphosphonic acid; HMPA: hydroxymethylphosphonic acid; -: not detected

B.8.2.3. Degradation in the saturated zone

No data are available or are considered to be required.

B.8.2.4. Impact of water treatment procedure

Applicant submitted data on water treatment processes through extensive review of published peer reviewed literature. The report of [redacted] (2020) summarizes this assessment. It includes the data from two Monsanto (Bayer) commissioned studies and from open literature data review updated for air V renewal. The applicant commissioned studies ([redacted], 2010, CA 7.5/081 and [redacted], 2012, CA 7.5/080) to address the fate of glyphosate and AMPA when subjected to low-chemical and chemical water treatment processes. The first study contains a review and some original work on removal rates. The same material has also been presented in a peer reviewed publication (Jönsson *et al*., 2013, CA 7.5/084). The second study is also a review, which looks at three low-chemical processes: bank filtration, slow sand filtration and biological activated carbon. RMS indicates that these applicants' studies were included in the RAR 2015. The data have been updated in [redacted], 2020, with updated open literature references for AIR V renewal.

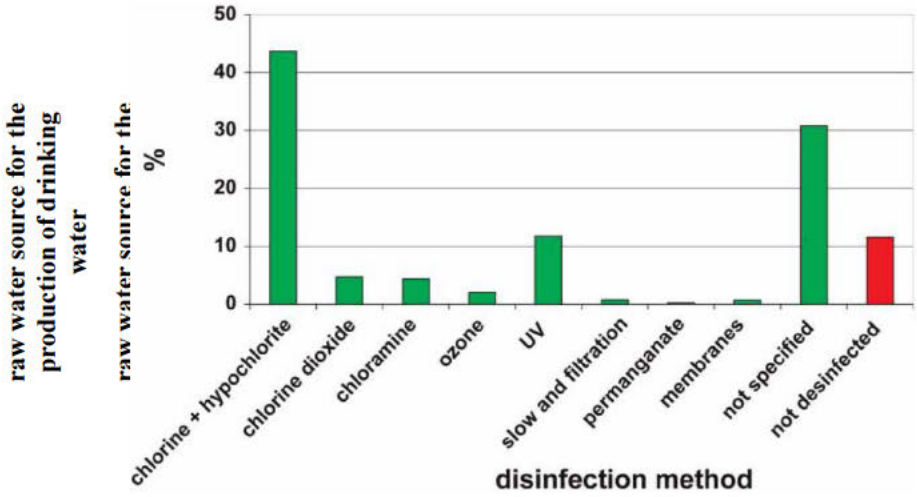
Overall RMS considers sufficient information were submitted to address the effect of water treatment processes on glyphosate and AMPA and the potential formation of by-products. Summary of the data provided is proposed below by RMS. Applicant studies and articles from open literature review are summarized after.

B.8.2.4.1. *RMS summary*

Water treatment prevalence

The prevalence across the EU of the treatment processes was inferred by in [redacted], 2020 from a publication of van der Hoek *et al*., 2014, CA 7.5/098. This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MSs and 405 million European citizens, in 2014. It came out that in 88% of the drinking water production a disinfection method is applied; almost all the raw water taken from surface water is subject to disinfection (99.9%). For bank filtration and artificial recharge (AR), the values are 90.1% and 92.2%, respectively. Where surface water is disinfected, the paper reports that chlorine disinfection is applied to 62% (30% is 'not specified', but it is very likely that as disinfection by chlorine is by far the most employed method, a significant portion of the 'not specified' is also likely to be chlorine based; hence, 62% should be considered a conservative minimum value.)

Figure 8.2.4.1-1: Raw water sources and treatment scheme (from van der Hoek *et al.*, 2014, CA 7.5/098)



Note: The green bars sum to 88%

Low-chemical Water Treatment and Bank Filtration

In summary of all the data submitted, removal rates for glyphosate and AMPA when subjected to low-chemical processes are very variable.

The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon. . Experiment using granular activated carbon are reported in some articles from the literature review, but no straighforward conclusion can be made on the efficiency of this process (data of variable reliability). From the data, it seems that it indeed does not provide an effective barrier to glyphosate or AMPA on its own.

Other processes commonly used in water treatment (bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration) would each contribute to some removal, but alone would not provide a secure barrier. Slow sand filtration is more common in Europe where it has been installed at several hundred treatment works. The use of bank filtration is relatively limited in Europe, with less than 50 sites specifically designed to utilize this technique. Van der Hoek *et al.*, 2014, (summarized in [redacted], 2020, CA7.5/002) reports that <10% of raw water for drinking water in the EU involves bank filtration processes, while however, there are several places in the EU where a significant proportion of drinking water involves bank filtration processes as reported in Gillefalk *et al.* (2018, CA7.5/097, in [redacted], 2020) : e.g. Paris, Berlin (60% of drinking water), Düsseldorf (100% of drinking water).

The glyphosate removal efficiency of aerobic sand filters used in three Danish waterworks was assessed in Hedegaard & Albrechtsen (2014, CA 7.5/083) .The degradation of ^{14}C-glyphosate in very wet filter sands was investigated at 10°C in the dark for up to 13 days,and reports decreased of the glyphosate concentration to 14% of initial after 13 days. However, the results reliability are questioned due to insufficient details, and the experimental conditions are clearly not representative of the *in situ* conditions. Indeed the residence time of contaminated water in the rapid sand filter in the treatment works was reported to be 7.5 to 12 minutes.

Technical scale semi-field investigations (bank filtration and slow sand filter experiments) were carried out with glyphosate and reported in Litz *et al.* (2011, CA 7.5/063). These slow sand filter experiments demonstrated that 70 – 80% reduction in glyphosate concentrations were achieved (for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L)

The fate of organic micropollutants during long-term/long-distance river bank filtration, at a temporal scale of several years, was investigated along a row of monitoring wells perpendicular to the Lek River in The Netherlands (Hamann *et al.*, 2016, CA 7.5/082). Only AMPA was part of the monitored susbtances. The

article indicate AMPA was non-detectable in the bank filtrate. However the initial oncentration of AMPA is not given and efficiency of the river bank filtration cannot be evaluated from these data.

In Ruel *et al.* (2012, CA 7.5/086) data from several waste water treatment plant was studied and it was concluded that the removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30%. However the results to support this conclusions are not clearly presented in the article.

Another investigation of the removal rates for glyphosate and AMPA was reported in Ruel *et al.*, (2011, CA 7.5/087). Various stages of treatment were studied (sand filtration, reverse osmosis, ozone) and the efficacy of sand filtration was reported to be 30 – 70% for glyphosate and AMPA.

The results from bank filtration experiments in Peschka, M. et al. (2006, CA7.5/072) showed that glyphosate was removed after a distance of about 200 m, whereas AMPA needed about 300 to 500 m to be completely eliminated. The experiments were carried out at the waterside of the Main, but the method and results are only briefly described.

Efficiencies of the low chemical treatments, from Jönsson *et al.* (2013, CA 7.5/084), and adjusted in the light of the summarised literature are summarized below.

Table 8.2.4.1-1: Summary of glyphosate and AMPA removal rates following low-chemical treatment processes (based on Jönsson *et al.*, 2013, CA 7.5/084, and adjusted for summarised literature)

Treatment process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminium coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 – 85
Slow sand filtration	The limited information available suggests that significant removal can be achieved but removal is likely to be highly dependent on conditions	
UV irradiation	Not effective alone at doses used in water treatment	
Activated carbon adsorption	10 – 90	20 – 70

Chemical Water Treatment

Glyphosate and AMPA are most likely to be exposed to chemical water treatment processes via the treatment of surface waters abstracted for the production of drinking water.

Among the existing chemical water treatment, ozonation and chlorination are highly effective in degrading both glyphosate and AMPA but parameters such as reduced temperature or reduced ozone concentration may reduce the efficiency. UV doses typically used for disinfection will not degrade significant amounts of either compound. Higher UV doses in combination with H_2O_2 showed good degradation of glyphosate, but not AMPA.

Ozonation is effective for glyphosate and AMPA degradation, with efficiency increasing with ozone concentration (an initial conentration of 5 mg/L glyphosate was reduced to <LOD within 25 minutes with ozone concentration of 1.5 mg/L, while within 20 minutes with initial ozone concentration of 2.0 and 3.0 mg/L) and with increasing pH (an initial concentration of 5 mg/L was reduced to <LOD within 25 minutes at pH 4.9, and within 15 minutes at pH 9.3) in Shen *et al.*, 2011 (CA 7.5/089). The same pH effect was observed in Assalin, M. *et al.* (2010, CA7.5/091) with higher removal rate at alkaline pH. Glyphosate was transformed to AMPA and other compound identified to glycolic acid, glycine, phosphoric acid, which were also subsequently degraded (Total Organic Carbon was reduced by >93% within 60 minutes). Investigations into the reactivity of glyphosate and AMPA when subjected to ozonation was also carried out at pilot-scale (Jönsson *et al.*, 2013, CA 7.5/084). These experiments under various conditions found that a 15-minute treatment period was enough to result in removal rates of >99% for both glyphosate and AMPA under all the tested experimental conditions. No effect of temperature was found on the removal rate within the 15 minutes (always>99% with temperature from 6°C to 15°C) but the O_3 demand increased with temperature.

Chlorine dioxide is effective for glyphosate degradation at around pH 6, but the efficiency decreases with increasing pH and decreasing temperature The highest degradation (removal of 93%) was seen for the low

pH samples (~pH 6) with high temperature (22°C) and high ClO_2 concentrations. The lowest degradation (removal of 17%) was seen for low temperature (~4°C) and low ClO_2 concentrations. For AMPA, removal rate was >99% whatever temperature and pH conditions. UV and UV H_2O_2 treatment can degrade significant amounts of glyphosate under the tested conditions (removal rates from 25% without H_2O_2 to 91% with H_2O_2).AMPA is poorly degraded by UV (6% to 32% removed) and degradation was little enhanced when using UV with H_2O_2 (between 8 to 49%). Highest removal was observed for highest UV dose tested (1240 mJ/cm²). It is worth noting that UV doses used in these experiments in Jönsson *et al.*, 2013, CA 7.5/084 (740 and 1,240 mJ/cm²) were all higher than those usually used for disinfection of drinking water treatment (indicated being usually 40-100 mJ/cm², although this latter information cannot be checked).

From literature review included in Jönsson *et al.*, 2013, CA 7.5/084, authors concluded that UV/TiO_2 can degrade significant amounts of both compounds but irradiation times are long Also ultrafiltration (UF), NF (nanofiltration) and RO (reverse osmosis) can also be effective in removing glyphosate and AMPA. However, no detailed information are reported to support these conclusions.

Removal rates for glyphosate and AMPA when subjected to disinfection processes are high as summarised below.

Table 8.2.4.1-2: Summary of removal rates for glyphosate and AMPA following disinfection processes (*after* Jönsson *et al.*, 2013, CA 7.5/084)

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

Transformation products

Chlorination and ozonation were demonstrated to remove glyphosate residues from water effectively and both are known for the formation of harmful disinfection by-products. Other chemical treatment like ultra-violet irradiation or ozonation/ozonolysis processes might also result in formation of other potential transformation products. Finally, treatment processes such as activated carbon filtration or reverse osmosis can be excluded as a potential source of transformation products.

Transformation products identified under chorination in two publications (Mehrsheikh *et al.*, 2006, CA 7.5/095; Brosillon *et al.*, 2006, CA 7.5/094) are the same as those formed from glycine and other amino acids under the same conditions (using stable isotopes and NMR spectroscopy to identify species generated when glyphosate and glycine are separately treated with aqueous chlorine).

A thorough investigation of the process of ozonation of glyphosate was reported in Shen *et al*, 2011 (CA 7.5/089). Under ozonation, glyphosate was transformed to AMPA and other compounds identified to glycolic acid, glycine, phosphoric acid, which were also subsequently degraded (Total Organic Carbon was reduced by >93% within 60 minutes). Also in Klinger *et al.* (2008, CA 7.5/096) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate.

Overall it is considered that the degradation pathway linked with water treatment processes has been sufficiently investigated and there are no indications that harmful disinfection by-products would be formed.

* * * *

All papers from literature review and company sponsored studies reviewed in [redacted], 2020 are listed in the following table and summarized hereafter.

Data point	**Study (Author, year)**	**Study type**	**Substance(s)**	**Status**
APPLICANT STUDIES				
Data point	**Study (Author, year)**	**Study type**	**Substance(s)**	**Status**

CA 7.5/002	[redacted], 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Acceptable
CA 7.5/080	[redacted], 2012	Review of sustainable water treatment	Glyphosate AMPA	Acceptable
CA 7.5/081	[redacted], [redacted] R., 2010	Removal of glyphosate and AMPA by water treatment	Glyphosate AMPA	Acceptable
RELEVANT LITERATURE ARTICLES				
CA 7.5/082	Hamann, E. *et al*., 2016	The fate of organic micropollutants during long-term/long-distance river bank filtration	Glyphosate AMPA	Reliable with restrictions
CA 7.5/083	Hedegaard, M., Albrechtsen, H., 2014	Microbial pesticide removal in rapid sand filters for drinking water treatment – Potential and kinetics	Glyphosate	Reliable with restrictions
CA 7.5/084	Jönsson J. *et al*., 2013	Removal and degradation of glyphosate in water treatment	Glyphosate AMPA	Reliable
CA 7.5/085	Malaguerra, F. *et al*., 2013	Computation model simulation for the contamination of drinking water wells	Glyphosate AMPA	Not relevant
CA 7.5/086	Ruel, S. *et al*., 2012	Occurrence and fate of relevant substances in wastewater treatment plants	Glyphosate AMPA	Reliable with restrictions
CA 7.5/027	Bruchet A. *et al*., 2011	Monitoring experiment in France	Glyphosate AMPA	Reliable with restrictions
CA 7.5/063	Litz, N.T. *et al*., 2011	Comparative studies on retardation and reduction during subsurface passage	Glyphosate AMPA	Reliable
CA 7.5/087	Ruel, S. *et al*., 2011	Evaluation of removal of 100 micropollutants through wastewater treatment processes	Glyphosate AMPA	Reliable
CA 7.5/088	Schoonenberg Kegel, F.S., 2010	Removal of 47 micropollutants from river bank filtrate	Glyphosate	Reliable with restrictions
CA 7.5/072	Peschka, M. *et al*., 2006	Trends in pesticide transport into the River Rhine	Glyphosate AMPA	Reliable with restrictions
CA 7.5/089	Shen, Y. *et al*., 2011	Ozonation	Glyphosate	Reliable
CA 7.5/090	Shen, Y. *et al*., 2011	Translation of CA 7.5/089	See above	See above
CA 7.5/091	Assalin, M. *et al.*, 2010	Degradation by several oxidative chemical processes	Glyphosate AMPA	Reliable
CA 7.5/092	Boucherie, C. *et al.*, 2010	Ozone and GAC filtration synergy	Glyphosate AMPA	Reliable with restrictions
CA 7.5/093	Manassero, A. *et al*., 2010	Degradation in water employing the H_2O_2/UVC process	Glyphosate	Reliable
CA 7.5/094	Brosillon, S. *et al.*, 2006	Chlorination kinetics of glyphosate and its by-products	Glyphosate AMPA	Reliable
CA 7.5/095	Mehrsheikh, A. *et al.*, 2006	Investigation of the mechanism of chlorination of glyphosate	Glyphosate	Reliable
CA 7.5/096	Klinger, J. *et al.*,2008	Formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra	Glyphosate AMPA	Reliable
CA 7.5/097	Gillefalk *et al. 2018*	Potential Impacts of Induced Bank Filtration on Surface Water Quality: A Conceptual Framework for Future Research	Not applicable	Reliable

CA 7.5/098	Van der Hoek *et al, 2014*	Practical Paper Drinking water treatment technologies in Europe: state of the art – challenges – research needs	Not applicable	Reliable

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B.8.2.4.1. *Applicant's studies*

New studies/assessments

[redacted], *2020*

The following report addresses the monitoring data collection and analysis for each environmental compartment (Soil, groundwater, surface water, tidal water, sediment, drinking water) so as the data to address the impact of water treatment. Only the part concerning the impact of water treatment is summarized below. All parts concerning monitoring data are reported under point B.5, reported in a dedicated appendix

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
GLP/Officially recognised testing facilities	No
Previous evaluation	Not previously submitted
Acceptability/Reliability:	Yes

Executive Summary

The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. In addition to this analysis, an assessment of water treatment processes was undertaken through review of published peer reviewed literature.

Removal of Glyphosate and AMPA by Water Treatment Processes

For surface water destined to be drinking water, there are almost always water treatment processes applied to remove bacteria and viruses and other organic micro-pollutants. The vast majority (88%) of raw water sources for drinking water production are subject to disinfection (Van der Hoek *et al*., 2014, CA 7.5/098). In particular, almost all (99.9% by volume) the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62% of the raw water (Van der Hoek *et al*., 2014, CA 7.5/098). Disinfection and oxidative processes are applied where needed and at predetermined rates for the removal of microbial and organic micro-pollutants, regardless of GLY and AMPA presence. GLY and AMPA are known to be very readily transformed by the most common disinfection methods, ranging from 25 to 95% for AMPA and 60 to 99% for GLY (the higher of these

values corresponding to chlorination; Jönsson *et al.*, 2013, CA 7.5/084). Transformation products are small molecules, often similar or identical to those found from natural sources. Other chemical treatment processes are also often applied as are low chemical processes (processes with either no involvement of chemicals or where the treatment is to occur via physical processes like complexation and adsorption) and bank filtration (infiltration of surface water from a river or lake into a groundwater system, induced by water abstraction close to the surface water). Drinking water treatment processes are carefully controlled and the water treatment process train at any given abstraction site optimised to ensure that quality standards are met at the tap of consumers (e.g. GLY < 0.1 µg/L).

INTRODUCTION

Under Article 4 of Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market, it is required that a plant protection product, *"…shall have no immediate or delayed harmful effect on human health, including that of vulnerable groups, or animal health, directly or through drinking water (taking into account substances resulting from water treatment), food, feed or air…*

An assessment of components potentially formed from drinking water treatment processes is therefore required. The assessment includes potential transformation of the active substance glyphosate and its metabolites AMPA and HMPA into other compounds and the relevance of those components to consumer risk assessment to drinking water.

However, the data requirements listed in Regulations (EU) 283/2013 and (EU) 284/2013 do not stipulate how to address the impact of water treatment processes. No EU agreed guideline or guidance has been adopted yet.

Glyphosate and its metabolite AMPA are considered for the environmental risk assessment of groundwater, and the metabolite HMPA is considered in addition for surface water. However, no data on the presence of HMPA is currently available from public monitoring data sources.

As strongly indicated by data on degradation and adsorption to soil, glyphosate and AMPA are unlikely to be found frequently in groundwater being abstracted as raw drinking water. This is supported by monitoring data available from EU MSs indicating that *ca.* 0.6% (from *ca.* 3.0% of sites) of the groundwater samples investigated showed residues of glyphosate at levels ≥ 0.1 µg/L. About 0.002% (from *ca.* 0.006% sites) of groundwater samples showed residues of AMPA at levels ≥ 10.0 µg/L.

In contrast, findings of Glyphosate and AMPA were more frequent in surface water monitoring when being referenced to a value of 0.1 µg/L, i.e. in 23% and 48% of total samples analysed, respectively, residues were beyond this threshold. It should be noted that the term 'surface water' is not strictly defined. Though the percentage of findings may appear high, it is not possible to distinguish readily between large scale and smaller scale surface waters and their overall use for drinking water abstraction. Large surface waters like rivers can be a source for raw drinking water by abstraction *via* bank filtration.

As such, an assessment of the likely fate of glyphosate and its metabolites when exposed to water treatment processes has been carried out and is presented below. For pragmatic purposes, differentiation has been made between 'low-chemical' and chemical methods of treatment of raw drinking water.

There are a very wide variety of water treatment processes that may be applied for a given raw drinking water including 'low-chemical' and 'chemical' options. The exact combination depends on the context including characteristics and origin and must be adapted to the source (the 'treatment train').

"Low-chemical" refers to processes with either no involvement of chemicals or, where the treatment is to occur *via* physical processes like complexation and adsorption. It also includes water treatment processes where it is very unlikely that metabolites known to be formed by microbial processes in soil or water/sediment are then transformed under the conditions of that process. For example, the abstracted raw water from most water sources must be cleaned and sieved to remove suspended materials, often achieved by filtration through sand and often followed by concomitant chemical coagulation/flocculation steps.

'Chemical treatment' following low-chemical processes in most 'treatment trains' for drinking water till the consumers tap represents a necessary disinfection step designed to remove hazardous biological

material such as bacteria and viruses before it is released. The latter measure is a major water quality objective, achieved, for example, by chlorination.

Chlorination was demonstrated to remove glyphosate residues from water effectively while having the potential to form transformation products. Other chemical treatment like ultra-violet irradiation or ozonation/ozonolysis processes might also result in formation of other potential transformation products. Finally, treatment processes such as activated carbon filtration or reverse osmosis can be excluded as a potential source of transformation products.

The information available in the form of publications or company-sponsored studies to investigate potential transformation routes of glyphosate, AMPA and HMPA under conditions simulating water treatment processes are summarised in the next two sections.

I. MATERIALS AND METHODS

An integral part of potentially understanding the patterns of exposure highlighted by the public monitoring data is how raw water sources are treated to produce drinking water. An assessment of water treatment processes was undertaken through review of published peer reviewed literature. This identified treatment processes and the degree to which they are effective at removing glyphosate (GLY) and AMPA during the water treatment process. These can be used to interpret the groundwater and surface water data within the context of drinking water production.

II. RESULTS AND DISCUSSION

Low-chemical Water Treatment and Bank Filtration

Low-chemical water treatment processes are frequently applied to water destined to become drinking water.

There are two Monsanto (Bayer) commissioned studies which address the fate of glyphosate and AMPA when subjected to low-chemical water treatment processes. The first of these [redacted], 2010, CA 7.5/081), contains a review and some original work on removal rates. The same material has also been presented in a peer reviewed publication (Jönsson *et al*., 2013, CA 7.5/084), and the relevant findings summarised below. The second study ([redacted], 2012, CA 7.5/080), is also a review which looks at three low-chemical processes: bank filtration, slow sand filtration and biological activated carbon. The use of bank filtration is relatively limited in Europe, with less than 50 sites specifically designed to utilize this technique. Slow sand filtration is more common in Europe where it has been installed at several hundred treatment works. Biological activated carbon is the most common technique of the three; possibly because it is the easiest to retrofit. The removal rates in this study are also summarised in the peer reviewed publication (Jönsson *et al*., 2013, CA 7.5/084), and the relevant findings will also be summarised below.

In bank filtration, surface water (in a river or lake) filters through the sediment floor or bank, and travels to an extraction well set back from the water body where, following further treatment processes, it is delivered as drinking water. Consequently, the transformation of glyphosate and AMPA when subjected to bank filtration is essentially that which would be expected following aerobic or anaerobic degradation in soil or sediment/water systems: that is, no novel transformation products would be expected as the same microbial and hydrolytic processes take place. As indicated above, in the EU the use of bank filtration is relatively limited, with less than 50 sites specifically designed to utilize this technique. Further, <10% of raw water for drinking water in the EU involves bank filtration processes (van der Hoek *et al*., 2014, CA 7.5/098)). However, as indicated in (Gillefalk *et al*., 2018, CA 7.5/097), there are several places in the EU where a significant proportion of drinking water involves bank filtration processes (e.g. Paris, Berlin (60% of drinking water), Düsseldorf (100% of drinking water)), such that research is available on the fate of glyphosate and AMPA when subjected to bank filtration.

The degradation of ^{14}C-glyphosate in very wet filter sands from three Danish waterworks was investigated at 10°C in the dark for up to 13 days (Hedegaard & Albrechtsen, 2014, CA 7.5/083). The residence time of water *in situ* in rapid sand filters in treatment works was reported as 7.5 – 12 minutes. Under the experimental conditions, glyphosate decreased to 7 - 14% of initial amounts after 13 days (complete mineralisation); indicating that glyphosate was intrinsically degradable under these conditions (although unlikely to be degraded significantly *in situ*).

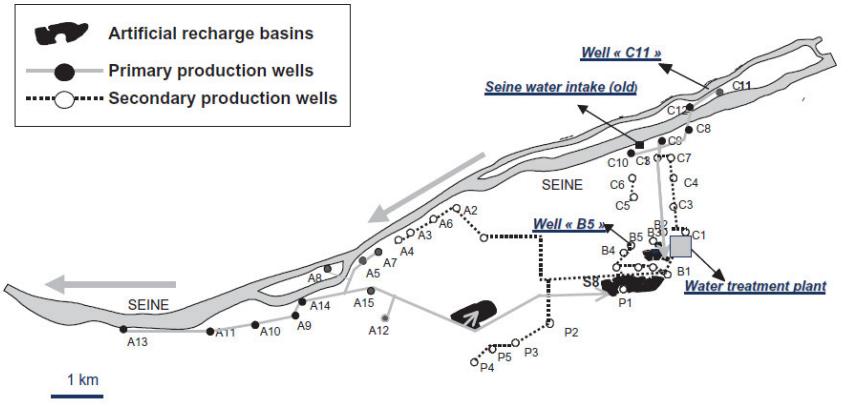
Technical scale semi-field investigations (bank filtration and slow sand filter experiments) were carried out with glyphosate and reported in Litz *et al*. (2011, CA 7.5/063). The experimental systems consisted of three enclosures (metal cylinders) of slow sand filter material, with an area of 1 m^2 and a height of 1.85 m (with a filtration length of 1 m) situated within an infiltration pond (area 90 m^2). The flow rate was set at 50 cm/day. Glyphosate was continuously dosed to the enclosures over a 14 day period, and water samples for glyphosate and AMPA analysis were taken for 34 days. These slow sand filter experiments demonstrated that 70 – 80% reduction in glyphosate concentrations were achieved (for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L). Modelling (using the VisualCXTFit model) generated a predicted required filtration length of 2.75 – 3.75 m (to give glyphosate concentrations below 0.1 µg/L), and using data from typical Berlin bank filtration sites yielded the same sufficient attenuation within a few days of travel time. Additional experiments on a slow sand filter planted with *Phragmites australis* and an unplanted control demonstrated that the planted slow sand filter enhanced retardation of glyphosate. Overall, the results showed that saturated subsurface passage has the potential to efficiently attenuate glyphosate, with aerobic conditions, long travel times and the presence of riparian boundary buffer strips.

A reactive transport model was developed to evaluate the potential for contamination of drinking water wells by surface water pollution (Malaguerra *et al*., 2013, CA 7.5/085). The model was designed to be applicable to a wide range of aquifers, especially in Denmark. The results of a tracer experiment conducted by other researchers using a river in Switzerland were used to test the model, which was found to adequately model the results of the tracer experiment. Sensitivity analysis showed that the characteristics of the clay aquitard (hydraulic conductivity and thickness) and well depth were the parameters governing the risk of contamination of the wells by pollution in streams. The authors also reported that their results showed that it is unlikely that glyphosate in streams will pose a threat to drinking water wells.

The fate of organic micropollutants during long-term/long-distance river bank filtration, at a temporal scale of several years, was investigated along a row of monitoring wells perpendicular to the Lek River in The Netherlands (Hamann *et al.*, 2016, CA 7.5/082). Analysis for a range of substances (including AMPA) in river and well water was carried out from 1999 – 2013. Models were constructed for transects from the river to three wells, calibrated using tracer experiments. Travel times from the river to the wells were found to be 1.7 to 3.7 years. Data for AMPA was presented (but not for glyphosate); which was fully removed by bank filtration under these conditions.

A detailed study of the fate of various contaminants (including glyphosate and AMPA) was carried out on a stretch of the Seine downstream of Paris (Bruchet *et al.*, 2011, CA 7.5/027). The investigated area is downstream of urban wastewater plants (Figure 8.2.4.1-1), in particular of a plant that treats effluent from 6.5 million people, and comprises 36 primary and secondary wells: the primary wells are located mostly along the river, naturally re-supplied under anoxic conditions through river bank filtration. The primary wells output is pumped and re-infiltrated through a sand-gravel artificial basin (under slightly aerobic conditions) to recharge secondary production wells. Water from the secondary wells is further treated in a drinking water plant that comprises settling with addition of powdered activated carbon, sand filtration, ozonation and final disinfection with chlorine. The plant production is equal to 144000 m^3/day.

Figure 8.2.4.1-1: Description of study site showing the four sampling points. Flow of the river is from right to left (from Bruchet *et al.*, 2012)



Grab samples were taken on five occasions during September and October 2008 from the Seine raw water, primary well C11, secondary well B5, and the treated water at the outlet of the drinking water plant. The sampling period covered both low flow conditions (220 m^3/s) and higher flow rates (up to 343 m^3/s). In the river, glyphosate was found at <0.1 – 0.12 µg/L, and AMPA at 0.25 – 0.65 µg/L: but, in both the primary well and the secondary well, concentrations of both substances were <0.1 µg/L, as they were in the drinking water samples. (It is worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85%, and 17% for glyphosate; whereas, it is clear from the context that removal is more likely to be 100%). Indeed, the authors state that "both these compounds are totally removed by bank filtration" in this case. With respect to glyphosate and AMPA, the study sheds light on the effectiveness of the water treatment train employed for a major surface water to drinking water plant, where the primary treatment process is bank filtration. It seems likely that similar arrangements associated with other major bank filtration complexes have equivalent effectiveness with respect to the removal of glyphosate and AMPA.

It is clear that bank filtration has been shown to be an effective process to reduce or remove glyphosate and AMPA from water destined to be drinking water.

Jönsson *et al.* (2013, CA 7.5/084) reports on some investigations conducted into the fate of glyphosate and AMPA when subjected to UV treatment, in a flow-through pilot reactor. The UV intensity used was significantly higher than typically used in water treatment for disinfection alone; and even then removal of glyphosate was only 36%, and AMPA was degraded even less.

The publication (Jönsson *et al.*, 2013, CA 7.5/084) also summarises attempts to remove glyphosate and AMPA using activated carbon (often utilized to remove organic micro-pollutants from water) where removal rates were found to be very variable, and reported new investigations using powdered activated carbon – but adsorption of glyphosate and AMPA was low (*ca*. 20% removal rate). Literature relating to other low-chemical processes (use of coagulants, slow sand filtration, air stripping and membrane filtration) was also summarised; although on some occasions high removal rates for glyphosate and AMPA were reported (e.g. 70% removal using an iron coagulant), the removal rates were variable. In Peschka *et al.*, (2006, CA 7.5/072), the removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminum salts, removal rate of 39±14% for glyphosate and 22±15% for AMPA; for gravel filtration removal rate of <10% for both compounds; and for activated carbon removal rates of <10% for glyphosate and 21±9% for AMPA. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30%, (reported in Ruel *et al.*, 2012, CA 7.5/086). An investigation of the removal rates for glyphosate and AMPA associated with various stages to be found across seven Waste Water Treatment Plants, was reported (Ruel *et al.*, 2011, CA 7.5/087): 30 – 70% for glyphosate and AMPA for sand filtration, <30% for AMPA for reverse osmosis and ozone treatment, but

>70% for glyphosate for reverse osmosis and ozone treatment; >70% for both glyphosate and AMPA for activated carbon filtration.

Summary

Removal rates for glyphosate and AMPA when subjected to low-chemical processes are very variable. Table 8.2.4.1-1 is summarised from Jönsson *et al.* (2013, CA 7.5/084), and adjusted in the light of the above summarised literature:

Table 8.2.4.1-1: Summary of glyphosate and AMPA removal rates following low-chemical treatment processes (based on Jönsson *et al.*, 2013, CA 7.5/084, and adjusted for summarised literature)

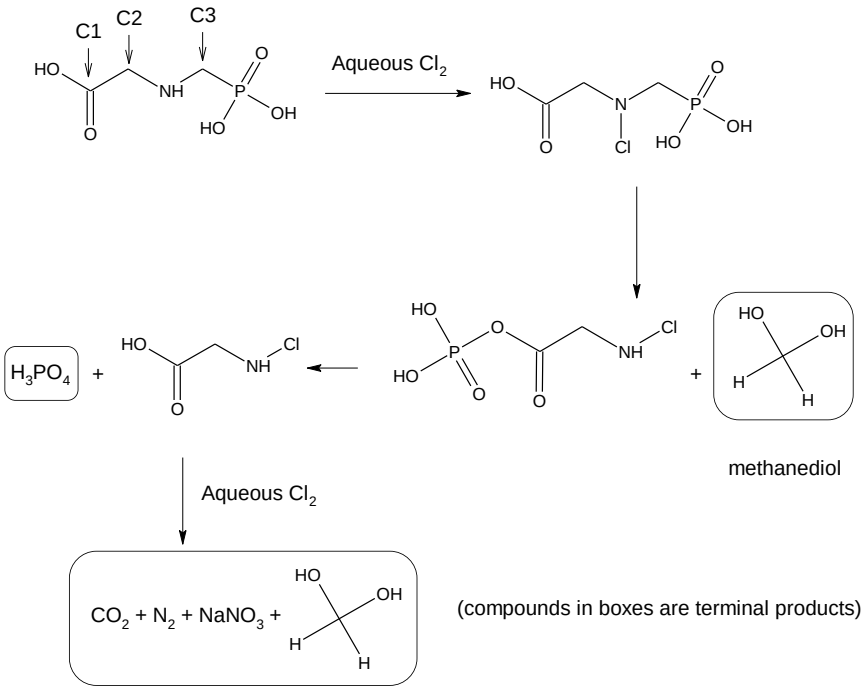
Treatment process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminium coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 – 85
Slow sand filtration	The limited information available suggests that significant removal can be achieved but removal is likely to be highly dependent on conditions	
UV irradiation	Not effective alone at doses used in water treatment	
Activated carbon adsorption	10 – 90	20 – 70

Of these processes, bank filtration, in particular, can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation; i.e. complete transformation of all the glyphosate/AMPA atoms to CO_2 or equivalent terminal products such as nitrate, phosphate etc.). Further, almost all water passing through bank filtration, and destined for drinking water is also subject to disinfection (see below) which is mostly chlorine-based, which rapidly and effectively removes glyphosate and AMPA.

<u>Chemical Water Treatment</u>

There is one Monsanto (Bayer) commissioned study ([redacted], 2010, CA 7.5/081) which addresses the fate of glyphosate and AMPA when subjected to water treatment chemical processes. This reviews original work on removal rates when glyphosate and AMPA are subjected to chemical treatment by ozone, chlorine, and chlorine dioxide. The same information has also been presented later in the form of a peer reviewed publication (Jönsson *et al.*, 2013, CA 7.5/084), and the relevant findings are summarised below. Neither of these report in detail on the transformation products of glyphosate and AMPA when subjected to water treatment processes. The mechanism of chlorination (when treated with aqueous chlorine) of glyphosate has been investigated exhaustively and reported in two linked publications (Mehrsheikh *et al.*, 2006, CA 7.5/095; Brosillon *et al.*, 2006, CA 7.5/094). Using stable isotopes and NMR spectroscopy to identify species generated when glyphosate and glycine are separately treated with aqueous chlorine, it was possible to generate a proposed route of degradation for glyphosate (Figure 8.2.4.1-2):

Figure 8.2.4.1-2: Proposed mechanism of glyphosate chlorination (compounds drawn in boxes are the terminal products) (from Mehrsheikh *et al.*, 2006, CA 7.5/095)



Glyphosate is totally degraded to small molecules common to the degradation of naturally occurring substances in raw water (e.g. amino acids), and the degradation pathway follows that of glycine. The C1 carboxylic acid carbon of glyphosate/glycine is converted to CO_2; the C2 methylene carbon is converted to CO_2 and methanediol; the nitrogen is transformed into nitrogen gas and nitrate; the C3 phosphonomethylene carbon is converted to methanediol; and the phosphorus moiety produces phosphoric acid. Kinetic models were constructed that allowed the temporal course of the reactions to be simulated; these predicted that under conditions similar to those found in water treatment plants, the chlorination of glyphosate is complete within seconds of contact with chlorine.

The very rapid reaction of glyphosate with aqueous chlorine was confirmed in the investigations reported in Jönsson *et al.* (2013, CA 7.5/084). In this work, incubation was for only 30 minutes, and at 20°C degradation of glyphosate reached 96-100%; although degradation was less complete at a lower temperature (71% at 5°C). AMPA degraded faster than glyphosate, >99% at all temperatures. The investigations indicated that chlorine dioxide is a less effective degrader of glyphosate (17-93%, 30 minutes, various temperatures/pH values) than aqueous chlorine, and an effective degrader of AMPA (>99% under all conditions tested).

Another approach to disinfection of drinking water sources is ozonation/ozonolysis, where ozone (O_3) is used to deactivate viruses, bacteria and some parasites. The operation of such processes in the context of treating surface water from three French rivers (Marne, Seine and Oise) to provide drinking water to 4 million people in the Paris region has been reported Boucherie *et al.* (2010, CA 7.5/092). A pilot plant was utilised for the investigations: glyphosate was found to be very rapidly degraded by ozone treatment (>91%, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88%, levels reduced to <0.1 µg/L); hence, the ozone treatment required to deliver disinfection targets was also effective in removing glyphosate and AMPA to levels below 0.1 µg/L. The use of ozone to degrade glyphosate and AMPA was also investigated in a batch reactor Assalin *et al.* (2010, CA 7.5/091). In these studies, it was clear that the pH of the test solution altered the reactivity of glyphosate and AMPA to ozonation. It was evident that AMPA was produced from glyphosate at all pH's. For glyphosate, at alkaline pH (pH 10) degradation was very rapid and AMPA was also completely degraded (but more slowly); indeed, total carbon content removal was measured to be 97.5%, indicating that transformation products were also completely degraded. At acidic

pH's (pH 6.5) glyphosate was 80% removed, with a build-up of AMPA, which didn't appear to be degraded under these conditions.

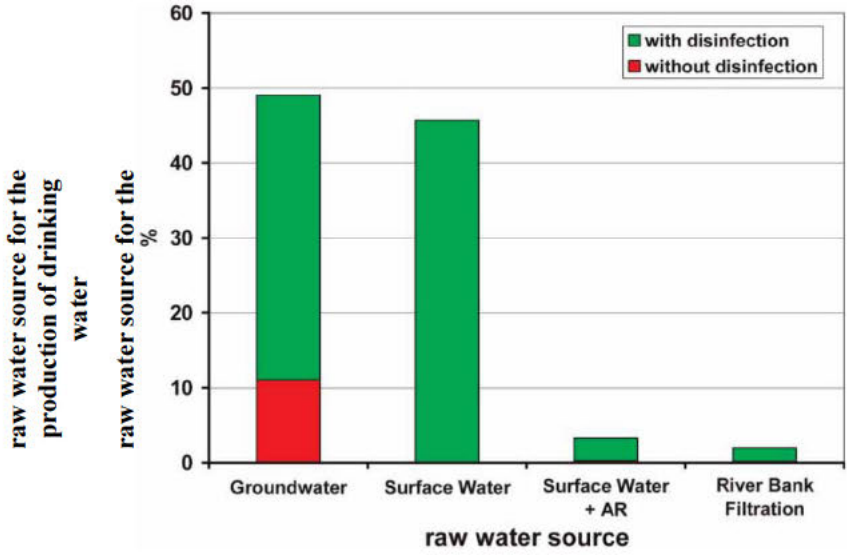
A thorough investigation of the process of ozonation of glyphosate was reported in (Shen, 2011, CA7.5/089), using batch, semi-continuous tests. It was found that with an initial glyphosate concentration of 5 mg/L, and an ozone concentration of 1.5 mg/L, glyphosate was completely degraded (LOD 0.1 mg/L) within 25 minutes. With an initial pH of 4.9, an initial glyphosate concentration was reduced to <LOD within 25 minutes, and at pH 6.8, was reduced to <LOD within 20 minutes. At a pH of 9.3, the time required to reduce glyphosate to <LOD was 15 minutes. It was demonstrated that as glyphosate was degraded by the oxidation reactions, the amount of AMPA increased, and then AMPA also decreased, and phosphate gradually increased. Indeed, the TOC (total organic carbon) content was degraded by 77.65% after 30 minutes (when glyphosate had been reduced to <LOD), and further reduced to 93.53% after 60 minutes of reaction time. Investigation of the presence of intermediates allowed glycolic acid, glycine, phosphoric acid and AMPA to be identified. Under the conditions investigated, it was clear that degradation of glyphosate when subjected to ozonation was rapidly degraded first to a range of intermediates which were in turn subsequently completely degraded.

Partial information on the route of degradation of glyphosate and AMPA, when subjected to ozonation, comes from Klinger *et al.* (2008, CA 7.5/096). The ozonation of a phosphonate complexation agent was investigated, and it was found that this produced glyphosate and AMPA. Consequently, ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions. An investigation was reported of the removal rates associated with various stages found across seven Waste Water Treatment Plants, including one ozone treatment module (Ruel *et al.*, 2011, CA 7.5/087). For this ozone treatment module, glyphosate was found to have a removal rate of >70%, whereas for AMPA the removal rate was <30%. Investigations into the reactivity of glyphosate and AMPA when subjected to ozonation was also carried out at pilot-scale (Jönsson *et al.*, 2013, CA 7.5/084). These studies found that a 15-minute treatment period was enough to result in removal rates of >99% for both glyphosate and AMPA under the experimental conditions.

Of less importance, from a water treatment perspective (due to rare implementation of the process) is the degradation of glyphosate in water by UV/H_2O_2. One investigation used a high concentration of glyphosate (50 mg/L) to look at the removal of glyphosate from water following the washing out of product containers in Argentina (Manassero *et al.*, 2010, CA 7.5/093). Due to the high concentration of glyphosate used it was possible to identify the compounds formed during the process. It was found that AMPA was not formed from glyphosate under the test conditions, as carbon-phosphate bond cleavage was the first step of the degradation, and after the oxidative removal of one carbon unit, glycine was formed. Glycine is a naturally occurring amino acid, and under the experimental conditions it went on to generate methanediol, formic acid, nitrate anion, ammonium and phosphate anions.

The prevalence across the EU of the treatment processes referred to above, can be inferred from a publication (van der Hoek *et al*., 2014, CA 7.5/098). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MSs and 405 million European citizens, in 2014. Figure 8.2.4.1-3 shows that the vast majority of raw water sources for drinking water production (88%) are subject to disinfection.

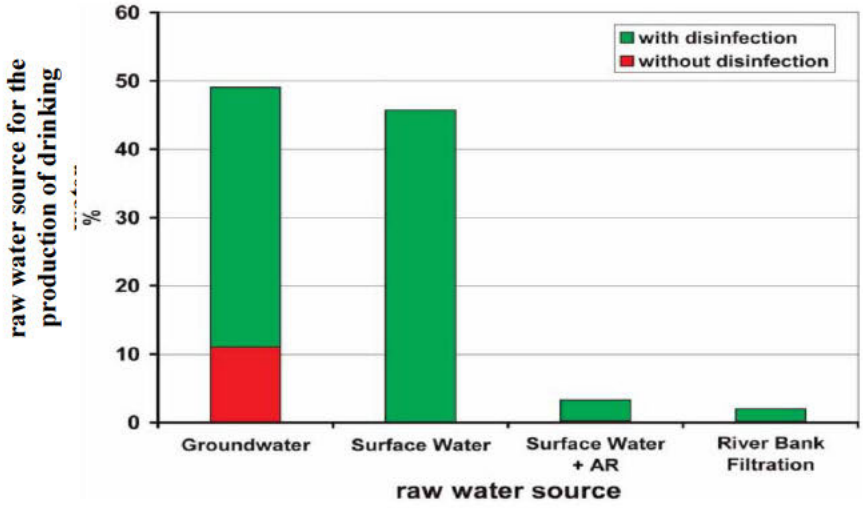
Figure 8.2.4.1-3: Raw water sources for drinking water production in Europe (from van der Hoek *et al.*, 2014)



Note: The green bars sum to 88%

The paper reports that almost all the raw water taken from surface water is subject to disinfection (99.9%). For bank filtration and artificial recharge (AR), the values are 90.1% and 92.2%, respectively. Figure 8.2.4.1-4 summarises the disinfection method employed – where surface water is disinfected, the paper reports that chlorine disinfection is applied to 62% (30% is 'not specified', but it is very likely that as disinfection by chlorine is by far the most employed method, a significant portion of the 'not specified' is also likely to be chlorine based; hence, 62% should be considered a conservative minimum value.)

Figure 8.2.4.1-4: Raw water sources and treatment scheme (from van der Hoek *et al.*, 2014, CA 7.5/098)



Note: The green bars sum to 88%

Chemical Water Treatment Summary

Glyphosate and its metabolites (AMPA and HMPA) are most likely to be exposed to chemical water

treatment processes via the treatment of surface waters abstracted for the production of drinking water. Such raw water is very likely to be subjected to a range of treatment processes, and to be subject to disinfection designed to ensure the subsequent drinking water is microbiologically safe to drink. Glyphosate and AMPA are known to be transformed by the most common disinfection methods, transformation products identified are the same as those formed from glycine and other amino acids under the same conditions. Removal rates for glyphosate and AMPA when subjected to disinfection processes are high as summarised in Table 8.2.4.1-2.

Table 8.2.4.1-2: Summary of removal rates for glyphosate and AMPA following disinfection processes (*after* Jönsson *et al.*, 2013, CA 7.5/084)

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

Furthermore, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised to ensure removal of these substances below the required threshold values.

Water Treatment Summary

For drinking water derived from surface water, there is almost always water treatment processes applied to generate the drinking water. The prevalence across the EU of the chemical treatment processes, can be inferred from a publication (van der Hoek *et al*., 2014, CA 7.5/098). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MS's and 405 million European citizens. The report indicates that the vast majority of raw water sources for drinking water production (88%) are subject to disinfection.

Further, almost all the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62% of the raw water. Glyphosate and AMPA are known to be transformed by the most common disinfection methods. Transformation products appear to be small molecules, often similar or identical to those found from natural sources.

Other chemical treatment processes are often applied (either for disinfection or for the explicit removal of micro-pollutants), and low chemical processes are also very frequently applied. Monitoring data is usually only available for raw water, before any water treatment processes have been applied, but for contextualising monitoring data, the effects of these processes should be included. Removal rates for glyphosate and AMPA, for various water treatment processes are summarised in Table 8.2.4.1-3.

Table 8.2.4.1-3: Summary of removal rates for glyphosate and AMPA following removal processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminum coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 - 85
Activated carbon adsorption	10 - 90	20 - 70
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

In addition to disinfection processes, bank filtration can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation).

Generally, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised, where necessary, to ensure removal of these substances below the required threshold values, and therefore, there is a low risk of exceeding the relevant thresholds in drinking water of 0.1 µg/L for glyphosate and 10 µg/L for AMPA, nor for exceeding the life-time health-based ADI concentrations of 1500 µg/L for GLY and 3960 □g/L for AMPA.

III. CONCLUSIONS

For surface water destined to be drinking water, there are almost always water treatment processes applied to remove bacteria and viruses and other organic micro-pollutants. The vast majority (88%) of raw water sources for drinking water production are subject to disinfection. In particular, almost all (99.9% by volume) the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62% of the raw water. Disinfection and oxidative processes are applied where needed and at predetermined rates for the removal of microbial and organic micro-pollutants, regardless of GLY and AMPA presence. GLY and AMPA are known to be very readily transformed by the most common disinfection methods, ranging from 25 to 95% for AMPA and 60 to 99% for GLY. Transformation products are small molecules, often similar or identical to those found from natural sources. Other chemical treatment processes are also often applied as are low chemical processes (processes with either no involvement of chemicals or where the treatment is to occur via physical processes like complexation and adsorption) and bank filtration (infiltration of surface water from a river or lake into a groundwater system, induced by water abstraction close to the surface water). Drinking water treatment processes are carefully controlled and the water treatment process train at any given abstraction site optimised to ensure that quality standards are met at the tap of consumers.

Assessment and conclusion by applicant:

The report describes the analysis of public monitoring data for key European countries for the compartments soil, water and sediment for Glyphosate and AMPA. An assessment of water treatment processes was undertaken through review of published peer reviewed literature. This identified treatment processes and the degree to which they are effective at removing glyphosate (GLY) and AMPA during the water treatment process. These can be used to interpret the groundwater and surface water data within the context of drinking water production.

The report is considered valid.

Assessment and conclusion by RMS:

This report summarized the review of open-literature publications or company-sponsored studies to investigate potential transformation routes of glyphosate, AMPA and HMPA under conditions simulating water treatment processes. Study authors have collated data from more than 20 articles or studies, on either chemical water treatment or lower chemical water treatment. This review identified the process used for water treatment and their efficacy to remove glyphosate and AMPA. It provides reliable information on efficiency of low-chemical and chemical water treatment, so as on the potential transformation products caused by chlorination.

Overall it is considered that the degradation pathway linked with water treatment process has been sufficiently investigated and there are no indications that harmful disinfection by-products would be formed.

All the publications cited in this review are summarized and commented hereunder.

The study is acceptable.

Existing studies/assessments

[redacted], *2012*

Data point:	CA 7.5/080
Report author	[redacted]
Report year	2012
Report title	Review of sustainable water treatment
Report No	UC8408v2; BVL No. 2316001
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Yes

Executive Summary

As the European water industry is moving towards 'simple treatments', a review of literature information on the performance of low chemical/energy processes - Bank Filtration (BF), Slow Sand Filtration (SSF) and Biological Activated Carbon (BAC) –for removal of glyphosate and AMPA was conducted. The limited information suggests that BF and SSF can remove glyphosate and AMPA, although the results are inconsistent between studies. No information is available for BAC, but significant removal is not expected through this treatment.

I. MATERIALS AND METHODS

The performance for removal of glyphosate and AMPA by "simple" water treatment process like bank filtration and variations thereof (BF), slow sand filtration (SSF) and biological activated carbon (BAC) was investigated based on literature review. The use of bank filtration is relatively limited in Europe, with less than 50 sites specifically designed to utilize this technique. Slow sand filtration is more common in Europe where it has been installed at several hundred treatment works. Biological activated carbon is the most common technique of the three; possibly because it is the easiest to retrofit.

II. RESULTS AND DISCUSSION

The results of the literature review for removal of glyphosate and AMPA by bank filtration, slow sand filtration and related processes are summarized in the table below. No information was found for biological activated carbon.

Table 8.2.4.1-4: Overview on different treatments and results

Compound	Redox conditions	Process	C_0 (µg/L)	Residence time (days)	Removal (%)	Reference
Glyphosate	Anaerobic	BF	0.07	30-300	>30	Post et al., 2000
Glyphosate	Anaerobic	BF	0.12	Unknown	17	Post et al., 2000
Glyphosate	Aerobic & anaerobic	BF and SSF	<0.05 - 0.09	Unknown	~50	Schlett et al., 2005
Glyphosate	Aerobic	SSF	<0.05 - 0.19	Unknown	>75	Schlett et al., 2005
Glyphosate	Aerobic	Soil column	10	25	>95	Lindner et al., 2000
Glyphosate	Aerobic	Batch river water	150000	72	40-72	Zaranyika and Nyandoro, 1993
Glyphosate	Aerobic	Batch soil samples	100 µg/g	50	95	Getenga and Kengara, 2004
Glyphosate	Initially aerobic	Batch river water	100	56	54 - 89	Wang et al., 1994
Glyphosate	Initially aerobic	BF	3.5, 11.6	Half life 7-10 days	80 [1]	Krause et al., 2009

Table 8.2.4.1-4: Overview on different treatments and results

AMPA	Anaerobic	BF	0.46	30-300	46 - 87	Post et al., 2000
AMPA	Anaerobic	BF	0.54	450-2000	85 - 94	Stuyfzand et al., 2004
AMPA	Anaerobic	BF	1.8	Unknown	90	Post et al., 2000
AMPA	Aerobic & anaerobic	BF and SSF	0.23 - 1.1	Unknown	≤95	Schlett et al., 2005
AMPA	Aerobic	SSF	0.08 - 0.7	Unknown	>89	Schlett et al., 2005
AMPA	Aerobic	SSF	0.04 - 0.48	Unknown	≤94	Hopman et al., 1995

BF=Bank Filtration, SSF=Slow Sand Filter, C_0=initial concentration

[1] 80% removal under test conditions, but removal to <0.1 µg/l identified from modelling for high initial concentrations with half life shown

This table shows that BF and SSF can remove glyphosate and AMPA. The general trend seems to be that the concentration of AMPA is higher than glyphosate but that AMPA is more readily degraded or removed. The degradation of glyphosate seems to benefit from aerobic conditions whereas AMPA is readily degraded both under aerobic and anaerobic conditions.

Krause et al. (2009) studied the removal of glyphosate from surface water using a variety of methods; adsorption experiments, degradation experiments, leaching experiments, enclosure experiments, and lysimeter experiments. Overall, the results from the tests carried out confirm that bank filtration should be effective for removal of glyphosate through the range of mechanism investigated.

III. CONCLUSIONS

Glyphosate and AMPA can be removed by sustainable water treatments like BF and SSF. Although no information is available for BAC, this treatment is not expected to effectively remove glyphosate and AMPA from raw water.

Assessment and conclusion by applicant:

A literature review is summarised on removal of glyphosate and AMPA by "simple" water treatment process like bank filtration (BF), slow sand filtration (SSF) and biological activated carbon (BAC). As there is no guideline on assessment of effects of water treatment procedures available, compliance cannot be assessed. Overall, results are sufficiently described.

The report was considered valid to address the data requirement.

Assessment and conclusion by RMS:

This study proposes a review of low-chemical water treatments.

It is considered acceptable and provides information on water treatment efficiencies for glyphosate and AMPA removal. Note that data from this review are included in the review provided in [redacted], 2020 summarised above.

[redacted], *2010*

Data point:	CA 7.5/081
Report author	[redacted]
Report year	2010
Report title	Removal of glyphosate and AMPA by water treatment
Report No	UC8154v2; BVL No. 2316003
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Previous evaluation	Yes, accepted in RAR (2015)

Acceptability/Reliability: Yes

Executive Summary

The first part of this study reports the performance of commonly used water treatment processes for the removal of glyphosate and AMPA from raw water during drinking water production. The results show that two of the most common oxidants used in water treatment, ozone and chlorine, can provide a high degree of removal (>95%) for glyphosate and AMPA under typical conditions used in water treatment. The majority of water treatment works use one (mainly chlorine) or both of these oxidants. The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon (GAC). However, this does not provide an effective barrier to glyphosate or AMPA. Other processes commonly used in water treatment (bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration) would each contribute some removal, but alone would not provide a secure barrier in relation to meeting a 0.1 µg/L standard.

The second part of this study assessed the removal of glyphosate and AMPA by a number of treatment processes in laboratory trials using oxidation and activated charcoal, as well as combinations of ozone, high dose ultraviolet (UV) and hydrogen peroxide in advanced oxidation pilot plant tests. Ozone (O_3) and ozone plus hydrogen peroxide (H_2O_2) are highly efficient in removing glyphosate and AMPA and better than 99% removal was seen for all conditions tested. Chlorine (Cl_2) was similarly efficient at higher temperature but removal decreased with decreasing temperature to about 70% at 5°C for glyphosate (but remained >99% for AMPA). The removal of glyphosate by chlorine dioxide (ClO_2) was not as efficient and more variable, 17-93% removed, whilst complete removal was achieved for AMPA under these conditions. PAC was the least efficient treatment for glyphosate & AMPA removal, with removals in the range 0-30%.

Advanced oxidation pilot plant tests with combinations of UV, ozone and hydrogen peroxide confirmed the result of the batch tests with ozone and ozone/peroxide. However, advanced oxidation using UV alone, or UV with peroxide, was less effective for glyphosate removal than ozonation based treatment, particularly with respect to AMPA formation and removal.

I. MATERIALS AND METHODS

The first part of the study was based on a literature review.

In the second part, laboratory batch tests were carried out to investigate the removal of glyphosate and AMPA by oxidation using O_3 alone or in combination with hydrogen peroxide (H_2O_2), Cl_2 and ClO_2, and by adsorption using PAC. In addition, pilot plant tests were conducted on advanced oxidation processes (AOP) to investigate the removal of glyphosate and AMPA by UV radiation and H_2O_2.

The stock solutions of glyphosate and AMPA were prepared by dissolving high purity solids in deionised water. For the AMPA tests using PAC and for all glyphosate tests, a 10 litre sample of Swindon tap water was spiked with 3 µg/L of either glyphosate or AMPA. Samples of the spiked water were taken for analysis to establish the initial concentration of pesticides, and the remainder of the spiked water was used in the tests. This concentration was agreed as the maximum concentration likely to be found in raw waters.

Ozonation alone: A one litre sub-sample of spiked water was ozonated using a pilot-scale O_3 generator and a bubble diffuser stone. Following ozonation for 10 s, the O_3 residual was measured immediately, and at 5 minute intervals, during a 15 minute contact time. At the end of the contact period, the residual ozone was quenched with sodium thiosulphate ($Na_2S_2O_3$).

Ozonation with hydrogen peroxide: A further set of tests were carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg/L. The ozonation conditions were identical to the test with O_3 alone with the temperature kept constant at 15 ±0.6°C. The O_3 residual was measured immediately after ozonation, and then at 5 minute intervals, during a 15 minute contact time. At the end of the contact period, the residual O_3 was quenched with sodium thiosulphate.

Chlorine: One-litre samples of the spiked water were dosed with sodium hypochlorite (NaClO) at 1.5 mg Cl_2/L. The dosed water was left for 30 minutes at the desired temperature. At the end of the contact period, the residual Cl_2 was measured and then quenched with sodium thiosulphate.

Chlorine dioxide: The tests with Cl_2 was repeated but with ClO_2 as the oxidant. The ClO_2 was added as crushed tablets, supplied by Accepta. The initial target concentration of ClO_2 was 1 mg/L.

Powdered Activated Charcoal (PAC): Tests were carried out to investigate the performance of 3 different types of coal based PAC. One litre samples of the spiked water were dosed with the three different PAC at 5, 15, and 25 mg/L. The dosed water was left stirring for 1 hour, to keep the PAC in suspension. The samples were then filtered through GF/C grade filter paper to remove the carbon, prior to analysis.

Advanced oxidation process (AOP) pilot plant test: The AOP pilot rig consisted of in-line hydrogen peroxide dosing, ozone dosing and a UV reactor, which could be used individually or in combination. The retention time in the unit was around 30-60 s, most of which was in the UV reactor. Two tests were performed, each with the same matrix of operating conditions. For the first test, the feed tap water was spiked with glyphosate to the same target concentration as previous tests, 3 µg/L. For the second test, the feed water was spiked with AMPA to a target concentration of 3 µg/L. The matrix of operating conditions was:

UV, dose 740 mJ/cm^2

UV, 1240 mJ/cm^2

UV, 740 mJ/cm^2, + H_2O_2, 5 mg/L

UV, 1240 mJ/cm^2, + H_2O_2, 5 mg/L

O_3, 2 mg/L + H_2O_2, 2 mg/L

O_3, 2 mg/L

O_3, 2 mg/L, with sample left standing for 9 minutes to provide ozone contact time

In the oxidation tests with glyphosate spiking, the treated water samples were also analysed for AMPA, to investigate whether any of the glyphosate was degraded only to AMPA by oxidation.

Workup and analysis:

All samples were analysed for glyphosate and AMPA using the following method. Water samples were treated with fmoc (9-fluorenylmethyl chloroformate) derivatising reagent prior to concentration by solid phase extraction. The extracts are then analysed by high performance liquid chromatography (HPLC) using primary mass spectroscopic (MS) detection in negative ion electrospray with selective ion monitoring. The reported limit of detection (LOD) for the method was 0.006 µg/L for glyphosate and 0.016 µg/L for AMPA.

II. RESULTS AND DISCUSSION

Literature review:

Chlorine, which is one of the most common disinfectants (oxidants) used in water treatment in Europe, can provide a high degree of removal (>95%) for glyphosate and AMPA under typical conditions used in water treatment. Ozonation, another oxidation process commonly used for pesticide removal, can also provide more than 95% removal of glyphosate and AMPA. Bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration, commonly used in water treatment, would each contribute some removal, but alone would not provide a secure barrier in relation to meeting a 0.1 □g/L standard. Depending on the treatment processes used, waterworks which include chlorine could deal with between 1 and 4 □g/L (glyphosate + AMPA) in the raw water to maintain less than 0.1 □g/L in the treated water, but if the works also includes ozonation total concentrations of above 30 □g/L could be treated. The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon (GAC). However, this does not provide an effective barrier to glyphosate or AMPA. The results of the literature review are summarized in the table below.

Table 8.2.4.1-5: Removal of glyphosate and AMPA by treatment processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 to 50	25 to 95

Aluminium coagulant and clarification	15 to 40	20 to 25
	Not a reliable barrier for Glyphosate and AMPA	
Iron coagulant and clarification	40 to 70	20 to 85
	Not a reliable barrier for Glyphosate and AMPA	
Slow sand filtration	Insufficient information but likely to be less effective than bank or dune filtration and therefore of little practical benefit	
Chlorination	74 to > 99	40 to >95
	Likely to provide the main barrier to Glyphosate and AMPA at most water treatment works	
Chlorine dioxide	Insufficient information but not expected to be effective	
Ozonation	60 to >99	25 to 95
	Provides an additional barrier at works where already installed for other pesticides and micropollutants	
UV irradiation	No information found. Highly unlikely to be effective alone at doses used in water treatment. May be effective at very high doses not currently used for water treatment.	
UV/hydrogen peroxide	Little direct information available, but indications that a combination of UV with hydrogen peroxide would be effective	
Advanced oxidation	No information found, but would be expected to be effective through free radical mechanisms. Little used for water treatment at the present time.	
Activated carbon adsorption	10 to 90	20 to 70
	Higher removals relate to virgin GAC and are unlikely to be achieved under practical conditions. Not a reliable barrier for Glyphosate and AMPA.	
Membrane filtration	>90 (NF/RO) >50 (UF)* *depending on membrane type	>95 (NF/RO) No information found for UF
	Membrane processes not widely used in water treatment, and unlikely to be installed solely as a barrier to pesticides and other organic micropollutants.	
Air stripping	No information found, not expected to be effective based on chemical characteristics.	

NF = nano filtration
RO = reverse osmosis
UF = ultra filtration

Laboratory batch tests:

Ozone was highly effective in removing both glyphosate and AMPA and virtually complete removal was achieved under all conditions tested. The combination of O_3/H_2O_2 was as effective as O_3 alone in removing glyphosate and complete removal was achieved under all conditions tested. The Cl_2 results indicate that changes in pH had little influence on the removal of glyphosate by chlorine; but that the temperature had a larger influence on the glyphosate removal with 71% being removed at 5°C compared to 96% at 20°C. The removal of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17 % to 93%. The highest removal was seen for the low pH samples (pH ~6) with high temperature (~22°C) and high ClO_2 concentrations. However, complete removal of AMPA was seen for all conditions tested, suggesting AMPA is readily removed by ClO_2. Although the results are somewhat scattered, it is clear the investigated PACs would not provide adequate removal of glyphosate and AMPA. The results of the laboratory batch tests are summarized in the table below.

Table 8.2.4.1-6: Removal of glyphosate and AMPA during laboratory batch tests

Treatment Process	Glyphosate		AMPA	
	Conditions	Removal (%)	Conditions	Removal (%)
Ozonation	T: ~7, 11, 15 °C Residual O_3: 0.41, 0.76 mg/L Conc.: 2.6 , 2.7 µg/L	>99	T: ~5, 10, 13 °C Residual O_3: 0.5 mg/L Conc.: 3.65 µg/L	>99
Ozonation + hydrogen peroxide	H_2O_2 : 0.5, 1.0 mg/L	98 - >99	H_2O_2: 0.5, 1.0 mg/L Residual O_3: 0.16, 0.04 mg/L	85 - 97

	Residual O_3: 0.09, 0.18, 0.24, 0.46 mg/L Conc.: 2.6, 2.7 µg/L		Conc.: 3.65 µg/L	
Chlorine	pH: 6, 7.5, 8.5 T: 5, 10, 20 °C Residual Cl_2: 1.4 mg/L Conc.: 2.17, 3.17 µg/L	71 - >99 (removal decrease with T°)	pH: 6, 7., 8.5 T: 6, 10, 20°C Residual Cl_2: 1.4 mg/L Conc.: 3.65 µg/L	>99
Chlorine dioxide	pH: 6-8.6 T: 4-23°C Residual ClO_2: 0.4-1.35 mg/L Conc.: 2.17, 2.47 µg/L	17 - 93 (removal decrease with T°)	pH: 6.2 - 8.4 T: 6, 10, 20°C Residual Cl_2: 1 - 1.4 mg/L Conc.: 3.65 µg/L	>99
Powdered Activated Charcoal	PAC conc.: 5, 15, 25 mg/L Conc.: 3.13 µg/L	0 - 22	PAC conc.: 5, 15, 25 mg/L Conc.: 3.13 µg/L	0-31

Advanced Oxidation Processes (AOP) pilot plant tests:

UV alone did not remove significant amounts of glyphosate or AMPA even at relatively high doses (1240 mJ/cm^2). UV in conjunction with H_2O_2 showed good removal of glyphosate (approximately 90%) but significant amounts of AMPA was also generated and AMPA was poorly removed by this treatment (<10 %).

An applied dose of 2 mg/L ozone removed greater than 95% of the glyphosate, this removal being essentially achieved within 1 minute contact time after the eductor. This indicates a very high rate of reaction with molecular ozone. This is consistent with the previous laboratory tests with ozone, but the earlier laboratory tests showed better removal of AMPA (literature search) by ozone alone. Near complete removal of glyphosate was also seen for the combination of ozone and H_2O_2, >95% was removed after 1 minute. Again, the removal of AMPA was not as good as in previous tests, but this was probably an effect of the short contact time (1 minute). The results are summarized in the table below.

Table 8.2.4.1-7: Removal of glyphosate and AMPA during AOP pilot plant tests

Treatment Process	Glyphosate		AMPA	
	Conditions	Removal (%)	Conditions	Removal (%)
UV (740 mJ/cm^2)	1 min contact time Conc.: 1.72 µg/L	25	1 min contact time Conc.: 2.31 µg/L	6
UV (1240 mJ/cm^2)	1 min contact time Conc.: 1.72 µg/L	36	1 min contact time Conc.: 2.31 µg/L	32
UV (740 mJ/cm^2) H_2O_2 (5 mg/L)	1 min contact time Conc.: 1.72 µg/L Residual H_2O_2: 5.5 mg/L	88	1 min contact time Conc.: 2.31 µg/L Residual H_2O_2: 4.98 mg/L	8
UV (1240 mJ/cm^2) H_2O_2 (5 mg/L)	1 min contact time Conc.: 1.72 µg/L Residual H_2O_2: 5.16 mg/L	91	1 min contact time Conc.: 2.31 µg/L Residual H_2O_2: 4.65 mg/L	6
O_3 (2 mg/L) H_2O_2 (2 mg/L)	1 min contact time Conc.: 1.72 µg/L	96 - >99 (duplicates)	1 min contact time Conc.: 2.31 µg/L	35
O_3 (2 mg/L)	1 min contact time Conc.: 1.72 µg/L Residual O_3: 0.83 mg/L	96	1 min contact time Conc.: 2.31 µg/L Residual O_3: 0.90 mg/L	63
O_3 (2 mg/L)	10 min contact time Conc.: 1.72 µg/L Residual O_3: 0.36 mg/L	97	10 min contact time Conc.: 2.31 µg/L Residual O_3:0.52 mg/L	>99

III. CONCLUSIONS

Literature review:

The majority of water treatment works worldwide use chlorine for disinfection, and therefore have an effective barrier for glyphosate and AMPA. Exceptions to this would be works in mainland Europe which use chlorine dioxide for disinfection and protection of the water in distribution, instead of chlorine. In this

situation, the removal of glyphosate would be more variable, but complete removal of AMPA (>99%) could be expected.

The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon. This system does not provide an effective barrier to glyphosate and AMPA. However, at many treatment works ozone is also installed for removal of pesticides or other organic micropollutants, and would be highly effective for glyphosate and AMPA removal under the dose and contact time conditions typically used. As expected, UV disinfection processes are not very effective in removing glyphosate and AMPA, but in combination with hydrogen peroxide could provide an efficient barrier for glyphosate (but not AMPA).

Other processes commonly used in water treatment (bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration) would each contribute some removal, but each process in isolation is unlikely to provide a secure barrier in relation to meeting a 0.1 □g/L standard.

Laboratory tests:

Ozone was highly effective in removing both glyphosate and AMPA and virtually complete removal was achieved under all conditions tested. No AMPA was detected in any of the treated samples from the glyphosate tests.

The combination of O_3/H_2O_2 was as effective as O_3 alone in removing glyphosate and complete removal was achieved under all conditions tested. For AMPA breakdown, the hydroxyl radical mechanism is less effective than free ozone.

The results of chlorine treatment indicate that changes in pH had little influence on the removal of glyphosate (96-100 % removal at 20 °C) while the temperature had a larger influence (removal of 71% at 5°C and 96% at 20°C. AMPA concentrations in samples from the glyphosate tests were all non-detectable, confirming the effective degradation of AMPA by chlorine seen in the investigation of the variable concentration of controls.

The removal of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17% to 93%. The highest removal was seen for the low pH samples (pH ~6) with high temperature (~22°C) and high ClO_2 concentrations. Low concentrations of AMPA were detected in the glyphosate test samples (1 – 5% of total glyphosate concentration), suggesting that AMPA was formed as a degradation product when glyphosate was oxidised by ClO_2. However, for AMPA alone, complete removal of AMPA was seen for all conditions tested, suggesting AMPA is readily removed by ClO_2.

PAC was ineffective as a removal treatment for glyphosate, even at the relatively high dose for water treatment of 25 mg/L. No more than 20% was removed. Removal of AMPA decreases with increasing PAC dose as PAC removes Cl_2 and this stops the degradation of AMPA by Cl_2 present in tap water. Overall, the PACs investigated would not provide adequate removal of glyphosate and AMPA.

Results from AOP tests indicate that advanced oxidation using UV alone, or UV with peroxide, is less effective for glyphosate removal than ozonation-based treatment, particularly with respect to AMPA formation and removal.

Assessment and conclusion by applicant:

In the study, conclusions from a literature review on removal of glyphosate and AMPA by drinking water treatment processes are combined with laboratory experiments on removal efficiency of different treatment procedures. As there is no guideline on assessment of effects of water treatment procedures available, compliance cannot be assessed. Overall, methods and results are sufficiently described. No detailed information is given about the identity and purity of the test items, but this does not have an impact on the results of the study.

The study was considered valid to address the data requirement.

Assessment and conclusion by RMS:

This study provides literature review on removal efficiency of water treatment for glyphosate and AMPA. It also provides additional results from laboratory test of different treatments.

The study is considered acceptable and provides information on water treatment efficiencies for glyphosate and AMPA removal. Data from this study are included in the review provided in [redacted] 2020 summarised above.

B.8.2.4.2. *Relevant literature articles*

Hamann et al., 2016

Data point:	CA 7.5/082
Report author	Hamann, E. *et al.*
Report year	2016
Report title	The fate of organic micropollutants during long-term/long-distance river bank filtration
Document No	Science of the Total Environment
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restriction

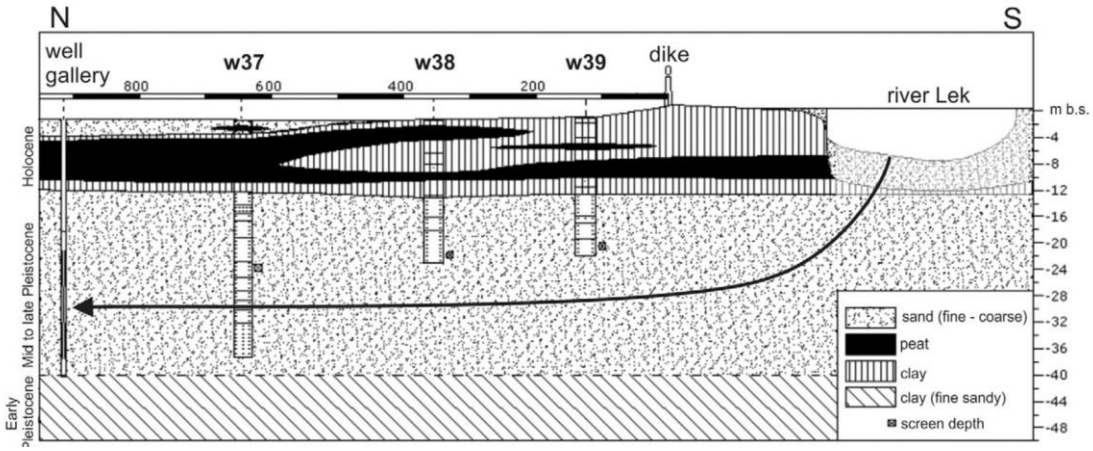
The fate of organic micropollutants during long-term/long-distance river bank filtration (RBF) at a temporal scale of several years was investigated along a row of monitoring wells perpendicular to the Lek River (the Netherlands). Out of 247 compounds, which were irregularly analyzed in the period 1999-2013, including AMPA, only 15 were detected in both the river and river bank observation wells. Out of these, 10 compounds (1,4-dioxan, 1,5-naphthalene disulfonate (1,5-NDS), 2-amino-1,5-NDS, 3-amino-1,5-NDS, AOX, carbamazepine, EDTA, MTBE, toluene and triphenylphosphine oxide) showed fully persistent behavior (showing no concentration decrease at all), even after 3.6 years transit time. The remaining 5 compounds (1,3,5-naphthalene trisulfonate (1,3,5-NTS), 1,3,6-NTS, diglyme, iopamidol, triglyme) were partially removed. Their reactive transport parameters (removal rate constants/half-lives, retardation coefficients) were inferred from numerical modeling. In addition, maximum half-lives for 14 of the fully removed compounds, including AMPA, for which the data availability was sufficient to deduce 100% removal during sub-surface passage, were approximated based on travel times to the nearest well. The study is one of very few reporting on the long-term field-scale behavior of organic micropollutants. It highlights the efficiency of RBF for water quality improvement as a pre-treatment step for drinking water production. However, it also shows the very persistent behavior of various compounds in groundwater.

Materials and methods

Field Site

The Rodenhuis RBF study site is located on the Lek River, a tributary of the Rhine River in the Netherlands, situated between the cities of Rotterdam and Utrecht. The transect with observation wells w37, w38 and w39 is aligned along the flow direction between the river and the public supply well field at Rodenhuis (Figure 8.2.4.2-1), as ascertained by a numerical groundwater flow model (Oasen Drinking Water Company, personal communication).

Figure 8.2.4.2-1: Hydrogeological conditions and position of the observation wells along the studied transect (modified from Segers (2006)). The arrow indicates the hypothetical groundwater flow path.



Hydrochemical data for the Lek River were taken from the public accessible annual reports of the Rhine River and its tributaries (RIWA, 1999-2013), where monthly analyses of the inorganic and organic compounds are given.

Hydrochemical data from the observation wells was taken from a database provided by the Water Company OASEN. The database includes physico-chemical parameters, major ions, some trace elements and a vast number of organic micropollutants measured from 1999 to 2013. The individual parameters were measured at irregular intervals between 1 and 18 times. Chloride was for example measured 7 times at w37 as compared to toluene which was measured 18 times at the same well.

Altogether, 247 organic substances present above the detection limit in the Lek River were also part of the measurement program at the transect. Out of those, 29 organic micropollutants, including AMPA, were selected for the detailed fate analysis during RBF.

Groundwater flow and reactive transport modeling

Along the transect three 1D models were built, one for each observation well representing the flow path from the river to the well. The use of three separate models was necessary in order to match the measured tracer breakthrough curves during the process of calibration, as insufficient hydrogeological data was available to account for changes in aquifer characteristics over such large distances. The flow and transport simulations were carried out with MODFLOW and MT3DMS, respectively. The extent of the respective models was 370, 606 and 906 m, matching the distance between the river and the observation wells w39, w38 and w37, respectively. The flow conditions were assumed to be steady-state in a homogenous medium. The flow boundaries representing the river and the pumping well were prescribed as 1st and 2nd order boundary conditions, respectively. The latter was adjusted to match the tracer breakthrough curves during calibration. Hydraulic conductivity and effective porosity were chosen according to typical values of medium-grained sands with values of 80 m/d and 0.25, respectively. The resolution of the model grid was 1 m. The temporal discretization was set to monthly time steps according to the availability of measured river data. When data was not available monthly but for longer time intervals, the data was linearly interpolated. The available concentration trends of the conservative tracer chloride in the river and in the observation wells were used to calibrate the flow and non-reactive transport models by adjusting the flow velocities via the production well pumping rates as well as the longitudinal dispersivity.

To simulate the attenuation of the organic micropollutants during RBF, linear adsorption and 1st order degradation were implemented in the models as follows:

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - \nu\frac{\partial C}{\partial x} - \lambda C$$

with R [-], retardation coefficient; C [M/L^3], aqueous concentration of a solute; x [L], spatial dimension in flow direction; t [T], time; v [L/T], pore velocity; D [L^2/T], longitudinal dispersion coefficient defined as $D = v\alpha_L$, with α_L [L], longitudinal dipersivity; and λ [T^{-1}], first-order degradation rate constant. The often used degradation half-life is defined as:

$$t_{1/2} = \frac{\ln 2}{\lambda}$$

Retardation by linear adsorption was considered in the model using the conventional linear distribution coefficient K_d [L^3/M]:

$$R = 1 + \rho_b \frac{K_d}{\theta}$$

with ρ_b [M/L^3], bulk density; and θ [-], total porosity. When included, retardation by sorption was assumed to act equally throughout the whole model domain.

First order degradation rate constants (λ's) were likewise uniformly prescribed to the whole model domain, independent of groundwater redox conditions or temperatures. Calibration was carried out by automatically estimating λ for the partially removed and persistent compounds with the model independent parameter estimation tool PEST and manually adjusting K_d to obtain the best fit between measured and modeled data. PEST generated 95% confidence intervals were provided to inform about the uncertainty of the estimated values of λ.

In cases where the observed concentration time series were not suitable to infer delayed transport by retardation, sorption properties based on the quantitative structure-activity relationship (QSAR) were used for interpretation. For that purpose and due to the fact that some of the investigated compounds will form ions at the prevalent pH conditions at the site, the pH-dependent octanol-water partition coefficient log D_{OW} at pH 7 to 8.5 was used.

Results

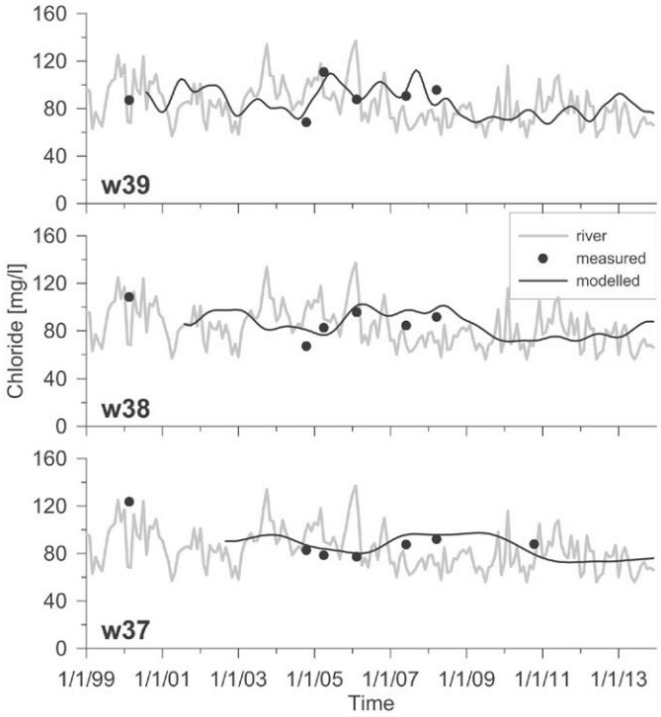
The resulting flow velocities were identical in the models w37 and w38, but slightly lower in model w39 (Table 8.2.4.2-1). Increasing dispersivity with increasing distance to the river reflects the scale dependence of dispersion. One limitation for calibration was the frequency of analysis of chloride in the groundwater. While high resolution chloride data was available for the river, chloride was only available for six times in 14 years in groundwater. In our modeling study, more chloride measurements were available for w37, further improving the calibration.

Table 8.2.4.2-1: Flow and transport parameters of the calibrated models

Observation well	Distance to river [m]	Flow velocity [m/year]	Travel time [years]	α_L [m]
w39	370	224	1.65	2
w38	606	248	2.44	5
w37	906	248	3.65	10

Following the calibration of the flow and non-reactive transport model, the physical parameters were left unchanged in the reactive simulations. According to their appearance in the observation wells as compared to the river, the compounds were classified into fully removed (no detection in the groundwater observation wells), partially removed (compounds detected in the bank filtrate in decreased concentrations as compared to the river) and fully persistent (no indication for removal at all, even after 3.65 years of sub-surface residence time). Chloride shows conservative behavior as expected (Figure 8.2.4.2-2).

Figure 8.2.4.2-2: Times series of chloride in the river (measured) and in the observation wells (measured and modelled)



Fully removed compounds

Out of the 29 compounds selected for modeling, 14 substances were non-detectable in the bank filtrate, namely 2-naphthalene sulfonate (2-NS), 2,6-NDS, amidotrizoic acid, AMPA, aniline, bezafibrate, diclofenac, ibuprofen, iohexol, iomeprol, iopromide, ioxitalamic acid, metoprolol and sulfamethoxazol. For these compounds, 1st order degradation rate constants were calculated based on the travel time between river and w39, the mean input concentration at the river and the detection limit at w39 as the maximum possible residual concentration after degradation, assuming that complete degradation takes place somewhere between river and w39. Employing the detection limits and the estimated travel time to w39, the calculated rate constants have to be regarded as minimum values (or rather the half-lives as maximum values). Furthermore, possible retardation was neglected in this calculation. Therefore the degradation rates may be overestimated for retarding substances. To determine the tendency of substances to retard, log D_{ow} values were used. Substances with high log D_{OW}'s are likely to sorb, i.e., aniline, bezafibrate, diclofenac and ibuprofen. Other substances with very low log D_{OW}'s, i.e., amidotrizic acid, AMPA, 2-NS, 2,6-NDS, iohexol, iomeprol, iopromide, ioxatalamic acid, metoprolol and sulfamethoxazol, have more likely been subject to degradation only.

Partially Removed Compounds

The concentrations of triglyme, iopamidol, 1,3,5-naphthalene trisulfonate (1,3,5-NTS) and 1,3,6-naphthalene trisulfonate (1,3,6-NTS) clearly decrease during the RBF. Assuming that no retardation of the mentioned substances takes place based on their low log D_{OW}'s, non-reactive model simulations to a great extent overestimate the measured concentrations at the transect.

Persistent Compounds

Some compounds (MTBE, carbamazepine, AOX, triphenylphosphineoxide (TPPO), toluene, EDTA, 1,5-naphthalene-disulfonate (1,5-NDS), 2-amino-1,5-naphthalene disulfonate (2-amino-1,5-NDS), 3-amino-1,5-naphthalene disulfonate (3-amino-1,5-NDS) and 1,4-dioxan) were present in river and bank filtrate in similar concentrations and the model simulations achieved a best fit when assuming non-reactive and non-sorptive behavior, suggesting persistence over long periods in the sub-surface. Thereby, non-reactive behavior was assumed when the PEST estimated half-life was $t_{1/2}$ >10 years.

Table 8.2.4.2-2: Investigated organic micropollutants classified according to their removability at the RBF site in fully removed, partially removed and persistent substances

Fully removed organic micropollutants
(Considered removal processes are degradation and retardation. 1st order degradation rate constants and half-lives of substances with high affinity to adsorb ($\log D_{ow} > 0$) are written in parentheses.)

Substance (CAS nr.)	Application	$\log D_{OW}^{(1)}$ at pH 7.0–8.5[2]	$R_{\_current\_study}$ [−]	$R_{literature}$ [−]	$\lambda_{\_current\_study}^{(4)}$ [year^{-1}]	$t_{1/2\_current\_study}^{(4)}$ [d]	$t_{1/2\_literature}$ [d] only field studies
2-Naphthalene sulfonate (532-02-5)	Industry	−0.23	1	–	>0.54	<469	Low[w]
2,6-Naphthalene disulfonate (581-75-9)	Industry	−3.43	1	–	>0.37	<683	Low[w]
Amidotrizoic acid (117-96-4)	X-ray contrast agent	−0.62 to −0.64	1	1[p]	>1.95	<130	25–∞[p]
Aminomethyl-phosphonic acid (AMPA) (1066-51-9)	Main metabolite of Glyphosat	−3.16 to −4.33	1	>1[v]	>1.96	<129	37 ± 11[m], 50–300[u]
Aniline (62-53-3)		1.14	1		(>0.33)	(<776)	
Bezafibrate (41859-67-0)	Pharmaceutical	0.97 to 0.49	1	1[a]	(>0.26)	(<969)	1–2.1[a]
Diclofenac (15307-86-5)	Pharmaceutical	1.37 to 0.77	1	1[e], >1[r], 4.8[k], 1.41[t], 1.04–1.81[a], >26[f]	(>0.91)	(<280)	1–1.8[a], 36[f], 0.3–∞[g], 3[o]
Ibuprofen (15687-27-1)	Pharmaceutical	1.71 to 0.56	1	1[a][d][e], 1.1[t], 3[k]	(>0.4)	(<638)	0.7–1.2[a], 1.9[g]
Iohexol 66108-95-0)	X-ray contrast	−1.95	1	1[a][n][p], 2.6[h]	>1.34	<189	1.4–2.5[a], <7[p]
Iomeprol (48649-41-9)	X-ray contrast	−1.45	1	1[a][p]	>2.16	<117	1.5–2[a], <6[p], 108[ak]
Iopromide (73334-07-3)	X-ray contrast	−0.44 to −0.45	1	1[a][p]	>1.82	<139	1.3–2[a], 0.1[g], 1.5–3[o], <7[p]
Ioxitalamic acid (28179-44-4)	X-ray contrast	−1.47 to −1.49	1	–	>0.72	<353	
Metoprolol (51384-51-1)	Beta blocker	−0.81 to 0.57	1	>9[j], 4.3–10.8[y]	>0.95	<268	
Sulfamethoxa-zol (723-46-6)	Antibiotic	0.14 to −0.14	1	1[a][e][f][ad]	>0.58	<433	∞[a], persistent[bg], 22[f], 30[o], 30–∞[p], 5.3[q]

Partially removed organic micropollutants
(Contemplable removal processes are degradation and retardation)

Substance (CAS nr.)	Application	Log $D_{OW}^{(1)}$ at pH 7.0–8.5[2]	$R_{\_current\_study}^{(3)}$ [−]	$R_{literature}$ [−]	$\lambda_{\_current\_study}^{(3)}$ [year^{-1}]	$t_{1/2\_current\_study}^{(3)}$ [d]	$t_{1/2\_literature}$ [d] only field studies
1,3,5-Naphthalene trisulfonate (6654-64-4)	Industry	−6.62	1	–	0.44[3] (0.35–0.54)[5]	569[3] (470–722)[5]	Poorly removable[b][z]
1,3,6-Naphthalene trisulfonate (86-66-8)	Industry	−6.62	1	–	0.24[3] (0.07–0.42)[5]	1044[3] (605–3800)[5]	Removable[z]
Diglyme (111-96-6)	Industrial solvent	0.03	1	Not likely[ae]	0.34[3] (0.26–0.42)[5]	740[3] (598–971)[5]	∞[p]
Iopamidol (60166-93-0)	X-ray contrast agent	−0.74	1	1[a][p]	>1.28[3] (1.15–1.42)[5]	<197[3] (179–221)[5]	1.8–3.5[a], 25–85, 140–∞[p], ≈15[z]
Triglyme (112-49-2)	Industrial solvent	−0.02	1	Not likely[ae]	>0.86[3] (0.69–1.02)[5]	<296[3] (248–367)[5]	Persistent[al]

Persistent organic micropollutants

Substance (CAS nr.)	Application	Log $D_{OW}^{(1)}$ at pH 7.0–8.5[2]	$R_{\_current\_study}$ [−]	$R_{literature}$ [−]	$\lambda_{\_current\_study}$ [year^{-1}]	$t_{1/2\_current\_study}$ [d]	$t_{1/2\_literature}$ [d] only field studies
1,4-Dioxan (123-91-1)	Industrial solvent	−0.09	–	1[p]	0	∞	Persistent[p][ac]
1,5-Naphthalene disulfonate (81-04-9)	Chemical industry	−3.43	–	1[f]	0.004[3] (−0.03–0.07)[5]	63,250[3]	Persistent[b][w], 309[f]
2-Amino-1,5-naphthalene disulfonate (117-62-4)	Industry	−4.26	–	–	0.04[3] (−0.1–0.19)[5]	6325[3]	Persistent[w]
3-Amino-1,5-naphthalene disulfonate (131-27-1)	Industry	−4.26	–	–	0	∞	–
AOX			–	1[f][af]	0.02[3] (−0.03–0.07)[5]	12,650[3]	286[f], <∞[x]
Carbamazepine (298-46-4)	Anticonvulsant	2.77	2.5[3]	≥1[e][n][r][ag][ah][ai][aj], 1.84[k], 13[h], 3.6–5.3[y], 1.04–1.16[a], 1.7[f], 2.2[p]	−0.02[3] (−0.08–0.06)[5]	∞[3]	3.5–7[a], persistent[b], 66[f], 14–∞[g], 35[o], >7300[p]
EDTA (60-00-4)	Chelating agent	−14.2 to −15.51	–	1[f]	−0.02[3] (−0.08–0.05)[5]	∞[3]	∞[f][o], ≤ ∞[x]
MTBE (1634-04-4)	Fuel component, industrial solvent	1.18	1[3]	1[f],[ab], 1.05[o]	−0.06[3] (−0.16–0.04)[5]	∞[3]	82[f]
Toluene (108-88-3)	Industrial feedstock, solvent	2.49	–	2.1[aa]	0.06[3] (−0.11–0.24)[5]	4216[3]	<700[c] (review study)
Triphenylphosphine oxide (791-28-6)	Industrial byproduct	4.76	–	–	0	∞	

Note: footnotes are available in the original article.

Conclusion

In this study, the behavior of a large number of organic micropollutants during river bank filtration was evaluated on the basis of measurements in the period 1999-2013 on a row of monitoring wells with travel times of 1.6-3.6 years. Field studies reporting on such long distance and long-term behavior are rare. Most quantitative information has so far been inferred from laboratory studies or from field-studies with considerably shorter residence times. While the sampling frequency was very high in the river, fewer data were available for the observation wells, somewhat limiting the approach with regard to detailed process understanding. The information whether or not a compound is fully, partially or not at all removed is nevertheless of great value and compounds were classified accordingly.

Overall, only 15 of the 247 compounds detected in the river and analyzed for in the bank filtrate were detected in the bank filtrate. Out of those, 10 were fully persistent (1,4-dioxan, 1,5-NDS, 2-amino-1,5-NDS, 3-amino-1,5-NDS, AOX, carbamazepine, EDTA, MTBE, toluene and TPPO) and 5 only partially removed (1,3,5-NTS, 1,3,6-NTS, diglyme, iopamidol, triglyme).

For compounds detected in the river but not in the observation wells of the transect, including AMPA, at least minimum degradation rate constants were inferred. Many previous studies used the decrease of the concentration of a substance along a flow path time-independently. The long-term time series in this data-set shows how sometimes temporal changes in the river and the time-shift caused by the groundwater travel time can lead to misinterpretations. Instead, numerical model-based interpretations of time-series, which take these variabilities into account are far more suitable to quantify reactive transport parameters such as degradation rate constants.

Assessment and conclusion by applicant:

The article describes a modelling approach to describe long-term/long-distance river bank filtration for 29 compounds including AMPA. There are no new experimental data generated but the modeling approach gives relevant and reliable information on the behavior of AMPA at drinking water abstraction points.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable with restrictions. It provides supportive information on river bank filtration efficiencies for AMPA removal, based on monitoring results at a temporal scale of several years in the Netherlands. However, no detailed measured concentrations for AMPA are reported in the article.

The study aimed at calibrating a flow and solute transport model between the Lek River and the drinking water abstraction wells through the river bank, based on hydrochemical data available from the public accessible annual reports of the Rhine River and its tributaries (RIWA, 1999-2013).
It is indicated that AMPA is part of the substances that were fully removed, non detectable in the bank filtrate. However, concentration of AMPA in the river before filtration are not given.

Hedegaard &Albrechtsen, 2014

Data point:	CA 7.5/083
Report author	Hedegaard, M., Albrechtsen, H.
Report year	2014
Report title	Microbial pesticide removal in rapid sand filters for drinking water treatment - Potential and kinetics
Document No	Water Research 48 (2014) 71-81
Guidelines followed in study	None
Deviations from current test guideline	Not applicable

GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Filter sand samples, taken from aerobic rapid sand filters used for treating groundwater at three Danish waterworks, were investigated for their pesticide removal potential and to assess the kinetics of the removal process. Microcosms were set up with filter sand, treated water, and the pesticides or metabolites mecoprop (MCPP), bentazone, glyphosate and p-nitrophenol were applied in initial concentrations of 0.03-2.4 µg/L. In all the investigated waterworks the concentration of pesticides in the water decreased – MCPP decreased to 42-85%, bentazone to 15-35%, glyphosate to 7-14% and p-nitrophenol 1-3% – from the initial concentration over a period of 6-13 days. Mineralisation of three out of four investigated pesticides was observed at Sjælsø waterworks Plant II – up to 43% of the initial glyphosate was mineralised within six days.

Materials and methods

Degradation potential of filter sand

Filter sand from three Danish waterworks - Islevbro, Sjælsø Plant I and Sjælsø Plant II - was investigated for the removal potential of the pesticides mecoprop (MCPP), bentazone, glyphosate, and the degradation product p-nitrophenol.

The investigations included filter sand from three different groundwater-based waterworks. Selected parameters for water quality can be seen in Table 8.2.4.2-3. In order to investigate the potential of filter sand to degrade pesticides, four ^{14}C-labelled pesticides (mecoprop, bentazone, glyphosate and p-nitrophenol) were selected. To investigate pesticide removal at concentrations close to water quality guidelines, pesticides were in general added to an initial concentration of 0.1 µg/L.

Table 8.2.4.2-3: Water quality data based on information from the waterworks. The range is given for each parameter for the given time period for wells and the effluent water from the filters. The waterworks monitors for more than 20 pesticides and degradation compounds, but this table only includes detected pesticides.

		Islevbro		Sjælsø Plant I		Sjælsø Plant II	
Dry filter sand							
TOC[a]	mg/g	2.71		78.8		0.517	
		Wells 2010–2011	Effluent water 2011–2012	Wells 2011–2012	Effluent water 2012	Wells 2011–2012	Effluent water 2012
Water							
Oxygen	mg/L	0.23–2.2	7.3	0.31–0.62	–	0.25–0.44	–
Nitrate	mg/L	0.018–3.7	2.57	0.043–0.378	–	<0.03–0.061	–
Nitrite	mg/L	0.005–0.017	0.06	<0.0016–0.012	<0.0016–0.0079	<0.0016	<0.0016–0.0077
Ammonium	mg/L	0.251–0.97	0.10	0.38–0.77	<0.004–0.01	0.92–1.26	<0.004–0.016
Manganese	mg/L	0.028–0.1	–	0.005–0.22	<0.001–0.003	0.012–0.054	<0.001
Iron	mg/L	0.01–3.6	0.3	0.2–4.3	<0.01–0.02	0.38–2.6	<0.01–0.05
Sulphate	mg/L	67–160	33.1	9–58	–	<0.5–14	–
Hydrogen sulphide	mg/L	0.012–0.02	–	0.01–0.04	–	0.03–1.19	–
Methane	mg/L	0.01–0.13	–	0.06–0.5	–	1.13–9.2	–
BAM[a]	µg/L	0.014–0.076	–	<0.01–0.018	–	<0.01	–
4-CPP[a]	µg/L	0.016	–	<0.01–0.11	–	<0.01	–
DCPP[a]	µg/L	0.02–0.20	–	<0.01–0.014	–	<0.01	–
2,6-DCPP[a]	µg/L	–	–	<0.01–0.025	–	<0.01	–
MCPP[a]	µg/L	0.04–0.13	–	–	–	–	–
Glyphosate	µg/L	0.022	–	–	–	–	–
Phenol	mg/L	–	–	–	–	<0.05	–
NVOC[a]	mg/L	2.33	2.41	2.2–3.5	1.7–3.9	2.1–3.9	2.7–4.5
Conductivity at 12 °C	mS/m	–	–	65–108	–	66–82	–
Alkalinity	meq/L	5.12–7.18	5.4	–	–	–	–
pH		7–7.9	–	–	–	–	–

– no data available.

a TOC = Total organic carbon; BAM = 2,6-Dichlorobenzamide; 4-CPP = (RS)-2-(4-chlorophenoxy)propionic acid; DCPP = (R)-2-(2,4-dichlorphenoxy)propionic acid; 2,6-DCPP = 2-(2,6-dichlorphenoxy)propionic acid; MCPP = (RS)-2-(4-Chloro-2-methylphenoxy)propanoic acid; NVOC = Non-volatile organic carbon.

Water was collected from the inlet connecting to the clean water tanks. Filter sand was collected from the top 20 cm of the filter bed with a specially designed aluminum bucket on an extendable shaft, which was disinfected with 1% hypochlorite. The filter sand was transported to the laboratory in an autoclaved plastic bag inside a clean bucket.

Within 2 h of collecting water and filter sand at the waterworks, 250 g wet filter material was transferred with a sterilized spoon to 300 mL serum bottles, which had been acid washed and heated to 555°C for 12 h. A total water volume of 100 mL was added, including volumes of dissolved chemicals.

Abiotic controls were set up with filter sand, which was either autoclaved three times (20 min, 1 bar and 121°C, the microcosms cooled for approx. 30 min - to less than 80°C - before autoclaving was repeated) or was mixed with sodium azide to a concentration of 2 g/L to inhibit all microorganisms.

Microcosms were closed with Teflon caps and aluminum lids, and they were left at 10°C in darkness overnight before sampling. Incubation conditions were static. The pH remained at 7 during the experiment and the oxygen concentration was measured before and after the experiment with an HACH HQ40d oxygen electrode.

Sampling was frequent in the initial stages of the experiments and lasted for one to six hours. In the second phase the removal potential of the filter sand was investigated, and sampling was less frequent and lasted for 2-13 days after the experiment started.

The microcosms were spiked with dissolved [^{14}C-]pesticide to a concentration of 0.03-2.4 µg/L (Table 8.2.4.2-4). When sampling, 3 mL atmospheric air was added to the microcosms and the 2-3 mL water samples were collected with a syringe through the cap of the microcosms. A 0.25 mm hydrophilic PTFE-filter was used to remove suspended matter from the water sample. The analysis for ^{14}C was based on a double vial system, whereby $^{14}CO_2$ produced in the collected water sample was stripped off and captured by a base trap (1 mL 2M NaOH). Thus, the produced $^{14}CO_2$ and the ^{14}C-activity of the pesticide in the water phase could be quantified.

Table 8.2.4.2-4: Initial conditions in the microcosms in the different experiments. Added amount of filter sand and water appear as well as the initial concentration of the added pesticides.

Waterworks	Potential of filter sand			Removal kinetics	Effect of oxygen
	Islevbro	Sjælsø Plant I	Sjælsø Plant II	Sjælsø Plant II	Sjælsø Plant II
Filter sand (g)	250	250	250	250	100
Water (mL)	100	100	100	100	100
Initial concentration					
Mecoprop (µg/L)	0.38	0.04	0.03	–	–
Glyphosate (µg/L)	–	0.05	0.05	–	–
p-Nitrophenol (µg/L)	–	0.16	0.16	–	–
[Carbonyl-^{14}C-]Bentazone (µg/L)	0.1	0.1	0.1	0.1/0.5/2.4	0.6
[Benzene-ring-U-^{14}C]Bentazone (µg/L)	–	–	–	0.16	–

– no data.

Due to frequent sampling in the first 1-6 h, experiments were processed at an ambient temperature (20°C). After this period, the microcosms were incubated at 10°C in darkness.

The water content of the filter material was quantified through weight loss after 24 h at 105°C. The bulk density of the dry filter sand was found by weighing 40 mL, without compressing the filter sand. The amount of total organic carbon (TOC) in the filter sands was measured for the sample. The TOC analysis was carried out by employing a total element carbon analyser (LECO Induction Furnace CS-200) after the removal of carbonates by adding 5% sulphurous acid (H_2SO_3).

Results

Degradation potential of filter sand

All of the investigated rapid sand filters removed the investigated pesticides partially, either by abiotic or microbial processes (Table 8.2.4.2-5), and concentrations in the microcosms decreased during the experiment between 6 and 13 days. MCPP decreased to 42-85%, bentazone to 15-35%, glyphosate to 7-14% and p-nitrophenol to 1-3% of the initial concentration. Due to the position of the ^{14}C-label in

glyphosate only a complete removal of the compound would be detected - partial degradation to the primary metabolite 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA) would not be detected.

Table 8.2.4.2-5: Fractionation of ^{14}C-bentazone after incubation with filter material from different filter sands. The fractionation of ^{14}C (or $^{14}CO_2$) of the initial amount of $^{14}C_0$ is shown at two selected times. Data are from microcosms (two replicates) and abiotic controls. The removal of MCPP at Islevbro was tested with both outlet water from filter (OW), and inlet water to filter (IW)

	Fraction of bentazone in water phase ($^{14}C/^{14}C_0$)				$^{14}CO_2$-production from degradation ($^{14}CO_2/^{14}C_0$)			
	Microcosms	Abiotic control	Microcosms	Abiotic control	Microcosms	Abiotic control	Microcosms	Abiotic control
Islevbro	**4 hours**		**13 days**		**4 hours**		**13 days**	
MCPP OW	60–61%	64%	42–48%	57–61%	–	–	–	–
MCPP IW	60%	73%	51–57%	73–75%	–	–	–	–
Bentazone	72–74%	81%	26–33%	74–83%	–	–	–	–
Sjælsø Plant I	**4 hours**		**6 days**		**4 hours**		**6 days**	
MCPP	103%	63%	67–74%	67%	–	–	–	–
Bentazone	62–75%	60%	31–35%	62%	–	–	–	–
Glyphosate	8–9%	9%	7–8%	4%	–	–	–	–
4-Nitrophenol	29%	56%	1–3%	22%	–	–	–	–
Sjælsø Plant II	**4 hours**		**6 days**		**4 hours**		**6 days**	
MCPP[a]	103%	112%	70–85%	92%	–	–	–	–
Bentazone	59–71%	101%	15–18%	103%	–	–	8–14%	–
Glyphosate	19–20%	17%	9–14%	8%	31–36%	–	42–43%	–
4-Nitrophenol	28–33%	102%	3%	96%	4%	–	7–10%	–

– No evident tendency in results.
a Low initial concentrations (0.033–0.036 µg/L) – uncertain results.

The mineralisation of pesticides in terms of $^{14}CO_2$ production was observed only at Sjælsø Plant II. After six days, $^{14}CO_2$ production from bentazone reached 8-14%, glyphosate 42-43% and p-nitrophenol 7-10% of the initially added pesticide (mineralisation of MCPP was not detected).

For Islevbro and Sjælsø waterworks Plant I, [^{14}C-]pesticide was removed from the water phase in the abiotic controls, so a part of the pesticide was removed by abiotic processes, such as sorption. For Sjælsø Plant I the removal of MCPP and glyphosate was merely abiotic, since there was no difference between abiotic controls and microcosms. Microbiological removal did not result in immediate mineralisation ($^{14}CO_2$ production), and removal must have been caused by a degradation to a metabolite, which was eliminated from the water phase by sorption or volatilisation, or the compound was taken up by the microorganism. At Sjælsø waterworks Plant II, evident mineralisation was measured for bentazone, glyphosate and p-nitrophenol. Microbiological removal was substantial in this filter, though abiotic processes also had an influence especially on the removal of glyphosate.

Conclusion

The investigations showed a clear removal potential of the pesticides MCPP, bentazone, glyphosate, and p-nitrophenol in rapid sand filters at Danish waterworks. The largest microbial removal was observed with filter material taken from Sjælsø Plant II. At Sjælsø waterworks Plant II bentazone concentration in the water phase decreased as a result of microbial removal to less than 50% of the initial concentration within 30 min for all tested start concentrations (0.1-2.4 µg/L).

Overall, this study showed that substantial pesticide removal is possible within the contact time of rapid sand filters at Danish waterworks, and that rapid removal is followed by a slower mineralisation of the compound. Hence, there is a potential for microbial removal of pesticides from contaminated groundwater in Danish waterworks. This is of commercial interest due to substantial attention given to the maintenance of today's water treatments.

Assessment and conclusion by applicant:

The article describes experiments on the removal potential of glyphosate in rapid sand filters at Danish waterworks. Under the experimental conditions, glyphosate decreased to 7 - 14% of initial amounts after 13 days (complete mineralisation); indicating that glyphosate was intrinsically degradable under these conditions (although unlikely to be degraded significantly *in situ*). The experiments are well described. However, no details on analytical methods are given. Further, sampling times and individual results are only reported for bentazone in graphical plots.

The article is considered reliable with restrictions

Assessment and conclusion by RMS:

This article describes the assessment of pesticide removal of aerobic sand filters used in three Danish waterworks. Microcosms were set up with filter sand, treated water, and ^{14}C-labelled pesticides or metabolites mecoprop (MCPP), bentazone, glyphosate and p-nitrophenol were applied. However, it is indicated in the study that "to investigate pesticide removal at concentrations close to water quality guidelines, pesticides were in general added to an initial concentration of 0.1 µg/L." and the mesocosm seems to be spiked with dissolved [^{14}C-]glyphosate at the intial concentration of 0.05 µg/L, which is very low.

Moreover, the results from the experiment are not clearly exposed. The text indicates that "Glyphosate decreased to 7 - 14% of initial amounts after 13 days", but there is no detailed results reported to support this. Results reported in table 14 are not clear, since related to "fraction of bentazone in the water phase", but results for each tested substance are presented. The 14% of glyphosate initial concentration seem to be reached within 6 days and not 13 days according to the results reported in this table. However, the experimental conditions are clearly not representative of the *in situ* conditions; the residence time of contaminated water in the rapid sand filter in the treatment works was reported to be 7.5 to 12 minutes.

In any case, considering the initial concentration of 0.05 µg/L, in the absence of description of analytical method accuracy, and of definition of LOQ, the reliability of the results is questioned.

The article is considered reliable with restrictions.

Jönsson et al., 2013

Data point:	CA 7.5/084
Report author	Jönsson, J. *et al.*
Report year	2013
Report title	Removal and degradation of glyphosate in water treatment: a review
Document No	Journal of Water Supply: Research and Technology-AQUA/62.7/2013
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable

Treatment methods such as ozonation and activated carbon are currently used for pesticide degradation and removal. This article provides a review of the reported efficiency in removal and degradation of glyphosate and aminomethylphosphonic acid (AMPA) by some commonly employed treatment options. Additional experiments have been carried out where knowledge gaps were identified. Oxidants used in water treatment, particularly Cl_2 and O_3, are highly effective in degrading glyphosate and AMPA. Removal by coagulation and activated carbon is ineffective as a barrier against contamination in drinking water. UV treatment is also ineffective for glyphosate and AMPA degradation, but the combination of UV/H_2O_2 provided significant degradation of glyphosate, but not AMPA, under the

conditions investigated. UV/TiO_2 treatment can degrade significant amounts of glyphosate, but the irradiation time needed is long. Removal or degradation by bank filtration, slow sand filtration, ClO_2 and membranes is variable, but can provide significant removal under the right conditions.

Materials and methods

Batch tests were carried out to investigate the degradation of glyphosate and AMPA by oxidation using Cl_2, ClO_2, O_3, O_3/H_2O_2, and by adsorption using PAC (powdered activated carbon). The stock solutions of glyphosate and AMPA were prepared by dissolving high purity solids in deionized water. Tap water, purged with air to remove residual chlorine, was spiked with stock solutions to achieve a concentration of 3 µg/L of either glyphosate or AMPA. This concentration was chosen to represent a moderately contaminated water. Samples of the spiked water were taken for analysis to establish the initial concentration of glyphosate and AMPA. In the oxidation tests with glyphosate spiking, the treated water samples were also analyzed for AMPA, to investigate whether any of the glyphosate was degraded only to AMPA.

For the ozonation tests, preliminary tests were carried out to find suitable settings to achieve a residual of approximately 0.2-0.4 mg O_3/L after a contact time of 15 min. A 1 L sub-sample of spiked water was ozonated using a pilot-scale O_3 generator (Labo II ozonator from Ozotech Ltd) and a bubble diffuser stone. Following ozonation, the O_3 residual was measured immediately, and at 5 min intervals, during a 15 min contact time. At the end of the contact period, the residual ozone concentration was quenched with sodium thiosulphate. A further set of tests was carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg/L of H_2O_2. The ozonation conditions were identical to the test with O_3 alone. At the end of the contact period, the residual O_3 and H_2O_2 were quenched with sodium thiosulphate as above.

For the chlorine tests, 1 L samples of the spiked water were dosed with sodium hypochlorite at 1.5 mg Cl_2/L. The dosed water was left for 30 min at the desired temperature. At the end of the contact period, the residual Cl_2 was measured and then quenched with sodium thiosulphate as above. The tests with Cl_2 were repeated, but with ClO_2 as the oxidant. The ClO_2 was added as crushed tablets (Accepta). The initial target concentration of ClO_2 was 1 mg/L.

Tests were carried out to investigate the performance of three different types of coal based PAC; Norit W35, Norit SA Super and Chemviron W. One litre samples of the spiked water were dosed with the three different PAC products at 5, 15, and 25 mg/L. The dosed water was left stirring at room temperature for 1 h to keep the PAC in suspension. The samples were then filtered through GF/C grade filter paper to remove the carbon prior to analysis for glyphosate and AMPA.

The initial results for AMPA showed large variations, even for the spiked untreated control samples. This was found to be caused by a rapid degradation of AMPA by the low concentrations of free chlorine present in the tap water used (<0.2 mg Cl_2/L). Tap water for the subsequent oxidation tests was thoroughly purged with air for 72 h to remove the free chlorine before addition of AMPA. This changed the pH from 7.5 to 8.4. The free chlorine concentration in the purged water was <0.02 mg/L. This rapid degradation of AMPA by chlorine in the control samples was not apparent for glyphosate.

The effects of UV, UV/H_2O_2, O_3, O_3/H_2O_2, and UV/ O_3/H_2O_2 were investigated in a flow through pilot reactor from ITT Wedeco, consisting of in-line H_2O_2 dosing, O_3 dosing and a UV reactor, which could be used individually or in combination. The retention time in the unit was 0.5-1 min, most of which occurred in the UV reactor which has a single low pressure, high output germicidal UV lamp (254 nm, input power to the lamp 330 W). Two tests were performed, each with the same matrix of operating conditions. The feed tank was filled with 2 m^3 of tap water and then left for a minimum of 7 days, during which the free and total chlorine residuals were monitored. Free chlorine residual declined to below the limit of detection (LOD) within 48 h. The feed tank was then spiked with glyphosate or AMPA at a target concentration of 3 µg/L and the water recirculated to ensure the compound was evenly distributed.

The concentrations of O_3, H_2O_2, Cl_2 and ClO_2 were analysed by test kits (Palintest). Samples were treated with 9-fluorenylmethyl chloroformate derivatising reagent prior to concentration by solid phase extraction. The extracts were analysed by high-performance liquid chromatography/mass spectrometry detection in negative ion electrospray with selective ion monitoring. The reported recovery up to 0.3 µg/L was 99% with a LOD of 0.006 µg/L. The results presented are for single samples.

Results and Discussion

Chlorination

In the tests carried out in this work, the free Cl_2 concentration was relatively stable over the 30 min that the experiments lasted (Table 8.2.4.2-6). The results indicate that changes in pH had little influence on the degradation of glyphosate by chlorine; 96-100% was degraded in the three samples tested at 20°C. The temperature had a larger influence on the glyphosate degradation with 71% being degraded at 5°C compared to 96% at 20°C. AMPA concentrations in samples from the glyphosate tests were all non-detectable, confirming the effective degradation of AMPA by chlorine.

Table 8.2.4.2-6: Results of chlorination tests in this work

			Free Cl_2 residual (mg L^{-1})			Final conc.		
Compound spiked	pH	Temp. (°C)	0 min	30 min	Initial conc. (µg L^{-1})	Glyph. (µg L^{-1})	AMPA (µg L^{-1})	Removal (%)
Glyphosate	6.00	20.5	1.46	1.46	2.17	0.017	<0.016	99
Glyphosate	7.66	20.5	1.38	1.13	3.17	0.141	<0.064	96
Glyphosate	8.60	20.5	1.46	1.38	2.17	0.007	<0.016	>99
Glyphosate	7.52	4.9	1.38	1.28	3.17	0.915	<0.064	71
Glyphosate	7.52	10.2	1.38	1.24	3.17	0.552	<0.064	83
AMPA	6.25	20.5	1.42	1.28	3.65	N/A	<0.016	>99
AMPA	7.08	20.5	1.46	1.31	3.65	N/A	<0.016	>99
AMPA	8.38	20.5	1.51	1.42	3.65	N/A	<0.016	>99
AMPA	8.38	6.2	1.56	1.46	3.65	N/A	<0.016	>99
AMPA	8.38	9.8	1.56	1.46	3.65	N/A	<0.016	>99

N/A = not analysed.

Chlorine dioxide

The results from the current work with ClO_2 as the oxidant are shown in Table 8.2.4.2-7. The degradation of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17 to 93%. The highest degradation was seen for the low pH samples (~pH 6) with high temperature (22°C) and high ClO_2 concentrations. The increased degradation as pH decreases could be due to changes in the speciation of glyphosate, rather than a direct influence on the oxidative potential of chlorine dioxide. Glyphosate has a second pK_a of 5.44 and the results suggest that the singly deprotonated form of glyphosate ($^{-}OOC\text{-}CH_2\text{-}NH_2^{+}\text{-}PO_3H^{-}$ or H_2L^{-}) could potentially be more readily oxidized by ClO_2 than the doubly deprotonated form ($^{-}OOC\text{-}CH_2\text{-}NH_2^{+}\text{-}PO_3^{2-}$ or HL^{2-}) that dominates between pH 5.44 and 10.13. At pH 6, the concentration of H_2L^{-} is about 30% of the total concentration of glyphosate, decreasing to about 1% at pH 7.5 and 0.1% at pH 8.5.

Low concentrations of AMPA were detected in the glyphosate test samples (1-5% of total glyphosate concentration), suggesting that AMPA was formed as a degradation product when glyphosate was oxidized by ClO_2. However, for AMPA alone, complete degradation of AMPA was seen for all conditions tested, suggesting AMPA is readily degraded by ClO_2.

Table 8.2.4.2-7: Results of chlorine dioxide tests in this work

Compound	pH	Temp. (°C)	ClO_2 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
			0 min	30 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	6.04	23	0.52	0.39	2.47	0.58	N/A	76
Glyphosate	7.96	23	0.39	0.20	2.47	1.35	N/A	45
Glyphosate	8.60	23	0.39	0.27	2.47	1.42	N/A	43
Glyphosate	8.05	5.2	1.35	1.35	2.47	1.64	N/A	34
Glyphosate	8.05	11.5	1.35	1.16	2.47	1.48	N/A	40
Glyphosate	6.05	21.1	1.23	1.03	2.17	0.16	0.097	93
Glyphosate	7.61	21.1	0.84	0.59	2.17	0.53	0.017	76
Glyphosate	8.56	21.1	1.10	1.03	2.17	0.53	0.093	76
Glyphosate	7.61	4.2	0.39	0.27	2.17	1.79	0.063	17
Glyphosate	7.61	11.6	0.91	0.84	2.17	1.16	0.039	46
AMPA	6.25	20.5	1.35	1.23	3.65	N/A	<0.016	>99
AMPA	7.08	20.5	1.03	0.39	3.65	N/A	<0.016	>99
AMPA	8.38	20.5	1.35	1.16	3.65	N/A	<0.016	>99
AMPA	8.38	6.2	1.42	1.10	3.65	N/A	<0.016	>99
AMPA	8.38	10.8	1.35	1.16	3.65	N/A	<0.016	>99

N/A = not analysed.

Ozone, UV and advanced oxidation processes (AOPs)

The ozonation treatment carried out in the current work degraded all of the glyphosate and AMPA to below the LOD after 15 min contact time (Table 8.2.4.2-8) and no temperature effect was seen. The initial O_3 concentration was similar between all of the tests and the O_3 demand increased with increasing temperature. Ozone was highly effective in degrading both glyphosate and AMPA and virtually complete degradation was achieved under the conditions tested. No AMPA was detected in any of the treated samples from the glyphosate tests.

Table 8.2.4.2-8: Results of ozonation test in this work

Compound spiked	Temp. (°C)	O_3 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
		0 min	15 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	6.7	0.76	0.48	2.76	< 0.014	N/A	>99
Glyphosate	10.8	0.76	0.44	2.76	<0.014	N/A	>99
Glyphosate	15.2	0.76	0.35	2.76	<0.014	N/A	>99
Glyphosate	6.8	0.42	0.24	2.59	<0.006	<0.016	>99
Glyphosate	11.9	0.41	0.18	2.59	<0.006	<0.016	>99
Glyphosate	15.0	0.41	0.19	2.59	<0.006	<0.016	>99
AMPA	5.1	0.51	0.16	3.65	N/A	<0.016	>99
AMPA	10.5	0.54	0.10	3.65	N/A	<0.016	>99
AMPA	13.4	0.55	0.10	3.65	N/A	<0.016	>99

N/A = not analysed.

A further set of tests was carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg/L (Table 8.2.4.2-9). The ozone concentrations quickly decreased indicating rapid breakdown of the ozone to produce hydroxyl radicals. The initial O_3 concentration was significantly lower in the presence of H_2O_2 due to the reaction between O_3 and H_2O_2 to generate hydroxyl radicals. The combination of O_3/H_2O_2 was as effective as O_3 alone in degrading glyphosate and complete degradation was achieved under the conditions tested. In the sample from the glyphosate tests with the highest H_2O_2 concentration, traces of AMPA were found at <2% of total glyphosate concentration. With the addition of H_2O_2 the degradation of AMPA seems to decrease with an increasing H_2O_2 dose, although 85% was still degraded at the highest H_2O_2 concentration. This is in line with the results from the glyphosate tests, where AMPA was detected at the highest H_2O_2 concentration.

Table 8.2.4.2-9: Results of ozonation with hydrogen peroxide at 15°C in this work

Compound spiked	H_2O_2 dose (mg L^{-1})	O_3 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
		0 min	15 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	0.5	0.46	0.04	2.76	<0.014	N/A	>99
Glyphosate	1.0	0.24	0.04	2.76	<0.014	N/A	>99
Glyphosate	0.5	0.18	0.05	2.59	<0.006	<0.016	>99
Glyphosate	1.0	0.09	0.06	2.59	<0.006	0.042	98
AMPA	0.5	0.16	0.02	3.65	N/A	0.11	97
AMPA	1.0	0.04	0.02	3.65	N/A	0.54	85

N/A = not analysed.

The use of UV, O_3 and AOPs was investigated in this work by the use of a flow through pilot reactor. The tap water used had a temperature of 22°C, pH between 7 and 7.2, alkalinity between 215 and 219 mg/L $CaCO_3$, and a UV transmittance of 96.7-96.8%. Measured concentrations of both glyphosate and AMPA were less than the target 3 µg/L (Table 8.2.4.2-10) and AMPA was present in the glyphosate stock solution. It has not been determined whether this was a result of decomposition in solution, or AMPA being present in the original glyphosate product. However, it does not impact on the quality of the results, as the test concentrations were high enough to provide reliable data, and were representative of those found in source waters.

The UV dose used in drinking water treatment is typically in the region of 40-100 mJ/cm when used for disinfection alone. Doses >1,000 mJ/cm are usually required for >50% degradation of organic micropollutants. The doses used in this work were 740 and 1,240 mJ/cm and this resulted in a degradation of 36% of the spiked glyphosate for the highest dose. The addition of 5 mg/L of H_2O_2 significantly increased the degradation of glyphosate to 88-91% using the same UV doses, while the AMPA concentration increased. This indicates that AMPA is not readily degraded by UV or UV/H_2O_2 at the conditions used.

The ozonation tests were run with 1 min contact time and confirmed the evidence of rapid degradation of glyphosate from previous tests. The AMPA concentration also decreased in the ozonation tests.

Repeating the tests in the flow through system with AMPA it was confirmed that AMPA is poorly degraded by UV and UV/H_2O_2 under the conditions tested; between 6 and 36% was removed at the doses used. The results from the ozonation tests showed lower degradation of AMPA (35-66%) than the previous results for 15 min contact time (>99%). This was due to the shorter contact time of 1 min as the degradation increased to >99% when the contact time in the flow through pilot plant was increased to 10 min. The results also confirmed the previous finding that the degradation of AMPA in the O_3/H_2O_2 system was reduced compared to the O_3 only system.

Table 8.2.4.2-10: Results of UV, O_3 and AOP tests for glyphosate and AMPA removal in this work

Compound spiked	Operating conditions	Initial conc. (µg L^{-1})	Final conc.		Removal (%)
			Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	Feed water	1.72	1.72	0.30	0
Glyphosate	UV 740 mJ cm^{-2}	1.72	1.29	0.34	25
Glyphosate	UV 1,240 mJ cm^{-2}	1.72	1.10	0.42	36
Glyphosate	UV 740 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	1.72	0.21	0.59	88
Glyphosate	UV 1,240 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	1.72	0.15	0.69	91
Glyphosate	O_3 2 mg L^{-1}	1.72	0.068	0.17	96
Glyphosate	O_3 2 mg L^{-1}, H_2O_2 2 mg L^{-1}	1.72	<0.006	0.22	99
AMPA	Feed water	2.31	N/A	2.31	0
AMPA	UV 740 mJ cm^{-2}	2.31	N/A	2.16	6
AMPA	UV 1,240 mJ cm^{-2}	2.31	N/A	1.57	32
AMPA	UV 740 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	2.31	N/A	2.13	8
AMPA	UV 1,240 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	2.31	N/A	1.18	49
AMPA	O_3 2 mg L^{-1} 1 min contact time	2.31	N/A	0.86	63
AMPA	O_3 2 mg L^{-1} 10 min contact time	2.31	N/A	<0.016	>99
	O_3 2 mg L^{-1}, H_2O_2 2 mg L^{-1}	2.31	N/A	1.50	35

N/A = not analysed.

Activated carbon

The removal of glyphosate and AMPA by PAC was investigated in this work (Table 8.2.4.2-11). Although the results are somewhat scattered, it is clear the PAC was ineffective as a removal treatment for glyphosate, even at the relatively high dose for water treatment of 25 mg/L no more than 20% was removed. This is not surprising considering the high water solubility (approximately 10 g/L) and low log K_{ow} for glyphosate. No major differences between the different PACs could be seen.

The tap water used for the PAC testing had not been thoroughly de-chlorinated, and the initial concentration of AMPA was therefore lower than expected. However, PAC removes Cl_2 and this stops the degradation of AMPA by Cl_2. This explains why the removal of AMPA seems to increase with decreasing PAC dose. The removal that actually occurs is degradation by Cl_2 and an increased PAC dose removes more Cl_2. A similar, though much less marked, effect is suggested for glyphosate. The conclusion is that the PACs investigated would not provide adequate removal of glyphosate and AMPA.

Table 8.2.4.2-11: Results of PAC tests for glyphosate removal in this work

Compound	PAC	PAC conc. (mg L^{-1})	Initial conc. ($\mu g\ L^{-1}$)	Final conc.	Removal (%)
Glyphosate	Norit W35	5.1	3.13	2.51	20
Glyphosate	Norit W35	15.2	3.13	2.76	12
Glyphosate	Norit W35	25.3	3.13	3.14	0
Glyphosate	Norit SA Super	5.2	3.13	2.86	9
Glyphosate	Norit SA Super	15.0	3.13	2.46	22
Glyphosate	Norit SA Super	25.0	3.13	3.03	3
Glyphosate	Chemviron W	5.1	3.13	2.57	18
Glyphosate	Chemviron W	15.1	3.13	2.79	11
Glyphosate	Chemviron W	25.2	3.13	2.72	13
AMPA	Norit W35	5.1	1.57[a]	1.09	31
AMPA	Norit W35	15.2	1.57[a]	1.39	12
AMPA	Norit W35	25.3	1.57[a]	2.19	0
AMPA	Norit SA Super	5.2	1.57[a]	1.92	0
AMPA	Norit SA Super	15.0	1.57[a]	2.28	0
AMPA	Norit SA Super	25.0	1.57[a]	3.23	0
AMPA	Chemviron W	5.1	1.57[a]	1.63	0
AMPA	Chemviron W	15.1	1.57[a]	1.49	5
AMPA	Chemviron W	25.2	1.57[a]	1.92	0

[a]Spiked at 3 $\mu g\ L^{-1}$.

A summary of removal efficiencies for glyphosate and AMPA (based on literature survey and studies reported in the paper) is given in Table 8.2.4.2-12.

Table 8.2.4.2-12: Summary of removal of glyphosate and AMPA

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 to 50	25 to 95
Aluminium coagulant and clarification	15 to 40	20 to 25
	Not a reliable barrier for Glyphosate and AMPA	
Iron coagulant and clarification	40 to 70	20 to 85
	Not a reliable barrier for Glyphosate and AMPA	
Slow sand filtration	The limited information suggests that significant removal can be achieved but removal is likely to be highly dependent on conditions	
Chlorination	74 to > 99	40 to >95
	Likely to provide the main barrier at most water treatment works	
Chlorine dioxide	17-93	>99
	Removal of glyphosate is variable and works best at lower pH and high temperature. Good removal of AMPA can be expected	

Table 8.2.4.2-12: Summary of removal of glyphosate and AMPA

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Ozonation	60 to >99	25 to 95
	Provides an additional barrier at works where already installed for other pesticides and micropollutants	
UV irradiation	Not effective alone at doses used in water treatment	
Advanced oxidation	O_3/H_2O_2 provides an additional barrier at works where already installed. UV/H_2O_2 show good removal of glyphosate but not AMPA UV/TiO_2 can degrade significant amounts of both compounds but irradiation times are long	
Activated carbon adsorption	10 to 90	20 to 70
	Higher removals relate to virgin GAC and are unlikely to be achieved under practical conditions. Not a reliable barrier	
Membrane filtration	>90 (NF/RO) >50 (UF)[1]	>95 (NF/RO) No information found for UF
	Membrane processes not widely used in water treatment, and unlikely to be installed solely as a barrier to pesticides	
Air stripping	Not expected to be effective based on chemical characteristics	

[1]Depending on membrane type

Conclusion

The literature review and laboratory tests showed that glyphosate and AMPA are both readily degraded or removed by a number of common treatment steps at drinking water treatment plants. Biodegradation and adsorption processes can be highly effective in degrading or removing glyphosate and AMPA in bank filtration and SSF. These processes could potentially be of importance in biologically active GAC (granular activated carbon), but the residence time is generally much shorter. Iron-based coagulants are generally more effective than Al-based coagulants in removing glyphosate and AMPA; coagulation is particularly effective if coagulant residuals are removed by filtration. Ozonation and chlorination are highly effective in degrading both glyphosate and AMPA but a decrease in temperature reduces the efficiency. Combining O_3 and H_2O_2 did not improve the degradation compared to O_3 alone; in fact a decrease was observed at high H_2O_2 concentrations. UV doses typically used for disinfection will not degrade significant amounts of either compound. Higher UV doses in combination with H_2O_2 showed good degradation of glyphosate, but not AMPA. Chlorine dioxide is effective for glyphosate and AMPA degradation at around pH 6, but the efficiency decreases with increasing pH and decreasing temperature. UV/TiO_2 treatment can degrade significant amounts of glyphosate, but the irradiation time needed is long. Ultrafiltration (UF), NF (nanofiltration) and RO (reverse osmosis) can also be effective in removing glyphosate and AMPA, but the cut-off for UF needs careful consideration. Activated carbon is not likely to provide a practical removal option for either compound.

Assessment and conclusion by applicant:

The article describes different methods used in drinking water treatment plants with regard to the degradation of glyphosate and AMPA, and presents a useful summary of removal efficiencies for glyphosate and AMPA.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on water treatment efficiencies for glyphosate and/or AMPA removal. Data from this study are included in the review provided in [redacted], 2020.

Batch tests were carried out to investigate the degradation of glyphosate and AMPA by oxidation using Cl_2, ClO_2, O_3,O_3/H_2O_2, and by adsorption using PAC (powdered activated carbon). Different test conditions (concentration, time of contact) are tested. Results are clearly reported. Removal efficiencies up to 99% under certain described conditions are reported.

For ozonation, a 15-minute treatment period was enough to result in removal rates of >99% for both glyphosate and AMPA under all the tested experimental conditions. No effect of temperature was found on the removal rate (always>99% with temperature form 6°C to 15°C) but the O_3 demand increased with temperature.

For chlorination: chlorine dioxide is effective for glyphosate degradation at around pH 6, but the efficiency decreases with increasing pH and decreasing temperature. The highest degradation (removal of 93%) was seen for the low pH samples (~pH 6) with high temperature (22°C) and high ClO_2 concentrations. For AMPA, removal rate was >99% whatever temperature and pH conditions.

UV and UV H_2O_2 treatment can degrade significant amounts of glyphosate (25% without H_2O_2 to 91% with H_2O_2), but the irradiation time needed is long. AMPA is poorly degraded by UV and UV/H_2O_2 under the conditions tested; between 6 and 49% was removed at the UV doses used. It is worth noting that it was indicated in Jönsson *et al.*, 2013, CA 7.5/084 that the doses of UV used in the experiments (740 and 1,240 mJ/cm²) were all higher than those usually used for disinfection of drinking water treatment (usually 40-100 mJ/cm²).

Furthermore, a review of water treatment removal and degradation by bank filtration, chemical coagulation, clarification/filtration, slow sand filtration, chlorination, degradation of glyphosate by chlorine dioxide, ozone, UV, AOPs, activated carbon, pressure driven membrane process and air stripping was performed. However, no sufficiently detailed information are reported.

Malaguerra et al., 2013

Data point:	CA 7.5/085
Report author	Malaguerra, F. *et al.*
Report year	2013
Report title	Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques
Document No	Journal of Hydrology 476 (2013) 321–331
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Not relevant

A reactive transport model is employed to evaluate the potential for contamination of drinking water wells by surface water pollution. The model considers various geologic settings, includes sorption and degradation processes and is tested by comparison with data from a tracer experiment where fluorescein dye injected in a river is monitored at nearby drinking water wells. Three compounds were considered: an older pesticide MCPP (Mecoprop) which is mobile and relatively persistent, glyphosate (Roundup), a newer biodegradable and strongly sorbing pesticide, and its degradation product AMPA. Global sensitivity analysis using the Morris method is employed to identify the dominant model parameters. Results show that the characteristics of clay aquitards (degree of fracturing and thickness), pollutant properties and well depths are crucial factors when evaluating the risk of drinking water well

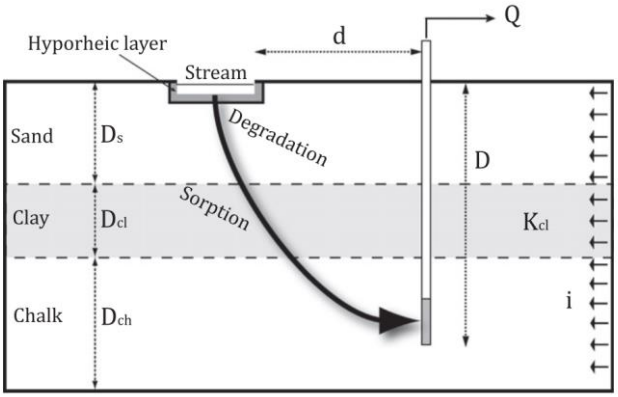
contamination from surface water. This study suggests that it is unlikely that glyphosate in streams can pose a threat to drinking water wells, while MCPP in surface water can represent a risk: MCPP concentration at the drinking water well can be up to 7% of surface water concentration in confined aquifers and up to 10% in unconfined aquifers. Thus, the presence of confining clay aquitards may not prevent contamination of drinking water wells by persistent compounds in surface water. Results are consistent with data on pesticide occurrence in Denmark where pesticides are found at higher concentrations at shallow depths and close to streams.

Materials & Methods

Conceptual model

In order to study the link between surface water and a nearby drinking water well, a generic model of contaminant transport from surface water into groundwater is established. The model is designed to quantify the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer. The conceptual model is illustrated in Figure 8.2.4.2-3. A pumping well is placed at a distance *d* (m) from a stream and pumps water at a constant pumping rate *Q* (m^3/d) from a depth *D* (m). The geology is simplified to be a 3-layer system: a hyporheic layer separates the stream from an underlying sandy aquifer, below which a clay aquitard overlies a chalk aquifer; *Ds*, *Dcl* and *Dch*, respectively, are the thicknesses of the three layers, and *Kcl* is the hydraulic conductivity of the fractured clay till. Clayey glacial tills are very wide- spread in the northern hemisphere, especially at higher latitudes, and represent a frequent aquiclude in countries such as Denmark, Canada or the United States. Thus, the configuration considered in the conceptual model is applicable to a wide range of aquifers. The natural flow in the aquifer is driven by a regional groundwater gradient *i* (m/m) and to simplify the system, the hydraulic gradient is assumed to be the same in both aquifers. During pumping the well modifies the natural water flow, lowering the water head in the aquifer, so that surface water from the stream can seep into the groundwater and reach the pumping well. Pollutants in the stream may be retarded by sorption and degraded by microorganisms during their travel to the well. Both the sandy and chalk aquifer are considered to be strictly anaerobic, while the hyporheic zone can be aerobic.

Figure 8.2.4.2-3: Conceptual model of the system considered



Model formulation

At steady state, the groundwater flow equation can be written as:

$$\nabla \cdot \mathbf{K} \cdot \nabla H - W = 0 \qquad (1)$$

where K is the hydraulic conductivity tensor, *H* is the hydraulic head and *W* is the sink term for water withdrawal. The fate of an aqueous component in groundwater is influenced by advection, mechanical dispersion and diffusion, as well as sources/sinks and geochemical reactions and can be described by the advection– diffusion equation:

$$\left(1+\frac{\rho_b}{n}K_d\right)\frac{\partial C}{\partial t}+\nabla\cdot(\mathbf{v}C)-\nabla\cdot(\mathbf{D}\nabla C)=-kC \qquad (2)$$

Where *qb* is the bulk density, *n* is the soil porosity, *Kd* is the sorption coefficient, v is the pore water velocity, D is the dispersion tensor and *k* is the degradation rate. Degradation kinetics are assumed to follow a first-order rate with half lives for aerobic and for anaerobic conditions. Despite the fact that for many pollutants sorption is often better described by non-linear isotherms, linear sorption isotherms were considered when calculating the retardation factor, because low concentrations are expected and computations are simplified by avoiding sorption related concentration shock fronts and rarefactions. Such a simplification is a common assumption in reactive transport modeling. The model is set up and solved using COMSOL Multiphysics, a finite-element modeling package for solving partial differential equations. The groundwater flow model is solved at steady state, subsequently the transport model is solved transiently, until the concentration of contaminant at the well reach the steady state.

Pesticides considered

Two pesticides and a pesticide metabolite were considered: an older pesticide MCPP (Mecoprop) which is mobile and persistent under anaerobic conditions, glyphosate (Roundup), a newer, readily degradable and strongly sorbing pesticide, and AMPA, which is a more mobile, less degradable glyphosate degradation product, which can therefore accumulate in groundwater. All three compounds have been regularly found in Danish drinking water wells. The three pesticides are known to be more quickly degraded under aerobic conditions than in anaerobic environments. Monitoring in agricultural streams reveals a high occurrence of MCPP in surface waters with detection rates as high as 78% in some catchments. Glyphosate and AMPA concentrations in streams and groundwater are not always measured because of the cumbersome analytical procedure. However, glyphosate is the most sold chemical used for weed control in agricultural, silvicultural and urban environments, is likely to be found in surface waters, and despite its high degradability under aerobic conditions, it can pose a threat to groundwater.

Model application to a tracer experiment

In order to test the ability of the model to simulate solute transport from surface water to nearby wells, data from a tracer experiment performed in 2002 on the river Aare, Switzerland (Wanner and Grunner, 2002) were modeled. The experiment investigated the vulnerability of drinking water wells in the riparian zone to river water contamination. A 10 m-thick highly permeable aquifer is pumped by two horizontal wells (ZPW1 and ZPW3) located at around 100 m from the river shore, and one vertical well (VB) located at approximately 50 m from the river shore. In 2002, the two horizontal wells were pumped at 7 and 9.5 m^3/ min respectively while the vertical well had a pumping rate of 2 m^3/min. A pumping test performed in the vertical well revealed a hydraulic conductivity of 5.5e-3 m/s.

A tracer pulse (fluorescein) was injected in the river near the study area, far enough upstream to ensure a good mixing of the tracer in the river close to the sampling area. The maximum river velocity was 1.27 m/s and the duration of the tracer concentration peak was about 2 h. Tracer concentrations at the pumping wells were measured every 2-4 h using in situ fluorometers. No aquitard was present between the river and the drinking water well, and so the geology of the experimental site was different from the geology considered in the conceptual model (Figure 8.2.4.2-3). A tracer experiment for the geology setting presented in Figure 8.2.4.2-3 would be very difficult to perform, because conventional tracer breakthrough test are difficult to perform for long travel times. Since a confined aquifer tracer test is not available in the literature, the unconfined tracer test is used instead to validate the conceptual model employed in this paper. It includes the main processes simulated by the model, with the exception of the low permeability aquitard. A three-dimensional model of the aquifer was developed: the size of the model domain was 2 by 1 km and the aquifer had a constant thickness of 10 m. The hyporheic zone was modeled as a 2-m thick layer under the stream. Two different isotropic hydraulic conductivities were assigned to the hyporheic zone and the highly permeable aquifer. A fixed head boundary condition was assigned to the river with the head being determined by the topography which had a slope of about 0.1%. No flow boundary conditions were assigned to the southern boundary since previous studies found water flow streamlines to be mostly in the East–West direction. A fixed hydraulic gradient parallel to the river at the downstream (west) boundary was considered.

The model was calibrated using the tracer breakthrough curves obtained at two pumping wells for five parameters: hydraulic conductivity of the hyporheic zone, hydraulic conductivity of the highly permeable aquifer, longitudinal and transverse dispersivity and hydraulic gradient parallel to the river at the downstream boundary. A Shuffled Complex Evolution Metropolis algorithm (SCEM) was used to determine optimal parameters and confidence intervals.

Sensitivity analysis

The aim of the sensitivity analysis was to determine which parameters most affect the risk of contamination of drinking water wells from pollutants in nearby streams for the conceptual model presented in Section *Conceptual model*. The sensitivity analysis is a generic study of groundwater/surface water interaction and is not restricted to the case study presented in the previous section. Moreover, sensitivity analysis provides information on how parameters influence the seepage of pollutants from the stream to the pumping well. Sensitivity analysis is often considered as a local measure of the effect of a given input on a given output, such as a simple or normalized derivative. Nevertheless local sensitivity relies on point measures, which can be inappropriate to describe the behavior of a model over the whole input parameter space. Here, a global sensitivity analysis (GSA) tool was used to analyze the model over the full extent of the model space.

Many global sensitivity analysis methods are available, most of- ten based on Monte Carlo methods in conjunction with a variety of sampling strategy and sensitivity measures. Since the finite-element solution is computationally expensive, the sensitivity analysis was performed using the Morris method, which belongs to the group of the derivative-based global sensitivity measures (DGSMs) and produces qualitative results with limited computational effort. The Morris method aims to determine the factors leading to negligible, linear and additive, or non-linear effects, and parameter interactions. The method is based on *elementary effects*, which are attributed to each input. For a model with *k* parameters, the parameter space Ω will be the k-dimensional hypercube with $x_i \in [x_{i_{min}}, x_{i_{max}}]$ for $i \cdots k$, where $x_{i_{min}}$ and $x_{i_{max}}$ are the minimum and maximum values of the a priori distribution for each parameter. In order to observe the model response in several places of the model spaces, a region of experimentation x included in X is constructed as a regular k-dimensional p-level grid, p being a fixed scalar representing the refinement of the grid. Each *xi* may only take on values from $\{x_{i_{min}}, x_{i_{min}} + \Delta, x_{i_{min}} + 2\Delta, \ldots, x_{i_{max}}\}$,

where Δ is a multiple of 1/(1 - *p*). For a given value of x, the elementary effect of the input factor i is defined as:

$$d_i(x) = \frac{[y(x_1, x_2, \ldots, x_{i-1}, x_i + \Delta, x_{i+1}, \ldots, x_k) - y(x)]}{\Delta} \qquad (3)$$

The finite distribution of elementary effects associated with the *i*th input factor, named *Fi*, is obtained by randomly sampling different x from Ω. The mean (μ) and the standard deviation (σ) of *Fi* are the most informative sensitivity measures. A high value of μ implies that the factor has a large effect on the output, while a high value of σ means that the elementary effects relative to this factor are significantly different from each other, i.e. the value of an elementary effect is strongly affected by the values taken by the other parameters. Campolongo et al. (2007) proposed that the distribution of the absolute values of the elementary effects, *Gi*, and its mean μ* should also be considered. In fact, if the distribution *Fi* contains negative values some effects may cancel each other when computing the mean, leading to an underestimation of their effect.

The percentage of pollutant in the stream that reached the well at the end of the simulation was chosen as model output considered for sensitivity analysis. Note that the ratio of concentrations at the pumping well to surface water concentrations are independent of initial concentrations since only first order degradation and linear sorption are considered.

Parameter sensitivity was studied on a standard three-dimensional model domain consisting of a 5-m wide stream surrounded by a 1-m thick hyporheic layer, placed in the middle of a 1 km by 1 km area. The abstraction well was modeled as a vertical well with a diameter of 150 mm and a screen length of 6 m (representative of a typical Danish drinking water well). Fixed head boundary conditions were set to vertical boundaries parallel to the stream, while no flux boundary conditions were chosen for the vertical boundaries perpendicular to the stream and for the bottom of the lower horizontal layer.

The effect of the domain size and the distance of boundaries on the stream solute seepage was investigated by performing several simulations with variable geometry of the model domain. Decreasing the distance between the two fixed head boundaries resulted in lower steady-state pollutant concentrations at the pumping well, since more uncontaminated water was coming from the fixed head boundaries. When wells neared the two no-flow boundaries, contaminant concentrations were higher since more water entered the model domain from the stream to compensate for the lower lateral water flow. The 1 km by 1 km domain chosen was the smallest domain ensuring negligible effect of boundaries on contaminant transport from the stream to the pumping well.

An optimal sensitivity analysis should investigate all model parameters, however, due to computational constraints, some parameters were kept fixed to reduce the number of model evaluations needed to obtain results. Values for sand and chalk horizontal saturated hydraulic conductivity were 8.64 and 5 (m/d) respectively, while a lower value (1 (m/d)) was assigned the horizontal hydraulic conductivity of the hyporheic zone. For each layer, the vertical hydraulic conductivity was assigned to be one tenth of the horizontal values. We choose relatively high values for longitudinal and transverse dispersivities (4 m and 0.4 m) since travel distances and water velocity were both high, and to decrease simulation times. Recharge was assumed to be 150 mm/yr, a typical value for a Danish groundwater.

To facilitate the computation of solutions in a reasonable amount of time, sorption coefficients and first-order degradation rates were kept constant. Half lives considered for aerobic conditions were higher than values found in the literature because the model assumed that the hyporheic layer has a thickness of 1 m while oxygen is usually depleted at depths of 5–40 cm. Thus, using low half lives for aerobic conditions in the whole hyporheic layer would overestimate the pesticide degradation. The fixed thickness of the hyporheic layer of 1 m was necessary to avoid model failures caused by a lack of available memory. The parameter space of the inputs is summarized in Table 8.2.4.2-13. Statistics from the Danish National Boreholes Database were used to identify the most representative values for Danish drinking water wells. Empirical cumulative distribution functions of 21,837 wells were employed to obtain intervals representative of 95% of the drinking wells in Denmark. Clay hydraulic conductivity values are typical for Danish clay tills, and are relatively high in order to consider the permeability increase due to clay till fracturing. Each model run simulated the system for 30 years, which corresponds to the time to remove 99% of the least degradable compound. When constructing the random model domain, the pumping well screen was always assumed extracting water below the clay layer.

Table 8.2.4.2-13: Parameters intervals used for sensitivity analysis

Parameter	Symbol	Unit	Range
Sand aquifer thickness	D_s	m	1–30
Clay layer thickness	D_{cl}	m	0–30
Chalk aquifer thickness	D_{ch}	m	1–100
Distance from the stream	d	m	3–150
Well depth	D	m	8–100
Aerobic hypoheric zone	O_2	–	Yes/no
Abstraction rate	Q	m^3/h	1–100
Natural hydraulic gradient	i	m/m	−1% to +1%
Clay hydraulic conductivity	K_{cl}	m/s	3e−7–1e−8

Results

Tracer experiment

It was not possible to find parameter values able to fit the breakthrough curves of tracer concentration at the two wells (VB and ZPW1) at the same time. Possibly assuming a uniform, isotropic aquifer is an extreme simplification of the system. However, the purpose of using the tracer experiment data was to test if the model can correctly simulate the transport of solutes from the river to a single pumping well, and not to create a reliable groundwater model of the data. Moreover, the measurements available did not justify a more complex model: the inclusion additional parameters would have led to an over-parametrized model.

Satisfactory results were obtained when considering breakthrough curves of one well at a time. Figure 8.2.4.2-4 shows the calibrated breakthrough curve of fluorescein concentrations at the two wells

with two optimized parameters sets. Both arrival times and peak concentrations were simulated correctly for the model calibrated with the vertical well (VB) dataset. The model calibrated with the horizontal well (ZPW3) dataset showed a poorer fit to experimental data, but it still could approximate the peak concentration time and the breakthrough mass (difference between observed and simulated breakthrough mass was about 2%). Parameters values obtained during the two calibrations are presented in Table 8.2.4.2-14. The calibration on the vertical well indicated a value of 5.45e-3 m/s for the hydraulic conductivity in the sand layer, which is extremely close to the value of 5.5e-3 m/s measured during the field pumping test.

Figure 8.2.4.2-4: Calibrated tracer breakthrough curves at the vertical well VB (A) and at the horizontal well ZPW3 (B)

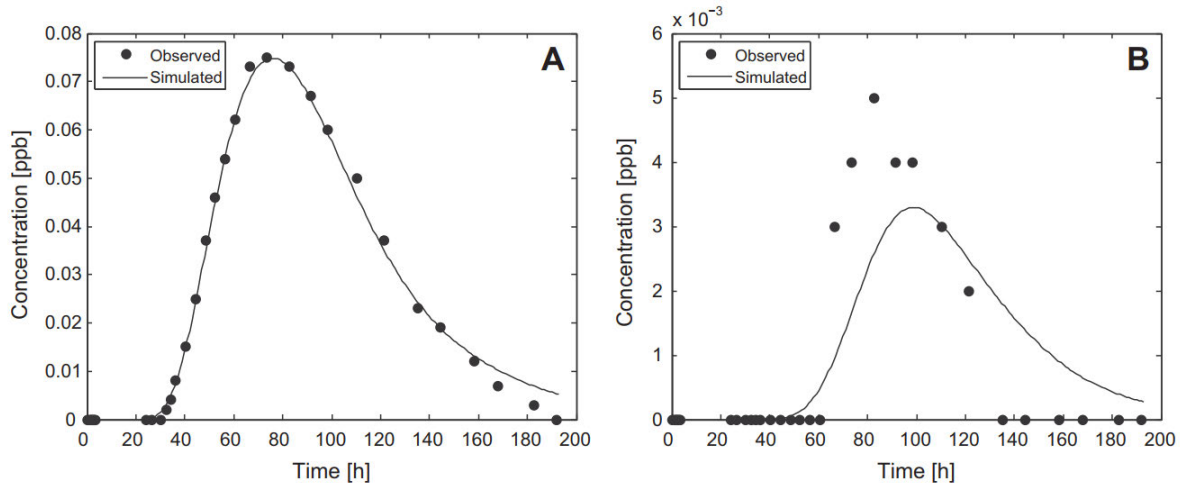


Table 8.2.4.2-14: Calibrated parameters for the tracer experiment and 95% confidence intervals

Parameter	Symbol	Unit	Best fit VB	CI 95%	Best fit ZPW1	CI 95%
Sand hydraulic conductivity	K_s	m/s	5.45e−3	5.23e−3–7.93e−3	7.61e−3	5.99e−3–9.89e−3
Hyporheic zone hydraulic conductivity	K_{hz}	m/s	6.68e−3	6.50e−3–7.78e−3	7.11e−3	5.87e−3–7.91e−3
Longitudinal dispersivity	α_l	*m*	5.18	5.17–5.32	7.24	6.35–7.89
Transverse dispersivity	α_t	*m*	0.286	0.285–0.291	0.272	0.253–0.308
Gradient at the downstream boundary	*i*	–	0.015	0.012–0.051	0.037	0.028–0.043

Sensitivity analysis

The model space was screened through 165 parameter paths (each one consisting in 10 model runs). Results of sensitivity analysis are presented in Table 8.2.4.2-15, where the parameters are ranked according to the absolute value of the mean of elementary effects. A more intuitive way to show the sensitivity analysis result is given by σ - μ andn σ - μ* plots as suggested by Morris (1991). For MCPP concentrations at the drinking water well, the hydraulic conductivity of the clay layer (*Kscl*) is the most influential parameter followed by the well depth (*D*), the thickness of clay layer (*dcl*) and the abstraction rate (*Q*). The mean value μ shows whether an increase of a parameter will induce a higher (μ > 0) or lower (μ < 0) contamination to the drinking water well. Results show that a more permeable clay layer (*Kscl* > 4e-8 (m/s)) or an higher abstraction rate (*Q* > 20 (m^3/h)) will lead to higher concentrations at the drinking water well, while deeper wells, longer distance between the stream and the well, and thicker geologic layers will result in lower pollution.

For most of the parameters, values of μ* are very close to the values of |μ|, which means that the parameters are always acting in the same 'direction', i.e. always influencing negatively or always positively the pollutant concentration at the drinking well, and there are no elementary effects that eliminate each other. However, this is not the case for parameter *i*, the regional hydraulic gradient. A given increase or decrease of the regional hydraulic gradient can lead to different consequences depending on the values of other parameters. In order to explain this behavior, we performed random simulations while looking for dependencies between couples of inputs and pesticide concentrations at the drinking water well. Figure 8.2.4.2-6 shows the relationship between well depth, regional hydraulic gradient, and MCPP concentrations at the drinking water well: each circle represents a random model

simulation and the black dots indicate a simulation for which the MCPP concentration exceeded 0.2% of the stream contamination. As can be seen, the black dots are located in a well defined triangular zone. Shallow wells can be contaminated with every regional gradient value, while contamination of deep wells only occurs if the regional hydraulic gradient is close to zero. This is probably due to the fact that for steep hydraulic gradients, the contaminant plume from the stream does not intercept the drinking water well. No interaction between the regional hydraulic gradient and the distance between the well and the stream was found, suggesting that the vertical profile is much more influential for the contaminant transport than the horizontal location of the well. Graphs representing the results for MCPP concentration at the drinking water well are presented in Figure 8.2.4.2-5, but similar graphs can be obtained for every model output. Results show that elementary effects for glyphosate and AMPA concentrations at the drinking water wells are very small. Because only very small concentrations of glyphosate and AMPA arrive at the drinking water well (see Figure 8.2.4.2-7), absolute changes in pesticide concentrations are very small and consequently Eq. (3) will produce very small elementary effects. Percentages of stream concentrations reaching the drinking water well as a function of the values of most important parameters for the three pesticides considered are shown in Figure 8.2.4.2-7. MCPP concentrations seem to be positively correlated to clay hydraulic conductivities values (Spearman q = 0.14, $p < 0.001$, Figure 8.2.4.2-7A) and negatively correlated to the drinking water well depth (Spearman q = -0.53, $p < 0.001$, Figure 8.2.4.2-7B). Results also show that MCPP steady state concentrations at the water well can be up to 7% of stream concentrations, if the well is shallow and the clay hydraulic conductivity is high. The relationship between thickness of the clay aquitard and maximum MCPP concentrations at the drinking well was also investigated. Results also indicate that the well is protected against MCPP leaching from the stream (concentrations in the water wells below 0.01% of stream water concentration) when clay layer thicknesses are greater than 20 m, for all values of clay hydraulic conductivity and well depth within the considered range.

Maximum concentrations in the drinking water well for glyphosate and AMPA are much lower than for MCPP: only up to 0.025% of glyphosate stream concentration can be found at the drinking water well (Figure 8.2.4.2-7C) and maximum AMPA concentrations are about 0.0024% of glyphosate stream concentrations (Figure 8.2.4.2-7E). Nevertheless, despite very low concentration at the drinking water well, trends between glyphosate and AMPA findings and well depth are found (Spearman q well depth - glyphosate concentrations = -0.44, $p < 0.001$, Spearman q well depth - AMPA concentrations = -0.32, $p < 0.001$) and can be highlighted by plotting concentrations on a logarithmic scale (Figure 8.2.4.2-7D and F). Lower glyphosate and AMPA concentrations are found in deep wells.

A separate sensitivity analysis was performed on a model considering only unconfined aquifers. The overall method was identical to the one used for confined pumping wells, but the geometry of the model was changed in order to have contact between the sand layer and the chalk layer. Results of sensitivity analysis indicated the depth of the well D as most influential parameter, followed by the natural hydraulic gradient i, the distance between the stream and the well d, and the thicknesses of the sand and clay layers. Maximum concentrations in the pumping well increased up to 10% of MCPP and 0.12% of glyphosate stream concentrations. Up to 0.043% of stream glyphosate could be found in the well as AMPA. Dependence of MCPP concentrations on well depth is more evident than for confined aquifers (Figure 8.2.4.2-8A); moreover, shallow wells are often contaminated with MCPP concentrations above 1% of stream concentrations. The distance between the well and the stream, which was determined to be an insensitive parameter for confined wells, seems to play a more important role in unconfined aquifers: wells close to the stream are more likely to be contaminated than wells placed far from the stream (Figure 8.2.4.2-8B).

Table 8.2.4.2-15: Parameter ranking according to the Morris screening. Parameters are ranked according to the mean of elementary effects absolute values μ

Rank	MCPP			Glyphosate			AMPA		
	Parameter	μ^*	σ	Parameter	μ^*	σ	Parameter	μ^*	σ
1	Ks_{cl}	2.871	7.828	K_{cl}	0.050	0.295	D_s	0.010	0.094
2	D	0.263	0.570	D_s	0.007	0.049	K_{cl}	0.005	0.027
3	D_{cl}	0.185	0.419	D	0.004	0.019	D	0.001	0.004
4	Q	0.178	0.397	Q	0.004	0.021	Q	0	0.004
5	D_{ch}	0.158	0.355	i	0.003	0.025	D_{cl}	0	0.002
6	D_s	0.131	0.255	D_{cl}	0.001	0.005	i	0	0.001
7	i	0.125	0.373	D_{ch}	0.001	0.004	O_2	0	0.001
8	d	0.073	0.195	O_2	0.001	0.005	D_{ch}	0	0
9	O_2	0.001	0.004	d	0.001	0.002	d	0	0

Figure 8.2.4.2-5: Results of sensitivity analysis for MCPP concentrations: the standard deviation (r) of the elementary effects is plotted against their mean l (A) and mean of absolute values μ (B).

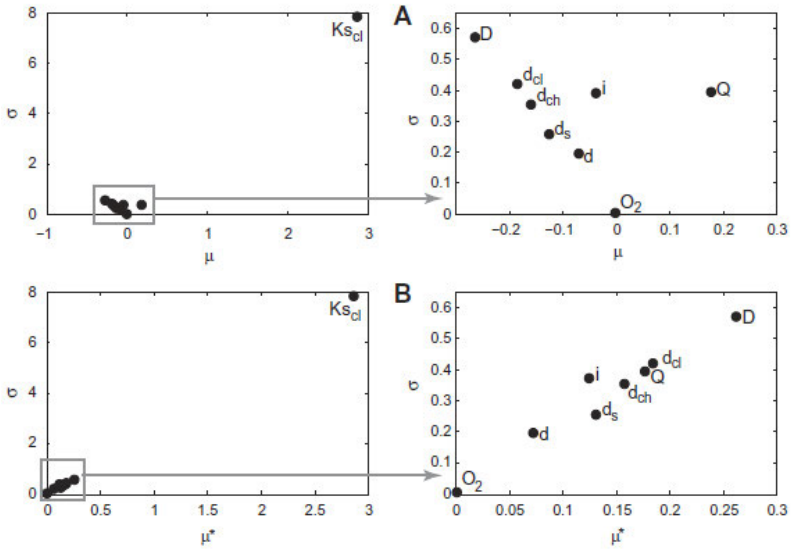
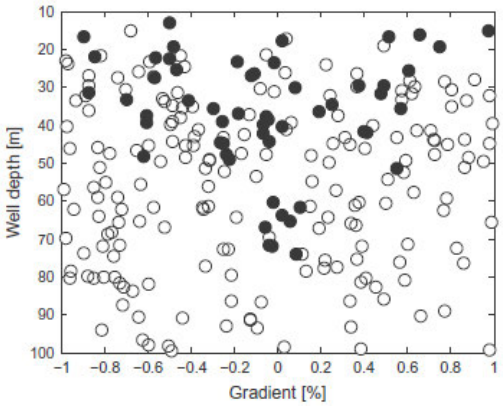


Figure 8.2.4.2-6: Relationship between well depth, natural hydraulic gradient and MCPP well contamination. In White the sampled points, in black, the point for which MCPP concentration at the drinking water well is higher than 0.2% of the stream concentration.



Discussion

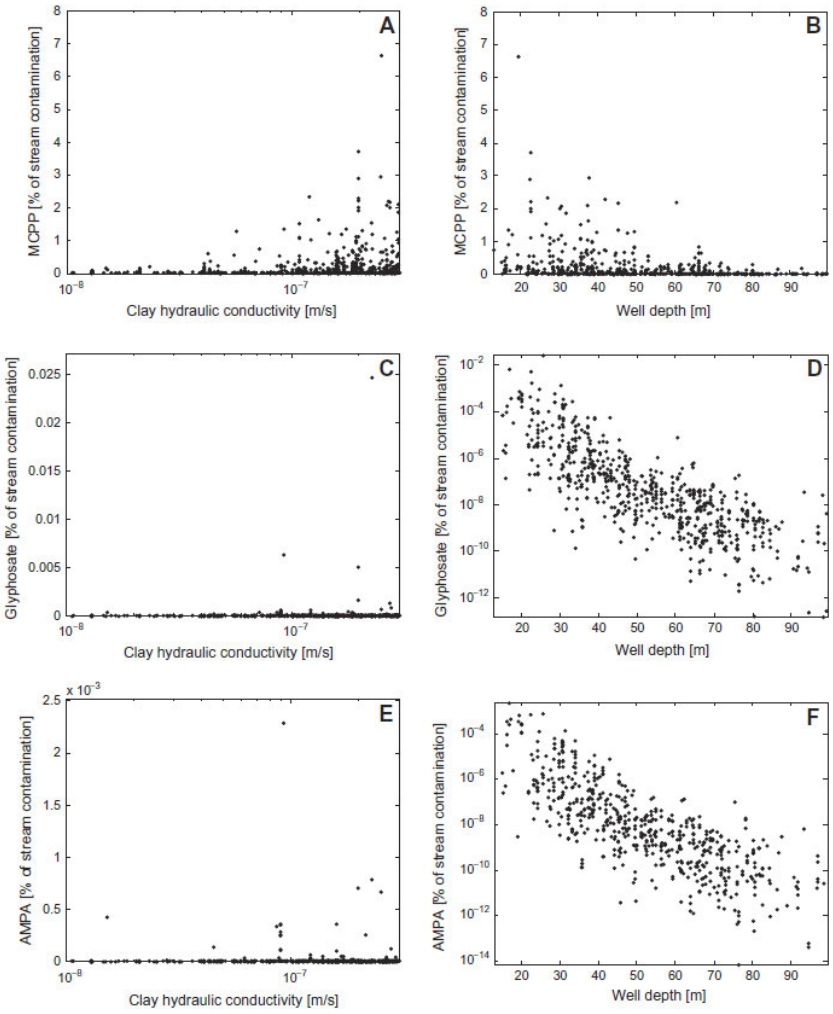
Sensitivity analysis

Steady state concentrations at the drinking water wells vary greatly depending on pesticide properties. Highly sorbable, readily degradable compounds like glyphosate and AMPA, reach the wells at very low concentrations due to transformation and dilution processes. The arrival time of such compounds at the drinking water well is delayed because their high sorption coefficients, and so bacteria have more time to degrade the pollutants. In contrast, persistent, mobile pesticides such as MCPP can travel faster from the stream water to the well and higher pollutants concentrations can be found in the drinking water. Results also indicate that the clay aquitard characteristics are the most important parameters controlling

infiltration of pollutants from surface water to drinking water wells. If fractures are present in the clay or if the clay layer is thin, pumping wells can be at risk of contamination, independently of the distance between the stream and the pumping well. In the absence of a clay aquitard, contamination from stream pollution is more likely to occur since sandy sediments are directly in contact with the chalk layer. In this case, the distance between the stream and the pumping well matters, and closer wells are more hydraulically connected to the stream. Sensitivity analysis indicated that deeper wells are less subject to contamination because water travel times are increased and dilution is more effective. However, deep wells are more expensive to drill and require higher operational costs, thus, the majority of drinking water wells (especially those privately owned) are relatively shallow.

The natural hydraulic gradient can also play a major role in contaminant transport from surface water to nearby wells, especially in case of pumping from unconfined aquifers. Depending on the natural hydraulic gradient, the capture zone of the pumping well intersects the stream and surface water can be transported to the drinking water well. The greater the hydraulic gradient; the more elongated the capture zone and so wells have to be shallow to intercept water coming from the stream. On the other hand, if the hydraulic gradient is very low, the capture zone extends vertically and stream water can travel into deep wells.

Figure 8.2.4.2-7: Percentages of stream pesticide concentrations leaching into the well plotted against the most sensitive parameters. Note the logarithmic *y*-axis in plots D and F.



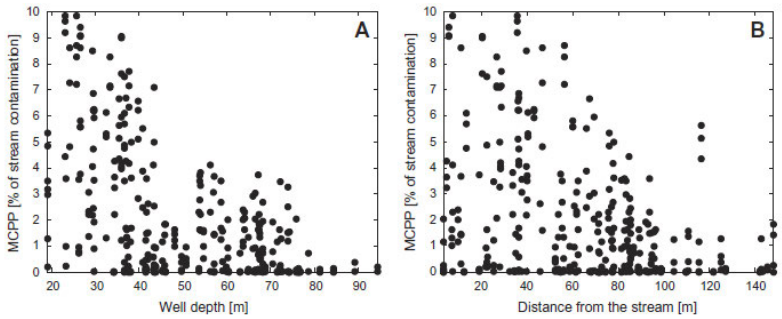
In natural systems, the hydraulic gradient can vary with time and thus increase the contaminant spread. Data on MCPP concentration in drinking water wells from the Danish National Boreholes Database show that both the occurrence and concentrations of MCPP is related to well depth. Thus, a similar trend has been found between simulation results and real data. The comparison also highlights that in both

field and simulated data MCPP concentrations are higher for wells shallower than about 50 m. Sensitivity analysis indicated that the hydraulic conductivity of the clay layer is the most important parameter affecting MCPP leakage into pumping wells (Table 8.2.4.2-15), but this relationship could not be verified by the real dataset, because parameters such as the degree of fracturing or the hydraulic conductivity of the clay layer are not included into the Danish borehole database. Real data also shows a relationship between MCPP concentrations measured in drinking water and the distance between the stream and the well. This relationship is not seen in the model results possibly because the streams are placed in valleys, where surface runoff water from cultivated land will flow to riparian zones during rain events, enabling more contaminated water to infiltrate and reach groundwater. Thus, higher pesticide concentration will be found in wells close to surface water. Moreover, near surface water the water table is usually shallower, and the unsaturated aerobic zone is relatively thin or inexistent. Anaerobic conditions will then be predominant and MCPP degradation does not occur.

Implications for drinking water quality

Simulation results indicate that up to 7% of stream MCPP pollution can reach the drinking water well in confined aquifers. Thus, the European Union drinking water limit for pesticides of 0.1 µg/L can be exceeded for stream MCPP concentrations above 1.5 µg/L. In the case of unconfined aquifers, when up to 10% of stream MCPP can potentially reach the drinking water well, MCPP stream concentrations of 1 µg/L are enough to threaten drinking water quality. Such concentrations are common in agricultural streams, where non-sorbable, relatively persistent pesticides like MCPP, bentazone and dichlorprop have been regularly found with monthly average concentrations above 2 µg/L. Although pesticides are among the most frequently detected micropollutants in surface waters, emerging contaminants such as pharmaceutical residues and other household residues are gradually becoming a serious issue. Such compounds can affect human health even when present in very small concentrations. Moreover, some of these compounds are mobile and poorly degradable. Studies have shown that some of these substances can reach drinking water wells during bank filtration, when the connection between surface water and pumping well is desired end evident. Our work showed that it is likely that this will occur in confined aquifers too.

Figure 8.2.4.2-8: Results of MCPP concentrations plotted versus well depth (A) and distance between the stream and the pumping well (B) in unconfined aquifers.



Model limitations

In the model considered here, constant concentrations over a long period of time are considered. In reality, high variability in pesticide stream concentrations are often observed, since pesticides are applied for a specific period of the year, and because most of pesticides fluxes are linked to soil flushing during rain events. In contrast, pesticides originating from landfill leachate plumes are more likely to have a constant effect on surface water if landfills are placed near streams or creeks. The influence of other parameters influencing the fate of pesticides in groundwater such as recharge, dispersion or degradation rates, where not assessed in the sensitivity analysis. Only parameters related to basic well geometry were considered because they are more easily linked to the available information on drinking water wells. Degradation rates values used in this study were close to the lower range of literature values and may lead to an overestimation of contaminant concentrations. On the other hand, the model does not consider the presence of preferential flow paths, which are known to play a major role in contaminant transport and can potentially lead to an underestimation of pesticides concentrations at the drinking

water well. The relative low complexity level of the model considered in this study makes it possible to consider the problem in a general way, neglecting site-specific conditions. More complex models would be physically more accurate, but more computationally expensive and include a higher number of parameters.

Conclusion

Results of the global sensitivity analysis showed that the characteristics of the clay aquitard (hydraulic conductivity and thickness) and well depth are the parameters governing the risk of contamination of drinking water wells by pollution in streams. Results also show that although it is unlikely that glyphosate in streams can pose a threat to drinking water wells, MCPP in surface water can pose a serious risk when pumping in confined and unconfined aquifers. Thus, the presence of confining clay aquitards may not prevent contamination of drinking water wells by persistent compounds in surface water. Comparison between simulation results and pesticides concentration data from the Danish National Boreholes Database showed a similar trend of decreasing MCPP concentrations with well depth. Real data also showed that wells located close to streams are more vulnerable to MCPP contamination, a result not simulated by the model. Some aspects of this study were limited due to computational constraints; therefore efforts should be made in future to enhance the model efficiency. Overall findings suggest that contamination of drinking water wells by pesticides in surface water is possible and may be a serious problem, especially for mobile and persistent compounds.

Assessment and conclusion by applicant:

The article reflects a computation model simulation for the contamination of drinking water wells with glyphosate and AMPA via filtration from surface waters. Generalized soil parameters were considered that reflect European agricultural soil characteristics. The derived results represents modelling results, no measured values.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

The study is not considered relevant to provide information on the impact of water treatment on glyphosate or AMPA. This study describes the construction of a generic model of contaminant transport from surface water into groundwater in order to study the link between surface water and a nearby drinking water well. The model is designed to quantify the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer. There is no usuable measured data.

Ruel et al., 2012

Data point:	CA 7.5/086
Report author	Ruel, S.M. et al.
Report year	2012
Report title	Occurrence and fate of relevant substances in wastewater treatment plants regarding Water Framework Directive and future legislations
Document No	Water Science & Technology/65.7/2012
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

The next challenge of wastewater treatment is to reliably remove micropollutants at the microgram per litre range. During the present work more than 100 substances were analyzed through on-site mass balances over 19 municipal wastewater treatment lines. The most relevant substances according to their occurrence in raw wastewater, in treated wastewater and in sludge were identified, and their fate in wastewater treatment processes was assessed. About half of priority substances of Water Framework Directive (WFD) were found at concentrations higher than 0.1 µg/L in wastewater. For 26 substances, potential non-compliance with Environmental Quality Standard (EQS) of Water Framework Directive has been identified in treated wastewater, depending on river flow. Main concerns are for Cd, DEHP, diuron, alkylphenols, and chloroform. Emerging substances of particular concern are by-products, organic chemicals (e.g. triclosan, benzothiazole) and pharmaceuticals (e.g. ketoprofen, diclofenac, sulfamethoxazole, carbamazepine). About 80% of the load of micropollutants was removed by conventional activated sludge plants, but about two-thirds of removed substances were mainly transferred to sludge. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30%.

Materials and methods

Substances studied and chemical analysis

In total, 117 substances have been selected: 45 substances with EQS and 72 other substances that were chosen according to their potential harmfulness, their reported occurrence and their expected resistance to treatments. The list includes 20 metals, and several organic substances like 4-nonylphenolethoxylates (mono- and di-ethoxylate), 4-nonylphenoxyacetic acid, aminomethylphosphoric acid (AMPA), triclosan, bisphenol A, 33 pharmaceuticals and five hormones.

Wastewater treatment plants selection and sampling

Overall, 19 WWTP treatment lines were studied, chosen as representative of various sizes (100 to 1,000,000 PE) and of various types of treatment processes:

- two primary treatments including primary settling, primary lamellar settling.
- 15 secondary treatments like activated sludge, fixed film processes like biofilter, trickling filter, biodisc, reed bed filter, one membrane bioreactor, one stabilisation pond.
- six tertiary treatment lines including sand filtration, activated carbon filter, ozone oxidation, reverse osmosis.

A total of seven plants were located in rural areas, and eight in urban areas. Half of the plants were equipped with combined sewer, and half with separate sewer. Sampling was performed in the influent and effluent during two or three successive 24 h-periods under dry weather flow conditions, with refrigerated samplers equipped with Teflon pipes and glass containers. Grab samples were collected for treated sludge. Strict procedures of cleaning, sampling, and field blanks were carried out.

Data processing and criteria for relevance determination

The results were described using:

- The frequency of quantification (Fq) and total concentration in influents, effluents and sludges.
- The specific daily average load received at WWTP (g/d/PE), calculated for each substance.
- The removal rate for different processes, with some calculation rules to take into account the variability of concentrations in raw wastewater and the analytical uncertainties associated with low concentrations of substances in complex matrices. If inlet concentration was not higher than 10 times the limit of quantification, removal efficiency was not calculated. Additionally, results were displayed as a removal efficiency range : 0-30%, 30-70% or 70-100%.

The substances with the following criteria were pointed out: Fq>70% in raw wastewater, removal rate below 30%, concentration >1 mg/kg DW (dry weight) in sludge. In treated wastewater the relevance of the substances was determined through the ratio between the effluent concentration (C) and the EQS

(noted C/EQS). Three levels of relevance were defined: 'high level' for substances with Fq>70% and C/EQS>1, 'medium level' for Fq>10% or C/EQS>1, and 'low level' for Fq<10% and C/EQS<1.

Results and Discussion

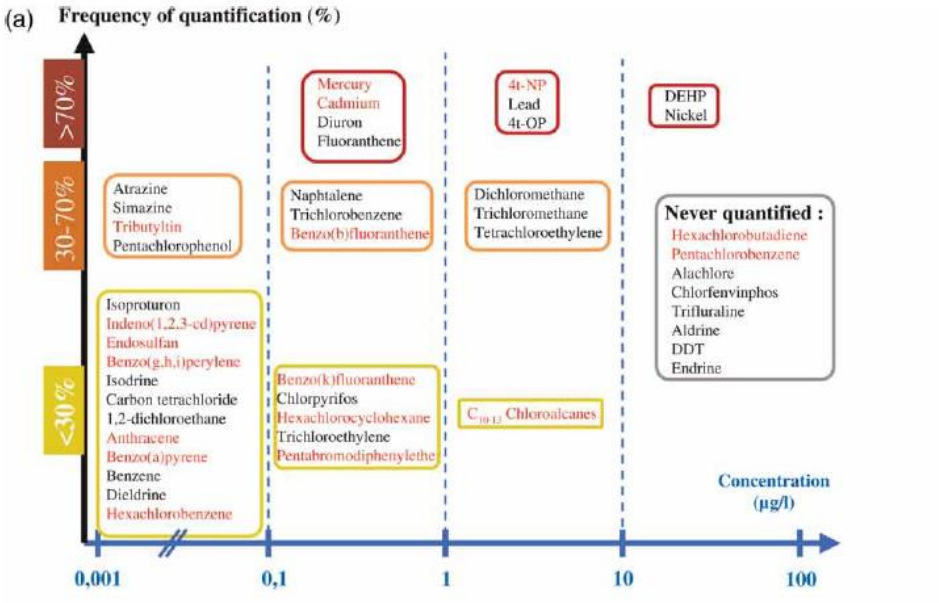
Relevant substances in raw wastewater

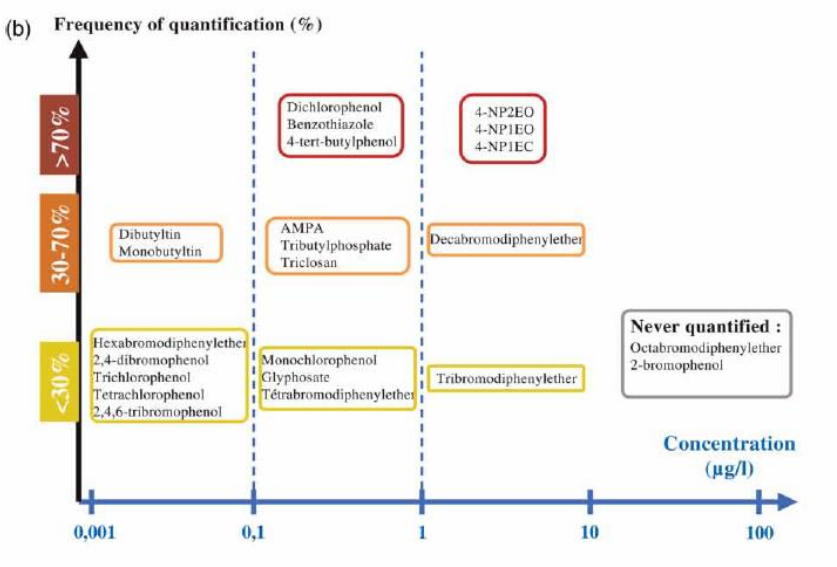
In raw wastewater, about half of the 45 substances with EQS and about 80 % of the 72 other studied substances were found at significant concentrations (>0.1 µg/L):

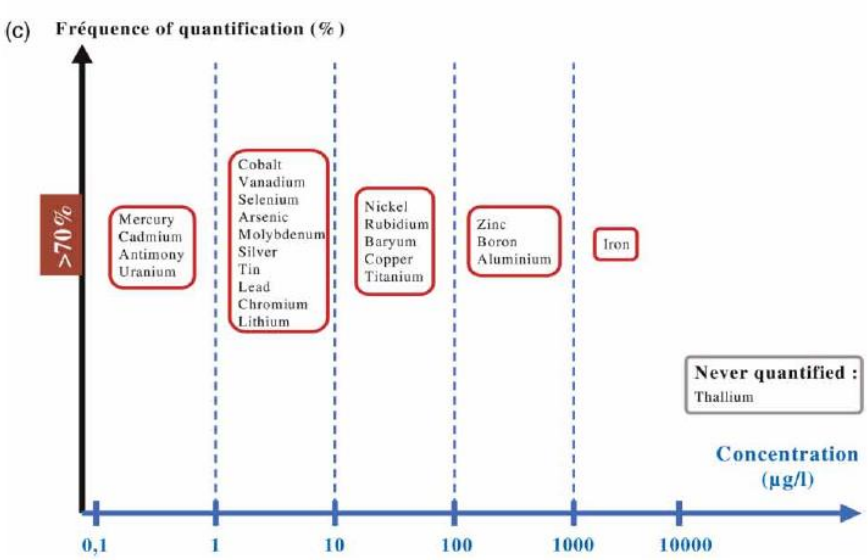
Substances with EQS (Figure 8.2.4.2-9):

- The highest Fq was found for DEHP (100%), which also had the highest mean concentration (67 µg/L). Alkylphenols were also present at high concentration (4.3 µg/L for 4-t-OP; 9.7 µg/L for 4-NP) and very frequently quantified (81% and 100% for 4-t-OP and for 4-NP, respectively). Light PAHs (naphthalene and fluoranthene) were frequently found in raw wastewater (59 and 81%, respectively), with a mean concentration higher than 0.1 µg/L.
- VOCs dichloromethane, trichloromethane and tetrachloroethylene combined a high mean concentration (1.4-2.9 µg/L), with a medium frequency of quantification (30-70%).
- Among pesticides, diuron was the most frequently quantified (81%) with a mean concentration of 0.25 µg/L. Atrazine and simazine were found in about half of the samples of wastewater, but their mean concentration was much lower (0.02 µg/L).
- All priority metals were systematically quantified, but with different mean concentrations: 10.6 µg/L for nickel, 5.7 µg/L for lead, 0.36 µg/L for mercury and 0.21 µg/L for cadmium.
- Are also worth mentioning the high mean concentration of C10-C13 chloroalkanes (5.5 µg/L) in the six samples where they were quantified (Fq of 20%), and the relatively high Fq for trichlorobenzene (47%) and pentachlorophenol (34%) with concentrations close to 0.1 µg/L.
- Eight substances with EQS were never quantified, either because their use is now prohibited (pesticides alachlor, aldrine, DDT, endrine, chlorfenvinphos, trifluraline), or because their use is very specialised (e.g., hexachlorobutadiene, pentachlorobenzene).

Figure 8.2.4.2-9: Frequency of quantification (%) and mean concentration (µg/L) in domestic raw wastewater (15 WWTP, 32 samples) of substances with EQS (a), other organic substances (b) and metals (c)







Other organic substances (Figure 8.2.4.2-9):

- Alkylphenol ethoxylates and carboxylates (4-NP1EO, 4-NP2EO and 4-NP1EC) were systematically quantified at mean concentrations between 2.1 and 6.1 µg/L, which is the same level as priority substances 4-NP and 4-t-OP.
- Benzothiazole (100 %), 4-tert-butylphenol (81%), dichlorophenol (78%), tributylphosphate (66%) and **AMPA (53%)** were frequently quantified, with mean concentrations between 0.1 and 1 µg/L.
- Very high concentrations of triclosan (up to 49 µg/L) and flame retardants (deca- and tri bromodiphenyl ether: 1.6-2.6 µg/L) were found in some samples (30%).

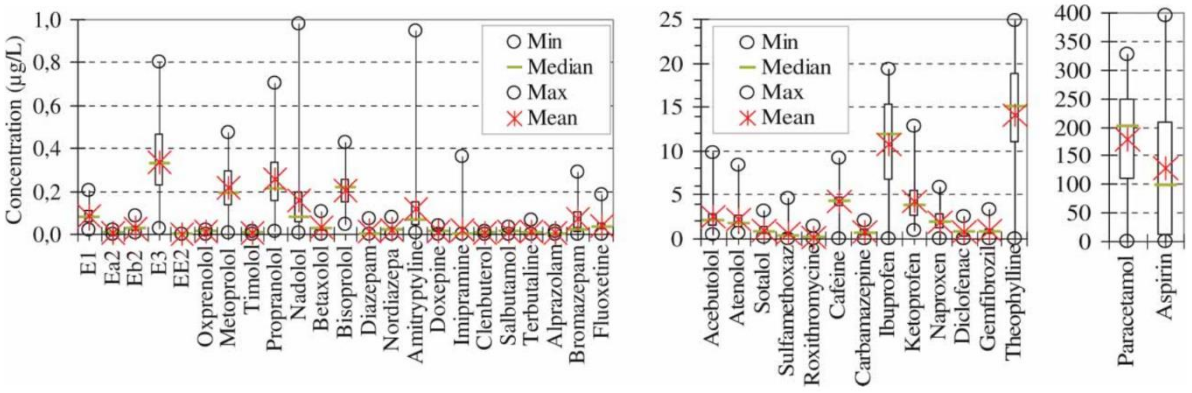
Metals (Figure 8.2.4.2-9):

- Metals were systematically quantified, except Ag and Se (occurrence of 70%).
- Mean concentration for Cd, Hg, Sb and U were between 0.1 and 1 µg/L; mean concentrations of other metals were higher than 1 µg/L, except for Fe, Al, B and Zn for which the mean concentrations were >100 µg/L.

Hormones and pharmaceuticals (Figure 8.2.4.2-10):

- Oestrone (E1), 17β-estradiol (Eb2) and estriol (E3) were systematically quantified in raw wastewater. Mean concentrations of E1 and Eb2 were lower than 0.1 µg/L, while mean concentrations of estriol reached 0.34 µg/L.
- A majority of pharmaceuticals were very frequently quantified in raw wastewater (Fq >80%). Paracetamol and aspirin presented the highest mean concentrations (>100 µg/L). Acebutolol, atenolol, sotalol, sulfamethoxazole, roxithromycine, caffeine theophylline, carbamazepine, ibuprofen, ketoprofen, naproxene, diclofenac and gemfibrozil presented mean concentrations between 0.1 and 10 µg/L. The other pharmaceuticals were never quantified above 1 µg/L.

Figure 8.2.4.2-10: Box-plot diagram of concentrations of hormones and pharmaceuticals in domestic raw wastewater (15 WWTP, 32 samples).



Sources of micropollutants can be very variable for each WWTP depending on the period of the year (summer/winter), the location (rural/urban), and the type of activities connected (hospitals, industries). Due to their industrial origin, some substances are quantified at higher concentrations in urban networks (with respect to rural ones): alkylphenols (except 4-NP1EC), VOCs (dichloromethane, trichloromethane trichloroethylene, tetrachloroethylene), chloroalkanes, dichlorophenol, bisphenol A, Ni, Cr and Ag. The concentration of alkylphenol polyethoxylates (adjuvants of detergents in textile industries, additives in paper industries) is two to three times higher in urban WWTPs; these compounds are responsible for the release of alkylphenols (4-NP and 4-t-OP) by biodegradation. **Glyphosate** is more frequently used as a herbicide in urban environments. Comparing the total load of micropollutants in raw wastewater to the load of micropollutants in treated wastewater in WWTPs with conventional activated sludge process, a reduction of about 80% was observed.

Fate of relevant substances in biological treatments

Three main removal mechanisms in WWTPs need to be considered: biodegradation, adsorption on sludge flocs and stripping to gas phase. The fate of micropollutants in biological treatments will mainly depend on their physicochemical properties. The dissolved phase of the substances in raw wastewater provides a first indication on their propensity to remain in wastewater or to be transferred to sludge. For many substances, the values calculated present a significant variability due to the variability of suspended solids (SS) concentration and to the variability of the volatile suspended solids (VSS) content among the wastewaters tested. Main results are the following:

- Hydrophobic substances (e.g. « heavy » PAH, PBDE, chloroalkanes) were only quantified in particulate phase; DEHP, 4-NP and 4-t-OP, quantified in almost all samples, presented a mean fdiss close to 50%, meaning that they should be relevant at the wastewater outlet and at the sludge outlet. The transformation product 4- NP1EC was mainly present in dissolved phase (f_{diss} 60%).
- Most pesticides are hydrophylic (log K_{ow}<3) and were mainly quantified in dissolved phase.
- Metals distribution varied according to their physicochemical properties: f_{diss} >70% for B, Li, Rb and Mo; f_{diss} <30% for Zn, Cd, Ag, Ti, Cr, Fe, Pb, Cu, Sn, Al and Hg.
- More than 90% of hormones were generally found in dissolved phase, in agreement with literature values. Most of the pharmaceuticals were also mainly present in the dissolved phase

as most of them are hydrophilic. However, some of them were more evenly distributed, in link with their lower log K_{ow} values. Substances with f_{diss} >70% (e.g. paracetamol, carbamazepine) have a log K_{ow} ranging between -0.39 and 2.87. At the opposite, f_{diss} of amitriptyline, doxepine and fluoxetine is about 50 % and their log K_{ow} is between 3.99 and 4.95.

Table 8.2.4.2-16 provides a classification of the different types of substances addressed, depending on the range of removal efficiencies observed in low load activated sludge process (data from 5 WWTPs). More than 30% removal efficiency was calculated for about 70% of the substances quantified in inlet raw water, and more than 70% removal efficiency for about 50% of the substances quantified:

- Removal rates were calculated for 23 substances with EQS. More than half of them were removed to more than 70% due to hydrophobic properties (DEHP, 4-NP, 4-t-OP, heavy PAH, PBDE, chloroalkanes) or volatile properties (chloroform, dichloromethane). Four priority substances (diuron, isoproturon, atrazine, simazine), with hydrophilic properties (log K_{ow}<3) and slow biodegradability (half-life constant >40 days), were found in treated wastewater without significant removal within WWTP.
- Other organic substances were mainly removed from water by adsorption, except for **glyphosate**, **AMPA** and 4-NP1EC that are hydrophilic and not biodegradable. Moreover, alkylphenol carboxylates are produced during biological oxidation of alkylphenol ethoxylates and **AMPA** is a degradation product of glyphosate or detergents, which may increase their concentration at WWTP outlet.
- Metals were distributed among the three ranges of removal rates: 11 metals were efficiently removed, particularly the ones adsorbed onto suspended solids of raw wastewater (Ag, Ti, Cr, Fe, Pb, Cu, Sn, Al, Hg), and also Zn and Cd; seven metals were not removed, in particular B, Li, Rb quantified in the dissolved phase of raw wastewater.
- Hormones were all removed by biotransformation. More than one-third of the studied pharmaceuticals were well removed from water (>70%) by both adsorption and biotransformation (caffeine, ibuprofen, theophylline, aspirin and paracetamol). One-third is hydrophilic and hardly biodegradable (e.g. carbamazepine, diclofenac), therefore refractory to biological treatments.

Significant differences of removal efficiencies have been measured between different biological treatment processes. Results of Table 8.2.4.2-16 should therefore be modulated for each biological process considered.

Table 8.2.4.2-16: Fate of substances through low load activated sludge plant (n = 5)

Low load conventional activated sludge	Removal efficiency range <30%	30–70%	>70%
Priority substances with Environmental Quality Standard	Diuron Isoproturon Atrazine Simazine	Nickel Cadmium Naphtalene Chlorpyrifos Trichlorobenzene	Lead Mercury Fluoranthene Benzo(b)fluoranthene Benzo(k)fluoranthene C10-13 Chloroalkanes Pentabromodiphenyl ether DEHP Dichloromethane Chloroform Tetrachloroethylene Trichloroethylene Nonylphenols Octylphenols
Other relevant organic substances	Glyphosate AMPA NP1EC	Mono-, di- chlorophenols Bisphenol A	Triclosan Tri-, tetra-, bromodiphenyl ether Hexa-, deca-, bromodiphenyl ether Benzothiazole 4 Tertbutylphenol Tributylphosphates NP1EO, NP2EO
Metals	Li, B, V, Co, As, Rb, Sb	Ni, Zn, Se, Cd, Ba, U, Mo	Al, Cr, Fe, Cu, Ag, Sn, Hg, Ti, Pb
Hormones			Estrone (E1) 17α-Estradiol (Ea2) 17β-Estradiol (Eb2) Estriol (E3)
Pharmaceuticals	Carbamazepine Diazepam Nordiazepam Doxepine Oxprenolol Propranolol Sotalol Diclofenac Salbutamol Terbutaline	Sulphamethoxazole Roxytromicine Metoprolol Timolol Atenolol Amitriptyline Fluoxetine	Nadolol Betaxolol Bisoprolol Acebutolol Imipramine Bromazepam Gemfibrozil Bromazepam Ibuprofen Paracetamol Aspirin Ketoprofen Naproxen

Relevant substances in sludge

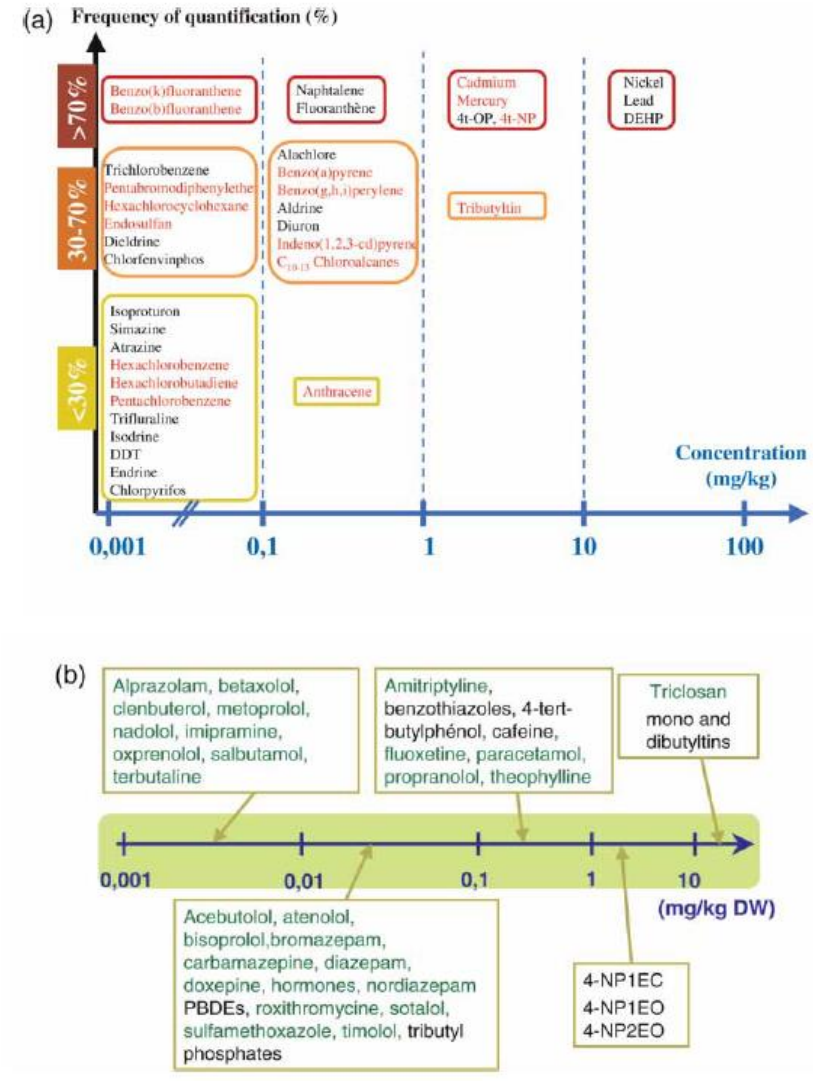
In biological treatment processes, the major removal mechanism was transfer to sludge for about two-thirds of the substances. Twenty-one substances with EQS were frequently measured in sludge, and about 35% of substances with EQS and 65 % of the others substances were found at concentrations higher than 100 mg/kg DW. All the substances quantified in raw wastewater were also measured in secondary sludge. Nevertheless, the concentrations of PAH (fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene) and metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) were always below the threshold limits for agricultural landspreading in France:

- Some substances with EQS were always quantified in sludge, with concentration levels of <1 mg/kg DW (PAH except anthracene), 1 to 10 mg/kg DW (alkylphenols, Cd, Hg), or >10 mg/kg DW (DEHP, Ni, Pb). Hydrophilic substances (e.g. pesticides) were hardly quantified (<30 %) with low concentrations (<0.1 mg/kg). Several substances with EQS that were never quantified in raw wastewater were sometimes quantified (i.e., hexachlorobutadiene, pentachlorobenzene, chlorfenvinphos, alachlor, DDT).
- Tributyltin (priority substance) was frequently found at a concentration higher than 1 mg/kg, and its degradation products (mono- and di-butyltin) were found at more than 10 mg/kg.
- Other organic hydrophobic substances were often quantified (>70%) due to high adsorption. Different ranges of concentration were observed: <0.1 mg/kg DW (tributylphosphate), 0.1-1 mg/kg DW (4-tert-butylphenol, benzothiazole), 1-10 mg/kg (4-NP2EO, 4-NP1EO), >10 mg/kg DW (4-NP1EC).
- Metals were quantified in all the samples at concentrations above 1 mg/kg DW, up to 10 or

100 mg/kg DW (Zn,Cu,Ti).

- Two pharmaceuticals (acebutolol, propranolol) were always quantified in sludge, at concentration levels of 0.078 and 0.126 mg/kg DW respectively, due to their high concentration in raw wastewater. Oestrone (E1), carbamazepine and amitriptyline were quantified in 70-90% of the samples at a concentration of 0.029, 0.075 and 0.195 mg/g DW, respectively. Caffeine, ibuprofen and fluoxetine were quantified in 67% of the samples at levels of 0.245, 0.245 and 0.104 mg/g DW. Other pharmaceuticals were quantified in less than half of the samples at low concentration levels (<0.1 mg/kg DW), except for aspirin and ketoprofene for which the concentration were 7.9 and 3.8 mg/kg DW respectively, due to high concentrations in raw wastewater (Figure 8.2.4.2-11).

Figure 8.2.4.2-11: Frequency of quantification (%) and sludge concentration range (mg/kg DW) for substances with EQS (a), and sludge concentration range (mg/kg DW) of other organic relevant substances (b) (17 WWTP, 17 samples).



Relevant substances in treated wastewater

In treated water released by biological treatments, 30 % of substances with EQS and 60% of other substances were still quantified at concentrations higher than 0.1 µg/L. Even if a significant decrease of the concentrations was observed through the WWTP, some concentrations higher than 1 µg/L still

prevailed for metals (among them Ni had a mean concentration of 5.6 µg/L), DEHP (mean concentration of 4.6 µg/L) and two by-products (4-NP1EC and AMPA with mean concentrations of 2.3 and 3.1 µg/L respectively).

Twenty-six substances with EQS may be a problem regarding the objectives of the WFD (Table 8.2.4.2-17):

- Four pesticides (diuron, isoproturon, atrazine, simazine) were classified as medium or high level due to their high Fq and to their poor removal in WWTP. Diuron appeared as the most relevant one as it was frequently quantified at concentrations above the EQS.
- Eight substances were found in almost all samples, sometimes with concentrations above EQS, due to their high concentration in raw wastewater, despite good removal efficiencies in WWTP: four metals, DEHP, two alkylphenols and chloroform.
- Nine substances less frequently found, but with some samples above EQS, due to their high concentration in raw wastewater or to low EQS values: three PAH (fluoranthene, anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene), of four pesticides/biocides (chlorpyrifos, endosulfan, hexachlorocyclohexane, tributyltin), and pentabromodiphenyl ether.
- Five substances were found at low concentrations below EQS, but were quantified in more than 10% of the samples. A medium risk of reaching EQS was then estimated, as their frequent presence increases the possibility of overcoming EQS. This was the case of naphthalene, pentachlorophenol, trichlorobenzene, dichloromethane and tetrachloroethylene.

It should be noted that for 17 substances (in brackets in Table 8.2.4.2-17) current analytical methods do not give reliable results for concentration levels as defined by EQS. According to the QA/QC Directive (2009/90/EC), the criterion for considering a method to be valid for WFD is a limit of quantification equal to or less than one-third of the EQS. Therefore, their emission must be completely eliminated. It should be noted that EQS have been defined for concentration compliance in receiving bodies, not for WWTP effluents.

Indeed, most of the non-regulated substances quantified in raw wastewater were also frequently measured at significant concentrations in treated wastewater. Special concern is related to 4-NP1EC (alkylphenol carboxylate) and AMPA, with higher concentrations at the outlet than at the inlet of WWTP. 4-NP1EC is formed by aerobic degradation of alkylphenols, and AMPA can result from the degradation of glyphosate or from phosphoric acid present in detergent.

Table 8.2.4.2-17: Frequency of quantification (Fq) and concentration of substances in treated wastewater released by activated sludge plants (n = 5)

	Priority substances (WFD)		
Relevance treated wastewater: frequency & concentration	**Priority substances (to be reduced)**	**Hazardous priority substances (to be stopped)**	**Additional substances with EQS**
Metals	Nickel Lead	Cadmium Mercury	
PAH	Fluoranthene Naphtalene	Anthracene (Benzo(a)pyrene) (Benzo(b)fluoranthene) (Benzo(g,h,i)perylene) (Benzo(k)fluoranthene) (Indeno(1,2,3-cd)pyrene)	
Pesticides	Alachlor Chlorfenvinphos Chlorpyrifos Diuron Isoproturon Atrazine (Trifluraline) Simazine Pentachlorophenol	(Hexachlorocyclohexane) (Endosulfan) (Tributyltin)	(Aldrin) DDT (Dieldrin) (Endrin) (Isodrin)
Industry	Benzene Trichlorobenzene DEHP	Hexachlorobutadiene (C10-13 Chloroalkanes) (Pentachlorobenzene) (Hexachlorobenzene) (Pentabromodiphenylether)	
Solvents and surfactants	Dichloroethane Dichloromethane Chloroform Octylphenol	Nonylphenol	Carbon tetrachloride Tetrachloroethylene Trichloroethylene

Dark grey: high (frequency >10% and C/EQS>1); light grey: medium (frequency >10% or C/EQS>1); no shading: low (frequency <10% and C/EQS<1); (…): analytical limitation: LQ>1/3 * EQS.

Conclusion

Relevant substances

- About half of substances with EQS were found at concentrations >0.1 µg/L in wastewater.
- Main loads of micropollutants were identified in raw wastewater: metals >pharmaceuticals >DEHP >alkylphenols > VOCs >other organics.
- For 26 substances with EQS, potential non-compliance with EQS of WFD has been identified in treated wastewater. Main concerns are for Cd, DEHP, diuron, alkylphenols and chloroform.
- Emerging substances of particular concern are by-products (AMPA, NP1EC), other chemicals (triclosan, benzothiazole, chlorophenols, PBDEs) and some pharmaceuticals [analgesics (e.g. ketoprofen, diclofenac), beta-blockers (e.g. sotalol), antibiotics (e.g. sulfamethoxazole), antidepressants (e.g. carbamazepine)].

Fate in WWTPs

- About 80% of the load of micropollutants are removed by conventional activated sludge plants.
- More than half of substances with EQS were removed to more than 70% due to hydrophobic or volatile properties. Other organic substances (with no EQS) are mainly removed from water by adsorption. Hormones and more than one third of the studied pharmaceuticals are well removed from water (>70%) by both adsorption and biotransformation.
- About two-thirds of removed substances were mainly transferred to sludge. All the substances quantified in raw wastewater were also measured in secondary sludge.
- Tertiary treatments may be applied to complete the removal of micropollutants, but this implies additional cost (up to þ 100% for reverse osmosis) and potential by-products and concentrates (advanced oxidation processes, activated carbon).
- The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30%.

Assessment and conclusion by applicant:

The article describes the occurrence of glyphosate and AMPA among other substances in different wastewater treatment plants in France. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30%.

The analytical methods are poorly described.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

Agrees with applicant's conclusions. The study is considered reliable with restrictions and provides supportive information on water treatment efficiencies for glyphosate and AMPA removal from waste water.

The analytical methods are not described within this article, but in a cited reference (not provided here) and could not be ckecked, especially for the limit of quantification. However, the study authors clearly indicated that "If inlet concentration was not higher than 10 times the limit of quantification, removal efficiency was not calculated".

It is noted that results were displayed as a removal efficiency range : 0–30%, 30–70% or 70–100%, and not detailed measures from the inlet and outlet are reported.

19 WWTP treatment lines were studied, chosen as representative of various sizes. Sampling was performed in the influent and effluent during two or three successive 24 h-periods under dry weather flow conditions. Grab samples were collected for treated sludge.

In raw wastewater, AMPA was frequently quantified (53%), with mean concentrations between 0.1 and 1 µg/L. Glyphosate was less quantified (<30%), also with mean concentrations between 0.1 and 1 µg/L.

Results for removal rates are less clear, no concentration for glyphosate or AMPA are reported in the activated sludge (figure 20) neither in the waster water from treated sludges (table 26). However, study concluded that "the removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30%.

Bruchet et al., 2011

Data point:	CA 7.5/027
Report author	Bruchet, A. *et al.*
Report year	2011
Report title	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge
Document No	European Journal of Water Quality 42 (2011) 123-133
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at an officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

The fate of various emerging contaminants as well as priority pollutants from the European Union Water Framework directive was examined along a complex combination of natural and engineered processes used to produce drinking water downstream of a major metropolitan area. The sampling points examined comprised Seine river water downstream of the Paris area, water from a primary well after bank filtration, water from a secondary well influenced by an artificial recharge process and water from the

mixture of secondary wells after drinkingwater treatment. More than 80 organic contaminants includingglyphosate and AMPA, were monitored during five campaigns. River bank filtration and to a lesser extent artificial recharge clearly decreased the variety of contaminants, in particular glyphosate and AMPA were reduced from <0.1 – 0.12 µg/L and 0.25 – 0.65 µg/L, respectively, in the river to <0.1 µg/L in both the primary and secondary wells.

Materials and methods

Study site

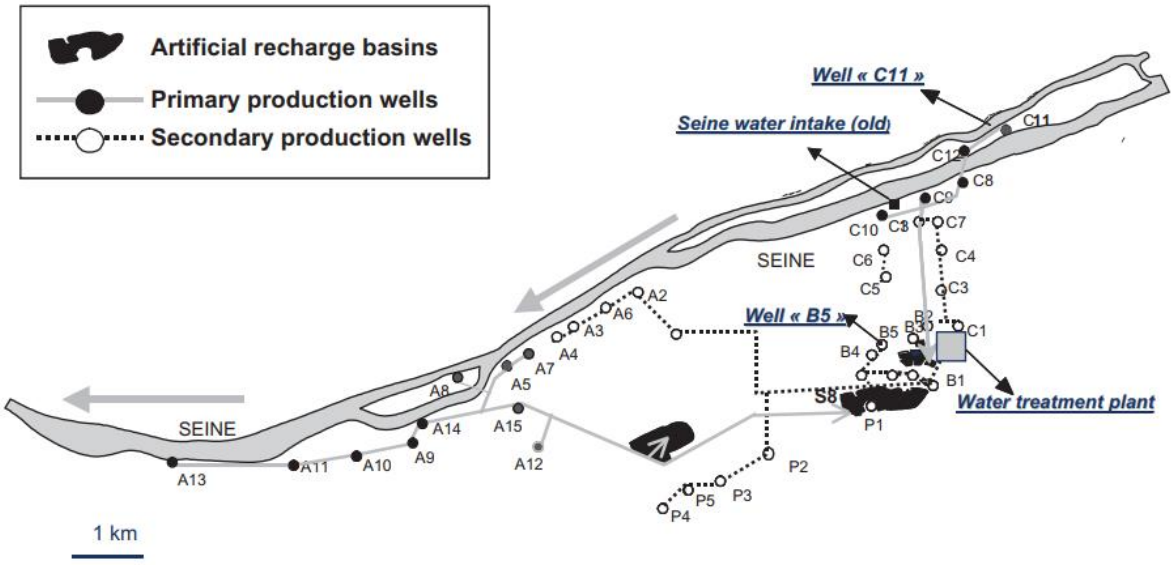
The aquifer studied (Figure 8.2.4.2-12) is located along the Seine river, downstream of Paris and its urban wastewater plants. In particular, it is located downstream of a wastewater plant that treats the effluents from 6.5 million people at a rate of 2 million m^3/day. This aquifer covers an area of 40 km^2 and comprises 36 primary and secondary wells. The primary wells are located mostly along the river, naturally re-supplied under anoxic conditions through river bank filtration.
The mixture of primary wells is pumped and re-infiltrated through a sand-gravel artificial basin in order to recharge secondary production wells. This artificial recharge takes place under slightly aerobic conditions. This process that comprises active biological interfaces in anoxicaerobic conditions allowed to replace drinking water treatment processes including settling, nitrification, iron and manganese removal and hence allowed a reduction in sludge production. Water from the mixture of secondary wells is further treated in a drinking water plant that comprises settling with addition of powdered activated carbon (PAC), sand filtration, ozonation and final disinfection with chlorine. The plant production is equal to 144 000 m^3/day.

The following points were sampled (grab samples) on five occasions during September and October 2008:

(1) the Seine raw water,
(2) primary production well C11 (one of the C wells in Figure 8.2.4.2-12) which is located on a small island on the Seine river and hence directly influenced by the river after bank filtration,
(3) secondary well B5 (one of the B wells in Figure 8.2.4.2-12) which is influenced by the main artificial recharge basin. However, due to the direction of underground flows, this well is also influenced by other areas of the aquifer and,
(4) the treated water at the outlet of the drinking water plant.

The sampling period covered both low flow conditions (220 m^3/s) and higher flow rates (up to 343 m^3/s).

Figure 8.2.4.2-12: Description of study site showing the four sampling points. Flow of the river is from right to left.



Analytical methods

A wide array of analytical methods was used to cover most priority pollutants and emerging contaminants. Volatile organic compounds (VOC's) were determined by Purge and Trap gas chromatography-mass spectrometry (GC/MS). Glyphosate and AMPA were determined by FMOC derivatization-HPLC-fluorescence.

Results and Discussion

Although the sampling point on the Seine river is located downstream of a metropolitan area with 11 million people, most EU priority compounds were never detected.

The only pesticide or degradate found at a level exceeding 0.1 µg/L in the Seine river is **glyphosate** (on one occasion) and its degradate **AMPA** (systematically in the range 0.25-0.65 µg/L). AMPA can also be present as a wastewater contaminant, from household detergent use. These two compounds are totally removed by bank filtration, in accordance with previous observations and do not reappear in the aquifer.

Table 8.2.4.2-18: Fate of priority and emerging contaminants during bank filtration (C11), artificial recharge (B5) and drinking water treatment.

Parameter	Unit	Seine river $n = 5$	C11 well $n = 5$	B5 well $n = 5$	Drinking water $n = 5$
Semi-volatiles compounds					
Fluoranthene	ng/L	9–14	<10	<10	<10
DEHP	ng/L	191–675	367–509	320–2013	243–521
Atrazine	ng/L	17–23	19–23	34–50	3–9
Diuron	ng/L	32–40	58–73	26–52	<1
Isoproturon	ng/L	<1–3	24–35	15–31	<1
Simazine	ng/L	3–9	6–10	7–15	<1
Glyphosate	µg/L	<0.1–0.12	<0.1	<0.1	<0.1
AMPA	µg/L	0.25–0.65	<0.1	<0.1	<0.1
Alkylphenols					
4-NP (Nonylphenols)	µg/L	0.06–0.21	0.45–1.71	0.06–0.29	0.05–0.18
4-t-OP (Octylphenols)	µg/L	<0.01–0.05	0.05–0.57	<0.01–0.02	<0.01–0.03
4-NP1EO	µg/L	0.02–0.11	0.04–0.19	0.02–0.04	0.002–0.04
4-NP2EO	µg/L	0.01–0.13	0.03–011	0.01–0.04	0.01–0.08
4-NP1EC	µg/L	0.08	0.19	0.03–0.01	<0.001–0.003
Beta-blockers					
Atenolol	ng/L	99.5–155.2	<LoQ-6.6	0.7–2.5	<LoQ-1.6
Sotalol	ng/L	65.9–117.1	3.3–12.1	<LoQ-3.8	<LoQ-0.6
Nadolol	ng/L	1–4	<LoQ	<LoQ	<LoQ
Timolol	ng/L	<LoQ-0.5	<LoQ	<LoQ	<LoQ
Acetobutolol	ng/L	32.9–75.6	0.6–1.6	3.3–6.2	<LoQ-1.2
Metoprolol	ng/L	8.5–13.5	<LoQ-0.9	<LoQ-0.4	<LoQ-0.4
Oxprenolol	ng/L	<LoQ-1.7	<LoQ	<LoQ	<LoQ
Propanolol	ng/L	8.8–30.6	<LoQ	<LoQ-0.6	<LoQ-0.5
Betaxolol	ng/L	<LoQ-1.7	<LoQ	<LoQ-0.5	<LoQ
Bisoprolol	ng/L	7.5–12.5	<LoQ-0.3	<LoQ-0.3	<LoQ-0.5

In the river, glyphosate was found at <0.1 – 0.12 µg/L, and AMPA at 0.25 – 0.65 µg/L: but, in both the primary well and the secondary well, concentrations of both substances were <0.1 µg/L, as they were in the drinking water samples. (It is worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85%, and 17% for glyphosate; whereas, it is clear from the context that removal is more likely to be 100%. Indeed, the authors state that "both these compounds are totally removed by bank filtration" in this case.

Conclusion

The present study allowed most priority substances from the EU Water Framework Directive to be measured, and also a wide variety of emerging substances in a surface water downstream of a major metropolitan area that treats the majority of its urban wastewaters (the Seine river downstream of Paris). The study site selected allowed the fate of the substances detected to be observed, during their infiltration into an aquifer primarily re-supplied by natural bank filtration. The fate of the substances reaching the aquifer was monitored along a natural recharge process and at the outlet of a drinking water plant treating a mixture of boreholes from this aquifer.

In a system influenced by urban wastewaters downstream of a major metropolitan area, a drinking water produced by a complex combination of natural bank filtration, artificial recharge, clarification with powdered activated carbon addition, ozonation and chlorination, complies with the current legislation. In particular, glyphosate and AMPA were reduced, by the bank filtration process, from <0.1 – 0.12 µg/L and 0.25 – 0.65 µg/L, respectively, in the river, to <0.1 µg/L in the primary and secondary wells. It is also worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85%, and 17% for glyphosate; whereas, it is clear from the context that removal is more likely to be 100%. Indeed, the authors state that "both these compounds are totally removed by bank filtration" in this case.

Assessment and conclusion by applicant:

The article describes a monitoring experiment with glyphosate and AMPA among different other substances from Seine river and a drinking water production area downstream of the Paris urban area. The study is well described, the analytical methods used are sufficient.

With respect to glyphosate and AMPA, the study sheds light on the effectiveness of the water treatment train employed for a major surface water to drinking water plant, where the primary treatment process is bank filtration. In this case, it is clear that bank filtration has been shown to be an effective process to remove glyphosate and AMPA to <0.1 µg/L from water destined to be drinking water.

The article is considered reliable.

Assessment and conclusion by RMS:

The article describes a monitoring experiment with glyphosate and AMPA among different other substances from Seine river and a drinking water production area downstream of the Paris urban area.

It includes measured concentrations of glyphosate in treated water from drinking water plant that comprises settling with addition of powdered activated carbon, sand filtration, ozonation and final disinfection with chlorine. However, these processes are not precisely described.

Comparison of measured concentrations before and after bank filtration shows that glyphosate and AMPA compounds are removed by bank filtration to level in the primary and secondary wells <0.1 µg/L (0.1 µg/L being the LOQ). Removal rates are calculated to be 85% and 17% for AMPA and glyphosate, based on the LOQ.

It is worth noting that the glyphosate concentration is <0.1 µg/L in the primary, secondary and WWTP effluent. Therefore the efficiency of the WWTP cannot be measured.

Also considering the measured concentration of glyphosate and AMPA close to the LOQ in the river, and since the accuracy of the analytical method is unknown, the reliability of any removal rate is questionable.

The study is considered reliable with restrictions.

Litz et al., 2011

Data point:	CA 7.5/063
Report author	Litz, N.T. et al.
Report year	2011
Report title	Comparative studies on the retardation and reduction of glyphosate during subsurface passage
Document No	Water research (2011), Vol. 45, No. 10, pp. 3047-54
Guidelines followed in study	None (for filter experiments)
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (German UBA, German KompetenzZentrum Wasser)
Acceptability/Reliability:	Reliable

The herbicide Glyphosate was detected in River Havel (Berlin, Germany) in concentrations between 0.1 and 2 µg/L (single maximum outlier: 5 µg/L). As the river indirectly acts as drinking water source for the city's 3.4 million inhabitants' potential risks for drinking water production needed to be assessed. For this reason laboratory (sorption and degradation studies) and technical scale investigations (bank filtration and slow sand filter experiments) were carried out. Batch adsorption experiments with

glyphosate yielded a low K_F of 1.89 (1/n = 0.48) for concentrations between 0.1 and 100 mg/L. Degradation experiments at 8°C with oxygen limitation resulted in a decrease of glyphosate concentrations in the liquid phase probably due to slow adsorption (half life: 30 days). During technical scale slow sand filter (SSF) experiments glyphosate attenuation was 70-80% for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L, respectively. Relevant retardation of glyphosate breakthrough was observed despite the low adsorption potential of the sandy filter substrate and the relatively high flow velocity. The VisualCXTFit model was applied with data from typical Berlin bank filtration sites to extrapolate the results to a realistic field setting and yielded sufficient attenuation within a few days of travel time. Experiments on an SSF planted with *Phragmites australis* and an unplanted SSF with mainly vertical flow conditions to which glyphosate was continuously dosed showed that in the planted SSF glyphosate retardation exceeds 54% compared to 14% retardation in the unplanted SSF. The results show that saturated subsurface passage has the potential to efficiently attenuate glyphosate, favourably with aerobic conditions, long travel times and the presence of planted riparian boundary buffer strips.

Materials and methods

In all experimental settings – laboratory batch, enclosure and SSF tests- the same filter material was used. The texture of the applied sandy substrate can be characterized as follows: on average 2% fine sand (0.1-0.2 mm), 43% medium sand (>0.2-0.5 mm), 49% coarse sand (>0.5-2.0 mm) and 6% fine gravel (>2 mm), no clay or silt with only traces of organic matter and an effective porosity of 0.38-0.4% (Table 8.2.4.2-19). The pH value of the percolated water was ~7.7. Solid glyphosate produced by Sigma-Aldrich with a purity degree of 98.7%, dissolved in a 0.01 M $CaCl_2$-solution, was used for the experiments. Glyphosate concentrations were analyzed according to the German Standard DIN 38407-22 (2001). The quantitative determination of AMPA and glyphosate was done using a Waters HPLC system with a fluorescence detector and two Knauer 64 as reagent pumps. The analytical column for glyphosate was a Supelco SAX column (25 x 4 mm), for the quantification of AMPA a cation exchange column (Pickering) was applied (15 x 4 mm), because in field samples the AMPA peak was interfered by matrix peaks. The run conditions were: 0.4 mL/min, isocratic, phosphate buffer pH 2.05 ± 0.1 at 50°C. Retention time for glyphosate was 13.6 min on the anion exchange column and for AMPA 13.9 min on the cation exchange column. The detection limits were 0.02 µg/L and 0.005 µg/L, the quantification limit 0.07 µg/L and 0.02 µg/L for glyphosate, for AMPA, respectively. The two analytes AMPA and glyphosate were detected after a 2-step post-column derivatization. The first step was an oxidation with a phosphate buffer containing sodium hypochlorite (0.4 mL/min) in a 10 m reaction coil of PEEK tubing (i. d. 0.25 mm, volume 500 µL) at 50°C, the second a transformation into fluorescing compounds by reaction with phthaldialdehyde and 2-mercaptoethanol in an alkaline borate buffer (0.3 mL/min) in a 2 m reaction coil of PEEK tubing (i.d. 0.25 mm, volume 100 µL) at ambient temperature. The excitation wavelength of the resulting compounds was 390 nm and the emission wavelength 450 nm. All solutions were degassed and filtered through 0.45 µm prior to use. Samples of the filter substrate were extracted according to methodology reported elsewhere: 10 g of the sample were brought into contact for 30 min with 25 mL of 1 M NaOH. Subsequently the mixture was centrifuged for 15 min at 3000 rpm. The supernatant was abstracted with a pipette and the extraction was repeated. 4.2 mL concentrated HCl was added to the combined supernatants. After dilution of the sample with deionized water to a volume of 200 mL the analytes glyphosate and AMPA were determined as described above. The cleanup of the water samples was also performed according to the abovementioned German standard method DIN 38407-22. Water samples obtained from laboratory-, and enclosure experiments (typically 100-500 mL) were filtrated through glass fiber filters and adjusted with hydrochloric acid to pH 2 ± 0.1. The filtrate was applied to a column filled with a cation exchange resin which had been loaded with Fe^{3+} ions. Subsequently the column was rinsed with 20 mL water and 40 mL 0.02 M HCl. The analyte-iron complex was eluted with 10 mL 6 M HCl and 4 mL 32% HCl were added to the eluate. This solution was applied to an anion exchange column. By elution of the column with 6 M HCl the iron was retained on the column.

Table 8.2.4.2-19: Characterisation of the enclosure filling material

Characteristics	Clogging layer	Filter substrate	Drainage stratum
Soil type	n.a.	mS, gS, fg	fG, mg
Thickness [m]	0.05[b]	1	0.25
CU[a]/CG[a]	n.a.	3.2/0.7	2.0/1.0
Fe(ox) [mg/kg]	605	275	n.a.
Mn(ox) [mg/kg]	68	11	n.a.
$C_{org}/C_{an.org}$ [%]	0.343/1.4	0.022/0.12	n.a.

a Parameters for classification of non-structured sediments (uniformity coefficient, coefficient of gradation).
b Clogging layer is situated in the upper layer of the filter substrate, n.a. = not analyzed.

Laboratory experiments

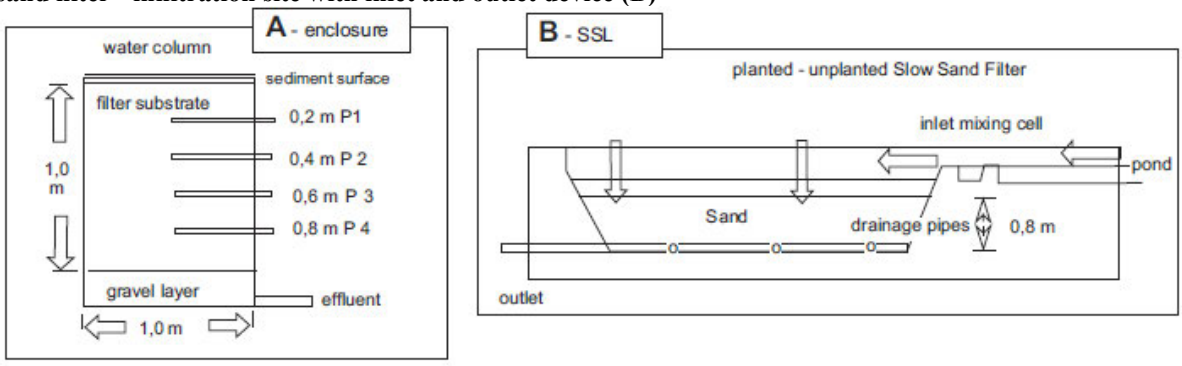
Batch experiments

The batch experiments were conducted according to OECD 106 using the filter substrate and deionized water with glyphosate concentrations of 0.1 mg/L, 1 mg/L, 10 mg/L and 100 mg/L and a soil/water-ratio of 1:2, shaking the mixture for 4 h to establish an equilibrium. The chosen concentrations were applied in three parallels. After centrifugation the supernatant was carefully extracted and prepared for measurement. The Freundlich adsorption isothermal model was used to describe the nonlinear water/sediment distribution relations (K_F) over the total concentration range. The equation's first differentiation was used to describe also the linear distribution coefficient (K_D) and to estimate retardation factors (R_F).

Degradation experiment

Degradation studies were carried out by taking a defined sediment sample of 450 g wet material and mixing it with 10 mg glyphosate per kg filter substrate. The vessels were stored in the dark at a temperature of around 8°C for a period of up to 73 days to allow for biological degradation processes to take place. The airtight stoppers of the vessels sealed the sample from the atmosphere. During the experiment the vessels were left undisturbed. The redox potential, oxygen content, pH value and the temperature in the supernatant were determined after the respective vessels were opened and sampled. At intervals (7, 14, 21, 28 and 73 days) two experiment vessels were opened at a time. This experimental arrangement was intended to simulate naturally deposited filter substrate under partly reducing conditions, as it would be expected in slowly flowing groundwater.

Figure 8.2.4.2-13: Schematic cross section and location of sampling ports in enclosures (A) and slow sand filter - infiltration site with inlet and outlet device (B)



Technical scale experiments

Enclosure experiments

Water production pre-treatment via bank filtration or/and slow sand filtration is commonly used if

drinking water is produced from surface water. In enclosure experiments the attenuation of compounds can be determined simulating conditions that occur during slow sand filtration or within the first meter of infiltration. The enclosures are three metal cylinders with an area of 1 m^2 and a height of 1.85 m (filtration length 1.00 m) (see Figure 8.2.4.2-13A). They are situated within an infiltration pond (area: 90 m^2) in order to be exposed to natural environmental conditions. Three different concentration levels of glyphosate were continuously dosed to the supernatant of the enclosures over a time period of 14 d from 20 October to 6 November 2007, yielding average inlet concentrations of 0.7, 3.5 and 11.6 µg/L. Water samples for glyphosate and AMPA analysis were taken for 34 days from the supernatant, from sampling points within the filter material and from the filter effluent. The flow rate was set at 50 cm/d and was controlled by adjustable pumps connected to the enclosure outlets. The depth of the supernatant was kept constant by siphoning the water out of the infiltration pond into the enclosure without additional pumping. The water in the infiltration pond originates from a large storage pond (volume of 7000 m^3) with relatively high mineralization (average electrical conductivity: 1000 µS/m) but low nutrient status (nitrate < 1 mg/L, orthophosphate < 1 mg/L, DOC 3-4 mg/L) thus representing oligotrophic surface water.

Slow and filter (SSF) experiments

The SSF experiments were conducted at two vertical-flow experimental SSFs: (Figure 8.2.4.2-13B) one without vegetation cover (average area 60 m^2, filter depth 0.8 m, filter volume 48 m^3) and the other with a 3 year old vegetation cover of *Phragmites australis* (average area 68 m^2, filter depth 1.2 m, filter volume 81.6 m^3) to simulate processes in grown planted bank filtration sites along rivers or surface water lakes. Due to the arrangement of inflow, water reservoir and drainage pipes, water flow through the SSFs was assumed to be predominantly vertical simulating conditions that occur during the first meter of bank filtration. The water fluxes of the unplanted and the planted SSF were regulated at the outlet and were regularly controlled by discharge measurements. Their yield amounted in average to approximately 0.41 and 0.45 m^3/h, respectively (corresponding to a filtration velocity of 0.16 and 0.18 m/d). Physico-chemical parameters of the water (pH, redox potential, and temperature) as well as DOC, PO_4^{3-} and NO_3^- concentrations were also monitored to gain insights into controlling processes. After an equilibration phase of 1 month during which nitrate and phosphate were dosed to target 10 mg/L N and 1 mg/L PO_4^{3-} in the supernatant, glyphosate was additionally applied for 22 days with a target concentration of 20 µg/L.

Results and Discussion

Batch experiments

Glyphosate exhibits under different site conditions a complex adsorption behavior in the environment which is influenced by pH and by variation of soil constituents and the chemical glyphosate species. In order to determine the distribution coefficient of glyphosate, degree of adsorption in the filter substrate batch experiments were conducted. The resulting linear regression with a Freundlich sorption coefficient (K_F) of 1.90 and a Freundlich exponential of 0.48 confirms the poor adsorptive characteristics of the sandy material and indicates beginning saturation at higher concentrations (Table 8.2.4.2-20). With sorption data from different concentration ranges a calculation of the adsorption coefficients (K_D-value) was carried out for different concentration ranges. Due to lower adsorption at high concentrations the K_D-values decrease by 3 orders of magnitude when regarding the complete range of concentrations from 0.1 to 100 mg/L. This is in agreement with comparable experiments of with sandy material reported elsewhere, which is comparable to the one used in this study, where a K_D-range of 1.5-2.9 L/kg was determined. Compared to other studies on glyphosate adsorption with soils showing K_D values that range from 62 to 410 L/kg these values are quite low. This is most probably due to the low content of clay, iron and aluminum oxide or organic matter content in the filter material. Only some iron and organic matter content may have influenced the sorption in the filter material and should be responsible for slightly elevated adsorption coefficients (5.4 L/kg) at least with low glyphosate concentrations (0.1-1 mg/L).

Table 8.2.4.2-20: Estimated retardation of glyphosate in the filter substrate on the basis of Freundlich distribution equation

Concentration (c_{aq}) [mg/L]	Gradient from first differentiation (G)[a] [L/kg]	Retardation factor (R_f)[b]
100	0.08	1.4
10	0.28	2.2
1	0.9	5
0.1	3	14
0.02	7	31

a $G = 1/n \times K_F \times (c_{aq})^{1/n-1}$ with K_F: 1.9 $mg^{1-1/n} \times L^{1/n} kg^{-1}$ and 1/n: 0.48.
b $R_f = 1 + (\rho_b/n_e) \times G$, with an effective porosity (n_e): 0,37 and bulk density (ρ_b): 1.59 kg/L.

Degradation experiment

It is well known that glyphosate degrades more easily under aerobic conditions compared to anaerobic conditions. Figure 8.2.4.2-14 shows the residual glyphosate concentrations, obtained from the analysis of the solvent samples in the batch degradation experiment under anaerobic conditions. As it is not clear, if the reduction of concentrations was due to degradation or adsorption, the term dissipation will be used in the following. The development of the redox potential and oxygen content during the degradation experiment showed that oxygen-free conditions were partially achieved. The oxygen in the supernatant was almost completely consumed (data not shown) whereas the pH value remained constant at around 7.7. Dissipation of 50% (DT_{50}) of the glyphosate in the supernatant was calculated to be achieved after 30.5 days yielding a rate of dissipation of 0.0227/d. A mass balance approach was carried out taking into account the initially applied amount of glyphosate, the concentrations measured in solution and the adsorbed fraction. During the first 30 days the decrease in dissolved concentration is due to a continuous adsorption in this time (data not shown). Degradation must therefore be initially negligible. Similar findings in anoxic substrate have been reported elsewhere. The results of laboratory degradation studies differed from the findings in the outside enclosure experiments, which were carried out under more aerobic and temperate conditions.

Enclosure experiments

By simulating slow sand filter conditions, enclosure experiments can help to verify the risk for groundwater pollution by contaminants entering from surface waters. Glyphosate and AMPA concentrations in enclosures II and III for the time of the experiment (34 days) are given in Figure 8.2.4.2-15 and Figure 8.2.4.2-16. Glyphosate was continuously dosed for 14 days to both enclosures reaching average concentrations of 3.5 and 11.6 µg/L, respectively, with a standard deviation of 20%. The two concentrations reflect medium and maximum levels generally observed in surface water. In enclosure II the glyphosate concentrations at the outlet reached a maximum value of 0.7 µg/L towards the end of the experiment (after 34 days). Since the experiment was terminated before the concentrations decreased again the point in time for the peak value could only be estimated. A break-through curve was observed in enclosure III, to which the highest glyphosate concentration was applied. The maximum outlet concentration for glyphosate of 2.7 µg/L occurred after 23 days. After 8 days (enclosure III) and after 17 days (enclosure II) nearly all observed glyphosate concentrations exceeded the European limit for pesticides in drinking water of 0.1 µg/L. AMPA concentrations above 0.1 µg/L were observed since day 6 in enclosure III and since day 12 in enclosure II. An example vertical concentration profile is illustrated for enclosure III in Figure 8.2.4.2-17. This shows sthat retardation and degradation processes are distributed almost linearly along the filtration depth as this was also observed in experiments elsewhere. Tracer and glyphosate concentrations at the outlets of enclosures II and III were modeled using the computer program VisualCXTFit. On the basis of the hydrodynamic properties of the filter substrate obtained from the tracer experiment (R^2 = 0.95 and 0.93 for enclosures II and III, respectively (data not shown)), it was possible to assess the retardation and degradation capacity of the enclosures for glyphosate. The modeled results of the glyphosate concentrations in enclosures II and III corresponds well compared to the observed breakthrough curves. Based on the recovered concentrations at the outlet the applied glyphosate was reduced by 78-80%. Modeling yielded a retardation factor of 25 and 18 and

a degradation rate of 0.0069/d and 0.092/d in enclosures II and III, respectively. The half-lives derived from the modeled degradation rates, amounted to 10 d (enclosure II) and 7.5 d (enclosure III), respectively, and correspond well to the values mentioned in literature with 2-14 d for aerobic conditions. The slightly higher degradation in enclosure III could be related to the higher glyphosate concentrations in the liquid phase and a resulting better access of microorganisms to glyphosate. With the obtained parameters data it was attempted to predict the necessary depth of filter substrate to ensure an attenuation of glyphosate to values below the European threshold for drinking water starting from source water concentrations of 3.5 µg/L (enclosure II) and 11.6 µg/L (enclosure III). The modeled filtration length for a sufficient attenuation in enclosure II and III would be about 2.75 m and 3.75 m, respectively (Figure 8.2.4.2-18). Model calculations assuming conditions occurring at existing bank filtration well fields yielded in all cases no contamination risk for the water used in drinking water production. Similar findings have been published elsewhere.

Figure 8.2.4.2-14: Glyphosate partitioning between solid and aqueous phases during degradation batch experiments (points represent samples from 2 replicates for each sampling date)

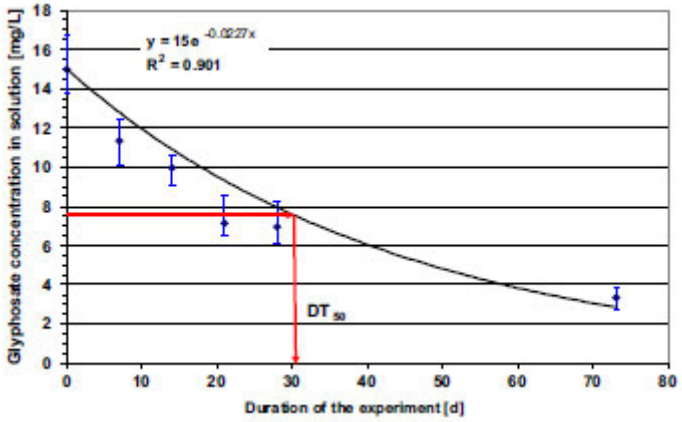


Figure 8.2.4.2-15: Glyphosate and AMPA concentrations in the outlet of enclosure II (with an average inlet glyphosate concentration of 3.5 µg/L)

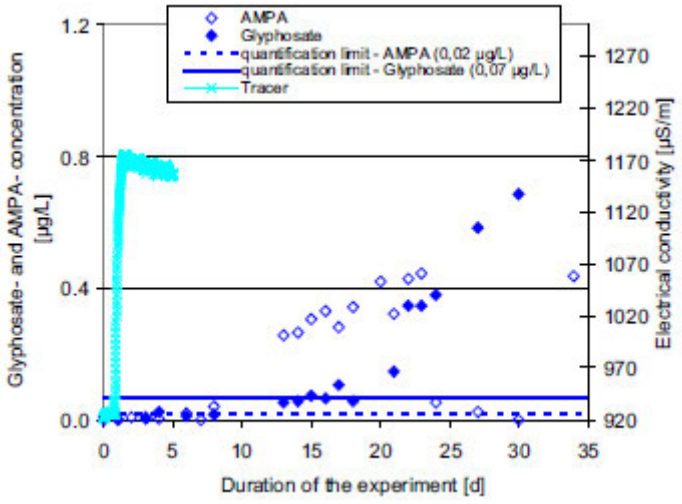


Figure 8.2.4.2-16: Glyphosate and AMPA concentrations in the outlet of enclosure III (with an average inlet glyphosate concentration of 11.6 µg/L)

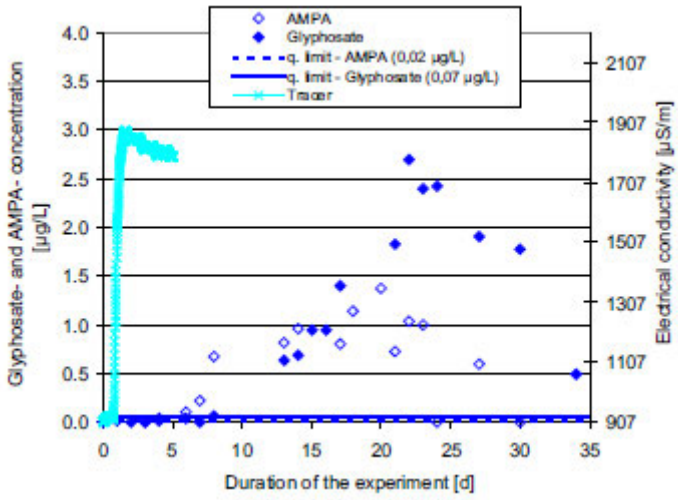
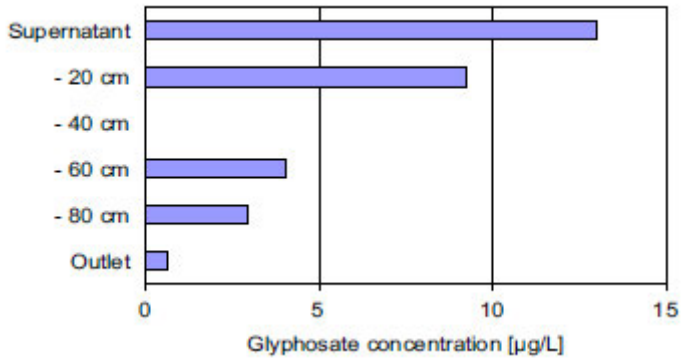


Figure 8.2.4.2-17: Vertical distribution of glyphosate concentrations in enclosure III on 05.11.2007 (16 days after dosing commenced)



Slow sand filter experiments

For simulating glyphosate attenuation in a riparian zone, studies with an adapted planted SSF and unplanted SSF were conducted. The hydro-chemical analyses (tracer tests, break-through curves of nitrate) indicated that the planted SSF does not show a homogeneous vertical flow pattern. Thus the planted SSF was divided into two zones (right and left) with different hydraulic and subsequently hydro-chemical characteristics and an estimation of the hydraulically effective surface area was carried out. These estimations showed a reduction in average surface area of the planted SSF to around 67% of the unplanted SSF, confirming that the flux in the planted SSF seems to be partly inhibited. The lowering to around 67% of the average surface area could be explained by collimation due to high production of biomass which at constant hydraulic head results in a decrease of pore velocities or even blocking of pore volume. The concentrations of glyphosate measured in the mixing cell, in the supernatant, in 40 cm depth and in the outlet of the planted SSF (left site) are given in Figure 8.2.4.2-19. In the mixing cell of the planted SSF the average glyphosate concentration of 21.2 µg/L was slightly higher than the targeted level of 20 µg/L. In the left zone of the planted SSF only little reduction was observed in the water reservoir above the SSF surface (19 µg/L in average). In 40 cm depth the maximum concentration of glyphosate was retarded by 11 days and reduced to approximately 7 µg/L (63% of the average concentration in the supernatant). In the right zone (data not shown) the concentrations decreased by more than 50% between mixing cell and surface water of the SSF. Glyphosate was completely removed from solution in 40 cm depth, which seems to be due to lower inlet concentrations, higher residence times and therefore higher efficiency of reduction. In the combined outlet (left and right zone) the fluxes of all sampling sites rejoined and resulted in a maximum concentration of 1.4 µg/L. The final measurements at the end of the experiment showed a reduction of about 93% of the applied glyphosate compared to the inlet concentration. While the planted SSF had to be divided into two zones the

unplanted SSF can be regarded as homogenous (Figure 8.2.4.2-20). The inlet concentrations of the unplanted SSF did not reach the targeted level of 20 µg/L. In average it was lower and characterized by strong fluctuations probably due to degradation processes in the stock solution (17.6 µg/L in average). The concentration gradient between the level of glyphosate in the mixing cell corresponds well to the concentrations measured in the supernatant. In contrast to the planted SSF where an increase in 40 cm depth was found only after 10 days, low concentrations of glyphosate were observed here from the very beginning in the unplanted SSF. This is clearly a result of enhanced attenuation and could be interpreted as retardation by the biomass of the root zone. Maximum glyphosate concentrations decreased to 9 µg/L after 40 cm of the filter passage (49% reduction of average supernatant concentration). The concentration in the outlet did not reach the climax of the breakthrough curve. The maximum concentration detected here was 4.5 µg/L. Comparing the concentrations in 40 cm depth and in the effluent of the unplanted SSF with those of the left zone as representative for the planted SSF there was slightly higher glyphosate reduction in the planted SSF (63% in 40 cm depth, compared to 49% in the unplanted filter), although the inlet concentrations were slightly higher and the residence time was lower. The higher reduction rate of glyphosate in the planted SSF could be due to the strong biological activity, which was concluded from the lower oxygen contents. The redox potential at 40 cm depth varied strongly in both SSFs and amounted to an average of -200 eV in the left zone as representative for the planted and +235 eV in the unplanted SSF. The decisive factor seems to be the availability of organic carbon, due to vegetal growth. The influence of *phragmites* buffer strips along surface water on glyphosate retardation has not been not studied by other experts before. Studies elsewhere on glyphosate attenuation during artificial recharge bank filtration have been carried out. Comparison of the results, demonstrated a high natural variability of subsurface mobility for glyphosate depending on site characteristics.

Figure 8.2.4.2-18: Modeled length of the filter substrate (from left to right: 1.25; 2.0; 3.0; 3.5 and 3.75 m) in order to ensure a reduction of the glyphosate concentrations below the European threshold for drinking water of 0.1 µg/L (enclosure III)

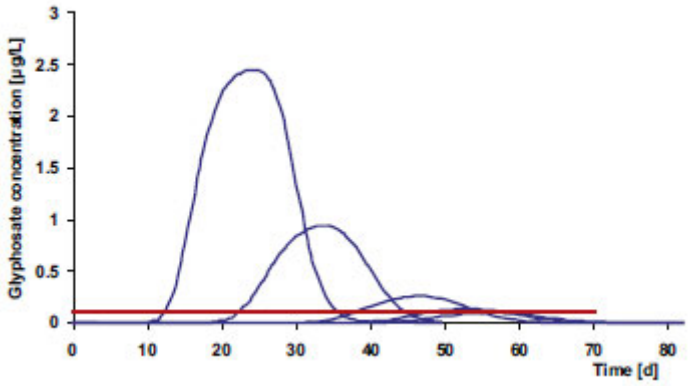


Figure 8.2.4.2-19: Glyphosate distribution in the left zone of the vegetated SSF

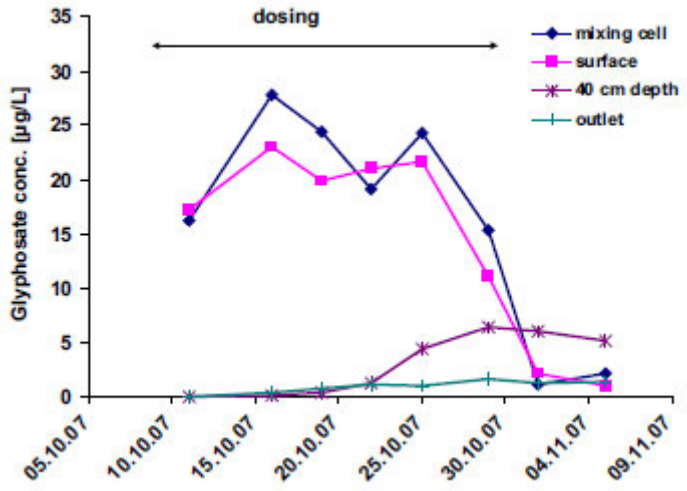
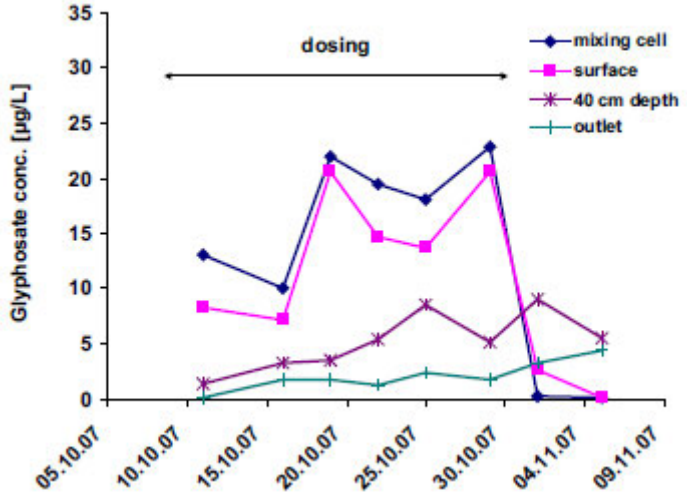


Figure 8.2.4.2-20: Glyphosate distribution in the unplanted SSF



Conclusion

Laboratory studies were conducted to characterize the substrate of the enclosures and the slow sand filters with regard to glyphosate removal processes. Batch adsorption studies yielded a very low adsorption capacity for glyphosate with a K_F of 1.9 in the sandy material. This is presumably due to the low organic matter content compared to studies carried out with soils, especially with those of a higher iron and aluminum oxide content. Anaerobic dissipation studies under laboratory conditions at 10°C resulted in a half-life of 30.5 d with dissipation rate of 0.023/d in the solvent phase. However, it could not be proven, that degradation is the main removal process for short subsurface passage as complete recovery was achieved from the solid phase after 30 d. In the further course of the experiment, however, significant degradation was observed. In the enclosure experiments a rapid degradation was observed due to the aerobic conditions and higher temperatures with a half-life of 7.5-10.5/d, with lower initial concentrations (3.5-12 µg/L) compared to the lab experiments. The enclosure experiments showed that between 78 and 80% of continuously applied glyphosate (3.5 µg/L or 11.6 µg/L in average) can be attenuated despite of low adsorption capacity of the filter substrate and high filtration velocity. The necessary length of the filter substrate in order to ensure a reduction of the glyphosate concentrations below the European threshold for drinking water of 0.1 µg/L was modeled with VisualCXTfit and must exceed 2.75 or 3.75 m for an initial glyphosate concentration of 3.5 µg/L (enclosure II) or 11.6 µg/L (enclosure III), respectively. In the SSF experiments the SSF covered with *P. australis* showed a 2-5 times higher removal capacity (57%) for glyphosate than the one without reed cover (14%). Thus, the following conclusions can be drawn for the attenuation of glyphosate during subsurface passage: At low

concentrations adsorption may play an important role, however, degradation needs to be considered as the main process for glyphosate attenuation. Favourable for glyphosate removal at bank filtration sites are oxic conditions, planted sediment surfaces and travel times of more than 10 days.

Assessment and conclusion by applicant:

The article describes experiments on subsurface passage of river water using so-called enclosures and semi-technical scale vertical slow sand filters (SSFs) to investigate the behavior of glyphosate and AMPA during bank filtration for drinking water supply. The filter experiments were supported by batch adsorption and degradation experiments with the filter material. Overall, the results showed that saturated subsurface passage has the potential to efficiently attenuate glyphosate, with aerobic conditions, long travel times and the presence of riparian boundary buffer strips. The main filter experiments and the analytical methods are well described and reported with sufficient details.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on water treatment efficiencies for glyphosate and AMPA removal. Data from this study are included in the review provided in [redacted], 2020.

Technical scale semi-field investigations (bank filtration and slow sand filter experiments) were carried out: The experimental systems consisted of three enclosures (metal cylinders) of slow sand filter material, with an area of 1 m^2 and a height of 1.85 m (with a filtration length of 1 m) situated within an infiltration pond (area 90 m^2). The flow rate was set at 50 cm/day. Glyphosate was continuously dosed to the enclosures over a 14 day period, and water samples for glyphosate and AMPA analysis were taken for 34 days.

These slow sand filter experiments demonstrated that 70 – 80% reduction in glyphosate concentrations were achieved (for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L).

Ruel et al., 2011

Data point:	CA 7.5/087
Report author	Ruel, S.M. et al.
Report year	2011
Report title	On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications
Document No	Water Science & Technology 63.11/2011
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable

The next challenge of wastewater treatment is to reliably remove micro-pollutants at the microgram per litre range in order to meet reuse applications and contribute to reach good status for water bodies. A hundred priority and relevant emerging substances were measured to evaluate at full-scale the removal efficiencies of seven advanced treatment lines (one membrane bioreactor process and six tertiary treatment lines) that were designed for reuse applications. To reliably compare the processes, specific procedures for micro-pollutants were applied for sampling, analysis and calculation of removal efficiencies. The membrane bioreactor process allowed to upgrade the removal efficiencies of about 20% of the substances measured, especially those that were partially degraded during conventional processes. Conventional tertiary processes like high rate clarification, sand filtration and polishing pond

achieved significant removal for some micro-pollutants, especially for adsorbable substances. Advanced tertiary processes, like ozonation, activated carbon and reverse osmosis were all very efficient to complete the removal of polar pesticides and pharmaceuticals; metals and less polar substances were better retained by reverse osmosis. For glyphosate and AMPA, removal rates were reported as being 30 – 70% for glyphosate and AMPA for sand filtration, <30% for AMPA for reverse osmosis and ozone treatment, but >70% for glyphosate for reverse osmosis and ozone treatment; >70% for both glyphosate and AMPA for activated carbon filtration.

Table 8.2.4.2-21: List of priority and emerging substances studied

	Priority pollutants (WFD) from EC (2008)			
	Priority pollutants	**Hazardous priority pollutants**	**Other dangerous pollutants**	**Other substances**
Metals	Ni, Pb	Cd, Hg		Li, V, Sb, B, Rb, Co, As, Mo, Zn, Ba, Se, U, Ti, Fe, Cu, Cr, Sn, Al, Ag
PAH		Anthracene, benzo(g,h,i) perylene		
	Naphthalene	Fluoranthene		
		Benzo(a)-, indeno(1,2,3-cd)-pyrene		
		Benzo(b)-, benzo(k)-fluoranthene		
Pesticides	Chlorfenvinphos, chlorpyrifos, pentachlorophenol	Hexachlorocyclohexane, endosulfan, tributyltin	Aldrin, DDT, dieldrin, endrin, isodrin	Triclosan mono-, di-, tri-, tetra-chlorophenols
	Diuron			Glyphosate, AMPA
	Alachlor, isoproturon, atrazine, trifluralin, simazine			Bromophenol, mono-, di-butyltin
Industry/solvents/ additives	Di-, tri-chloromethane, DEHP			Tri-, tetra-, hexa-bromodiphenylether
	Dichloroethane, chloroform, benzene		Carbon tetrachloride	Bisphenol A
				Octa-, deca-bromodiphenylether
	4-t-OP (octylphenol)	4-NP (nonylphenol)	tri- tetra-chloroethylene	4-NP1EO and 4-NP2EO (nonylphenol polyethoxylates)
	Trichlorobenzene			4-NP1EC (alkylphenol polyethoxyacids), benzothiazole, 4-tertbutylphenol, tributylphosphate
Pharmaceuticals				
Antibiotics				Sulfamethoxazole and Roxitromicin
Hypolipemiants				Gemfibrozil
Bronchodilatants				Clenbuterol, salbutamol, terbutalin
Analgesics				Ibuprofen, paracetamol, aspirin, diclofenac, ketoprofen, naproxen
Antidepressants				Diazepam, nordiazepam, doxepine, alprazolam
				Amitriptyline, imipramine, bromazepam, carbamazepin
Betablockers				Acetobutolol, atenolol, sotalol, metoprolol, propanolol, nadolol, bisoprolol
				Oxprenolol, timolol, betaxolol
Others				Caffeine, theophylline
Hormones				Oestrone, 17a-, 17b-oestradiol, oestriol, ethinylestradiol

Data from (Martin Ruel *et al.* submitted; Choubert *et al.* 2011; Gabet-Giraud *et al.* 2010).
Cinfluent < 0.1 µg/L; light grey shaded 0.1 < Cinfluent < 1 µg/L; dark grey shaded Cinfluent > 1 µg/L.
DDT, dichlorodiphenyltrichloroethane; DEHP, diethyl hexyl phthalate; PAH, polycyclic aromatic hydrocarbon.

Materials and methods

Micro-pollutants studied

The list of the 33 priority pollutants of the Water Framework Directive (WFD) was considered in this study together with the eight additional pollutants for which an environmental quality standard (EQS) has been defined. Additional substances have been chosen according to their potential harmfulness and their reported occurrence in waters based on French national inventories on dangerous and priority pollutants (see Table 8.2.4.2-21). Pharmaceutical compounds (emerging substances) were chosen considering their consumption and their occurrence in wastewater and surface water. A total of 127 micro-pollutants has been selected (including glyphosate and AMPA) but only 100 were quantified at least once in treated wastewaters of activated sludge.

Chemical analysis techniques

Various analytical methods were developed and applied to quantify the selected micro-pollutants (Table 8.2.4.2-22). Volatile pollutants were analysed in raw samples. For others, the dissolved phases were analysed due to low suspended solids concentrations (<5 mg/L). Limits of quantification (LoQ) are provided for the dissolved phase. The conventional parameters have also been analysed (total organic carbon, total nitrogen, total phosphorus) to determine if the operating conditions were correct.

Table 8.2.4.2-22: Analytical methods applied

Group of micro-pollutants	Extraction	Chromatographic analysis	LoQ in dissolved phase (µg/L)	Reference
Semi-volatile organics (Multiresidue)	Liquid-liquid + florisil	GC-MS-MS	0.010–0.050	Internal protocol
Volatile organic compounds (VOC)	Purge & trap	GC-MS	0.1	ISO 15680 (2004)
Chlorophenols	SPME	GC-MS	0.050–0.150*	Internal protocol
Pesticides-antibiotics	SPE	HPLC-MS-MS	0.001–0.002*	Internal protocol
Glyphosate/AMPA	SPE	HPLC-MS-MS	0.1	ISO 21458 (2009)
Chloroalkanes	SBSE	TD-GC-ECD	0.5	Internal protocol
PBDEs/bisphenol A	SBSE	TD-GC-MS	0.001–0.1*	Internal protocol
Alkylphenols + ethoxylates	SPE	LC-ESI-MS	0.01	Internal protocol
Betablockers, hormones, analgesics, broncho-dilatants, hypolipemiants, antidepressants	SPE	LC-MS-MS UPLC-MS-MS GC-MS	0.0005–0.002	Miège *et al.* 2009b Togola & Budzinski 2008
Mercury	–	AFS	0.0005	U.S. EPA (2002)
Metals and metalloids	–	ICP-MS	0.01–5	ISO 17294-2 (2005)

AFS, atomic fluorescence spectrometry; ECD, electron capture detector; ESI, electrospray ionisation; GC, gas chromatography; HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma – mass spectrometry; LC, liquid chromatography; MS, mass spectrometry; SPE, solid phase extraction; SPME, solid phase microextraction; TD, thermal desorption; UPLC, ultraperformance liquid chromatography.

Wastewater treatment plants (WWTP) selection and sampling

Seven WWTP of various sizes were studied (Table 8.2.4.2-23), which included various types of treatment: one full-scale membrane bioreactor (MBR); five full-scale conventional tertiary treatments, including high rate clarification, sand filtration or polishing pond; two advanced tertiary treatments at full-scale (ozonation and micro-filtration (MF) + reverse osmosis (RO)) and two advanced tertiary treatments at pilot-scale (activated carbon filtration and silex filtration + ultrafiltration + RO). The upstream treatment stages achieved both carbon and nitrogen removal to meet regulatory requirements. Influent and effluents of the studied processes were collected under dry weather flow conditions during two successive 24 h or 2 h periods (see Table 8.2.4.2-23). Automatic refrigerated samplers, equipped with Teflon pipes and glass containers, were used. Strict procedures of cleaning, sampling and field blanks were carried out. An ISCO bubble flowmeter was used to measure the flow released when a Venturi canal was available at the facility.

Table 8.2.4.2-23: Characteristics and operating conditions of the studied process

	Code size (PE)	Upstream treatment (carbon + nitrogen removal)	Process type	Operating conditions	Sampling period of composite sample
MBR	A – 24,000	–	MBR Ultrafor (4 Zenon ZWD500 modules, 10,000 m^2 of membrane surface)	F/M = 0.06 kg BOD_5/kg VSS/d, SRT = 17 d, T = 24 °C, membrane flux = 23 L/h/m^2	2 * 24 h
Tertiary processes	B – 300,000	AS	High rate clarification	Fast settling: 30 mg/L $Al_2(SO_4)_3$, 0.8 mg/L polyelectrolyte, lamellar velocity = 3.2 m/h	2 * 2 h
			+ sand filter	sand filter: velocity = 20 m/h	
	C – 97,000	AS	Sand filter	Sand filter: 3.6 m/h;	2 * 2 h
			+ ozone contact zone	ozone dosage = 10 mg O_3/L, contact time = 40 min	
	D – 25,000	AS	Sand filter	Sand filter: 3.5 m/h	2 * 2 h
			+ MF (84 modules, 1,350 m^2, hollow polypropylene fibres)	MF: 80 L/m^2/h, 1.6 bar	
			+ RO (90 modules filmtec BW30-365 polyamide)	RO: 20 L/m^2/h, 21 bar	
	E – 500 (pilot)	AS	Silex filter	Filter: 7.2 m/h	2 * 2 h
			+ UF (Norit X-flow, membrane 8″ SXL-225 FSFC Aquaflex, polyestersulfone)	UF: 50 L/m^2/h, 2 bar	
			+ RO (Trisep, three membranes 8″ 8040-X201-TSA, polyamide urea)	RO: 23 L/m^2/h, 8 bar	
	F – 470,000	AS	High rate clarification (full-scale)	Dosage: 70 mg/L $FeCl_3$, 1 mg/L polyelectrolyte, 1 g/L sand dosage, lamellar velocity = 6 m/h;	2 * 2 h
			+ activated carbon filter (pilot) (Filtrasorb-400, height = 1.4 m)	activated carbon: velocity 0.33 m/h, contact time = 2.1 h	
	G – 1,000	Biodisc + reedbed filter	Polishing pond (three tanks, total surface 2,570 m^2)	Contact time = 15 d	2 * 24 h

AS: Low load activated sludge process (SRT: 15–25 days); MBR: membrane bioreactor; PE: population equivalent; O_3: ozone contact zone; MF: microfiltration; UF : ultrafiltration; RO: reverse osmosis; F/M: loading rate (kg BOD_5/kg VSS/d); VSS: volatile suspended solids; SRT: sludge retention time; T: temperature.

Data processing

Mass balances were performed based on wastewater flow and micro-pollutant concentration data at the inlet and at the outlet of the studied processes. The removal efficiencies (R) were calculated with the following rules to obtain robust information:

– High and low levels of concentration were defined for each substance with respect to the LoQ. Low confidence level was for concentrations between LoQ and 2.5-5 times the LoQ (depending on the substance). High confidence level was for concentrations higher than 2.5-5 times the LoQ, depending on the substance. From analytical practice, at low confidence level, an analytical uncertainty in the range of 50-100 % is a regular value for most substances whereas an analytical uncertainty below 30 % is usual a high confidence level.

– When both inlet and outlet concentrations were lower than the LoQ or within the low level, the removal efficiency value was not calculated.

– When only one concentration, either inlet or outlet concentration, was lower than the LoQ, a value equal to half of the LoQ was adopted and the removal efficiency was calculated.

In addition to these criteria, removal efficiency data was displayed as a removal range (<30 %, 30-70 % and >70 %), since the analytical uncertainty and the variability of the concentrations related to micro-pollutants in wastewater do not allow to certify precise values.

Table 8.2.4.2-24: Concentrations (C, µg/L) in the effluent of the conventional AS process, in the effluent of MBR process and in the effluent of each type of tertiary process, and removal efficiency ranges (R, %) for tertiary processes

	Substances		CONCENTRATIONS															
			Effluent of secondary treatments		Effluent of advanced secondary treatment	Effluent of tertiary treatments						Conventional tertiary treatment			Advanced tertiary treatment		
						Conventional treatment			Advanced treatment								
			– 6 WWTP		– 1 WWTP	Fast settling – 3 WWTP	Sand filter – 3 WWTP	Polishing pond – 1 WWTP	Reverse osmosis – 1 WWTP	Ozone – 1 WWTP	Activated carbon filter – 1 WWTP	High rate clarification – 3 WWTP	Sand filter – 3 WWTP	Polishing pond – 1 WWTP	Reverse osmosis – 2 WWTP	Ozone – 1 WWTP	Activated carbon filter – 1 WWTP
			C (µg/L) Mean	SD	C (µg/L)	C (µg/L) Mean	C (µg/L) Mean	C (µg/L)	C (µg/L)	C (µg/L)	C (µg/L)	R	R	R	R	R	R
Trace organics	Benzene	VOC [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0,070	<LoQ						
	1,2-Dichloroethane	VOC [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Dichloromethane	VOC [a]	0.09	0.01	<LoQ	0.180	0.100	<LoQ	0.100	<LoQ	0.250	++					+
	Trichloromethane	VOC [a]	0,7	0.69	<LoQ	0.330	1.200	<LoQ	1.100	0.300	<LoQ	+	+		++		
	Carbon tetrachloride	VOC [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Trichloroethylene	VOC [a]	0.05	0,000	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Tetrachloroethylene	VOC [a]	1,1	1.3	<LoQ	1.100	1.000	<LoQ	<LoQ	<LoQ	0.530	++	+				+
	Glyphosate	Pesticides	0.43	0.06	<LoQ	0.310	0.510	0.560	<LoQ	0.160	<LoQ	++	++	+	+++	+++	+++
	AMPA	Pesticides	5,2	2.4	0.36	3.100	1.700	8.300	0.460	1.700	0.720	+	++	+	+	+	+++
	Pentabromodiphenylether	PBDE [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Octabromodiphenylether	PBDE	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Decabromodiphenylether	PBDE	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Hexabromodiphenylether	PBDE	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Tetrabromodiphenylether	PBDE	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Tribromodiphenylether	PBDE	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Bisphenol A	other	<LoQ		<LoQ	<LoQ	0.020	<LoQ	<LoQ	0.060	<LoQ						
	Triclosan	Biocide	<LoQ		0.02	0.020	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	C10-13 Chloroalcanes	Chlorinated organic [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Hexachlorobutadiene	Chlorinated organic [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Hexachlorobenzene	Chlorinated organic [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Pentachlorobenzene	Chlorinated organic [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Trichlorobenzene	Chlorinated organic [a]	0.04	0.001	0.08	<LoQ	<LoQ	<LoQ	0.020	<LoQ	<LoQ						
	Anthracene	PAH [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Fluoranthene	PAH [a]	0.09	0.09	<LoQ	0.140	0.070	<LoQ	<LoQ	<LoQ	<LoQ	+	+				
	Naphtalene	PAH [a]	0.01	0.002	<LoQ	<LoQ	0.010	<LoQ	<LoQ	<LoQ	<LoQ						
	Benzo(a)pyrene	PAH [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Benzo(b)fluoranthene	PAH [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Benzo(g,h,i)perylene	PAH [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Benzo(k)fluoranthene	PAH [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Indeno(1,2,3-cd)pyrene	PAH [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Di(2-ethylhexyl)phtalate (DEHP)	Phtalates [a]	2.2	1.8	1.40	5.200	1.100	2.300	0.340	0.750	4.600			+++		+++	++
	Alachlor	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Endosulfan	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Hexachlorocyclohexane	Pesticides [a]	0.06		<LoQ	<LoQ	0.120	<LoQ	<LoQ	<LoQ	<LoQ						
	Aldrine	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	DDT	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Dieldrine	Pesticides [a]	0,01		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Endrine	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Isodrine	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Chlorfenvinphos	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Chlorpyrifos	Pesticides [a]	0,07	0,04	<LoQ	0.090	0.050	<LoQ	<LoQ	<LoQ	<LoQ						
	Trifluraline	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						

	Substances		CONCENTRATIONS															
			Effluent of secondary treatments		Effluent of advanced secondary treatment	Effluent of tertiary treatments						Conventional tertiary treatment			Advanced tertiary treatment		
						Conventional treatment			Advanced treatment								
			– 6 WWTP		– 1 WWTP	Fast settling – 3 WWTP	Sand filter – 3 WWTP	Polishing pond – 1 WWTP	Reverse osmosis – 1 WWTP	Ozone – 1 WWTP	Activated carbon filter – 1 WWTP	High rate clarification – 3 WWTP	Sand filter – 3 WWTP	Polishing pond – 1 WWTP	Reverse osmosis – 2 WWTP	Ozone – 1 WWTP	Activated carbon filter – 1 WWTP
			C (µg/L) Mean	SD	C (µg/L)	C (µg/L) Mean	C (µg/L) Mean	C (µg/L)	C (µg/L)	C (µg/L)	C (µg/L)	R	R	R	R	R	R
Trace organics	Atrazine	Pesticides [a]	0.01	0.000	<LoQ	0.050	0.030	<LoQ	<LoQ	0.010	<LoQ	+	+			++	+++
	Simazine	Pesticides [a]	0.005	0.003	<LoQ	0.020	0.010	<LoQ	<LoQ	0.005	<LoQ	+	+		+++	++	+++
	Diuron	Pesticides [a]	0.17	0.11	0.01	0.150	0.310	0.060	0.010	0.050	<LoQ	+	+	+	+++	+++	+++
	Isoproturon	Pesticides [a]	<LoQ		<LoQ	0.005	0.010	<LoQ	<LoQ	<LoQ	<LoQ	+	+			+++	+++
	Pentachlorophenol	Chlorophenols [a]	0.05		<LoQ	0.040	<LoQ	<LoQ	<LoQ	0.030	<LoQ						
	Monochlorophenols	Chlorophenols	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Dichlorophenols	Chlorophenols	0.29	0.12	<LoQ	0.120	0.110	<LoQ	<LoQ	<LoQ	<LoQ	++	+				
	Trichlorophenols	Chlorophenols	0.1	0.04	<LoQ	0.090	0.120	<LoQ	0.120	<LoQ	<LoQ	+	+		+		
	Tetrachlorophenols	Chlorophenols	0.08	0.01	<LoQ	<LoQ	0.150	0.050	<LoQ	<LoQ	<LoQ						
	2-bromophenol	Bromophenols	0.06		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	2,4-dibromophenol	Bromophenols	0.21		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	2,4,6-tribromophenol	Bromophenols	0.81	1.0	<LoQ	0.070	0.930	0.060	0.370	<LoQ	<LoQ		++		++		
	Monobutyltin	Pesticides	0.01	0.002	0.002	0.004	0.020	<LoQ	<LoQ	0.010	<LoQ	++					
	Dibutyltin	Pesticides	<LoQ		0.01	<LoQ	0.010	<LoQ	<LoQ	0.004	<LoQ						
	Tributyltin	Pesticides [a]	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Tributyl phosphate	other	0.13		<LoQ	<LoQ	0.010	<LoQ	<LoQ	0.020	<LoQ	+					
	Benzothiazole	other	0.08	0.05	0.07	0.080	0.030	0.050	0.020	0.020	0.010	+	+	+	++		+++
	4-tert-butylphenol	Alkyphenols	0.06	0.03	<LoQ	0.140	0.060	0.070	<LoQ	<LoQ	<LoQ	+	+	+			
	4-NP (nonylphenols)	Alkyphenols [a]	0.66	0.51	0.14	0.390	0.250	0.700	0.220	0.140	0.020	+	++	+	++	++	+
	4-t-OP (octylphenols)	Alkyphenols [a]	0.04	0.02	<LoQ	0.070	0.020	<LoQ	0.030	0.010	0.030	+	++				
	4-NP1EO (nonylphenols poly-Ethoxylates)	Alkyphenols	0.46	0.70	0.05	0.210	0.060	0.140	0.010	<LoQ	0.010	+	++	+	+	+++	+
	4-NP2EO (nonylphenols poly-Ethoxylates)	Alkyphenols	1.6	3.7	0.04	0.180	0.130	0.120	0.020	0.100	0.020	+	++	++	+++	+	
	4-NP1EC (acides alkylphenol-polyethoxy-phenoxyacetiques)	Alkyphenols	5.7	7.2	0.19	1.500	2.700	19.200	0.320	0.170	0.004	+	+	+	+++	+++	+++
Metals	Hg	Metals [a]	0.07	0.07	0.001	0.002	0.001	0.004	<LoQ	0.001	<LoQ	++		++			
	Li	Metals	21	15.1	11.0	6.600	8.500	6.500	1.900	8.500	13.500	+	+	+	+++	+	+
	B	Metals	438	218	400	147.000	159.000	218.000	224.000	230.000	47.600	+	+	+	++	+	++
	Al	Metals	159	235	<LoQ	1.900	15.400	<LoQ	71.300	<LoQ	77.100	+++	++	+	+		
	Ti	Metals	21.9	12.7	1.60	1.900	7.600	11.800	2.000	10.800	2.800	++	+	+	+++	+	+
	V	Metals	6.4	7.6	0.46	1.700	2.700	1.300	0.660	6.500	17.200	++	+	+	+++	+	
	Cr	Metals	2.7	4.3	0.28	0.360	0.340	0.150	0.130	0.290	<LoQ	++	+	++	+++	+	+++
	Fe	Metals	59.8	36.0	51.1	47.300	45.800	8.100	3.200	62.400	2.500	++	+	+	+++	+	+++
	Ni	Metals [a]	5.2	5.1	4.2	6.300	1.900	2.000	0.200	1.600	5.100	+	+	+	+++	+	+
	Co	Metals	0.47	0.11	0.33	0.840	0.540	0.310	<LoQ	0.730	1.100	+	+	+	+++	+	+
	Cu	Metals	16.0	15.7	10.1	2.000	1.400	15.500	0.840	0.880	1.100	+	+	++	++	+	+
	Zn	Metals	26.6	27.2	66.1	17.700	14.000	6.400	2.800	24.500	0.740	++	+	++	+++	+	+++
	As	Metals	1.3	0.72	0.11	0.760	1.700	1.700	0.260	0.740	30.200	+	+	+	+++	+	
	Se	Metals	1.10	0.46	<LoQ	0.920	1.100	0.600	<LoQ	<LoQ	0.820	++	+		+++		
	Rb	Metals	17.6	3.5	14.8	17.200	16.100	12.500	1.600	14.700	15.600	+	+	+	+++	+	+
	Mo	Metals	2.9	1.4	1.10	3.600	1.900	1.400	0.200	0.450	58.100	+	+	+	+++	+	
	Ag	Metals	0.66	0.01	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.020	<LoQ	+++					
	Cd	Metals [a]	0.06	0.05	0.09	0.020	0.010	0.040	0.010	<LoQ	0.120	++		++			+
	Sn	Metals	0.82	0.91	<LoQ	0.330	0.260	<LoQ	<LoQ	0.110	<LoQ	++	+	+++	+++		
	Sb	Metals	0.58	0.26	0.28	0.650	0.560	0.240	<LoQ	0.310	3.100	+	+		+++	+	
	Ba	Metals	16	8.0	5.70	15.100	15.300	5.300	<LoQ	17.700	85.100	++	+		+++	+	
	Tl	Metals	<LoQ		<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ						
	Pb	Metals [a]	1.5	1.8	0.11	0.150	0.230	0.140	0.640	0.110	<LoQ	++	++	++	+		+++
	U	Metals	1	0.63	<LoQ	0.890	0.830	0.160	0.130	0.220	6.600	+	+	++	+++	+	

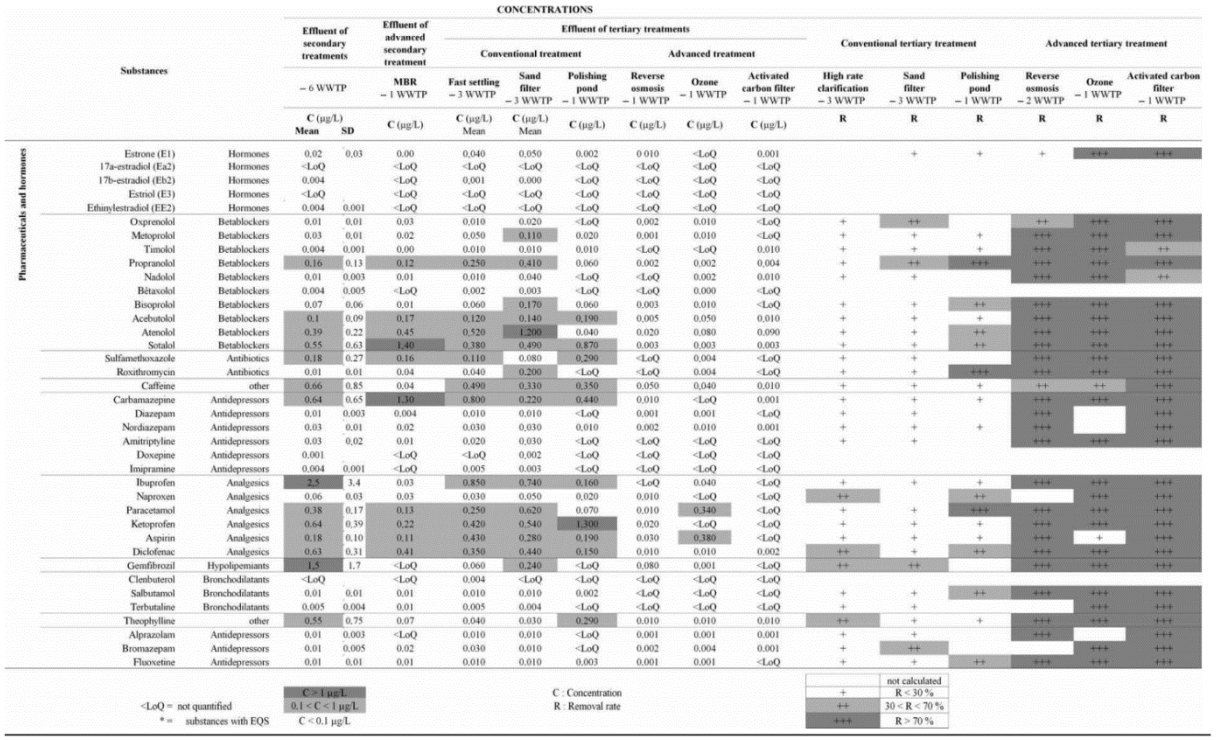


Table 8.2.4.2-25: Numbers of substances quantified in effluents of conventional AS process, MBR process, conventional and advanced tertiary processes (calculated from data of Table 8.2.4.2-24, total 127 substances)

	Effluent of secondary treatments		Effluent of tertiary treatments					
			Conventional treatment			Advanced treatment		
Parameter	Conventional treatment: AS – six WWTP	Advanced treatment: MBR – one WWTP	Fast settling – three WWTP	Sand filter – three WWTP	Polishing pond – one WWTP	Reverse osmosis – one WWTP	Ozone – one WWTP	Activated carbon filter – one WWTP
Number of quantified substances (C > LoQ)	88	58	81	85	54	51	61	42
Number of non-quantified substances (C < LoQ)	39	69	46	42	73	76	66	85
Number of substances with C > 1 µg/L	23	13	16	IS	16	8	10	16
Number of substances with C > 0.1 µg/L	48	30	46	49	35	22	26	22
Total	127	127	127	127	127	127	127	127

Results and Discussion

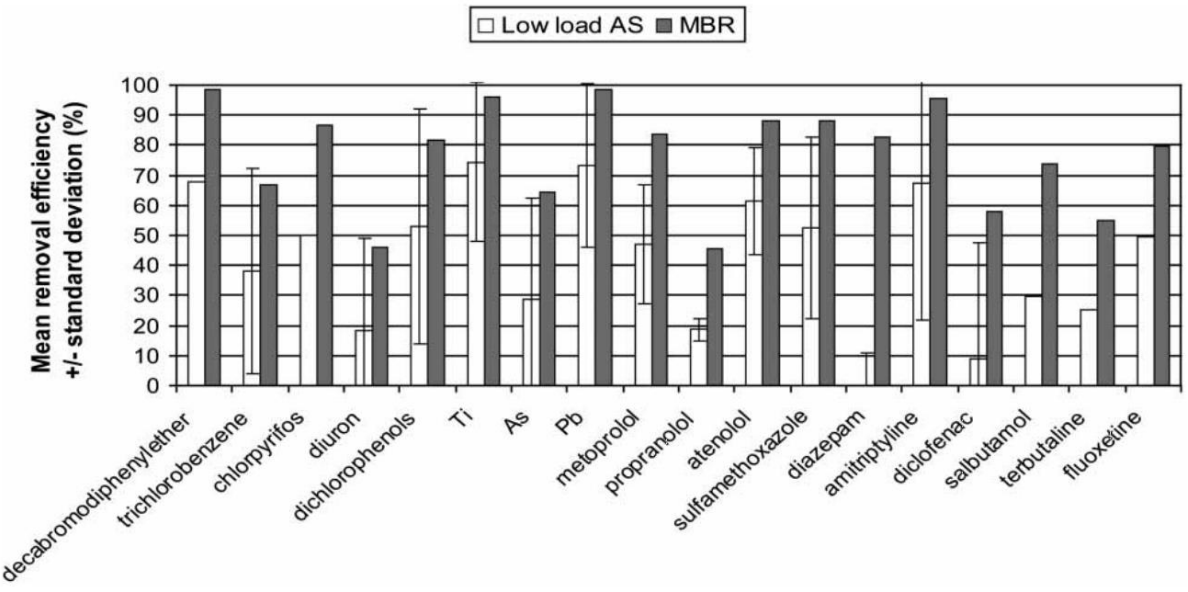
Table 8.2.4.2-24 presents, for each micro-pollutant, the mean concentration (C) in the effluents of the six conventional secondary stages (activated sludge) upstream of the tertiary treatment lines studied, in the effluent of the MBR and in the effluent of each type of tertiary process (conventional and advanced). The removal efficiency ranges (R) of the tertiary processes are also presented. In addition, Table 8.2.4.2-25 summarises the numbers of substances quantified in effluents of these processes in order to achieve a better comparability.

Efficiency of the MBR process

In the treated water of MBR process, the concentrations were below the LoQ for 69 micro-pollutants (39 were non quantified for low load activated sludge (AS)) as shown in Table 8.2.4.2-25; 30 micro-pollutants were measured at concentrations higher than 0.1 µg/L, instead of 48 micro-pollutants for the effluents of AS; and 13 micro-pollutants were measured at concentrations higher than 1 µg/L like DEHP, some metals and two pharmaceuticals sotalol, carbamazepine) (23 for AS). Compared to the effluent of AS, lower concentrations were measured for adsorbable micro-pollutants like decabromodiphenylether, Pb, Hg, 4-NP. These trends suggest a higher level of micro-pollutant retention with MBR compared to AS process. In addition, removal efficiencies at the MBR plant were calculated and compared to the mean removal efficiencies from six low load activated sludge plants, obtained with the same methodology for sampling, analysis and data processing. For 18 substances, removal efficiencies of the MBR were significantly higher than individual values obtained with the AS plants (more than 20 % difference compared to the mean values of AS or above the upper limit of the confidence interval). This suggests a potential improvement of removal efficiency for specific compounds that should be confirmed by other studies. The substances concerned are trichloromethane, naphthalene, chlorpyrifos, AMPA, diuron, sulfamethoxazole, ibuprofen, alprazolam, amitriptyline, and several betablockers (Figure 8.2.4.2-21).

Some studies have already shown higher removal for MBR for a limited selection of micro-pollutants referring to the effect of higher sludge retention time. But at almost similar SRT as AS, and with a 20 % higher sludge concentration (5 g mixed liquor total suspended solids/L in MBR instead of 2-4 g/L in AS), the specific bacterial population and the presence of exopolysaccharides in the biological tank of the MBR process may favour adsorption and biodegradation processes.

Figure 8.2.4.2-21: Comparison of removal efficiencies of the MBR process with removal efficiencies from low load conventional AS process



Efficiency of conventional tertiary processes

When applying high rate clarification or sand filter to a secondary effluent, the number of quantified micro-pollutants was only slightly reduced from 88 to 81-84 micro-pollutants in the effluents (depending on the plant). The number of quantified micro-pollutants was reduced from 60 to 54 between the inlet and the outlet of the polishing pond (this process was located in a rural area where less micro-pollutants were quantified). Depending on the process, 35 to 49 micro-pollutants are still present at concentration levels >0.1 µg/L. Between 16 and 19 micro-pollutants were quantified at concentrations higher than

1 µg/L. Three priority pollutants were found at concentrations exceeding the EQS in tertiary treated water (DEHP, 4-NP, chlorpyrifos), which could be a matter of concern when the flow of the receiving body is very low. Differences of removal efficiencies have been measured between the studied conventional tertiary treatment processes. With fast settling tank, removal efficiencies higher than 70% were measured for two metals (Ag and Al), while 30-70% removal was calculated for several metals (Zn, Ti, Cr, Pb, Cd and Hg), organic compounds (glyphosate, diclofenac, naproxen, aspirin, gemfibrozil and dichlorophenols) and VOCs (tetrachloroethylene, dichloromethane). Removal efficiencies below 30% were measured for all other micro-pollutants, in particular for pharmaceuticals and for polar pesticides. For priority pollutants, similar results were recently shown for one fast chemical settler. Through the sand filtration stage, a removal efficiency between 30-70% was measured for alkylphenols (4-NP, 4-t-OP and ethoxylates), glyphosate/AMPA and some betablockers, whereas high rate clarification had removal efficiencies below 30 % for these substances. With the polishing pond process, removal efficencies lower than 30 % were measured for most micro-pollutants except for some compounds like DEHP, paracetamol, roxithromicin and some betablockers (with removal efficiency higher than 70%); and bisoprolol, nadolol, sotalol, naproxen, diclofenac, salbutamol and fluoxetine, that were removed with removal efficiencies between 30 and 70%. In this case, photodegradation and high hydraulic retention time could be the main removal factors.

Efficiency of advanced tertiary processes

The number of quantified micro-pollutants in the effluent of tertiary treatment was reduced from 88 to 42-61 depending on the process. As many as 13 micro-pollutants were never quantified in the effluents of all types of advanced tertiary treatments: chlorobenzene, di-chlorophenols, tetra-chlorophenols, bromophenols, dibromophenols, naphthalene, trichlorobenzene, hexachlorocyclohexane, pesticides (chlorpyrifos, dieldrin), pharmaceuticals (doxepine) and hormones (17β-oestradiol, ethinyl-estradiol). Depending on the process, 22 to 27 micro-pollutants were relevant (concentration levels >0.1 µg/L), that is 50% less than for AS. Eight to 16 micro-pollutants were quantified with concentrations higher than 1 µg/L. Only DEHP was found at concentrations close to the EQS. Removal efficiencies higher than 70% were measured for 40-45 micro-pollutants for reverse osmosis and activated carbon filtration. For ozonation, due to low efficiencies of treatment on metals, 31 micro-pollutants were removed at R> 70%. Ozone oxidation allowed high removal for DEHP (75%) with double bonds accessible to ozone and hydroxyl radicals, but was not efficient for metals or alkylphenols, confirming previous studies. Reverse osmosis led to the retention of an extended range of micro-pollutants (especially metals and VOCs). DEHP was not retained by reverse osmosis or activated carbon filtration in this study. However, these results should be considered with care since the concentration levels of DEHP in tertiary processes were close to the analytical blanks. Except for metals and VOCs, the activated carbon filtration proved to retain a comparable number of micro-pollutants to reverse osmosis, but with slightly lower removal efficiencies. With the activated carbon filtration AMPA was well removed. For all of these treatments, several pesticides (diuron, simazine, glyphosate) were removed with efficiencies higher than 90%, and almost 100% for most pharmaceuticals (including refractory betablockers).

Conclusion

From on-site investigations carried out on seven wastewater treatment plants, the removal efficiencies of conventional and advanced tertiary processes have been assessed for 100 micro-pollutants quantified in secondary effluents.

– Ultrafiltration membrane in biological processes (MBR) could improve removal efficiency for some micro-pollutants in addition to disinfection capacities and suspended solids retention. This is an additional advantage when reuse of wastewater is expected.

– Conventional tertiary processes like fast tertiary settling and sand filtration can already achieve significant (30-70 %) removal for adsorbable micro-pollutants and could therefore be considered as a first complement to the activated sludge process.

– Advanced tertiary processes, like ozone oxidation, activated carbon filtration and reverse osmosis filtration, are efficient to complete the removal of polar pesticides and pharmaceuticals. Reverse osmosis provides a removal of a wider range of micro-pollutants, including metals and less polar organic micro-pollutants, that were not retained by other processes. However, it is also the most expensive

technology and the fate of the concentrate should be mastered to get a sustainable process. Ozone oxidation is the less expensive technology but the fate and toxicity of by-products still remains an issue to be investigated. Activated carbon filtration appears as an interesting alternative, but the reliability and the life duration of adsorbing material needs to be further investigated.

– Removal rates for glyphosate and AMPA were: 30 – 70% for glyphosate and AMPA for sand filtration, <30% for AMPA for reverse osmosis and ozone treatment, but >70% for glyphosate for reverse osmosis and ozone treatment; >70% for both glyphosate and AMPA for activated carbon filtration

The choice of the most appropriate technology should be made by matching the affordable cost in relation to water quality objectives, either to preserve the receiving water bodies or to secure the reuse of treated wastewater.

Assessment and conclusion by applicant:

The article describes the efficiency of different wastewater treatment processes to remove glyphosate and AMPA among other substances from wastewater for reuse application. Different processes are described and their specific efficiency is reported. Removal rates for glyphosate and AMPA were: 30 – 70% for glyphosate and AMPA for sand filtration, <30% for AMPA for reverse osmosis and ozone treatment, but >70% for glyphosate for reverse osmosis and ozone treatment; >70% for both glyphosate and AMPA for activated carbon filtration.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on different water treatment efficiencies for glyphosate and AMPA removal from wastewater. Results are obtained from seven WWTP of various sizes, which included various types of treatment, which are thoroughly described in table 34. Influent and effluents of the studied processes were collected under dry weather flow conditions during two successive 24 h or 2 h periods

Analytical method are described.

Removal rates were reported as being 30 – 70% for glyphosate and AMPA for sand filtration, <30% for AMPA for reverse osmosis and ozone treatment, but >70% for glyphosate for reverse osmosis and ozone treatment; >70% for both glyphosate and AMPA for activated carbon filtration.

It is worth noting that, as indicated by the authors, the removal efficiency data are displayed as a removal range (<30 %, 30-70 % and >70 %), since the analytical uncertainty and the variability of the concentrations related to micro-pollutants in wastewater do not allow to certify precise values.

Data from this study are included in the review provided in [redacted], 2020.

Schoonenberg Kegel et al., 2010

Data point:	KCA 7.5/088
Report author	Schoonenberg Kegel, F. et al.
Report year	2010
Report title	Reverse osmosis followed by activated carbon filtration for efficient removal of organic micropollutants from river bank filtrate
Document No	Water science and technology (2010) Vol. 61, No. 10, pp. 2603-10
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No

Acceptability/Reliability:	Reliable with restrictions

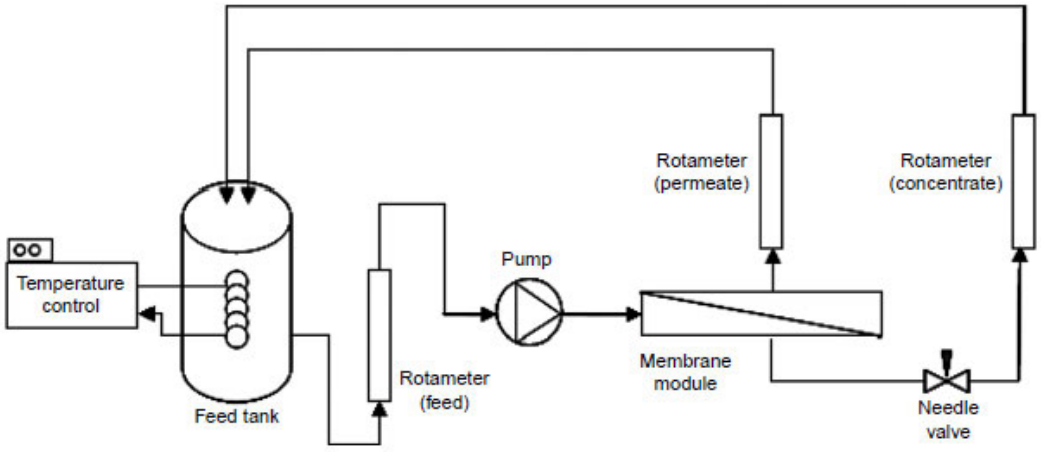
Drinking water utilities in Europe are faced with a growing presence of organic micropollutants in their water sources. The aim of this research was to assess the robustness of a drinking water treatment plant equipped with reverse osmosis and subsequent activated carbon filtration for the removal of these pollutants. The total removal efficiency of 47 organic micropollutants was investigated. Results indicated that removal of most organic micropollutants was high for all membranes tested. Some selected micropollutants were less efficiently removed (e.g. the small and polar NDMA and glyphosate, and the more hydrophobic ethylbenzene and napthalene). Very high removal efficiencies for almost all organic micropollutants by the subsequent activated carbon, fed with the permeate stream of the RO (reverse osmosis) element were observed except for the very small and polar NDMA and 1,4-dioxane. RO and subsequent activated carbon filtration are complementary and their combined application results in the removal of a large part of these emerging organic micropollutants. Based on these experiments it can be concluded that the robustness of a proposed treatment scheme for the drinking water treatment plant Engelse Werk is sufficiently guaranteed.

Materials and methods

Filtration equipment and protocol

The water used for the RO experiments was sampled after the pre-treatment of DWTP (Drinking Water Treatment Plant) Engelse Werk. For the activated carbon experiments, a batch of permeate was used as feed water. The 4-inch, single spiral wound membrane element filtration set-up and protocol for the RO experiments is schematically depicted in Figure 8.2.4.2-22. The feed water was fed from a 600 L stainless steel vessel. The feed solution was delivered to a pressure vessel, accommodating a single 4040-membrane element, by a pump. Applied transmembrane pressure was regulated using a needle valve in the concentrate stream, with transmembrane pressure measured with a precision manometer. Adsorption on test unit parts was ruled out, since no significant loss of solutes was observed when the water was recirculated over the installation without a membrane installed. All experiments were performed in a recycle mode with a single batch of water, with both permeate and concentrate recycled back into the feed reservoir. An immersed stainless-steel coil with cooling liquid fed from a cooling system was used to maintain a constant feed water temperature. Membrane filtration experiments were carried out at a constant cross-flow velocity of 0.2 m/s, which corresponds to a feed flow of 1,500 l/h and a concentration polarization factor of 1.07. The recovery is kept constant at approximately 10%. Permeate flux and temperature were set to approximately 20 l/(m^2.h) and 20 ± 1°C, respectively. Feed, permeate and concentrate samples were taken after 4 days of filtration and analyzed for organic micropollutants: 4 days was sufficient to reach adsorption equilibrium and ensure that steady-state rejection values were obtained. The granular activated carbon column was 1 m in height and had an inner diameter of 35 mm. The column contained approximately 0.7 L of carbon resulting in a filter bed depth of 0.7 m. The column was fed with a batch of RO permeate from a stainless steel tank. The hydraulic loading was set to 14 L/h resulting in an empty bed contact time of 3 min. Samples of feed and effluent of the column were taken after treatment of 1200 bed volumes to see whether break-through of some micropollutants could already be observed after this time period.

Figure 8.2.4.2-22: Membrane filtration set-up for rejection experiments.



RO membranes and activated carbon

The membranes used in this study were all commercially available reverse osmosis membranes: Trisep X20 and ACM5 (Trisep Corp., Goleta CA, USA) and Hydranautics ESPA1 and ESPA4 (Nitto-Denko/Hydranautics, Oceanside CA, USA). All membranes are thin film composite membranes with an aromatic polyamide top layer. Before use, all membranes were rinsed with Milli-Q water for two hours in order to remove preservation liquids present in the membranes. Afterwards, the membranes were characterized for pure water permeability with Milli-Q water and for NaCl rejection with a 1,500 ppm NaCl solution in Milli-Q water. Membrane properties are summarized in Table 8.2.4.2-26. Membrane contact angles were determined using the sessile drop method. The Zeta potentials were measured in a background solution containing 10 mM NaCl and 1 mM $NaHCO_3$. Membranes with different membrane properties were chosen (e.g. pure water permeabilities), to be able to select the most suitable membrane for the application, based on both energy demand and organic micropollutant removal. The granular activated carbon was supplied by Norit Nederland B.V. (Amersfoort, the Netherlands). The extruded grade Norit Row Supra 0.8 was chosen based on its multi-purpose adsorption characteristics, low hydraulic resistance and high resistance to attrition during regeneration. The bed density of the carbon is 345 kg/m^3 and the raw carbon material is peat. Freshly regenerated activated carbon was used in the experiments, so no pre-loading of natural organic matter (NOM) or other organic pollutants was present on the carbon before the start of the experiments.

Selected organic pollutants and analysis

The spiked organic pollutants were selected for two main reasons. Firstly, some emerging micropollutants, already occurring in Dutch surface- and ground waters were chosen. Examples include glyphosate, carbendazim, bentazon and MTBE. Since the dosing tests were carried out for a drinking water utility, assessment of the removal of problematic substances from the source waters was a necessity. Secondly, other organic solutes were also dosed, and these were mainly selected for their different physico-chemical properties. In a previous publication (Verliefde et al. 2008), it was shown that solute charge, solute hydrophobicity and solute size may all have an influence on solute rejection by NF/RO treatment. Therefore, solutes were divided in different categories of increasing hydrophobicity (expressed as log K_{ow}). Within each category of hydrophobicity, different solutes were chosen with increasing size (expressed as molar mass). Moreover, some charged solutes (positively, as well as negatively charged) were included. All micropollutants were dosed in concentrations that were 200 times higher than the limit of quantitation (LOQ) of the respective analysis method for that pollutant. Thus 99.5% removal could be quantified. The cocktail of organic micropollutants was prepared as a concentrated stock in 10 L of Milli-Q water. In order to prevent co-solvent effects and possible problems with biological growth in the system, no methanol was used to facilitate dissolution of the pharmaceuticals. For the RO rejection experiments, the desired volume of this stock solution was then added to the feed tank, containing the Engelse Werk ground water. For the activated carbon experiments, the desired volume of the stock solution was added to a tank containing 750 L of RO permeate (the RO

permeate did not contain any residuals of trace organic contaminants). Information on the analytical protocol can be found in (Sacher et al. 2001).

Table 8.2.4.2-26: Membrane properties for selected membranes for comparison of organic micropollutant rejection

Membrane	Pure water permeability (m/(s.bar))	Contact angle (°)	% NaCl rejection	Zeta-potential at pH 7 (mV)
Trisep ACM5	1.4×10^{-6}	35	98.5	−20
Trisep X20	9.9×10^{-7}	43	99.5	−10
Hydr. ESPA4	2.1×10^{-6}	70	99.0	n.d.
Hydr. ESPA1	1.5×10^{-6}	25	99.3	−27

Results and Discussion

Reverse osmosis rejection experiments

It was apparent that for almost all solutes rejection values were very high (.95%). However, some solutes showed low rejection. The low rejection of these solutes was consistent for all four membranes. Especially the removal of NDMA was low. This was probably due to the very small size and very compact structure of NDMA. The removal of the hydrophobic solutes ethylbenzene and naphtalene was very low, even though these solutes were larger than, for example, NDMA. This was probably due to hydrophobic interactions of these solutes with the hydrophobic membrane matrices, resulting in an increased partitioning of these solutes into the membranes and thus an increased transport through the membranes. Also the rejection value of glyphosate was lower than expected. Glyphosate is a very polar molecule that has several polar functional groups (positively, as well as negatively charged). At pH 7, there is a high positive charge density in the middle of the molecule, leading to a very high dipole moment (6.7 Debye) in the molecule and charge attraction towards the negatively charged membrane surface. Moreover, since glyphosate is a stretched molecule, steric hindrance is also lower and glyphosate permeates through the membrane quite easily. Both Hydranautics membranes showed lower rejection values for most solutes compared to the Trisep membranes. Comparing the performance of the two Trisep membranes, it was interesting to notice that rejection values were slightly higher for most solutes with the ACM5 membrane than with the X20 membrane, even though the ACM5 has a higher pure water permeability and a lower NaCl rejection (Table 8.2.4.2-26). The reason for this difference in organic solute rejection is probably the higher hydrophobicity of the X20 membrane (as shown in the contact angle measurements). The ACM5 membrane is more hydrophilic, which results in an increased transport of water and thus higher fluxes of this element at similar feed pressures, but also results in a decrease of hydrophobic interactions between hydrophobic solutes and the membrane matrix. This results in increased rejection values for hydrophobic solutes. Based on these results, it was decided that the ACM5 membrane will be applied in the new treatment scheme. All experimental rejection data for all solutes on the ACM5 membrane are shown in Table 8.2.4.2-27. The experimental rejection values were compared to predicted rejection values, using a QSAR (quantitative structure-activity relationship) model. The modelled rejection values predicted experimental rejection data quite well. As can be seen in Table 8.2.4.2-27, the rejection values of some solutes (e.g. dibutylphthalate) could not be determined. This was due to the low feed concentrations of these solutes on the fourth day of the experiments (when rejection is measured), probably due to volatilisation or adsorption of the solutes on the membrane polymer matrix. Adsorption on other test unit parts was ruled out, since almost all test parts were made out of stainless steel.

Based on the rejection values obtained on the single ACM5 membrane element in the laboratory-scale unit, some rough estimations were made for a full-scale installation, operating 80% recovery. These rough estimations were based on a full-scale rejection model. It was apparent that the ACM5 element performs extremely well in organic micropollutant removal applications: except for NDMA, most problematic organic pollutants (e.g. the pesticides diglyme, triglyme, atrazine, metamitron, bentazon and glyphosate and the pharmaceuticals phenazon, carbamazepine and ibuprofen) were expected to be removed for more than 90%. However, 90% removal is still not complete removal, and a subsequent activated carbon filtration step might still be necessary.

Activated carbon adsorption experiments

The removal of the selected organic pollutants after treatment of 1,200 bed volumes (carbon was freshly regenerated before use) on the ACF column is also summarized in Table 8.2.4.2-27. It was apparent that, even with the short contact times used, removal of most micropollutants was high (> 95%). However, removal of some pollutants, such as NDMA; 1,4-dioxane and 2-methylisoborneol (2-MIB) was more problematic. For NDMA and 1,4-dioxane, this low removal could be expected, due the hydrophilic character of these substances. Moreover, NDMA experienced significant competition from the other organic pollutants because it was dosed in extremely low concentrations (200 ng/l). For 2-MIB, no breakthrough of the solute through the column was expected. 2-MIB is very hydrophobic, and since ACF adsorption mainly occurs through hydrophobic van der Waals interactions, a high removal was expected. Especially the small size of the molecule should make it easy for this molecule to diffuse into the small micropores of the carbon, where it should adsorb readily. Maybe the contact time of 3 min was not enough to allow this pore-diffusion for 2-MIB. Also, log K_{ow} is apparently not always the most suitable parameter to describe adsorption inter-actions. This is because log K_{ow} measures the differences in interactions of a solute in a water phase and an octanol phase, and octanol does not represent the carbon surface very well. Despite the low removal for NDMA; 1,4-dioxane and 2-MIB, no breakthrough of any other substance through the column was observed. This was partly due to the freshly regenerated carbon, which should have a high adsorption capacity anyway. However, 1,200 bed volumes have already been treated, so the carbon capacity would already be lower than for freshly regenerated carbon. The removal capacity of the carbon was also high, because of the removal of NOM which would normally compete with the organic micropollutants for adsorption sites on the carbon, in the reverse osmosis step. This NOM removal not only diminishes the competition between NOM and the micro-pollutants for adsorption sites on the activated carbon, but also reduces the carbon pore blocking by large NOM molecules. As a consequence short empty bed contact times can be used for the ACF, or the time before regeneration of the column can be extended. This reduces investment costs for the ACF considerably. Compared with full stream RO treatment, split stream RO treatment will result in an increased preloading with NOM of the ACF. Nevertheless split stream RO treatment decrease the NOM preloading of the ACF significant.

Reverse osmosis and subsequent activated carbon filtration

The combined removal efficiency for organic micropollutants of split treatment with the ACM5 membrane and the subsequent activated carbon filter was calculated. The results are also shown in Table 8.2.4.2-27. The calculation was based on a RO installation equipped with the ACM 5 membrane, operating at a recovery of 80% on a by-pass stream of 50% of the total feed stream. Removal efficiency for all solutes was extremely high, except for the smallest hydrophilic solutes (NDMA and 1,4-dioxane). Fortunately, these two pollutants are absent in the raw water of DWTP Engelse Werk. Moreover, results of ongoing research suggests that small, hydrophilic solutes are preferentially removed by biological degradation in processes such as river bank or dune filtration. The combination river bank filtration RO – ACF would thus be able to remove almost all organic micropollutants. Therefore, we do expect that the proposed treatment scheme can remove these substances if they would be present in the river IJssel.

Table 8.2.4.2-27: Solute physico-chemical characteristics, initial feed concentrations, experimental rejection by the ACM5 membrane, experimental removal efficiency by ACF filtration and calculated values for the rejection at 80% recovery and for the combination of RO (by-pass 50%) and subsequent ACF

	Properties			Experimental data		Calculated data	
Compound	MW (g/mol)	log K_{ow} (–)	Initial feed concentration (µg/L)	RO Recovery = 10%	ACF	RO Recovery = 80%	RO (by-pass 50%) subsequent ACF
NDMA	74	−0.57	0.2	74	21	60	45
1,4-dioxane	88	−0.27	2,000	96	18	92	56
NMOR	116	−0.44	0.2	99	99	97	99
Diglyme	134	−0.36	30	99	n.d.	97	n.d.
Glyphosate	169	−4.00	10	90	>99	82	>99
Triglyme	178	−0.76	50	99	n.d.	97	n.d.
Caffeine	194	−0.07	10	99	>99	97	>99
TBA	74	0.35	20,000	99	n.d.	97	n.d.
MTBE	88	0.94	10	>99	>99	>97	>99
Phenazon	188	0.38	2	>99	>99	>97	>99
Metamitron	202	0.83	10	>99	>99	>99	>99
Terbutaline	225	0.90	2	>97	>99	>93	>99
Sulfamethoxazol	253	0.89	2	>99	>99	>97	>99
Sotalol	272	0.24	2	>98	>99	>95	>99
Pentoxifylline	278	0.29	2	>99	>99	>97	>99
ETBE	102	1.92	10	n.d.	>99	n.d.	n.d.
TAME	102	1.92	20	>97	>99	>93	>99
2,4-dinitrophenol	184	1.67	20	>98	99	>95	99
Carbendazim	191	1.52	3	99	99	97	99
Monuron	199	1.94	10	99	>99	97	>99
Metribuzin	214	1.70	10	99	>99	97	>99
Metoxuron	229	1.64	10	99	>99	97	>99
Pirimicarb	238	1.70	10	99	>99	97	>99
Bisphenol-S	250	1.65	10	99	>99	97	>99
Metoprolol	267	1.88	2	>99	>99	>99	>99
TCEP	285	1.44	10	n.d.	n.d.	n.d.	n.d.
Benzene	78	2.13	100	88	>99	79	>99
Isoproturon	206	2.87	10	99	>99	97	>99
Chlorotoluron	213	2.41	10	99	>99	97	>99
Atrazine	216	2.61	10	99	>99	97	>99
Diethylphthalate	222	2.42	100	>99	n.d.	>99	n.d.
Diuron	233	2.68	10	n.d.	>99	n.d.	n.d.
Carbamazepine	236	2.45	2	99	>99	97	>99
Bentazon	240	2.34	10	>99	>99	>99	>99
Metobromuron	259	2.38	10	99	>99	97	>99
Dimethenamid	276	2.15	5	99	>99	97	>99
Ethylbenzene	106	3.15	40	18	n.d.	9	n.d.
Naphthalene	128	3.30	50	68	n.d.	53	n.d.
2-MIB	168	3.31	50	99	97	97	99
Ibuprofen	206	3.97	2	>99	>99	>97	>99
Mecoprop (MCPP)	215	3.13	10	>99	>99	>97	>99
Bisphenol-A	228	3.32	2	99	>99	100	>99
Linuron	249	3.20	10	>99	>99	>97	>99
Estrone	270	3.13	2	99	>99	100	>99
Dibutylphthalate	278	4.50	100	n.d.	n.d.	n.d.	n.d.
Diclofenac	296	4.51	2	>99	>99	>97	>99
Bezafibrate	361	4.25	2	>99	>99	>97	>99

n.d.: not determined.

Conclusion

The capability of RO and ACF to remove emerging organic pollutants was studied. RO offers very high rejection values for almost all solutes. Lower rejection values are observed for hydrophilic solutes with a negative log K_{ow} value and a low molecular weight, and for relatively low molecular weight hydrophobic solutes. The removal efficiency of the solutes by activated carbon filtration, even with the low contact times used, is extremely high as well. Still, removal of some pollutants, such as NDMA; 1,4-dioxane and 2-methylisoborneol (2-MIB) is more problematic. RO and subsequent activated carbon filtration are complementary and their combination results in removal of a large part of the emerging organic micropollutants, since almost the whole range of solute hydrophobicity is covered. The barrier against micropollutants in the treatment scheme of DWTP Engelse Werk is based on a split stream treatment with reverse osmosis, followed by full-stream activated carbon filtration. The results of this research will be used as input for the analysis program that Vitens performs to monitor the water quality of the river IJssel. The removal capabilities of this treatment scheme are very high for almost all organic micropollutants dosed in this study, except for the smallest hydrophilic solutes (NDMA and 1,4-dioxane). Fortunately, these two pollutants are absent in the raw water of Engelse Werk. The robustness of the treatment scheme is therefore sufficiently guaranteed. Moreover, there is an opportunity to upgrade the scheme by introducing full-stream RO treatment.

Assessment and conclusion by applicant:

The study describes the removal of glyphosate among other substances from drinking water by reverse osmosis followed by activated carbon filtration. The substance properties and analytical methods are insufficiently described. The examined method focus on conservative filtration methods, no degradation products or processes are described.

The study is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

Agrees with applicant's conclusions.

The study is considered reliable with restrictions. It provides supportive information on glyphosate and AMPA removal from reverse osmosis, followed by activated carbon filtration, with the limitations exposed by applicant in its conclusion above.

Peschka et al., 2006

Data point:	CA 7.5/072
Report author	Peschka, M. *et al.*
Report year	2006
Report title	Trends in pesticide transport into the River Rhine
Document No	Hdb Env Chem Vol. 5, Part L (2006): 155–175 DOI 10.1007/698_5_016
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

The occurrence of relevant pesticides in the River Rhine and two of its tributaries is presented over a period of ten years. Trace determinations of 66 target pesticides and their metabolites in water from the River Main and the River Nidda were performed on continuously sampled wastewater and surface water utilizing different solid phase extraction protocols and detection by gas chromatography mass spectrometry, directly or after derivatization. The transport rates of pesticides in municipal wastewater treatment plant (WWTP) effluents and surface waters were determined from data obtained in 1994, and

these show that WWTPs contribute significantly to the pesticide pollution in the surface water. A trial education program providing improved methodology, spraying equipment and support to farmers living close to a single WWTP lead to a drastic reduction (more than 90%) in the total pesticide transport caused by this WWTP.

During two extensive sampling campaigns in 1999 and 2000, mixed samples from a total of 106 (for 1999) and 35 (for 2000) WWTPs in agricultural used areas from Hesse (Germany) were investigated for selected priority pesticides and metabolites. In this case, the mitigation measures mentioned above were found to be unsuccessful overall, which is most likely attributable to less interaction with the pesticide users as compared to projects in small villages with high public attention.

Methods

A total of 62 pesticides were selected including glyphosate and its metabolite AMPA.

Sampling:

Receiving streams (the Main and the Nidda) and WWTP located in agricultural areas were chosen for study in Hesse, Germany. Mainly grain and maize, but also rape and sugar beet are grown over the catchment area of the River Nidda. No companies discharging industrial waste containing pesticides were located on the river. At Frankfurt-Nied, the Nidda joins with the River Main, which subsequently joins with the River Rhine close to Bischofsheim. The Main receives discharges from many chemical industries, including those producing pesticides. The period from April to May was selected for sampling, as this time frame reflects the peak period for pesticide application.

River samples were taken twice a week from the Rhine during a period of ten years (1993–2003). In the period from 6th April to 17th May 17 1999, a total of 106 WWTP effluent samples were collected twice as three-week mixed samples. The sites found to be most polluted in 1999 were then sampled again over the same period in 2000. During the same time period, mixed samples from the WWTP at Woelfersheim, Hesse, Germany were also taken daily from 1994 to 1998. Mixed weekly surface water samples were collected automatically from the Main during pesticide application time (April to June), and collected as two-week mixed samples for the rest of the year.

Analysis:

Rhine samples were filtered if necessary and then enriched over C-18 cartridges. Main, Nidda and WWTP samples were passed through glass fiber filters, prewashed with methanol and Milli-Q water before solid phase extraction (SPE) was performed. Analysis was by GC/MS.

Results and discussion

Glyphosate was present in the river Main from April to September at a concentration of up to 0.1 µg L^{-1}. In the Nidda it was present over the whole year at a maximum concentration of 0.4 µg L^{-1}, which is due to the higher amount of waste water in the Nidda. The concentration of the metabolite AMPA exceeded the glyphosate concentration by several times.

The results from bank filtration experiments showed that glyphosate was removed after a distance of about 200 m, whereas AMPA needed about 300 to 500 m to be completely eliminated. The experiments were carried out at the waterside of the Main.

Glyphosate and AMPA were not detectable in groundwater, even though they had been applied in massive amounts around rail tracks since 1991.

Water treatment at the WWTP included several steps, namely floc filtration, gravel filtration, and activated carbon filtration. In order to evaluate the efficiencies of those steps, samples were taken before and after each step so that the glyphosate and AMPA could be quantified. The first step, flocculation with activated silicic acid and addition of potassium permanganate and aluminum salts, gave an elimination rate of 39 ± 14% for glyphosate and 22 ± 15% for AMPA. Gravel filtration reduced both by less than 10%. Activated carbon filtration also reduced glyphosate by < 10%, and AMPA by 21 ± 9%. These results showed that glyphosate and its metabolite were not completely removed in a raw water treatment facility.

Conclusion

Sampling from sewage drains leading to WWTPs showed that farms connected to sewage drains are the most important source of pollution. Analysis of puddles on roads and paths also showed pesticide contamination, which will also be a source of pesticide entry into sewage drains through rainfall wash-off. It can be assumed, however, that the main sources of pollution are the cleaning of spraying tools in farmyards and the pesticide lost from spraying machines traveling by road.

The diffuse pollution problem is a difficult one to tackle. Mitigation measures to circumvent diffuse pollution, even those resulting from many small point pollutions (such as those that were partially successful in this study) depend strongly on the motivation of the pesticide users and the level of interaction with them achieved, since a measurable result will only be obtained through the responsible application and use of pesticides by the farmers.

The removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminium salts, removal rate of 39±14% for glyphosate and 22±15% for AMPA; for gravel filtration removal rate of <10% for both compounds; and for activated carbon removal rates of <10% for glyphosate and 21±9% for AMPA.

Assessment and conclusion by applicant:

The article investigates urban sources of glyphosate in surface water in the Main and Nidda Rivers in Germany. The methods and results are briefly described. The removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminium salts, removal rate of 39±14% for glyphosate and 22±15% for AMPA; for gravel filtration removal rate of <10% for both compounds; and for activated carbon removal rates of <10% for glyphosate and 21±9% for AMPA.

Glyphosate was found in surface water at a concentration of up to 0.4 µg/L.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

The study is considered reliable with restrictions (no analytical method described). It provides supportive information on glyphosate and AMPA removal from bank filtration experiments. Data from this study are included in the review provided in [redacted], 2020.

The results from bank filtration experiments showed that glyphosate was removed after a distance of about 200 m, whereas AMPA needed about 300 to 500 m to be completely eliminated. The experiments were carried out at the waterside of the Main. It is noted that the flow velocity is not indicated. Also the measured concentration of glyphosate are not precisely reported, it is only indicated that Glyphosate is present in the river Main from April to September at a concentration of up to 0.1 µg/L. In the Nidda it is present over the whole year at a maximum concentration of 0.4 µg/L.

The removal rates for glyphosate and AMPA for some low-chemical processes were reported, however the detailed measurements used to estimate these rates are not given within the study.

- flocculation with activated silicic acid and addition of potassium permanganate and aluminium salts, removal rate of 39±14% for glyphosate and 22±15% for AMPA;
- for gravel filtration removal rate of <10% for both compounds; and
- for activated carbon removal rates of <10% for glyphosate and 21±9% for AMPA.

Shen et al., 2011

	CA 7.5/090 (Translation)
Report author	Shen, Y. et al.
Report year	2011
Report title	Ozonation of Herbicide Glyphosate (translated from the original Chinese-language paper)
Document No	Acta Scientiae Circumstantiae,31(8): 1647-1652
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

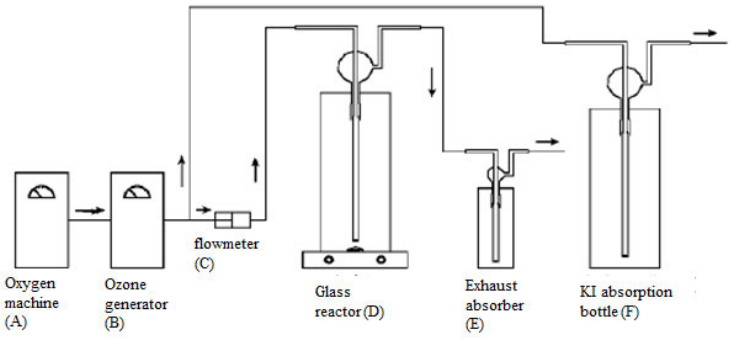
In this work, the influence of pH, ozone dosage and initial concentration of glyphosate on the degradation of glyphosate by ozone was investigated in detail. The pathway for the glyphosate degradation by ozone is also discussed. The results showed that the degradation rate of glyphosate by ozone increased with increasing ozone dosage, and decreased with increasing initial concentration of glyphosate. Under different pH conditions, the removal rate of glyphosate decreased in the following order: basic > neutral > acidic. The degradation of glyphosate by ozone was found to be accomplished by hydroxyl radicals. Intermediates of glycolic acid, glycine, AMPA, and orthophosphoric acid were identified during the ozonation of glyphosate. AMPA accumulated in the initial reaction time and decreased subsequently. Phosphate ions accumulated as reaction time increased.

Materials and methods

Experimental setup

The test adopted batch and semi-continuous test methods, and the test device is shown in Figure 8.2.4.2-23. High-purity oxygen was produced from an air source in medical oxygen machine (A), and ozone gas was produced by discharge of ozone generator (B). After measuring by flowmeter (C), through the bottom of the glass reactor (D), the microporous sand core diffuser ensured that the ozone gas was dissolved in the water. Remaining ozone was absorbed by the potassium iodide absorption bottle (F) after stirring. The reactor was made of quartz with a diameter of 100 mm, a height of 300 mm and a volume of 2.5 L. At the beginning of the test, the valve was first placed into the equilibrium position, the ozone was passed into the KI absorption bottle (F) and stabilized for a few minutes before the valve was transferred to the reaction. The air flow in the equilibrium phase was A-B-F, and the air flow in the test reaction phase was A-B-C-D-E.

Figure 8.2.4.2-23: Experimental set-up



Reagents and analytical methods

Glyphosate (purity 99.99%) and AMPA (aminomethylphosphoric acid) were purchased from Dima; H_3PO_4, NaH_2PO_4, HCl, NaOH were all analytical reagents and purchased from Beijing Chemical Reagent Co.; Water pH was adjusted by 1 mol L^{-1} NaOH and determined by 720APLUS Benchtop pH meter (Thermo Orion Co. USA); The UV-vis absorption was determined by U-3010 UV-vis

spectrometer (Hiachi Co. Japan); TOC was determined by N/C3000 TOC analyser (Jena, Germany); and the ozone dissolved in water was determined by the indigo method.

The principle of the indigo method for measuring the dissolved ozone concentration in water is to mix the ozone-containing water samples with acidic indigo reagents, and ozone degrades the solution's blue colour. The specific steps are: prepare indigo reagents according to the national standard method, add 9 mL samples to the colorimetric cup with 1 mL indigo reagent, mix and measure with a spectrophotometer.

Glyphosate was determined by HPLC with a pre-column derivatization. The pre-column derivatization was conducted as follow: add 0.5 mL sodium borate buffer solution (0.5 mol L^{-1}, pH = 9), 1 mL 4-toluene sulfonyl chloride ($C_7H_7ClO_2S$) acetonitrile solution (1 g L^{-1}) to 1.5 mL water sample, mix well and react at room temperature overnight. Then the reaction solution was filtered through 0.45 μm membrane and detected by HPLC. The mobile phase was methanol/50 mmol·L^{-1} NaH_2PO4 solution (pH = 5.5 adjusted by NaOH) (*v*/*v*, 20/80), flow rate was 1 mL min^{-1}, wavelength was 240 nm, injection volume 20 μL, and HYPERSIL GOLD column (250 mm × 4.6 mm × 5 μm, Thermo U.S.) was used. The retention time of glyphosate was 6.303 min, and the limit of detection was 0.1 mg·L^{-1}. The maximum limit of glyphosate in drinking water was specified as 0.7 mg L^{-1} by national standard GB 5749—2006, therefore the established method fully satisfied the requirement of this study.

The simultaneous detection of glyphosate and AMPA was also performed by HPLC with pre-column derivatization. The pre-column derivatization is the same as the above method. The mobile phase was acetonitrile /50 mmol·L^{-1} ammonium acetate aqueous solution (*V*/*V*, 20/80), flow rate was 1 mL min^{-1}, wavelength was 240 nm, the injection volume was 20 μL, and HYPERSIL GOLD column (250 mm ×4.6 mm, id: 5 μm, Thermo, USA) was used. The retention time of glyphosate was 3.6 min and that of AMPA was 3.2 min. The limit of detection of glyphosate was 0.1 mg L^{-1} and that of AMPA was 0.2 mg L^{-1}.

Ozone-oxidized glyphosate intermediates were determined by GC-MS. The specific treatment and heating procedures were as follows:

Pre-treatment method: 100 mL water samples at different reaction time was freeze-dried, the obtained solid powder was dissolved in 2.5 mL dichloromethane, and 0.1 mL BSTEA/TMCS silanizing reagents were added for silanization in 60°C water bath for 60 min. Then the anhydrous sodium sulfate calcined at 500°C was used for dehydration. The sample was then filtered with 0.45 μm organic membrane and concentrated to 0.5 mL by nitrogen before injection onto the GC-MS.

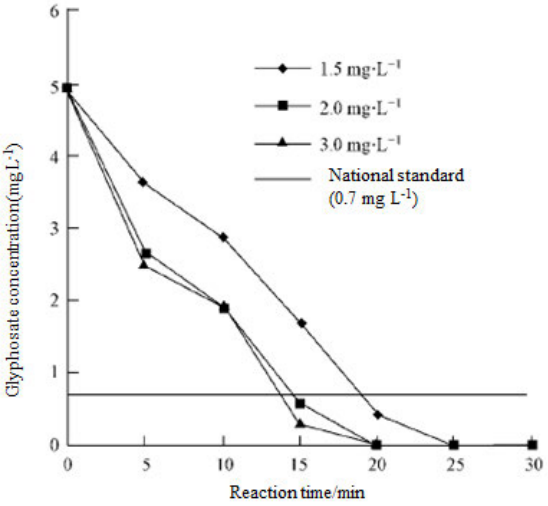
Heating procedures: 50°C for 3 min, heated up to 150°C over 5°C ·min^{-1} and for 5 min, then heat up to 250°C by 5°C ·min^{-1} and keep for 20 min. Injector temperature was 280°C, carrier gas was high purity helium, and the gas flow was 1 mL min^{-1}.

Results

Effect of ozone dosage on oxidative removal of glyphosate

In the study, the initial concentration of glyphosate was 5 mg/L, and the dosage of ozone was 1.5, 2.0 and 3.0 mg/L. The reaction was carried out for 30 min, and sampled every 5 min. The residual ozone in the sample was quenched by $NaSO_3$ to study the effect of different ozone dosage on the glyphosate concentration. Figure 8.2.4.2-24 shows that glyphosate was almost completely removed after 30 min. The larger the amount of ozone, the shorter the time it took for glyphosate to be completely removed. At 1.5 mg/L of ozone, glyphosate was completely removed at 25 min, while at 2.0 mg/L and 3.0 mg/L of ozone, glyphosate was completely removed at 20 min. There was not much difference for the reaction rates between 2 mg/L and 3 mg/L of the ozone dosage.

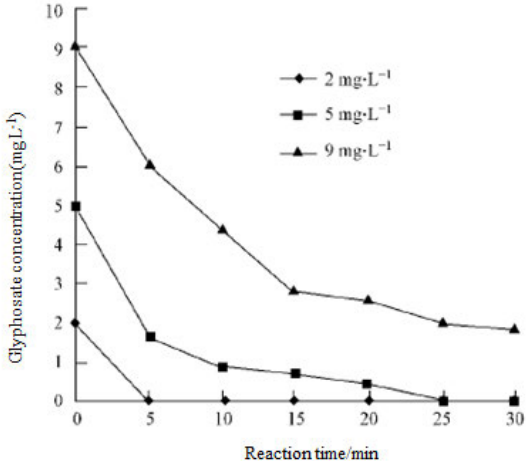
Figure 8.2.4.2-24: Degradation of glyphosate with different amounts of ozone



Effect of different initial concentration of glyphosate on the removal of glyphosate by ozone

In order to study the effect of different initial glyphosate concentration on their removal, three initial concentrations of glyphosate 2, 5 and 9 mg/L were applied, and the dosage of ozone was 1.5 mg/L. The reaction was kept for 30 min and the sampling was done every 5 min. The residual ozone in the sample was quenched with Na_2SO_3. It can be seen from Figure 8.2.4.2-25 that at the initial concentration of 2 mg/L, glyphosate was completely removed at 5 min, while at the initial concentration of 5 mg/L, glyphosate was completely removed at 25 min, however at the initial concentration of 9 mg/L, glyphosate was not completely removed and its concentration was still 2 mg/L at 30 min. Also, at the beginning of the reaction, the degradation rate was fast, while the degradation rate slowed down gradually as the reaction proceeded. This is because in the beginning the dissolved ozone concentration in the water was relatively high, as the reaction proceeded some of the ozone participated in the reaction and some decayed, then the concentration of ozone in the aqueous solution gradually decreased and the reaction rate slowed down.

Figure 8.2.4.2-25: Degradation of glyphosate at different initial concentrations of glyphosate

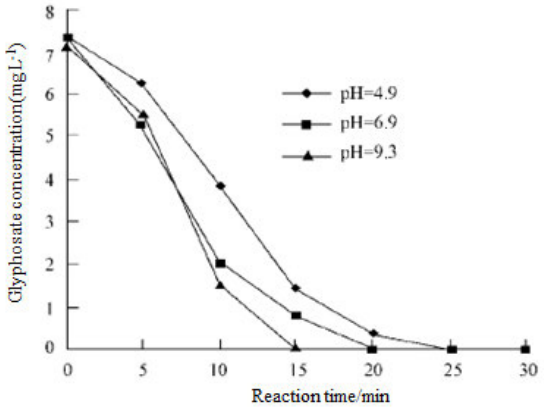


Effect of different initial pH on glyphosate oxidation

In the study, three initial pH values were selected, i.e. pH 4.9, pH 6.8, pH 9.3, to investigate the effect of pH on the glyphosate concentration. The initial concentration of glyphosate was 7.2 mg/L, and the ozone dosage was 1.5 mg/L. The reaction was kept for 30 min, sampling was done every 5 min, and the residual ozone was quenched with Na_2SO_3. The results (Figure 8.2.4.2-26) showed that glyphosate could always be removed in 30 min even at different initial pH values. The removal was the fastest in the alkaline system, i.e., completely removed at 15 min; it was slower under the neutral condition, i.e., removed completely at 20 min; the removal was the slowest under the acid conditions, i.e., removed

completely at 25 min. This is because there are more $OH^{\square}$ in alkaline systems, which can cause the reaction system to produce many hydroxyl radicals. And hydroxyl radicals produce more active radicals by chain reaction, accelerating the rate of oxidation of glyphosate by ozone.

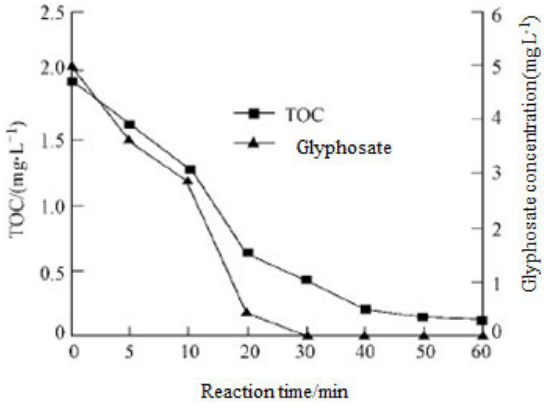
Figure 8.2.4.2-26: Degradation of glyphosate at different initial pH



Changes of TOC in the Ozone Oxidation of Glyphosate

The initial concentration of glyphosate was 5 mg/L, ozone dosage was 1.5 mg/L, reaction time was 60 min, sampling time was 0, 5, 10, 20, 30, 40, 50, 60 min, respectively. After sampling, the residual ozone was quenched with Na_2SO_3 to study the change of TOC in the process of glyphosate oxidation. As shown in Figure 8.2.4.2-27, the removal of glyphosate by ozone is quite complete, at 60 min the degradation rate of TOC reached 93.52%. When glyphosate was completely removed at 30 min, the degradation rate of TOC was 77.65%. This indicates that in the early phase glyphosate is oxidized by ozone to small molecular organics, which are then gradually oxidized until completely mineralized.

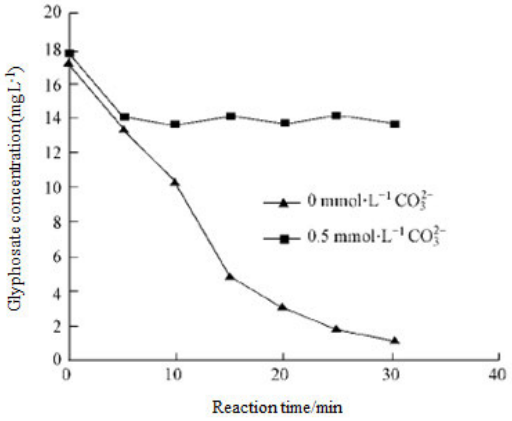
Figure 8.2.4.2-27: TOC change during ozonation of glyphosate



Effect of carbonate ions on glyphosate oxidation by ozone

Carbonate ions are typical hydroxyl radical quenchers, which have strong quenching effect on hydroxyl radical. The effect of carbonate ions on ozone oxidation of glyphosate was investigated. Figure 8.2.4.2-28 showed that carbonate ions obviously inhibited the rate of glyphosate oxidation by ozone. This indicates that hydroxyl radicals play a major role in glyphosate oxidation by ozone.

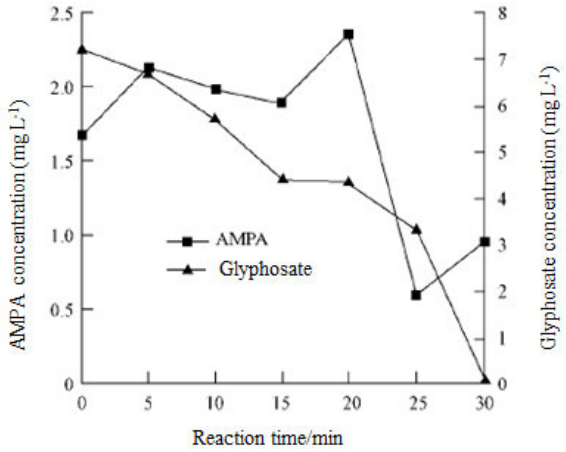
Figure 8.2.4.2-28: Effect of carbonate ions on the ozonation of glyphosate



Concentration of product AMPA during glyphosate oxidation

In the study, glyphosate with initial concentration of 7.2 mg/L was selected to investigate the concentration change of product AMPA during ozonation. The reaction time was kept at 30 min, the sampling was conducted every 5 min. The residual ozone was quenched by Na_2SO_3. AMPA was the first product generated from ozonation of glyphosate, as shown in Figure 8.2.4.2-29, the concentration of AMPA first increased and then decreased as the concentration of glyphosate decreased. Glyphosate was first oxidized to AMPA, which was then gradually oxidized by ozone to other small molecules.

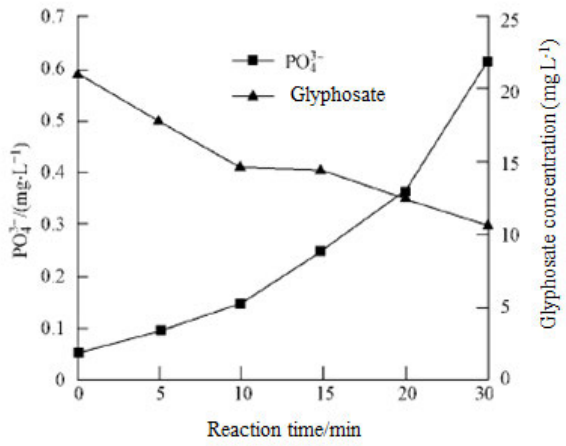
Figure 8.2.4.2-29: Degradation of glyphosate and production of AMPA during ozonation



Concentration of PO_4^{3-} during the process of glyphosate oxidation by ozone

20 mg/L was selected as the initial concentration of glyphosate. The reaction time was kept at 30 min, 20 mL was sampled at 0, 5, 10, 15, 20, 30 min, respectively, and the residual ozone was quenched by Na_2SO_3 to investigate the change of concentration of PO_4^{3-} during ozone oxidation of glyphosate. As was shown in Figure 8.2.4.2-30, along with oxidation of glyphosate by ozone, the concentration of glyphosate decreased gradually and the concentration of PO_4^{3-} increased gradually. PO_4^{3-} was detected in the initial stage of reaction, indicating the P-C bond was first attacked by ozone molecules and hydroxyl radicals during ozone oxidation of glyphosate, the phosphorus-containing groups were rapidly oxidized to PO_4^{3-} and the remaining groups continued to be oxidized by ozone molecules and hydroxyl radicals.

Figure 8.2.4.2-30: Degradation of glyphosate and production of PO_4^{3-}



GC-MS Analysis of intermediates of glyphosate oxidation by ozone

In order to investigate the intermediate products produced in the process of ozone degradation of glyphosate and then propose a more accurate degradation pathway, a qualitative determination was conducted on the intermediates using GC-MS. The initial concentration of glyphosate for the test was 100 mg/L, ozone was continuously provided, the reaction time was kept at 30 min, sampling was conducted every 5 min, and the residual ozone was quenched by Na_2SO_3. After the pretreatment, the sample was measured with GC-MS, and the total ion flow of ozone oxidation of glyphosate after 60 min is shown in Figure 8.2.4.2-31 (Peaks 1, 2, 3, 4 correspond to the products in Table 8.2.4.2-28).

Figure 8.2.4.2-31: Total ion current during ozonation of glyphosate

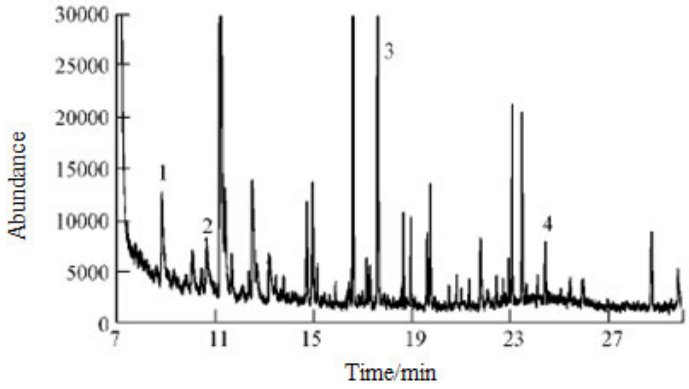


Table 8.2.4.2-28 gives the intermediates of reaction at 60 min by GC-MS measurement. The intermediates of glyphosate ozonation included glycolic acid, glycine, phosphoric acid and AMPA. By the analysis of intermediate products, the degradation pathway of glyphosate ozonation was proposed (see Figure 8.2.4.2-32). There are four main pathways for the oxidation of glyphosate by ozone, including: cleavage of C-N bonds, producing glycine and glycolic acid; cleavage of C-P bonds, generating phosphoric acid; cleavage of C-C bonds, forming AMPA.

Table 8.2.4.2-28: Intermediate products determined by GC-MS

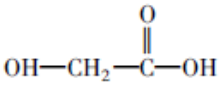
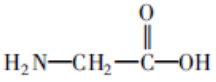
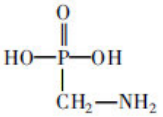
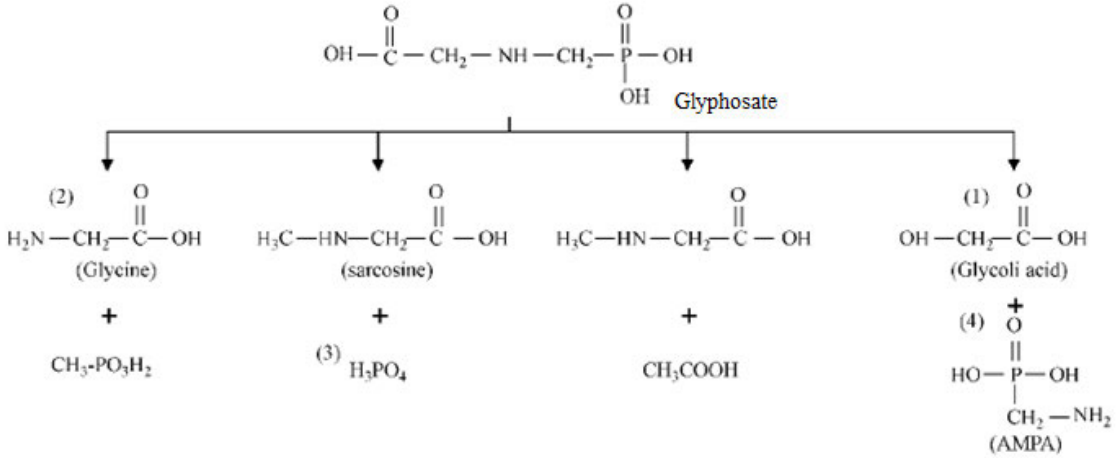
Retention time (min)	Product name (No.)	Structural formula
8.861	Glycolic acid (1)	
10.675	Glycine (2)	
17.627	Phosphoric acid (3)	H_3PO_4
24.367	AMPA(4)	

Figure 8.2.4.2-32: Glyphosate degradation pathway



Conclusion

The removal rate of glyphosate by ozone is related to the dosage of ozone, initial concentration of glyphosate and initial pH. The higher the ozone dose, the faster the reaction rate of glyphosate ozonation. The removal rate of glyphosate in a weak alkaline system (pH = 9.3) was faster than that in the medium system (pH = 6.8), with that in the acidic system (pH = 4.9) being the slowest; and the pH of the reaction system changed obviously in the first 20 min, at the later stage of reaction the changes were not apparent.

Ozone oxidation of glyphosate showed a high degree of mineralization, at 30 min the degradation rate of TOC was 77.65% and at 60 min it was 93.52%. At the initial stage of the reaction, ozone mainly oxidizes glyphosate to AMPA. After glyphosate is completely removed, most intermediates are completely mineralized to carbon dioxide and water.

The ozone oxidation process follows the reaction mechanism of hydroxyl radical. CO32- is a good hydroxyl radical quenching reagent, the rate of ozone oxidation of glyphosate was significantly reduced in the system containing CO32- compared to that without the addition of CO32-, which indicates that hydroxyl radicals play a major role.

The main intermediates of glyphosate oxidation by ozone were glycolic acid, glycine, AMPA and H3PO4, and there were 4 main degradation pathways. The main products of the initial reaction were AMPA and phosphoric acid. AMPA accumulated gradually and then decreased gradually. PO43- accumulated gradually from the initial period of reaction.

Assessment and conclusion by applicant:

The article describes the degradation of unlabeled glyphosate during ozonation in water with different initial concentrations and different pH values. The degradation products resulting from the ozonation process are described as well.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on water treatment efficiencies for glyphosate removal. Data from this study are included in the review provided in [redacted], 2020.

This study aimed at testing the influence of pH, ozone dosage and initial concentration of glyphosate on the degradation of glyphosate by ozone. The pathway of degradation is also described.

Protocol and analytical methods are well described.

Influence of ozone dosage

Initial concentration of glyphosate was 5 mg/L, and the dosage of ozone was 1.5, 2.0 and 3.0 mg/L. The reaction was carried out for 30 min, and sampled every 5 min. Glyphosate was almost completely removed after 30 min. At 1.5 mg/L of ozone, glyphosate was completely removed at 25 min, while at 2.0 mg/L and 3.0 mg/L of ozone, glyphosate was completely removed at 20 min.

Effect of initial glyphosate concentration

Three initial concentrations of glyphosate 2, 5 and 9 mg/L were applied, and the dosage of ozone was 1.5 mg/L. The reaction was kept for 30 min and the sampling was done every 5 min.

At the initial concentration of 2 mg/L, glyphosate was completely removed at 5 min, while at the initial concentration of 5 mg/L, glyphosate was completely removed at 25 min. At the initial concentration of 9 mg/L, glyphosate was not completely removed and its concentration was still 2 mg/L at 30 min. Also, the degradation rate slowed down gradually as the reaction proceeded due to decrase of Ozone concentration throughout the experiment.

Influence of pH

Three initial pH values were selected (4.9, 6.8 and 9.3). Initial concentration of glyphosate was 7.2 mg/L, and the ozone dosage was 1.5 mg/L. The reaction was kept for 30 min, sampling was done every 5 min. Glyphosate could always be removed in 30 min at all initial pH values. The removal rate of glyphosate decreased in the following order: basic (complete removal in 15 min) > neutral (complete removal in 20 min) > acidic (complete removal in 25 min).

Degradaton pathway of glyphosate under ozonation

The pathway for the glyphosate degradation by ozone is also discussed. The intermediates of glyphosate ozonation included glycolic acid, glycine, phosphoric acid and AMPA There are four main pathways for the oxidation of glyphosate by ozone, including: cleavage of C-N bonds, producing glycine and glycolic acid; cleavage of C-P bonds, generating phosphoric acid; cleavage of C-C bonds, forming AMPA.

Assalin et al., 2010

Data point:	CA 7.5/091
Report author	Assalin M., et al.
Report year	2010
Report title	Studies on degradation of glyphosate by several oxidative chemical processes: Ozonation, photolysis and heterogeneous photocatalysis
Document No	Journal of Environmental Science and Health Part B (2010) 45, 89–94
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

Several different Advanced Oxidation Processes (AOPs) including ozonation at pH 6.5 and 10, photolysis and heterogeneous photocatalysis using TiO_2 as semiconductor and dissolved oxygen as electron acceptor were applied to study the degradation of glyphosate (N-phosphonomethyl glycine) in water. The degree of glyphosate degradation, the reaction kinetics and the formation of the major metabolite, aminomethyl phosphonic acid (AMPA), were evaluated. Ozonation at pH 10 resulted in the maximum mineralization of glyphosate. It was observed that under the experimental conditions used in this study the degradation of glyphosate followed first-order kinetics. The half-life obtained for glyphosate degradation in the O_3/pH 10 process was 1.8 minutes.

Materials and methods

Chemicals

Glyphosate (purity 99.8 %) and AMPA (purity 99.1%) were obtained from Monsanto and used without further purification. Analytical grade organic solvents were used for high performance liquid chromatography (HPLC) analysis. Ultra pure distilled-deionized water from a Milli-Q (Millipore Corp.) system was used throughout this study. Commercially available TiO2 (Degussa P-25) was obtained from Degussa Chemical. All reagents used were of analytical-reagent grade.

Samples

A stock solution containing 1000 mg/L of glyphosate was prepared in deionized water and diluted to the required concentration (42.275 mg/L) for the degradation experiments. The original pH of this solution was about 6.5. The pH was adjusted to 10 by the addition of a NaOH solution for the ozonation experiment.

Ozonation process

Ozone was generated from pure oxygen using an OZO-CAV ZT-2 generator (Inter Ozone Ingenieria Ecologica, Santiago-Chile). The amount of ozone produced was determined spectrophotometrically at 258 nm (ϵ= 3.000 L/mol cm) in the gas phase by passing the mixture of oxygen and ozone through a flow cell. The system reached a steady-state production of ozone in 10 minutes. An ozone concentration of 14 mg/L was applied for 30 minutes in a batch reactor. Samples (42.275 mg/L glyphosate solution, 400 mL) were submitted to ozonation at pH 6.5 and at pH 10 (pH adjusted with a sodium hydroxide solution) at room temperature, using a tubular 500 mL reactor fitted with a sintered glass dispenser that released the gas from the bottom of the reactor. For all experiments, the excess of ozone was passed from the reactor into a glass flask containing a 2% solution of KI.

Heterogeneous photochemical process

Titanium dioxide (80% anatase and 20% rutile, average particle size of 30 nm and BET Method– Brunauer, Emmett and Teller [BET] surface of 50 ± 15 m2/g) was used without any pretreatment. Aqueous suspensions of 0.1 g of TiO2/L were used in this experiment. A volume of 200 mL of glyphosate solution (42.275 mg/L, original pH) was placed in the 250 mL cylindrical photoreactor. Illumination was provided by a high-pressure mercury lamp (Philips HPL-N, 125 W; λ>290 nm) with the glass bulb removed. The lamp was fixed in the center of the reactor and cooled by a water jacket, at room temperature. The suspension was bubbled with oxygen (through a sintered glass disk placed in the bottom of the reactor) at a flow rate of about 6 ± 0.2 L/h for 30 minutes. For analytical control, samples were removed and centrifuged at 3500 rpm.

Photolysis process

The same experimental set up, including the passage of oxygen, was used as in the previous section, but without the addition of TiO2 suspension.

Analytical determinations

Mineralization was followed by measuring the total organic carbon (TOC) through direct injection of filtered samples (pore size of 0.45 μm) into a Shimadzu-5000A TOC analyzer provided with a non-dispersive infrared (NDIR) detector and calibrated with standard solutions of potassium phthalate.

The glyphosate and AMPA concentrations were determined by HPLC, with a Merck-Hitachi HPLC system, model D-7000, with fluorescence detection (excitation at 350 nm and emission at 440 nm). A 300 × 4.6 mm I.D Aminex Glyphosate column and a 100 × 4.6 mm I.D HRLC-Glyphosate guard column (both from Bio Rad) were used.

The flow rate of mobile phase (0.68 g/L KH2PO4) was adjusted to 0.7 mL/min. After exiting the column, glyphosate and AMPA were then post column derivatized using 1,2 phthalic dicarboxaldehyde and 2-mercaptoethanol. The retention times for glyphosate and AMPA were 17 and 30 minutes, respectively. The limit of detection (LOD) was established at 0.0075 µg/L, using a signal to noise ratio of 3 for glyphosate and AMPA.

Results

The different treatment processes were applied for the degradation of glyphosate in aqueous solution. The processes studied were photolysis, heterogeneous photocatalysis (TiO2/UV) and ozonation at two different pH values (6.5 and 10.0). Glyphosate was the only organic compound initially present in the aqueous solutions used in this study.

Ozonation process

In Figure 8.2.4.2-33, the variation of the C/C0 ratio as a function of ozonation treatment time is represented. As can be seen, the ozonation carried out at alkaline pH was more effective for glyphosate degradation. After 17 minutes of treatment the glyphosate was totally removed while, in the ozonation carried out at pH 6.5, after 30 minutes of treatment about 80% of the glyphosate initially present in solution was removed. Due to the oxidation potential of hydroxyl radicals being much higher than that of the ozone molecule, radical oxidation was faster than direct oxidation and higher glyphosate degradation was therefore observed at pH 10. The HPLC chromatograms of the O_3/pH 10 process at 0, 15 and 30 minutes of treatment time are given in Figure 8.2.4.2-34.

Figure 8.2.4.2-33: Glyphosate degradation by ozone (pH 6.5) and ozonation based on the hydroxyl radical (pH 10).

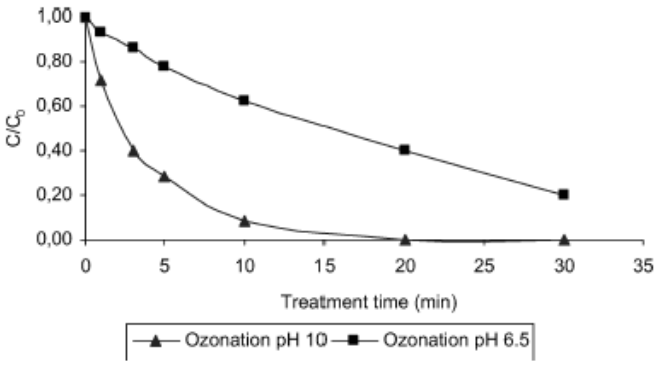


Figure 8.2.4.2-34: High performance liquid chromatography (HPLC) chromatograms of samples subjected to O3/pH 10 at 0, 15 and 30 minutes.

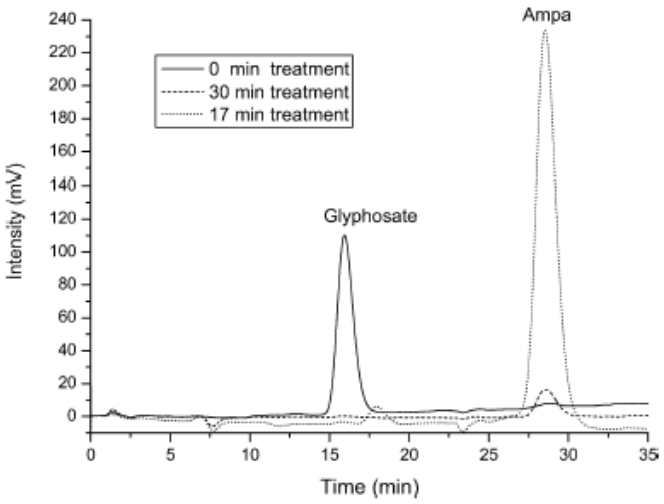
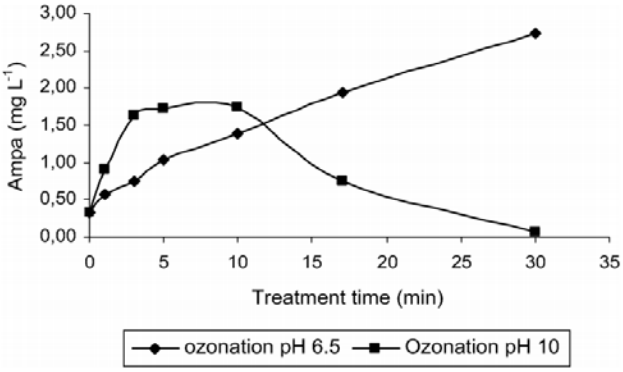


Figure 8.2.4.2-35: Aminomethyl phosphonic acid (AMPA) concentration during the ozonation processes



Aminomethyl phosphonic acid (AMPA) is the major metabolite of glyphosate produced by microbial degradation, and is found in plants, water and soil. The results of the present study indicate that the chemical oxidation processes O3/pH 6.5 and O3/pH10 produced this metabolite (Figure 8.2.4.2-35). Nevertheless, degradation by hydroxyl radicals also removed most of the AMPA produced in 30 minutes of treatment at pH10. For the O3/pH 6.5 degradation process, this metabolite was continually produced and, apparently, not further degraded.

The degree of pesticide degradation and mineralization can be measured by the reduction of the total organic carbon content of the solution. The results indicate 20% TOC reduction by application of the O3/pH 6.5 process. This indicates that other decomposition products, besides AMPA, can be produced during the ozonation process. The ozonation process carried out at pH 10 resulted in 97.5% TOC removal. These results were very important because they indicate that intermediate compounds (that might be more toxic than the parent compound) were almost totally removed.

Photolytic and photocatalytic degradation

In order to compare the efficiency of the photocatalytic degradation (UV-TiO2) with direct photolysis (UV), experiments were carried on using the same initial concentration of pesticide, at pH 6.5. The amount of catalyst used was 0.1 g of TiO2/L.

Figure 8.2.4.2-36 shows the disappearance of glyphosate by photocatalysis and photolysis in relation to illumination time. As expected, the direct photolysis was less effective than photocatalysis for glyphosate removal. After 3 minutes of irradiation without TiO2, only 10.9% of the initial amount of the compound was degraded while the glyphosate degradation for the same treatment time was 38.7% for the photocatalytic process. The literature reports that direct photolysis is usually not an option due to the low quantum efficiency for most pesticides. After 30 minutes of UV irradiation in the presence of TiO2, the residual concentration of glyphosate was 0.06 mg/L (99.9 % efficiency removal). TOC removal for the UV/TiO2 process achieved 92% after 30 minutes of treatment time.

Figure 8.2.4.2-36: Glyphosate degradation by the ultraviolet (UV) and TiO2/UV processes.

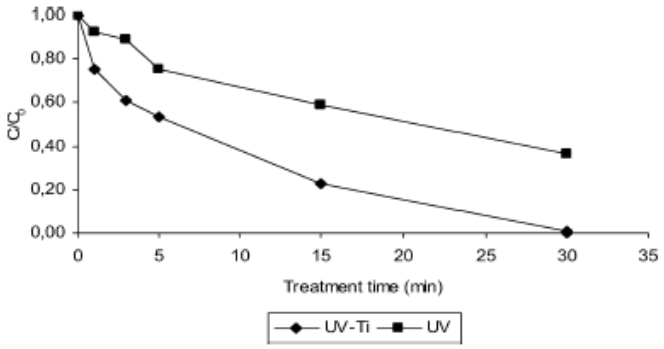
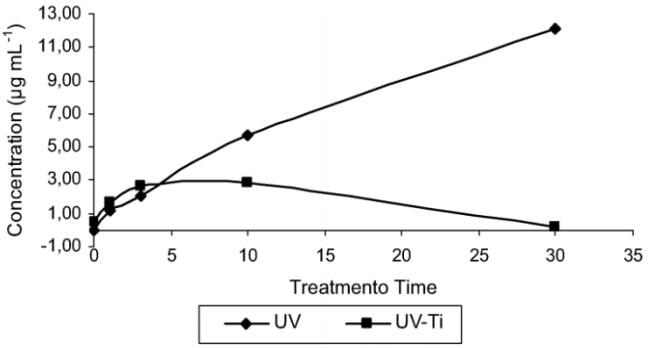


Figure 8.2.4.2-37: Aminomethyl phosphonic acid (AMPA) concentration during the ultraviolet (UV) and UV/TiO2 processes.



For both photo-induced processes formation of the AMPA intermediate was also observed (Figure 8.2.4.2-37). The amount of AMPA formed during the photocatalytic process was less than the amount formed during the UV process, 2.85 mg/L after 10 minutes of treatment, but this was completely degraded after 30 minutes. For the UV process, the amount of AMPA formed increased during the treatment. At the end of the UV treatment without TiO2 (30 minutes) the AMPA concentration was 12.1 mg/L indicating that this compound is less easily degraded by UV radiation than glyphosate.

It is believed that the photocatalytic degradation reaction of organic pollutants occurs on the surface of TiO2 and that O2 and H2O are necessary for photocatalytic degradation. Under UV illumination, electron-hole pairs are created on the TiO2 surface. Oxygen adsorbed on the TiO2 surface prevents the electron-hole pairs from trapping electrons. Superoxide radical-ions (•O2−) are thus formed. The •OH radicals are formed from holes reacting with either H2O or OH− adsorbed on the TiO2 surface. •OH and •O2− are widely accepted as primary oxidants in heterogeneous photocatalysis. The oxidizing power of the •OH radicals is strong enough to completely oxidize glyphosate adsorbed on the surface of TiO2.

Comparison between O3/pH 10 and TiO2/UV processes

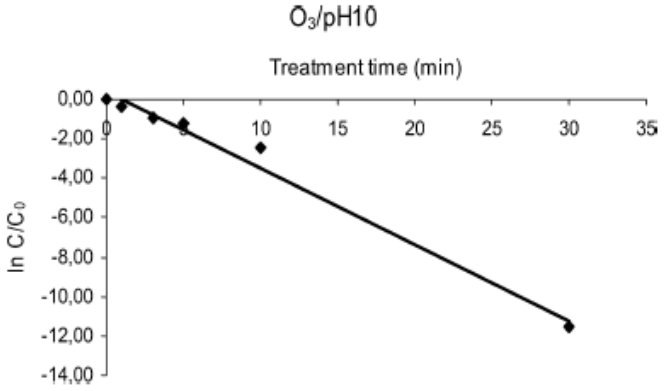
The processes that showed the highest rates for degradation of glyphosate in water were O3/pH 10 and TiO2/UV. Both processes were able to efficiently remove glyphosate and also the AMPA generated during the degradation processes. Knowledge of the kinetics and direct comparison of chemical oxidants are required to assess the efficiency of systems engineered for the oxidation of a variety of pollutants. Reliable kinetic studies require obvious substrate decay measurements. Thus, for comparison of the efficiency of these treatment processes, kinetic studies of glyphosate decomposition were carried out.

As several authors have previously reported, the reaction of ozone with organic compounds is second order, first order with respect to each reactant. Therefore, the glyphosate disappearance rate equation can be expressed as:

$$\frac{d[glyphosate]}{dt} = k[O_3][glyphosate]$$

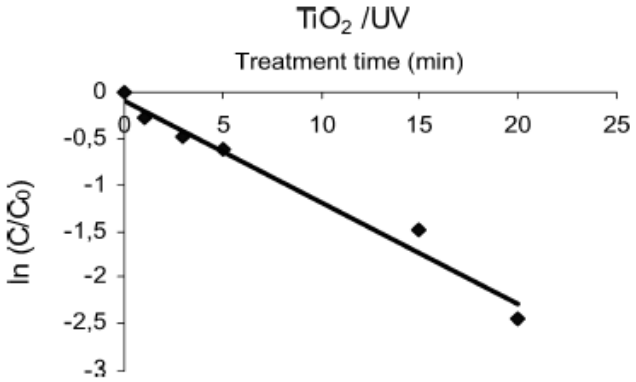
where k is the second order rate constant. In addition, as the initial ozone concentration was in excess with respect to glyphosate, the ozone concentration through each experiment can be considered almost constant. Then, the reaction rate can be reduced to pseudo-first-order kinetics with respect to the ozone concentration. In order to evaluate this pseudo rate constant, the data obtained for glyphosate degradation by O3/pH 10 were plotted as ln (C/C0) versus reaction time, and after linear regression analysis (R2 = 0.9836), the slope can be attributed as the apparent first-order rate constant k′ (Figure 8.2.4.2-38).

Figure 8.2.4.2-38: The pseudo-first-order decay of glyphosate by ozonation at pH 10.



Several experimental results have indicated that the photocatalytic degradation rates of pesticides over illuminated TiO2 follow the Langmuir-Hinshelwood kinetic model. In our investigation, by plotting ln [C/C0] as a function of time, a straight line was obtained (Figure 8.2.4.2-39) that confirms the apparent first-order kinetic law (R2 = 0.9743).

Figure 8.2.4.2-39: The pseudo-first-order decay of glyphosate by TiO2/UV process.



The half-life obtained for glyphosate was 1.8 and 6.2 minutes for O3/pH 10 and TiO2/UV, respectively. This indicates that for the ozonation carried out at pH 10 a faster rate of glyphosate decomposition was observed under the experimental conditions studied.

Conclusion

The degradation of aqueous solutions containing glyphosate can be realized by oxidative advanced processes. Processes based on the formation of hydroxyl radical, such as Ti/UV and O_3/pH 10, were effective for the degradation of glyphosate and its degradation intermediates, including AMPA, after a short treatment time. Under the experimental conditions used in this study the degradation of glyphosate

followed a pseudo first-order kinetic law for both processes studied. The half–lives obtained for glyphosate degradation were 1.8 and 6.2 minutes for O_3/pH 10 and TiO_2/UV, respectively.

Assessment and conclusion by applicant:

The article describes the removal of glyphosate by ozonation and photocatalysis (Ti/UV) process in water. The results are mainly shown as graphical plots. Thus, insufficient details were reported to evaluate the validity of the rate constants reported.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

The study provides reliable information on water treatment efficiencies for glyphosate and AMPA removal under Advanced Oxidation Processes Results are not provided as table results but should be read on the graphs. It however provides a sufficient level of information. Data from this study are included in the review provided in [redacted], 2020.

This study evaluated the degree of glyphosate degradation, the reaction kinetics and the formation of the major metabolite, aminomethyl phosphonic acid (AMPA) under different Advanced Oxidation Processes (formation of hydrocyl radical), including ozonation at pH 6.5 and 10, photolysis and heterogeneous photocatalysis using TiO_2.

It however does not give further information on potential degradation produts other than AMPA.

Protocol and analytical process are well described.

Ozonation

An ozone concentration of 14 mg/L was applied for 30 minutes in a batch reactor. Samples at concentration of 42.275 mg/L glyphosate solution were submitted to ozonation at pH 6.5 and at pH 10 at room temperature.

The ozonation carried out at alkaline pH was more effective for glyphosate degradation. After 17 minutes of treatment the glyphosate was totally removed at pH10, while in the ozonation carried out at pH 6.5, after 30 minutes of treatment about 80% of the glyphosate initially present in solution was removed.

These results are consistent with those of Shen, Y. et al. (2010) with degradation faster at more alkaline pH values.

It is worth noting that AMPA was formed by ozonation in the study, at both pH. Nevertheless, most of the AMPA produced was removed within the 30 min treatment at pH10 while it was continually produced and, apparently, not further degraded within 30 min at pH 6.5.

Photolytic and photocatalytic degradation

In order to compare the efficiency of the photocatalytic degradation (UV-TiO2) with direct photolysis (UV), experiments were carried on using the same initial concentration of pesticide, at pH 6.5. The amount of catalyst used was 0.1 g of TiO2/L.

The direct photolysis was less effective than photocatalysis for glyphosate removal. After 3 minutes of irradiation without TiO_2, only 10.9% of the initial amount of the compound was degraded while the glyphosate degradation for the same treatment time was 38.7% for the photocatalytic process.

The literature reports that direct photolysis is usually not an option due to the low quantum efficiency for most pesticides. After 30 minutes of UV irradiation in the presence of TiO_2, the residual concentration of glyphosate was 0.06 mg/L (99.9 % efficiency removal). TOC removal for the UV/TiO_2 process achieved 92% after 30 minutes of treatment time.

Boucherie et al., 2010

Data point:	CA 7.5/092
Report author	Boucherie, C., et al.
Report year	2010
Report title	"Ozone" and "GAC filtration" synergy for removal of emerging micropollutants in a drinking water treatment plant?
Document No	Water Science and Technology: Water Supply (2010), Volume 10, Number 5, pp. 860-868
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Veolia Water)
Acceptability/Reliability:	Reliable with restrictions

Ozonation plays an essential role in water disinfection to inactivate viruses, bacteria and some parasites. Ozone treatment rates to attain disinfection goals also result in oxidation reactions of emerging pollutants. Glyphosate, AMPA, amitrole and diuron – the four major pesticides in the Seine, Marne and Oise rivers – are reactive to ozone. Twenty-one pesticides are only partially reactive to ozone and an additional "GAC filtration" is needed to remove them.

Materials and methods

The pilot unit consists of an ozonation-deozonation step linked to a Granular activated carbon (GAC) filtration column. The system is continuously fed by Sand Filtered Water (SFW) from the Neuilly-sur-Marne drinking water plant. Bromide or micropollutants are injected into the feeding line via a static mixer. Moreover the pH can also be automatically controlled by online sodium hydroxide or sulphuric acid injection. The pilot geometry is a rectangular tank with one transfer chamber and three contact chambers and the following characteristics: hydraulic efficiency ratio of 0.70 and hydraulic residence time of 17 minutes for a mean flow rate of 12 m3/h. Gas/Liquid ozone transfer is achieved in the first chamber working as a bubble column with two porous diffusers and counter current ozonated gas and SFW flows. The 300 mm diameter GAC column contains a two meters GAC filter bed. The substrate of this filter bed comes from one full scale GAC filter in Neuillysur-Marne plant with an operating life equivalent to 21,000 bed volumes processed. The column is fed with ozonated-deozonated water from the ozonation unit with 750 L/h mean flow rate and 11 minutes mean contact time between water and filtering medium. The GAC unit is backwashed weekly (air, water and air + water back-wash steps).

The operator of the pilot unit uses a man-machine interface system. This system includes specific automatic regulation loops to control SFW Flow rate, SFW pH, ozone production or ozone residual outlet and ozone quenching upstream GAC filtration. At the beginning of each test, the operator can select a specific test level of ozone or instruct the pilot unit to fix the end-level of ozone. Then spiking of micropollutants is carried out to simulate medium or maximum concentrations found in the Seine, Marne and Oise rivers.

Samples are collected for analysis after a period of two hydraulic residence times, in order to reach a steady state. Some analyses – pH, temperature, alkalinity (AT), UV254, ozone gas and liquid residual – are carried out in situ. Ozone concentration measurements in "Air" and "Vent" gases are continuously monitored with sensors "Uvozon" and "BMT 964". Daily controls are also carried out using an iodometric method to assess the ozone concentrations in gases. A sensor "Depolox" continuously monitors the ozone concentration of the water at the pilot outlet. Micropollutants analyses are carried out using High Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC) methods followed by either fluorescence, or mass spectrometry or UV detection.

Results

Pesticides tests

Six tests were carried out with an ozone treatment level ranging from 0 to 2.3 g/m3 and with the following experimental conditions: pH = 7.3, 16.9 < T (°C) < 17.7, 0.143 < UV254 (cm-1) < 0.184 and

AT = 4.4 meq/L. Table 8.2.4.2-29 shows the concentrations of pesticides tested in the spiked SFW. These concentrations remained constant during the test runs which lasted three days.

Table 8.2.4.2-29: Average pesticides concentrations in spiked Sand Filtered, ozonated and GAC filtered water matrix

			Water flow rate = 12 m^3/h & pH = 7.3 & 16.9 < T (°C) < 17.7 & 0.143 < UV_{254} (cm^{-1}) < 0.184 & A_T = 4.4 meq/L					
			Ozone treatment rate = 0 g/m^3		Ozone treatment rate: 1.2–2.3 g/m^3 Dissolved ozone residual: 0.13–0.68 mg/L CT: 1.1–5.4 mg min/L			
Pesticides	Limit of quantification (µg/L)	Average SFW* concentration (µg/L) (n = 3)	SCAG1† concentration (µg/L) τ ~ 5 min	SCAG2† concentration (µg/L) τ ~ 10 min	SC1‡ concentration (µg/L) Minimum–Maximum (n = 5)	SC4§ concentration (µg/L) Minimum–Maximum (n = 5)	SCAG2† concentration (µg/L) Minimum–Maximum (n = 5)	Ozone removal (%) Minimum–Maximum
Acetochlore	0.02	0.28	0.05	<0.02	0.14–0.19	0.08–0.14	<0.02	50–71
Alachlore	0.02	0.17	0.03	<0.02	0.09–0.12	0.02–0.11	<0.02	34–88
Amitrole	0.10	1.17	<0.10	<0.10	<0.10	<0.10	<0.10	>91
AMPA‖	0.10	0.80	0.70	0.60	0.20–0.70	<0.10	<0.10	>88
Atrazine	0.02	0.16	<0.02	<0.02	0.12–0.15	0.10–0.13	<0.02	19–38
Azoxystrobine	0.02	0.22	0.04	<0.02	<0.02	<0.02	<0.02	>91
Bentazone	0.02	0.26	0.12	0.10	<0.02	<0.02	<0.02	>92
Bromuconazole	0.02	0.20	<0.02	<0.02	0.06–0.16	0.08–0.16	<0.02	21–61
Carbendazime	0.02	0.41	<0.02	<0.02	<0.02	<0.02	<0.02	>95
Carbetamide	0.02	0.35	<0.02	<0.02	0.15–0.21	0.07–0.11	<0.02	69–80
Carbofuran	0.02	0.97	0.29	0.06	0.18–0.54	<0.02–0.07	<0.02	93–> 98
Chloridazone	0.10	1.40	<0.10	<0.10	0.24–0.73	<0.10–0.21	<0.10	85–> 93
Chlortoluron	0.02	0.23	<0.02	<0.02	<0.02	<0.02	<0.02	>91
DCPMU¶	0.02	0.09	<0.02	<0.02	<0.02	<0.02	<0.02	>77
DEA**	0.02	0.29	<0.02	<0.02	0.27–0.32	0.29–0.54	<0.02	–
DEDIA††	0.02	0.27	<0.02	<0.02	0.28–0.33	0.30–0.35	<0.02	–
Deethylterbumeton	0.02	0.26	0.03	<0.02	0.21–0.28	0.22–0.26	<0.02	1–16
DIA‡‡	0.02	0.28	<0.02	<0.02	0.23–0.30	0.22–0.26	<0.02	6–20

*Spiked sand filtered water.
†GAC filtration outlet.
‡Transfer chamber outlet.
§Contact chambers outlet and.
‖Aminomethylphosphoric acid.
¶3,4-dichlorophenyl-methylurea.
** Deethylatrazine.
††Deethyldeisopropylatrazine.
‡‡Deisopropylatrazine.

			Water flow rate = 12 m^3/h & pH = 7.3 & 16.9 < T (°C) < 17.7 & 0.143 < UV_{254} (cm^{-1}) < 0.184 & A_T = 4.4 meq/L					
			Ozone treatment rate = 0 g/m^3		Ozone treatment rate: 1.2–2.3 g/m^3 Dissolved ozone residual: 0.13–0.68 mg/L CT: 1.1–5.4 mg min/L			
Pesticides	Limit of quantification (µg/L)	Average SFW* concentration (µg/L) (n = 3)	SCAG1† concentration (µg/L) τ ~ 5 min	SCAG2† concentration (µg/L) τ ~ 10 min	SC1‡ concentration (µg/L) Minimum–Maximum (n = 5)	SC4§ concentration (µg/L) Minimum–Maximum (n = 5)	SCAG2† concentration (µg/L) Minimum–Maximum (n = 5)	Ozone removal (%) Minimum–Maximum
Dichloroprop	0.05	0.20	0.05	<0.05	0.14–0.18	0.11–0.13	<0.05	35–45
Difenoconazole	0.04	0.19	<0.04	<0.04	0.04–0.16	<0.04–0.14	<0.04	25– >79
Dimetachlore	0.02	0.22	0.04	<0.02	0.08–0.14	0.06–0.11	<0.02	50–73
Diuron	0.02	0.47	<0.02	<0.02	<0.02–0.13	<0.02	<0.02	>96
Ethofumesate	0.02	0.28	<0.02	<0.02	0.16–0.24	0.10–0.21	<0.02	26–65
Fluquinconazole	0.02	0.14	<0.02	<0.02	<0.02–0.11	<0.02–0.07	<0.02	50– >86
Flusilazole	0.02	0.15	<0.02	<0.02	0.03–0.10	0.04–0.10	<0.02	32–73
Glyphosate	0.10	1.07	0.60	0.50	<0.10	<0.10	<0.10	>91
Hydroxyatrazine	0.02	0.12	<0.02	<0.02	0.11–0.12	0.09–0.10	<0.02	19–27
Imazamethabenz-methyl	0.02	0.21	0.07	0.02	0.15–0.21	0.14–0.19	<0.02	10–33
Isoproturon	0.02	0.45	<0.02	<0.02	<0.02	<0.02	<0.02	>96
MCPA‖	0.05	0.42	0.06	<0.05	0.12–0.19	<0.05–0.06	<0.05	86– >88
Mecoprop	0.05	0.15	0.05	<0.05	<0.05–0.06	<0.05	<0.05	>67
Metazachlore	0.02	0.13	0.03	<0.02	0.03–0.08	0.02–0.07	<0.02	46– >85
Metolachlore	0.02	0.30	0.06	<0.02	0.17–0.20	0.11–0.15	<0.02	50–63
Piclorame	0.05	0.15	<0.05	<0.05	0.10–0.16	0.09–0.14	<0.05	7–40
Prochloraze	0.02	0.14	<0.02	<0.02	<0.02	<0.02	<0.02	>86
Propazine	0.02	0.16	0.03	<0.02	0.13–0.16	0.11–0.16	<0.02	0–30

*Spiked sand filtered water.
†GAC filtration outlet.
‡Transfer chamber outlet.
§Contact chambers outlet and.
‖(4-chloro-2-methylphenoxy)acetic acid.

The presence of glyphosate and AMPA downstream of the GAC filtration unit indicated that neither compound was adsorbed by the column.

The tests with ozone show that three groups of components could be distinguished according to their reactivity to ozone:

- Very reactive molecules are removed as early as transfer chamber outlet: this included glyphosate.
- Less reactive molecules are removed at contact chambers outlet: including AMPA,
- Weak reactive molecules are only partially removed at contact chambers outlet.

Glyphosate and AMPA were not adsorbed in the GAC filter unit, but were reactive or very reactive to ozone.

Conclusion

In the context of a multi-barrier DWTP, ozonation remains an essential disinfection step: its capacity to inactivate viruses is necessary to control health risks. The ozone treatment levels needed to reach disinfection targets can also remove several emerging pollutants by oxidation like pesticides, pharmaceuticals, phthalates, nonylphenols and hormones.

Glyphosate was found to be very rapidly degraded by ozone treatment (>91%, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88%, levels reduced to <0.1 µg/L).

Assessment and conclusion by applicant:

The article describes the removal of glyphosate and AMPA among other substances from spiked drinking water with a combined ozonation – deozonation - filtration approach. Glyphosate was found to be very rapidly degraded by ozone treatment (>91%, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88%, levels reduced to <0.1 µg/L); hence, the ozone treatment required to deliver disinfection targets was also effective in removing glyphosate and AMPA to levels below 0.1 µg/L. However, no information about potential break-down products were provided.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

The study is considered reliable with restrictions and provides supportive information on water treatment efficiencies for glyphosate and AMPA removal. Data from this study are included in the review provided in [redacted], 2020.

This study described experiment of ozonation and GAC (granular activated carbon) on several pesticides.

Study authors indicate that "Glyphosate and AMPA were not adsorbed in the GAC filter unit, but were reactive or very reactive to ozone." However from table 38 it can be read that for an initial concentration of glyphosate of 1.07 µg/L, concentration after GAC was 0.5 µg/L after 10 minutes of contact. Also it is RMS understanding that the overall removal rate of 91% for glyphosate reported by authors, includes the fist step of GAC filtering, and cannot be attributed to ozonation alone.

Manassero et al., 2010

Data point:	CA 7.5/093
Report author	Manassero A. et al.
Report year	2010
Report title	Glyphosate degradation in water employing the H2O2/UVC process
Document No	Water research, (2010 Jul) Vol. 44, No. 13, pp. 3875-82
Guidelines followed in study	None

Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

Glyphosate is the organophosphate herbicide most widely used in the world. Any form of spill or discharge, even if unintentional, can be transferred to the water due to its high solubility. The combination of hydrogen peroxide and UV radiation could be a suitable option to decrease glyphosate concentration to acceptable limits. In this work, the effects of initial pH, hydrogen peroxide initial concentration, and incident radiation in glyphosate degradation were studied. The experimental device was a cylinder irradiated with two tubular, germicidal lamps. Conversion of glyphosate increases significantly from pH = 3-7. From this value on, the increase becomes much less noticeable. The reaction rate depends on the initial herbicide concentration and has an optimum plateau of a hydrogen peroxide to glyphosate molar concentration ratio between 7 and 19. The expected non-linear dependence on the irradiation rate was observed. The identification of critical reaction intermediaries, and the quantification of the main end products were possible and it led to a proposal of a plausible degradation pathway. The achieved quantification of the extent of mineralization is a positive indicator for the possible application of a rather simple technology for an in situ solution for some of the problems derived from the intensive use of glyphosate.

Materials and methods

Chemicals

The following reagents were used: (a) glyphosate (AccuStandard) as standard chromatographic, (b) glyphosate 95% provided by Red Surcos, (c) hydrogen peroxide (Ciccarelli p.a., >99%), (d) sarcosine (≥97.5%, Sigma-Aldrich), (e) glycine (97.3%, Merck), (f) aminomethylphosphonic acid, AMPA (≥99%, Sigma-Aldrich), (g) formic acid (98-100%, Merck), (h) acetic acid (100%, Merck), (i) glycolic acid (solution 70% in water, Merck) and (j) catalase from bovine liver, >2000 units/mg (Fluka, 1 unit decomposes 1 μmol H2O2 per minute at pH 7.0 and 25°C). Ultra pure water (0.055 μS/cm) was used in all experiments. This water was obtained from an OSMOION purification system made of several filters to eliminate particulate matter, chlorinated compounds, and low molecular weight organic substances. Two reverse osmosis membranes and an ion exchange resin completed the equipment.

Table 8.2.4.2-30: Experimental program.

Variable	Value
Glyphosate initial concentration (mM)	0.16–0.54
H_2O_2 initial concentration (mM)	0–11.82
Photon fluence rate ($E_{p,0}$) (Einstein $cm^{-2} s^{-1}$) × 10^9	
Heraeus 40 W lamp (100%)	23.3
Philips 15 W lamp	10.4
Heraeus 40 W lamp (with filter) (16%)	4.2
Reaction time	5 h
Initial pH	3.5–7–10
Temperature	25 °C

Experimental setups and procedures

The photodegradation of glyphosate was carried out in a cylindrical reactor made of Teflon TM, with two parallel, flat windows made of quartz (VReactor = 110 cm3). Each window was irradiated with a tubular, germicidal lamp (λ = 253.7 nm) placed at the focal axis of a parabolic reflector made of mirror finished aluminum. The small reactor operated in the loop of a batch recycling system that included a

pump, a heat exchanger (for temperature control) and a large volume, well stirred tank with provisions for sampling, temperature control and pH measurements (VTotal = 2000 cm3). Further details on the experimental device can be found elsewhere. Experiments were carried out changing the following variables: (i) initial glyphosate concentrations, (ii) initial hydrogen peroxide concentrations, (iii) initial pH and (iv) incident radiation on the windows of radiation entrance (or, according to IUPAC, the photon fluence rate, Ep,0) measured with potassium ferrioxalate actinometry (Table 8.2.4.2-30). Most of the experiments were done at 0.30 mM of glyphosate initial concentration. Lower and higher concentrations were used to study the behavior of glyphosate degradation at different initial concentrations. Values between 0.30 and 0.45 mM are important from an environmental point of view since they are the average values of glyphosate concentrations found in wastewaters which result from rinsing herbicide containers.

Analytical methods

Glyphosate was analyzed by ion chromatography with a suppressed conductivity detector and employing an Ion Pac AG4A-SC guard column, an AS4A-SC separating column, and an ion self-regenerating suppressor (Alltech DS-Plus) with electrochemical methods. A solution of Na2CO3 (9 mM) and NaOH (4 mM) was used as eluent at a flow-rate of 1.5 mL/min. The injection volume was 20 µL. Under this condition the retention time for glyphosate was 4.77 min. The aminomethylphosphonic acid (AMPA) standard could be identified under the same operating conditions. pH was monitored with a HI 98127 Hanna pH meter. Hydrogen peroxide was analyzed using a colorimetric method following techniques reported elsewhere, and employing a Cary 100 Bio UV visible spectrophotometer. Total organic carbon (TOC) was analyzed in order to compare glyphosate degradation rate with total mineralization rate and also in order to provide more accurate information about possible reaction intermediates. The instrument used was a Shimadzu TOC-5000A. End products were monitored by ion chromatography, and following a procedure similar to the one employed for glyphosate analysis. The identification of glycine, sarcosine and NH3 was done employing a specific test for free amino acids according to methodology published elsewhere. The presence of formaldehyde was also confirmed using a specific colorimetric method (NIOSH, 1994). Though the possible degradation products monitored were: glycine, sarcosine, AMPA, formaldehyde, acetic acid, formic acid, nitrate anion, ammonium and phosphate anion, only nitrate and phosphate ions were quantified.

Operations

The experimental run was started after every variable of the operating conditions had reached its steady-state and/or uniformity: concentrations, temperature, irradiation rates, etc. The employed equipment permitted the reactor to be isolated from the irradiating system until the starting time was reached. It should be noted that due to the type of equipment used in this work (an irradiated reactor in a recycle that includes a large volume tank) the reaction time plotted in the figures does not represent the irradiation time of the active reaction volume. The real reaction time is the reaction time measured in every experiment and multiplied by the ratio VReactor/VTotal which is a factor <<1.

Results and discussion

Preliminary runs

Two types of previous experiments were carried out in order to investigate the effects of UVC and H2O2 separately. The first run was performed employing C0Glyph: = 0:30 mM, (50 mg/L); C0H2O2 = 2:20 mM; (75 mg/L) and without UV radiation. After 3 h of total time, no noticeable changes in glyphosate concentration were observed. A similar run was performed with C0Glyph: = 0.30 mM (50 mg/L) and using 40 W Heraeus UVC lamps turned on during 3 h of total time. No signs of direct photolysis were observed, as it had been previously reported elsewhere. This is in agreement with the absorption spectrum of glyphosate, at least in the range from 200 to 400 nm.

Effects of initial pH values

The experiments were carried out at different initial pH: 3.5 (which results from the preparation of the reacting mixture), 7 and 10, and at initial concentrations of glyphosate and hydrogen peroxide of 0.30 mM and 2.20 mM, respectively. pH adjustment was accomplished by the addition of the required amount of 1 N NaOH. The results have shown that the best condition for degradation took place at the

highest pH value. However, there are no significant differences between pH 7 and pH 10 (Figure 8.2.4.2-40).

Effects of initial H_2O_2 concentration

It is known that there is an optimum concentration of H2O2 in the UV/H2O2 process. The results, for a total reaction time of 5 h, were analyzed using the final glyphosate conversion under the following operating conditions: C0Glyph: = 0.30 mM (50 mg/L), pH = 7, two 40 W lamps and H2O2 concentration range from 0 to 12.4 mM (Figure 8.2.4.2-41a). It is clear that 2.2-5.9 mM (75-200 mg/L) is the range of higher reaction rates. These values are related to the H2O2/glyphosate molar ratio between 7.3 and 19.7. Within this plateau, conversion of glyphosate after 5 h was almost 70%. For a run under the best operating conditions for degradation, Figure 8.2.4.2-41b shows the temporal progression of the participating species concentrations. The existence of this optimum is a well-known phenomenon which results from the scavenging effect of the excess of OH radicals on the hydrogen peroxide. The glyphosate decay follows a first-order kinetics with an observed rate constant k = 0.20/h ± 0.01 (3.68/h total process time) with a correlation coefficient of 0.9986. Also, the half-life value was calculated, resulting t1/2 = 0.19 h (3.5 h total process time).

Figure 8.2.4.2-40: Experiments made under the following conditions: C0Glyph = 0.30 mM; (50 mg/L), C0H2O2 = 2.20 mM; (75 mg/L) at different initial pHs and using a UV lamp of 40 W input power: ●, pH 3.5; ■, pH 7 and ▲, pH 10

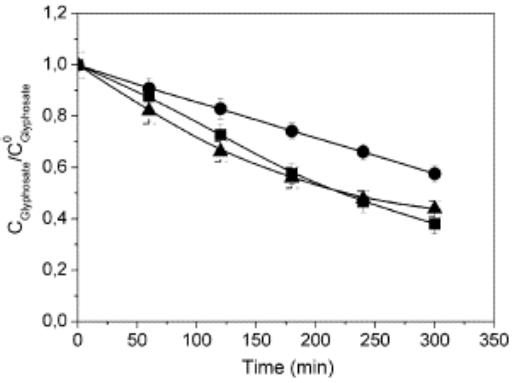
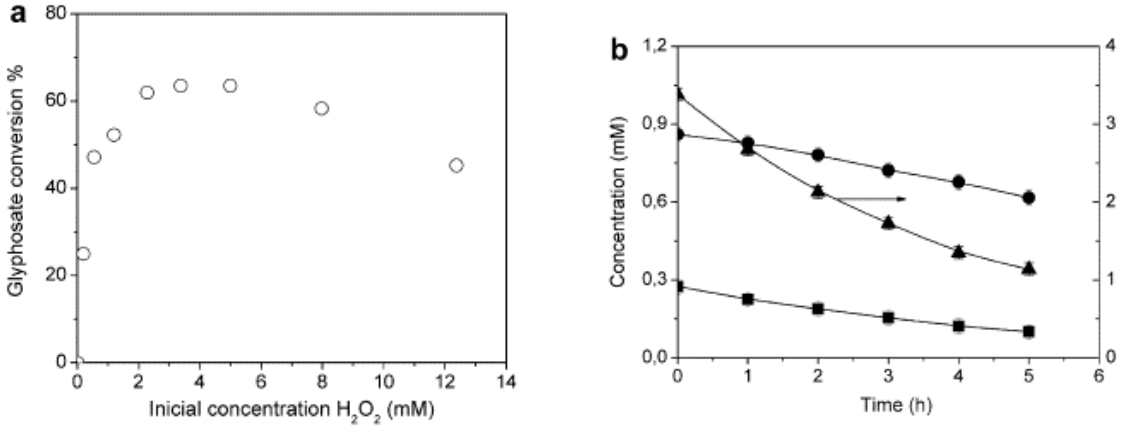


Figure 8.2.4.2-41: (a) Glyphosate conversion, for a fixed reaction time (5 h) vs. initial H2O2 concentration. C0Glyph: 0.30 mM; (50 mg/L), pH = 7 and UV lamp of 40W input power. (b) Glyphosate, H2O2 and TOC concentration evolution as a function of time. C0Glyph:0:27 mM; (46.4 mg/L), C0H2O2 = 3:38 mM; (114.9 mg/L) and UV lamp of 40W input power: ■, glyphosate ▲, :, H2O2 and ● C, TOC.



Effects of glyphosate initial concentration

The glyphosate degradation for different initial glyphosate concentrations - between 0.16 and 0.54 mM - and the same hydrogen peroxide initial concentration is shown in Figure 8.2.4.2-42. The degradation

rate is pseudo-first order with respect to initial concentration.

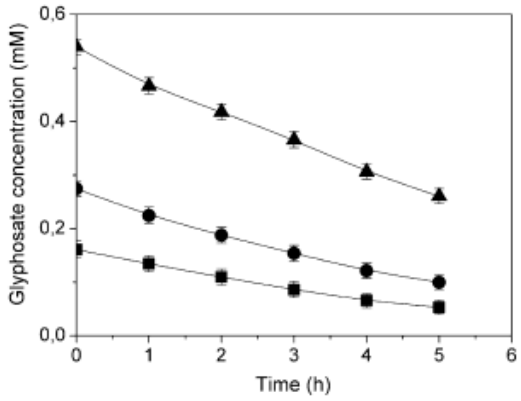
Effect of UV incident radiation intensity

The change on glyphosate concentration under different UV incident radiation rates at the reactor windows, at pH = 7, and for initial glyphosate and hydrogen peroxide concentrations of 0.30 mM and 3.38 mM, respectively, is shown in Figure 8.2.4.2-43. For a reaction time of 5 h, with two 40 W lamps (Photon fluence rate, Ep,0 = 2.3 x 10-8 Einstein/(cm2s), a glyphosate conversion of 63.5 % was reached while conversions with two 15W lamps (Ep,0 = 10.4 x 10-9 Einstein /(cm2s)) and two 40 W lamps with neutral density filters (Ep,0= 4.2 x 10-9 Einstein/(cm2s), were 36.3% and 20%, respectively. Please note that this is not a direct indicator of the reaction rate dependence with respect to the absorbed photons because, from the kinetic point of view, the exact information is provided by the average value of the local volumetric rate of photon absorption by H2O2 (sometimes called photon absorption rate) and not the fluence rate at the reactor walls.

Total Organic Carbon (TOC) evolution

The total organic carbon (TOC) concentration at every elapsed time is important from two points of view: (i) because it is one of the best indications to conclude that complete mineralization has been achieved. When the TOC concentration is zero, it is certain that the glyphosate and all the reaction byproducts have been entirely degraded. (ii) Because it is always possible to calculate the equivalent theoretical TOC value from the experimentally measured glyphosate concentration at each reaction time. This result can be compared with the above-mentioned experimental TOC. This information is very useful to have an indicator of the existence of stable reaction intermediates; i.e., other organic, carbon-containing compounds, during the progress of the reaction. Figure 8.2.4.2-44 depicts the result of a representative run. It proves the existence of different reaction intermediates. It was also observed that, under these experimental conditions, TOC conversion after 5 h was 29%.

Figure 8.2.4.2-42: Glyphosate concentration as a function of time. Initial glyphosate concentration is the parameter: C0Glyph: 0.54 mM;●, C0Glyph: 0:27 mM, ■, C0Glyph: 0.16 mM; UV lamp of 40 W input power and pH = 7.



Formation of byproducts

In order to confirm the extent of glyphosate oxidation and to obtain a better understanding of the reaction mechanism involved, a byproduct evaluation is needed. However, given the complex variety of photoproducts that can be produced, an exhaustive identification and quantification of all intermediate products would be very difficult. Hence, this study primarily focused on the major stable byproducts of the reaction. As shown in Figure 8.2.4.2-45, the mineralization of glyphosate under a longer run time using UV/H2O2 process is evidenced by the evolution of inorganic anions at the highest oxidation states, i.e., phosphate and nitrate. For each mol of glyphosate that is decomposed, one mol of phosphate appears at each reaction time (in the run shown in Figure 8.2.4.2-45, after 10 h of total reaction time, the difference between the theoretical and the experimental phosphate concentration was 7%). However, for nitrate ion the concentration of this end product was below the expected stoichiometric value. In fact, under the operating time shown in Figure 8.2.4.2-45, less than 20% of initial nitrogen is under the nitrate form. In addition to mineral ions, formic acid was detected in the degradation samples. However, other

organic acids such as acetic and glycolic acids were not found in this study.

Figure 8.2.4.2-43: Effect of irradiation rates on the reaction rate. Dimensionless glyphosate concentration vs. time. The parameter is the lamp input power for C0Glyph = 0.30 mM; (50 mg/L), C0H2O2 = 3:38 mM; (115 mg/L) and pH = 7: ●, Heraeus, 40W input power with filter, ▲, Philips, 15W input power, ■ Heraeus, 40W input power.

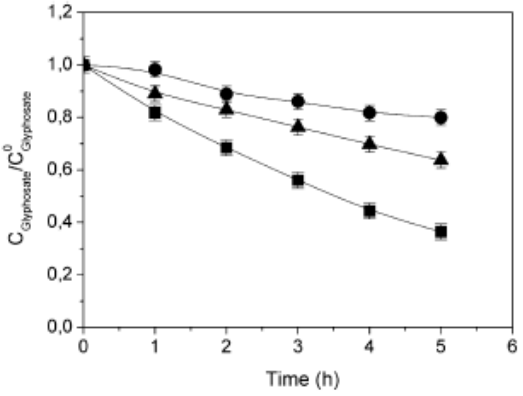


Figure 8.2.4.2-44: Total organic carbon evolution at pH 7 and UV lamps of 40W input power. Conditions: C0Glyph. = 0:30 mM; (50 mg/L), C0H2O2 = 2:35 mM; (80 mg/L). ○, calculated TOC ●, experimental TOC.

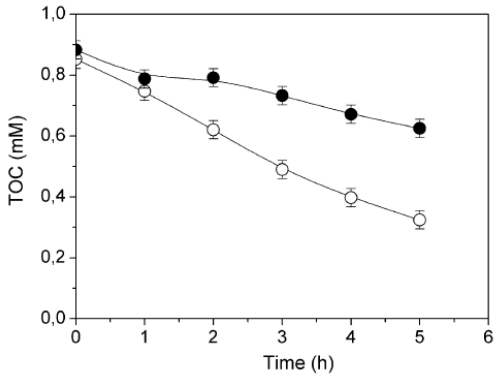
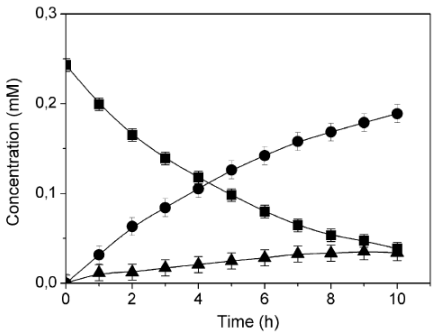


Figure 8.2.4.2-45: Evolution of glyphosate and end products during an extended run made under the best operating conditions for degradation: ■, glyphosate; ●, phosphate and ▲ nitrate. Conditions: C0Glyph. = 0:24 mM; (41 mg/L), C0H2O2 = 2:4 mM; (83 mg/L).

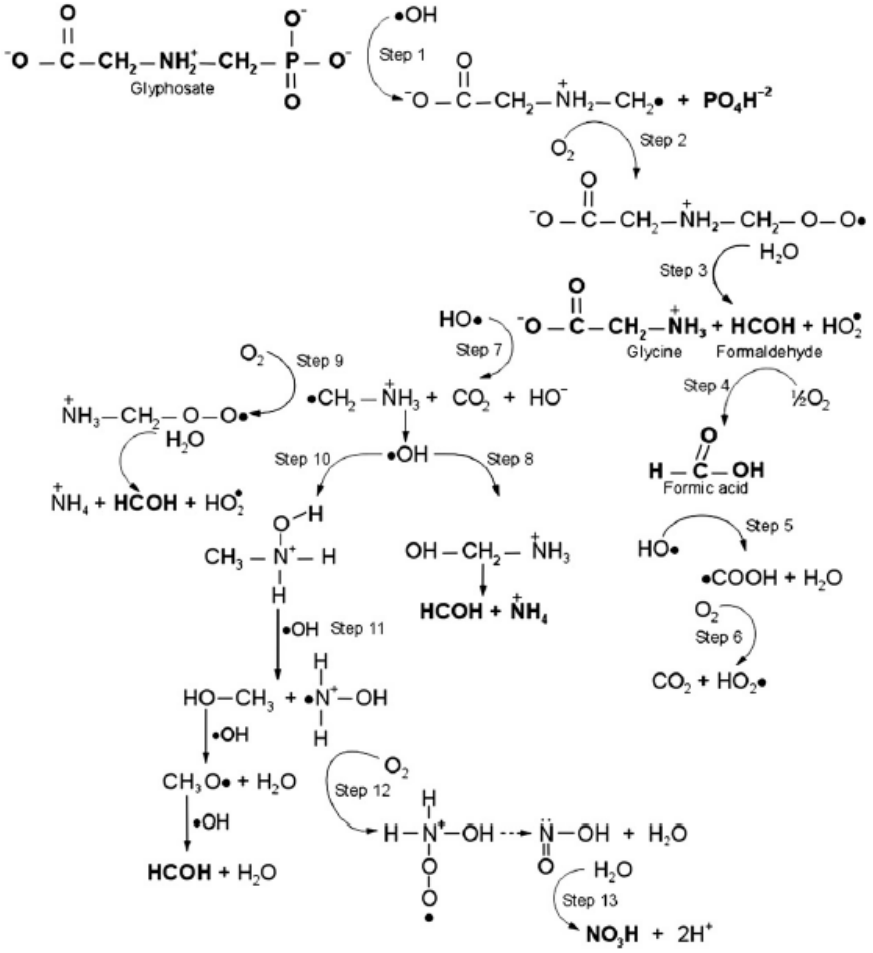


A reaction pathway proposal

A plausible reaction pathway of glyphosate decomposition with the H2O2/UV system is proposed (Figure 8.2.4.2-46). At pH 7 the glyphosate has the three hydroxyl groups ionized and the amino group protonated. The OH formation follows the classical mechanism related to hydrogen peroxide decomposition under illumination. The OH radical attacks glyphosate, which leads to the formation of a carbon centered radical •CH2-NH2+-CH2-COO- and phosphate. Since evolution of phosphate occurred during the initial stages of glyphosate decomposition, it may be inferred that C-P cleavage led to formation of phosphate (Step 1). The generated radical can react with molecular oxygen present in

the medium at high concentration to give a new radical COO--CH2-NH2+-CH2-O-O (Step 2), which reacts directly with water to form glycine, formaldehyde and HO2 radical (Step 3). The direct formation of glycine without the sarcosine generation was proposed due to verified absence of this compound in the described analytical procedures. The experimental results indicated that when this process was applied, only glycine was present. Furthermore, the absence of AMPA in all samples was confirmed. The generation of formaldehyde was also confirmed as described before. The formaldehyde generated (in all steps) can be directly oxidized to formic acid by the dissolved oxygen under UV light as proposed elsewhere. The steps corresponding to formic acid degradation have been proposed taking into account that the hydroxyl radical formed produces a hydrogen abstraction from the H-C bond to give rise to a •COOH radical. This radical, combined with the existing oxygen in the medium, result in CO2 and the hydroperoxyl radical HO2 (Step 5).

Figure 8.2.4.2-46: A proposal of a reaction scheme for glyphosate degradation with the UV/H2O2 process



For the next oxidation step of glycine in aqueous solution, it is proposed the decarboxylation of the a-amino acids due to the presence of the •OH radical. It results in CO2 and •CH2NH3+ radical (Step 7). This step is suggested elsewhere to degrade amino acids upon exposure to aqueous titania suspensions and irradiated with UV. The combination of •CH2NH3+ and •OH radicals produces formaldehyde and NH4+ (Step 8). The generation of NH3 has been detected as mentioned in the analytical section. There is also another possible step: the addition reaction of molecular oxygen to the •CH2NH3+ radical to produce NH4, formaldehyde and HO2 radical (Step 9). The nitrate formation would follow an alternative path to the NH4 formation. The nitrogen radical also reacts with •OH radical in Step 10 to give a protonated hydroxylamine intermediate. This oxidation path is proposed elsewhere as one of various steps during the photodegradation of an amino acid catalyzed by irradiated TiO2. Afterwards, a possible reaction is that the protonated hydroxylamine reacts with •OH radical to produce methanol and •NH2+-

OH radical (Step 11). This nitrogen radical can react with molecular oxygen to generate •O-O-NH2+-OH nitrogen radical. A similar step is proposed elsewhere in the removal of hydroxylamine by means of processes which generate •OH radicals in aqueous solution. The •O-O-NH2+-OH radical, under reorganization, yields nitrous acid (or nitrite) (Step 12). Then, the nitrous acid, by means of hydrolysis, is transformed into nitric acid (or nitrate) (Step 13). The nitrite and nitrate formation from hydroxylamine is also proposed elsewhere. The nitrate evolution for the longer run time shows that other forms of nitrogen compounds, such as glycine and nitrite, may be present. The methanol formed in Step 11 can be oxidized by •OH radical to generate, first, the CH3O• radical and then, formaldehyde. This oxidation pathway is described elsewhere as a possible mechanism where methanol is oxidized by the free •OH in solution.

In summary, the glyphosate decomposition stable compounds identified in this study were: glycine, formaldehyde, formic acid, nitrate anion, ammonium and phosphate anion (see compounds in bold face in Figure 8.2.4.2-46). The authors note that one of the most interesting outputs of this process is that, at a first glance, it seems that none of the formed byproducts is toxic.

Conclusion

1. As shown in this work, the study of the resulting effects of the most significant operating variables on glyphosate degradation would indicate that the combination of hydrogen peroxide and UV radiation may become a suitable and very simple process to remove glyphosate from water.

2. A proposal for a degradation path based on the observed experimental data has been possible.

3. In a first approach, it seems that glyphosate degradation does not lead to stable toxic end products.

Assessment and conclusion by applicant:

The article describes the degradation of glyphosate under H_2O_2/UVC processes and the generation of breakdown products. The experiment is well described. A degradation pathway is proposed.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on water treatment efficiencies for glyphosate removal and description of degradation pathway under H_2O_2/UVC processes. Data from this study are included in the review provided in [redacted], 2020.

This study used a high concentration of glyphosate (50 mg/L) to check the removal of glyphosate under H_2O_2/UVC processes and identified formation of by-product.

The protocol and analytical method are well described.

However, results of removal efficiencies are not easy to read. It can be retained that there is no pH effects. There is an optimum H_2O_2 concentration, that is in the range 2.2-5.9 mM (75-200 mg/L): within this plateau, conversion of glyphosate after 5 h was almost 70%.

The degradation pathway appears very complex due to formation of many photoproducts. It was found that AMPA was not formed from glyphosate under the test conditions, as C-P bond cleavage was the first step of the degradation, and after the oxidative removal of one carbon unit, glycine was formed. Glycine is a naturally occurring amino acid, and under the experimental conditions it went on to generate methanediol, formic acid, nitrate anion, ammonium and phosphate anions.

Brosillon et al., 2006

Data point:	CA 7.5/094
Report author	Brosillon, S. et al.
Report year	2006

Report title	Chlorination kinetics of glyphosate and its by-products: Modeling approach
Document No	Water Research 40 (2006) 2113-2124
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

Chlorination reactions of glyphosate, glycine, and sodium cyanate were conducted in well agitated reactors to generate experimental kinetic measurements for the simulation of chlorination kinetics under the conditions of industrial water purification plants. The contribution of different by-products to the overall degradation of glyphosate during chlorination has been identified. The kinetic rate constants for the chlorination of glyphosate and its main degradation products were either obtained by calculation according to experimental data or taken from published literature. The fit of the kinetic constants with experimental data allowed the authors to predict consistently the concentration of the majority of the transitory and terminal chlorination products identified in the course of the glyphosate chlorination process. The simulation results conducted at varying aqueous chlorine/glyphosate molar ratios have shown that glyphosate is expected to degrade in a fraction of a second under industrial aqueous chlorination conditions. Glyphosate chlorination products are not stable under the conditions of drinking water chlorination and are degraded to small molecules common to the degradation of amino acids and other naturally occurring substances in raw water.

Methods

Analytical conditions

Glyphosate and glycine were analyzed by HPLC fluorescence after pre-column FMOC derivatization using diethylether. Aqueous formaldehyde was analysed by HPLC–UV after a pre-column 2,4-DNPH derivatization. For the detection of anions (cyanate, nitrate and phosphate ions), samples were analysed using a Dionex AS9-HC ion chromatography (IonPac) column and suppressed conductivity detection.

Kinetics experiments

For the kinetic measurements, 10-4 M solutions of glyphosate, glycine, or sodium cyanate were chlorinated in a 1000 mL well-agitated reactor using HOCl/substrate molar ratios of approximately 4 and 50. At scheduled times, 5 mL portions of reaction mixture were withdrawn and quenched with an adequate volume of sodium thiosulfate solution, derivatized and analysed.

Results and discussion

Glyphosate chlorination

The dissipation of glyphosate and the formation of its chlorination products after 24 h of reaction conducted in a well-agitated reactor at various chlorine/glyphosate molar ratios at pH 7 indicated glyphosate decay was complete at chlorine/glyphosate molar ratios close to 2 or higher. The phosphonic acid moiety of glyphosate was converted into phosphoric acid at all ratios of applied chlorine. Nitrate ion was first detected at a chlorine/glyphosate molar ratio of approximately 10 and a maximum concentration was obtained at chlorine levels of 50 M equivalents or higher. In addition to nitrate, nitrogen gas was also a product of glyphosate chlorination. Hydrated formaldehyde (methanediol) and cyanogen chloride (V; CNCl)) were also formed.

The comparison of the products of glyphosate chlorination conducted at pH 7 and 8 showed no significant differences within the pH range relevant to the purification of natural water commonly sourced for drinking water.

To obtain kinetic rate constants, glyphosate chlorination was monitored for 24 h under the reaction conditions in which the chlorine concentration was limited to 4 M equivalents. Glyphosate degradation was complete and very fast, i.e. at the first measurements (10 min), no glyphosate was detected. The concentrations of methanediol and phosphoric acid reached maximums early in the reaction and remained unchanged during the remaining 24 h reaction period. The kinetics of the production of the transitory product cyanate/cyanogen chloride showed two steps: a sharp increase up to 30 min of contact and a slight increase over the remaining 24 h reaction period. Nitrate is not produced in significant amounts under the low excess chlorine chlorination conditions. The chlorination kinetics were carried out in purified water. However, chlorination of glyphosate conducted in typical environmental water samples, from actual water treatment plants, produced identical by-products.

Glycine chlorination

In order to confirm the proposed pathway for glyphosate chlorination, the chlorination of the related amino acid, glycine, was carried out. At low chlorine/glycine molar ratios (2 and 2.5) the cyanogen chloride/cyanate concentration appeared to reach a maximum value after 24 h of reaction, indicating that all of the active chlorine was consumed, hence all the chlorinating reactions were stopped and only hydrolysis could occur. Runs conducted at chlorine/glycine molar ratios of 5–7 mol mol-1 were strikingly different from those conducted at lower ratios. Indeed the amount of cyanogen chloride/cyanate was found to increase to maximum concentrations early in the reaction and then to decline quickly, most likely due to the chlorine-assisted catalytic hydrolysis of cyanogen chloride.

The nitrate concentration reached a plateau for chlorine/ glycine molar ratio of 5–7 mol mol-1 after 2 h. These results confirmed the hypothesis that nitrate is the terminal product of glyphosate/glycine chlorination and cyanogen chloride/cyanate can be considered as transitory intermediates.

Cyanate (VI) chlorination

The chlorination reaction of sodium cyanate(VI) was also investigated for further insight into the kinetic pathway of glyphosate/glycine chlorination. Fast dissipation of cyanate(VI) occurred when chlorination reactions were carried out at a chlorine/cyanate molar ratio of 5 mol mol-1. The cyanate(VI) chlorination reaction solution contained nitrate and inorganic carbons as the only carbon containing product, suggesting carbon dioxide formation under the reaction conditions. It is believed that the aqueous chlorine reaction with cyanate produces carbon dioxide, hydrochloric acid, nitrate, and nitrogen gas.

Chlorination kinetic model

Chlorination kinetics were simulated at the same glyphosate concentration used for the experimental kinetic measurements (10-4 M) to facilitate comparisons. A typical concentration of glyphosate in raw water is expected to be at the 10-8 M level (1 mg L-1). Nevertheless, the simulation provided a predictive tool for the estimation of kinetic rate constants and the establishment of overall rate of formation and decline of the short-lived transitory and final products of glyphosate chlorination in an industrial plant environment.

The simulations were computed with a glyphosate concentration of 10-4 M in aqueous chlorine solution at an initial pH 7, for different chlorine/glyphosate molar ratios. The model prediction is consistent with a fast decay of glyphosate as observed in experimental runs. For all simulation runs, the mass balance drawn around chlorine, carbon, nitrogen, and phosphorous showed excellent conformity with the initial chlorine and glyphosate concentrations, indicating that the resolution process of the proposed model worked well. Nitrate production showed the greatest differences between the observed and the computed concentrations of over 15% for the higher chlorine/glyphosate molar ratios. The model fits the experimental results quite well as highlighted by the remarkable similarity of the pattern of the evolution of the various glyphosate chlorination products. Therefore, the simulation results support the proposed chemical pathway for glyphosate chlorination and the assumptions made on the reaction rate orders and kinetic rate constants.

Conclusion

It was possible to quantitatively define the decomposition kinetics of glyphosate chlorination and to establish the overall rate of formation and decline of transitory and final chlorination products of glyphosate. The data generated were used to develop mathematical models for predicting glyphosate chlorination and understanding of the nature and lifetime of its transient chlorination products. The simulations of chlorination of glyphosate and model compounds under conditions similar to those used by water treatment plants have shown that chlorination of glyphosate is complete within seconds of contact with chlorine. The initial products of glyphosate chlorination are not stable under the conditions of drinking water chlorination and are degraded to small molecules, such as CO2, phosphoric acid, nitrate, nitrogen gas, and methanediol, similar to the degradation of amino acids and other naturally occurring substances in raw water.

Assessment and conclusion by applicant:

The article generated experimental kinetic measurements for the simulation of chlorination kinetics under the conditions of industrial water purification plants.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on glyphosate degradation pathway under chlorination. Data from this study are included in the review provided in [redacted], 2020.

This article describes the reaction of glyphosate under chlorination and the formation of by-products.

It aimed at defining the decomposition kinetics of glyphosate chlorination and to establish the overall rate of formation and decline of transitory and final chlorination products of glyphosate.

It is a very technical and detailed study, but the following information can be retained.

The first step was to study the degradation of glyphosate under chlorination. This was tested at different molar ratio of chlorine/glyphosate and the dissipation of glyphosate after 24h at pH7 was complete at molar ratio of 2 or higher. The product were not different at pH7 and 8.

Glyphosate chlorination products are not stable under the conditions of drinking water chlorination and are degraded to small molecules such as CO_2, phosphoric acid, nitrate, nitrogen gas, and methanediol, common to the degradation of amino acids and other naturally occurring substances in raw water.

Mehrsheikh et al., 2006

Data point:	CA 7.5/095
Report author	Mehrsheikh, A. et al.
Report year	2006
Report title	Investigation of the mechanism of chlorination of glyphosate and glycine in water
Document No	WATER RESEARCH 40 (2006) 3003 - 3014
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

The chlorination reactions of glyphosate and glycine in water were thoroughly studied. Utilizing isotopically enriched (13C and 15N) samples of glycine and glyphosate and 1H, 13C, 31P, and 15N NMR spectroscopy all significant terminal chlorination products of glycine and glyphosate were identified, and it was shown that glyphosate degradation closely parallels that of glycine. It has been demonstrated that the C1 carboxylic acid carbon of glycine/glyphosate is quantitatively converted to CO2 upon chlorination. The C2 methylene carbon of glycine/glyphosate is converted to CO2 and methanediol. The relative abundance of these two products is a function of the pH of the chlorination reactions. Under near neutral to basic reaction conditions (pH 6–9), CO2 is the predominant product, whereas, under acidic reaction conditions (pH <6) the formation of methanediol is favoured. The C3 phosphonomethylene carbon of glyphosate is quantitatively converted to methanediol under all conditions tested. The nitrogen atom of glycine/glyphosate is transformed into nitrogen gas and nitrate, and the phosphorus moiety of glyphosate produces phosphoric acid upon chlorination. In addition to these terminal chlorination products, a number of labile intermediates were also identified including N-chloromethanimine, N-chloroaminomethanol, and cyanogen chloride. The chlorination products identified in this study are not unique to glyphosate and are similar to those expected from chlorination of amino acids, proteins, peptides, and many other natural organic matters present in drinking water.

Methods

NMR experiments:

NMR spectra were recorded using a spectrometer. The proton and carbon-13 chemical shift scales were in parts per million downfield from external tetramethylsilane at 0.0 ppm. Phosphorus-31 proton decoupled NMR spectra were referenced to external phosphoric acid in D2O and 15N spectra were referenced to an external solution of 15NH4Cl in D2O.

NMR solutions were prepared by dissolving the appropriate amounts of each test material in NMR solvent (neat D2O or buffered D2O) in NMR tubes, with concentrations of glycine and glyphosate for the NMR experiments in the range of 0.38–6.25 mg/mL. Chlorination was conducted in un-buffered D2O at initial pHs of 8, 7 and 5. Additionally, the chlorination reactions were carried out in a 0.48 M borate buffer in D2O at pH 8 and 9. An appropriate amount of dilute NaOCl solution in D2O or buffered D2O was added to the sample in the NMR tube and the sample was sealed, mixed, and analysed immediately by NMR.

High Performance Liquid Chromatography (HPLC) experiments:

Analyses of the radiolabeled experiments were performed using HPLC. A strong cation exchange column was eluted (flow rate: 0.5 mL min-1 at 50°C) with a 0.005 M solution of KH2PO4 (adjusted to pH 2.0 with H3PO4) containing 4% methanol for 35 minutes and the HPLC effluent was passed through a radioactive flow detector. Some samples were analyzed by a second HPLC method using an IonPac column, eluting with a 0.009 M sodium carbonate solution at a flow rate of 1.0 mL min-1 for 35 min at ambient temperature using either a radioactivity detector or suppressed conductivity detection.

Chlorination products of glycine and glyphosate were monitored by HPLC using the corresponding 14C-labelled test materials in unbuffered water at initial pHs of 9, 8, 7, 6, and 5 with aqueous chlorine at a chlorine to substrate molar ratio of 100:1. Additionally, the chlorination reactions were carried out in a 0.05 M borate buffer at pH 8 and 9 or a 0.05 M phosphate buffer at pH 7, 6, and 5 in separate experiments. For these experiments, appropriate amounts of the dilute aqueous chlorine solution were transferred into a 2-mL amber coloured autosampler vial equipped with a Teflon septum cap. An aliquot of each 14C-stock solution of glyphosate or glycine was added to each autosampler vial containing dilute aqueous chlorine solution (chlorine to substrate molar ratio of 100:1) kept at room temperature in order to achieve a final concentration of 3.51–7.25 µM (0.27–0.55 µg/mL) for [2-14C]glycine; 4.29–13.03 µM (0.73–2.2 µg/mL) for [3-14C]glyphosate, and 3.61–13.25 µM (0.61–2.3 µg/mL) for [2-14C]glyphosate. Aliquots of the reaction mixture were then analysed by HPLC after 2 h and at about 24 h of contact.

Results and discussion

Figure 8.2.4.2-47 illustrates the proposed mechanism for the reaction of glycine with aqueous chlorine. N-chloroglycine (I) is formed when one equivalent of aqueous chlorine is reacted with glycine. N-chloroglycine appears to be stable under the reaction conditions in the absence of excess chlorine. When chlorination is conducted with more than 1 equivalent of aqueous chlorine, N,N-dichloroglycine (II) is detected as the predominant product immediately after contact. Decarboxylation and elimination of HCl of the labile N,Ndichloroglycine will provide N-chloromethanimine (III) as a transitory product, which was detected by NMR. From the product distribution reported in this study, it is postulated that a second mole of HCl is eliminated from N-chloromethanimine (III) to possibly form cyanide, which has not been detected in the experiments. The lack of cyanide detection is indicative of its facile chlorination under the reaction conditions to form CNCl (VII) as has been reported. CNCl (VII) undergoes hypochlorite-assisted catalytic hydrolysis to form CO2. Alternatively, it is postulated that N-chloromethanimine (III) is hydrated to form N-chloroaminomethanol (IV), favoured under acidic reaction conditions. It should be noted that hydration of N-chloromethanimine to form N-chloroaminomethanol is analogous to the widely known hydration of formaldehyde in water to form methanediol. N-chloroaminomethanol (IV) appears to be quantitatively converted to methanediol within 24h of formation as determined by the NMR experiments. Based on the 15N-NMR work it is proposed that the nitrogen atom of glycine, which is initially released as NH4Cl and chloramines from the hydrolysis of CNCl and/or decomposition of chloroaminomethanol, is eventually converted to N2 and nitrate due to further reactions with excess aqueous chlorine. The formation of N2 and NaNO3 from CNCl chlorination with 5.7 excess molar equivalents of aqueous chlorine has been reported previously.

Figure 8.2.4.2-48 illustrates the proposed mechanism for the reaction of glyphosate with aqueous chlorine. Analogous to the chlorination of glycine, and based on NMR evidence, N-chloroglyphosate (VIII) is postulated to be the first intermediate in the glyphosate chlorination. Rearrangement of N-chloroglyphosate through a six-membered ring transition state results in transfer of the phosphorus moiety to the carboxylate oxygen and ultimately formation of the acylphosphate intermediate IX and methanediol. Decomposition of intermediate IX by hydrolysis would lead to the formation of phosphoric acid and glycine/N-chloroglycine. Further chlorination of glycine/N-chloroglycine, according to the reaction scheme depicted for glycine would lead to a mixture of methanediol, CO2, N2, and nitrate as the final chlorination products.

With the exception of the formation of phosphoric acid, the final chlorination products of glyphosate are identical to those observed for glycine chlorination. The phosphorus-31 NMR study revealed that addition of one or more equivalents of aqueous chlorine to glyphosate in buffered and unbuffered D2O, and in the pH ranges of 5–9, produced phosphoric acid as the only P-containing terminal chlorination product.

Figure 8.2.4.2-47: Proposed mechanism of glycine chlorination (compounds in boxes are terminal products and intermediates are in brackets)

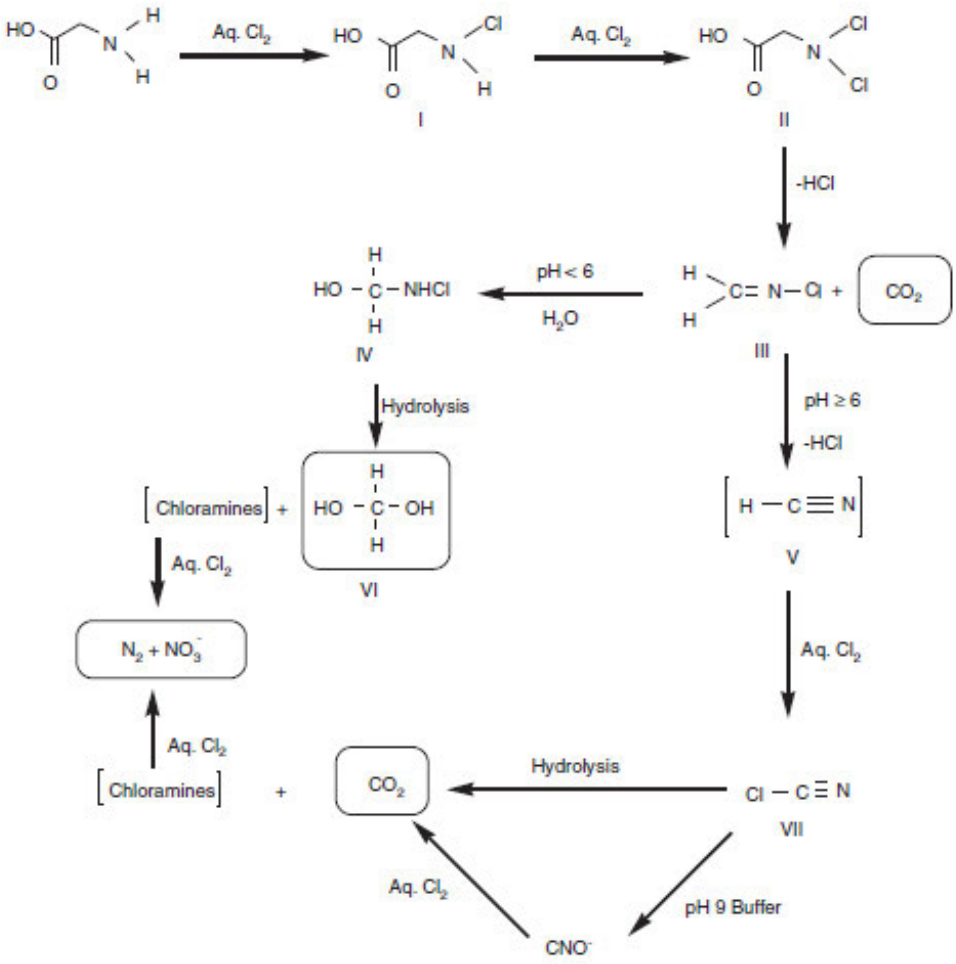
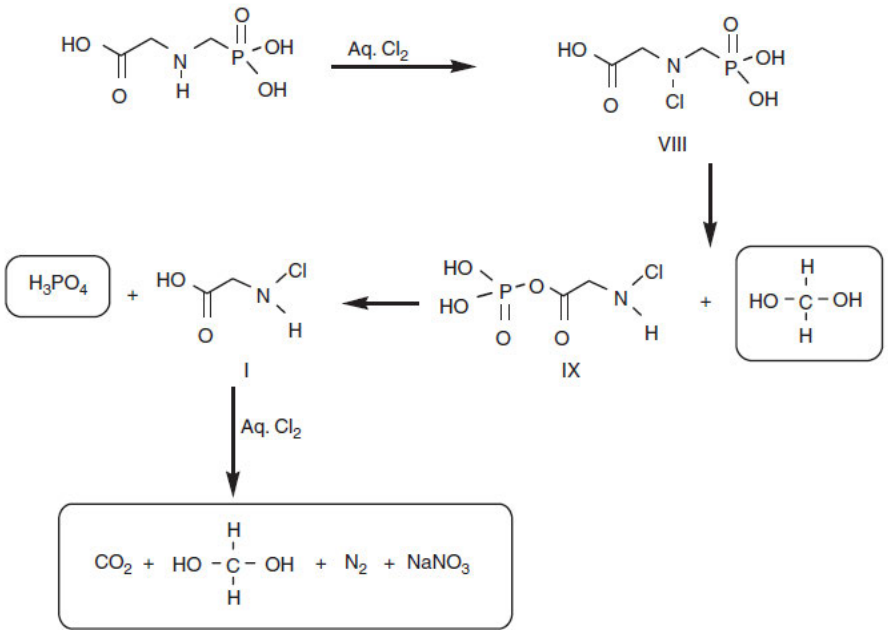


Figure 8.2.4.2-48: Proposed mechanism of glyphosate chlorination (compounds in boxes are terminal products)



Conclusion

The results of this study have shown that under aqueous chlorination conditions, glyphosate is totally degraded to small molecules common to the degradation of naturally occurring substances in raw water, and that the degradation pathway follows that of glycine. Utilizing stable isotopes and NMR spectroscopy we were able to identify all significant chlorination products of glycine and glyphosate after total breakdown. It has been demonstrated that upon chlorination the C1 carboxylic acid carbon of glycine/glyphosate is converted to CO2; the C2 methylene carbon of glycine/glyphosate is converted to CO2 and methanediol; the nitrogen of glycine/glyphosate is transformed into nitrogen gas and nitrate; the C3 phosphonomethylene carbon of glyphosate is converted to methanediol; and the phosphorus moiety of glyphosate produces phosphoric acid. The terminal glyphosate chlorination products identified in this study (phosphoric acid, CO2, methanediol, N2 and nitrate) are not unique to glyphosate and would also be expected as products from chlorination of other natural organic matter present in raw water.

Assessment and conclusion by applicant:

The article investigates the mechanism of chlorination of glyphosate and glycine in water. The methods and results are sufficiently described.

The article is considered reliable.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on glyphosate degradation pathway under chlorination. Data from this study are included in the review provided in [redacted], 2020. The chlorination reactions of glyphosate and glycine in water were thoroughly studied. The methods and results are sufficiently described.

Analyses were performed using HPLC. Chlorination products of glycine and glyphosate were monitored by HPLC using the corresponding 14C-labelled test materials

With the exception of the formation of phosphoric acid, the final chlorination products of glyphosate are identical to those observed for glycine chlorination

Klinger et al., 2008

Data point:	CA 7.5/096
Report author	Klinger, J. et al.
Report year	2008
Report title	Formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra (methylenephosphonic acid)
Document No	OZONE SCIENCE & ENGINEERING Vol 20, 99-110
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

Because of its widespread use and its low biodegradability, ethylenediaminetetra(methylenephosphonic acid) (EDTMP) might be found in river waters and could even be present in raw waters of drinking water treatment plants. In Europe, average surface water concentrations in the low µg/L range are predicted. Therefore, it is of interest for drinking water supplies whether EDTMP can be eliminated during water treatment processes. Since many water treatment plants have an ozonation step, this paper

deals with the behaviour of EDTMP during ozonation. Due to its chemical structure, a reaction scheme for the ozonation of EDTMP similar to the reaction pathway for the ozonation of EDTA was predicted.

The experimental results confirmed the predicted mechanism as well as the formation of glyphosate and AMPA during ozonation of waters containing EDTMP. Ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions.

Methods

Ozone was produced from high purity oxygen using an ozone generator Ozomat COM 6000. The reactor was a glass bottle with a working volume of 2 L. Ozone concentration in the gas stream was measured at the reactor inlet using an ozone measuring instrument GM 6000. Ozone was transferred into the liquid sample for three minutes with a gas stream of 40 L/h containing about 35 mg/L ozone. While stirring continuously, this time period was long enough to reach equilibrium conditions for dissolving ozone in the aqueous phase. After three minutes, the concentration of dissolved ozone was determined, and the target chemical was added. All reactions were carried out at room temperature in distilled water. Initial concentration of the target chemical was 1 mg/L and initial concentration of dissolved ozone was about 3 mg/L. This ozone dose is close to water works conditions. The pH value after addition of phosphonic acids was constant at pH 5 without adding any buffer solutions. Additionally, experiments in tap water from Karlsruhe were carried out at pH 7. In all cases, total reaction time was 10 minutes. Samples were taken after different reaction times and ozonation was stopped by adding sodium thiosulfate.

EDTMP was preconcentrated by evaporating the sample to dryness, methylated with diazomethane and determined by liquid chromatography and mass spectrometry coupled by a thermospray interface. A Merck LiChrospher 100 Diol (5 um) 125 x 4 mm separation column with a gradient mobile phase containing isopropyl alcohol and n-hexane was used.

Glyphosate and AMPA were determined after extraction on an ion exchange resin by liquid chromatography, post-column derivatization using orthophthaldialdehyde and N,N-dimethyleneaminoethanethiol and fluorescence detection. A strong basic cation exchange separation column with an isocratic aqueous mobile phase containing 0.005 M potassium dihydrogen phosphate and 4% methanol was used.

Dissolved ozone concentration was determined by the indigo method, which is based on the decolorization of the blue indigo trisulfonate solution by ozone measured spectrophotometrically at 600 nm. Orthophosphate also was determined spectrophotometrically. This method is based on the formation of a molybdenum blue complex which is measured at 880 nm. Phosphonoformic acid was analyzed by ion chromatography with conductivity detection. Standard deviations of the chromatographic methods were about 10%.

Results and discussion

Within one-minute reaction time, EDTMP is completely eliminated, but orthophosphate is formed up to only 50% yield. Because orthophosphate formation rises up to 60% within the entire reaction time of 10 minutes, one-half of the initial EDTMP concentration reacts very fast to orthophosphate and the other half is oxidized to further phosphorus-containing metabolites, such as phosphonoformic acid (PFA) and aminomethylphosphonic acid (AMPA) which may not react or react only very slowly with ozone. This is in accordance with ozone degradation during ozonation of EDTMP. After three minutes reaction time, no further significant ozone consumption takes place. Thus, after 10 minutes reaction time, the concentration of dissolved ozone is still 1.2 mg/L.

Due to the similar chemical structures of EDTMP and ethylenediaminetetraacetic acid (EDTA), an analogous reaction pathway and, consequently the formation of analogous intermediates during ozonation, seems to be quite probable. In order to verify the formation of the predicted ozonation products, not only concentration of orthophosphate but also concentrations of glyphosate, AMPA and phosphonoformic acid were determined during ozonation of EDTMP. Furthermore, the identified

oxidation products were treated with ozone in order to check their behaviour during ozonation separately.

Glyphosate, AMPA and phosphonoformic acid could be clearly identified under respective experimental conditions. With an initial concentration of 1 mg/L EDTMP, after 10 minutes reaction time, 1.2 µg/L glyphosate, 100 µg/L AMPA and 63 µg/L phosphonoformic acid were found. As can be seen from the mass balance data given in Table 8.2.4.2-31, up to now not all phosphorus-containing oxidation products are identified. This is due to the reaction pathway given in Figure 8.2.4.2-49, where other phosphorus-containing intermediates might be formed which are not amenable to analytical methods. Furthermore, the mass balance data in the table shows that no significant reaction takes place after 30 seconds, although dissolved ozone is still present. As already mentioned, this might be due to the low reactivity of the identified phosphorus-containing oxidation products. To verify this assumption, glyphosate, AMPA and PFA were treated with ozone.

Figure 8.2.4.2-49: Suggested reaction pathway for the oxidation of EDTMP by ozone

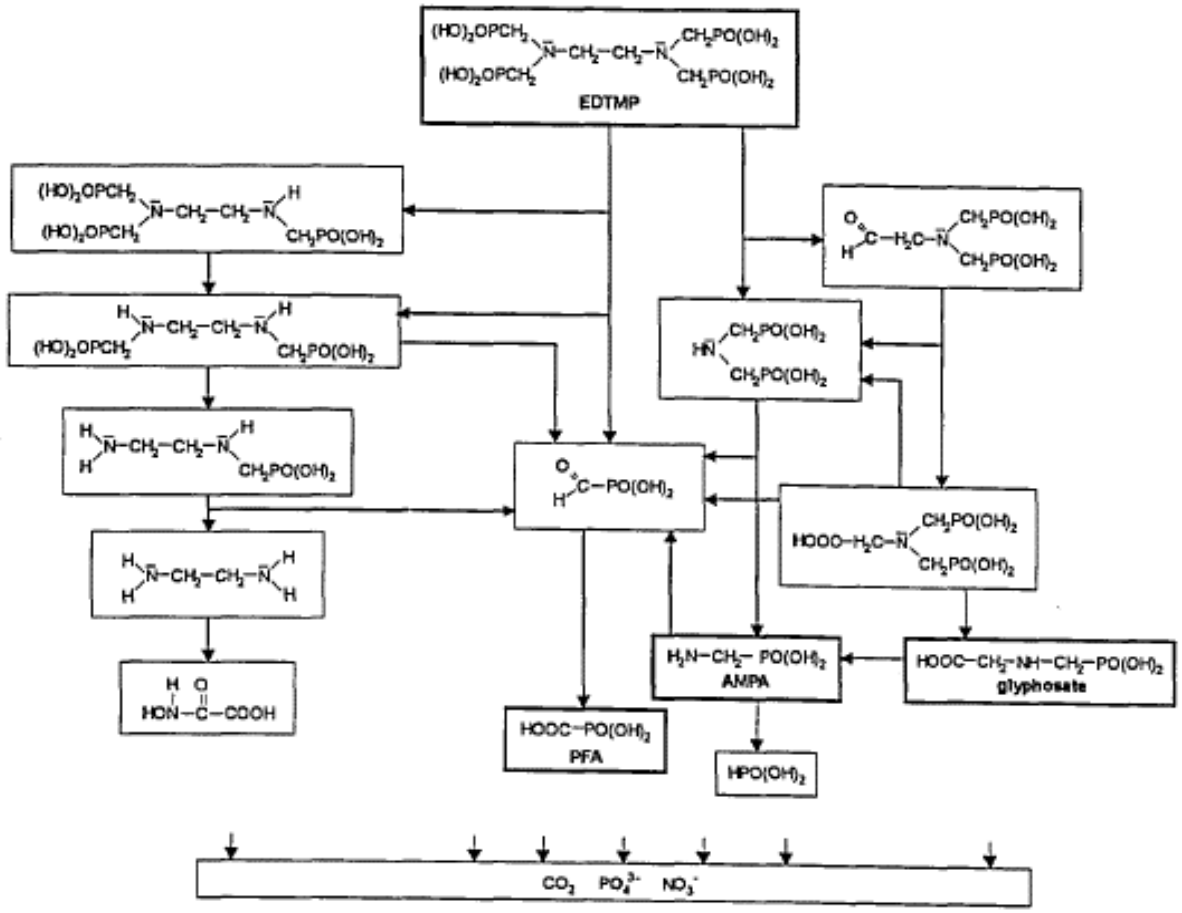


Table 8.2.4.2-31: Phosphorus mass balance for the ozonation of EDTMP

reaction time in sec	0	30	300	600
EDTMP in µmol P/L	9.2	0.62	0	0
glyphosate in µmol P/L	0	0.01	0.007	0.007
AMPA in µmol P/L	0	0.84	0.90	0.90
phosphonoformic acid in µmol P/L	0	0.66	0.58	0.55
orthophosphate in µmol P/L	0	4.3	5.4	5.7
sum in µmol P/L	9.2	6.4	6.9	7.2
identified oxidation products in % P	100	70	75	78

In Figure 8.2.4.2-50 oxidation of glyphosate and formation of orthophosphate as dependent on ozonation time is shown. It can be seen from this figure, glyphosate is eliminated up to 50% after ten minutes reaction time. The low reactivity might be due to the amine group of glyphosate which is protonated and therefore inert at pH 5. As orthophosphate concentration is raised up to only 30%, further phosphorus-containing oxidation products, such as AMPA, phosphonoformaldehyde and PFA must be formed.

In Table 8.2.4.2-32 the phosphorus mass balance at different reaction times for the ozonation of glyphosate is listed. Besides orthophosphate, AMPA is clearly identified as an oxidation product, but PFA could not be detected. From this table it can be seen that nearly all phosphorus-containing oxidation products are identified, so the reaction AMPA -phosphonoformaldehyde - phosphonoformic acid might not be a favoured pathway.

In Figure 8.2.4.2-52, oxidation of AMPA and formation of orthophosphate is shown. Within a reaction time of ten minutes AMPA is not totally eliminated. Formation of orthophosphate corresponds nearly with elimination of AMPA and PFA is not identified again. This corresponds to the conclusion that the reaction AMPA – phosphonoformaldehyde - phosphonoformic acid is not a favoured pathway.

In Figure 8.2.4.2-53, phosphonoformic acid is eliminated only up to 20% and elimination of PFA corresponds very well with formation of orthophosphate.

As glyphosate, AMPA and PFA are not totally eliminated by ozone under these experimental conditions, they can be identified as oxidation products during ozonation of EDTMP. Thereby the formation of orthophosphate up to only 60% is explainable. However, PFA was not detected during ozonation of glyphosate and AMPA. This might be due to the high detection limit of 50 µg/L; but this means on the other hand, that formation of PFA, which is clearly identified during ozonation of EDTMP, is also possible by other reaction pathways. This is according to the predicted reaction pathways in Figure 8.2.4.2-49. But obviously, the reaction AMPA - phosphonoformaldehyde - phosphonoformic acid is not favoured as 50% of the EDTMP reacts very fast to orthophosphate, other phosphorus-containing metabolites must be formed, which react very fast and are converted into orthophosphate. Moreover, one can divide the reactions in Figure 8.2.4.2-49 into the two main pathways dephosphonomethylation and C-N-cleavage.

Because formation of glyphosate and AMPA during ozonation of EDTMP is of particular importance, additional experiments were carried out in tap water in order to prove whether or not glyphosate and AMPA might be formed under these conditions. In Table 8.2.4.2-33 the determined concentrations of glyphosate and AMPA are listed. As can be seen from this table, glyphosate and AMPA are clearly identified also during ozonation of EDTMP in tap water. Comparing Table 8.2.4.2-33 with Table 8.2.4.2-31, it seems that more glyphosate and less AMPA is formed during ozonation of EDTMP at pH 7 in tap water than at pH 5 in model solutions.

Figure 8.2.4.2-51: Ozonation of glyphosate at pH5

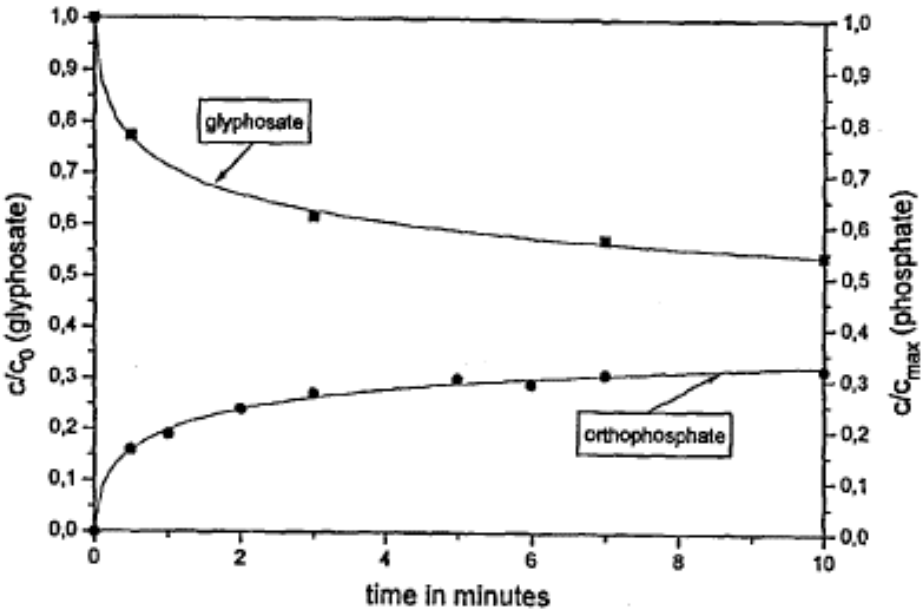


Table 8.2.4.2-32: Phosphorus mass balance for the ozonation of glyphosate

reaction time in sec	*0*	*30*	*180*	*600*
glyphosate in µmol P/L	5.92	4.57	3.65	3.20
AMPA in µmol P/L	0	0.41	0.62	0.69
orthophosphate in µmol P/L	0	0.95	1.61	1.91
sum in µmol P/L	5.92	5.94	5.88	5.80
identified oxidation products in % P	100	100	99	98

Figure 8.2.4.2-52: Ozonation of AMPA at pH5

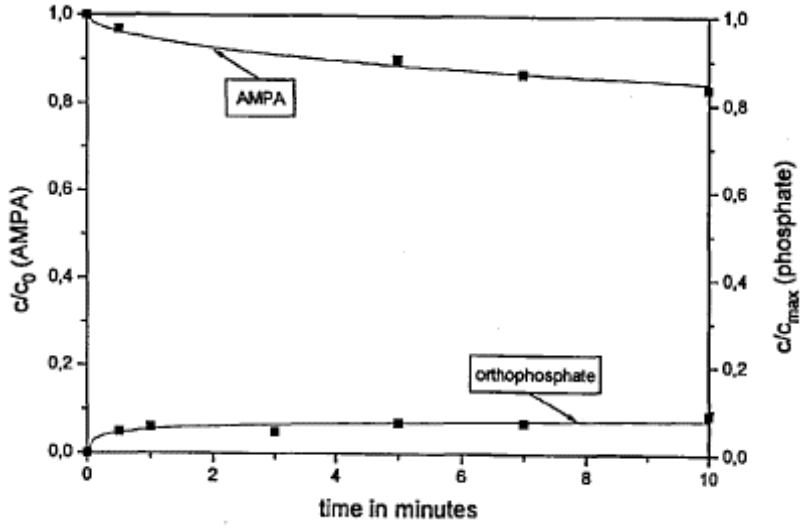


Figure 8.2.4.2-53: Ozonation of phosphonoformic acid at pH5

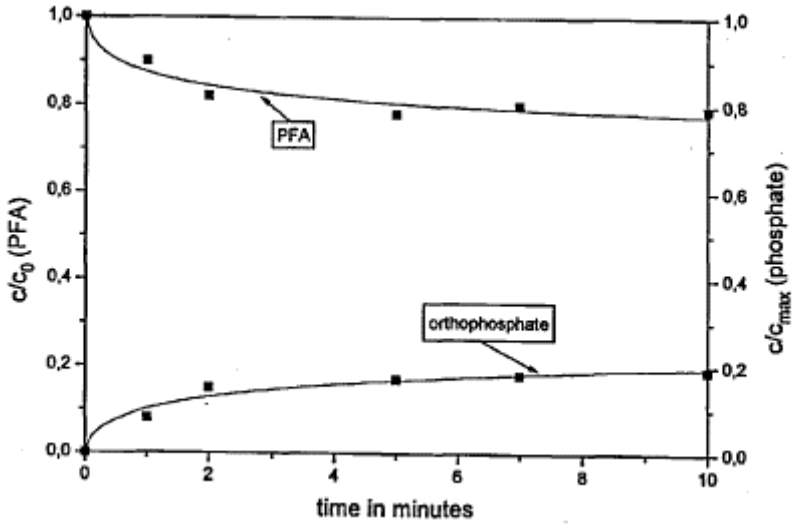


Table 8.2.4.2-33: Formation of glyphosate & AMPA during ozonation of EDTMP in tap water (pH 7)

reaction time in sec	30	180	600
glyphosate in µmol/L	0.03	0.05	0.06
AMPA in µmol/L	0.52	0.34	0.36

Conclusion

The experimental results confirmed the predicted mechanism as well as the formation of glyphosate and AMPA during ozonation of waters containing EDTMP.

Assessment and conclusion by applicant:

The article investigates the formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra(methylenephosphonic acid). Ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions.

The methods and results are sufficiently described.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

The study is considered reliable to provide information on water treatment efficiencies under ozonation for glyphosate and AMPA removal. Data from this study are included in the review provided in [redacted], 2020.

The study investigates the formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra(methylenephosphonic acid).

The methods and results are sufficiently described, and the study provides reliable information on the degradation of glyphosate under ozonation, in the experimental conditions that are decribed.

Ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded (about 50%) orthophosphate and presumably AMPA within 10 minutes;

This is consistent with results from other studies on ozonation, showing that dissipation of glyphosate may be less effective at acidic pH. In Shen, Y *et al.* 2011 complete removal was achieved in 25 min at acidic pH (4.9).

AMPA was partially degraded to orthophosphate within 10 minutes at pH5.

Hoek et al., 2014

Data point:	CA 7.5/098.
Report author	Hoek *et al.*,
Report year	2014
Report title	Drinking water treatment technologies in Europe: state of the art – challenges – research needs
Document No	Journal of Water Supply: Research and Technology—AQUA – Vol 63 p. 124-130
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (supportive information on drinking water treatment technologies applied in Europe. Does not explicitely focuses on glyphosate. Not summarized)

Assessment and conclusion by RMS:

This study provides a survey focusing on raw drinking water sources and drinking water treatment technologies applied in Europe.
Although not focusing on glyphosate, it is considered to provide supportive information on drinking water treatment technologies, and all relevant information from this study is included in the study summary of [redacted], 2020, CA7.5/002.

Gillefalk et al., 2014

Data point:	CA 7.5/097
Report author	Gillefalk *et al.*
Report year	2014
Report title	Potential Impacts of Induced Bank Filtration on Surface Water Quality: A Conceptual Framework for Future Research
Document No	Water 2018, 10, 1240
Guidelines followed in study	None
Deviations from current test guideline	Not applicable

GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (supportive information on Indiced bank filtration. Does not explicitely focuses on glyphosate. Not summarized)

Assessment and conclusion by RMS:

This study focuses on induced bank filtration (IBF), but does not provides information on pesticide removal rate. It aimed at studying the potential effects of IBF on several physical, chemical and biological processes in both the sediment and open water column.
It provides resliable information on percentage of induced bank filtration (IBF) in drinking water supply of different countries, which have been cited in [redacted], 2020.

B.8.3. FATE AND BEHAVIOUR IN AIR

B.8.3.1. Route and rate of degradation in air

The behaviour of glyphosate in air was investigated in 7 studies (6 existing studies and 1 new study provided for renewal).

Table 8.3.1-1: List of existing studies on route and rate of degradation in air

Annex point	Study	Previous evaluation in RAR (2015) / DAR (2001)	Status in RAR 2021	Remark
CA 7.3.1/001	[redacted], 2020	-	Acceptable	Calculated for glyphosate acid
CA 7.3.1/002	[redacted], 2012	Accepted in RAR 2015 (Phys-Chem section)	Acceptable	Calculated for glyphosate acid as well as for salts
CA 7.3.1/003	[redacted], 1997	Accepted in RAR 2015	Acceptable for plant experiment only	
CA 7.3.1/006	[redacted], 1993	Accepted in RAR 2015	Acceptable	
CA 7.3.1/007	[redacted], 1992	Not mentioned in RAR 2015 or DAR 1998	Acceptable	
CA 7.3.1/004	[redacted], 1996	Accepted in RAR 2015	Supportive	Analytical phase report to [redacted], 1995
CA 7.3.1/005	[redacted], 1995	Accepted in DAR 1998	Supportive	Field phase report related to [redacted], 1996

B.8.3.1.1. Atmospheric half-life

[redacted], 2020

Data point:	CA 7.3.1/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate: Calculation of the Chemical Half-Life in the Troposphere
Report No	110054-016
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Previous evaluation	Not, not previously submitted
Acceptability/Reliability	Yes

I. METHODS

The half-life of glyphosate in air was estimated according to structure-activity relationship (SAR) methods developed by [redacted] The approach of [redacted] was based on a comprehensive set of experimental data to result in a quantitative structure-activity relationship (QSAR) mathematic model that allows for estimation by calculation, starting from the molecular structure of a compound. The calculation procedure has been transferred into the personal computer program "Atmospheric Oxidation Program" (AOP) by Meylan & Howard. The version AOPWINTM 1.92a (U.S. EPA, 2008) was used for the calculations being part of the EPI SuiteTM set of programs.

Considering the chemical structure of glyphosate, it can be concluded that reactions with photochemical produced hydroxyl radicals will mainly determine its degradation rate (Ktotal, indirect photoreaction ≈ kOH) in the air. No significant ozone reaction is expected and therefore not included in the assessment. The diurnally and annually averaged 12-h daylight hydroxyl radical concentration of 1.5 × 106 molecules (radicals)/cm3 was used.

II. RESULTS AND DISCUSSION

The overall reaction rate of glyphosate with hydroxyl radicals is estimated to be 79.008 x 10 12 cm3 / (molecule x s). This rate is derived mainly from incremental reactions like hydrogen abstraction (15.2009 x 10-12 cm3 / (molecule x s), value estimated) and reactions to OH groups (assumed value of 63.8000 x 10-12 cm3 / (molecule x s)).

Based on the overall hydroxyl radical reaction rate constant in combination with the concentration of these radicals in the atmosphere (i.e. 1.5 x 106 OH radicals/cm3), the half-life of glyphosate in air is derived to 0.135 days (1.625 hours). This estimate should be regarded as worst-case assumption as the approach does not consider the contribution of any other reactive species than hydroxyl radicals to the overall atmospheric degradation of glyphosate in air.

III. CONCLUSIONS

The active substance glyphosate is considered to be susceptible to reactions with hydroxyl radicals which contribute to the overall degradation of the substance in the atmosphere. An attack by hydroxyl radicals should result in the formation of multiple primary radicals. Their formation may be followed by secondary oxidation products that can be eliminated from the atmosphere by wet and/or dry deposition.

The half-life of glyphosate in air was estimated with 0.135 days (1.625 hours).

Assessment and conclusion by applicant:

The study is considered valid to address the data point. The half-life of glyphosate in air was estimated with 0.135 days (1.625 hours).

Assessment and conclusion by RMS:

The study is considered acceptable.

[redacted], *2012*

Data point:	CA 7.3.1/002
Report author	[redacted]
Report year	2012
Report title	Atmospheric Oxidation of Glyphosate Salts - Atkinson Calculation
Report No	MSL0024050
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable

Previous evaluation	Yes, accepted in RAR (2015) (Phys-Chem section)
Acceptability/Reliability:	Yes

I. MATERIAL AND METHODS

The reaction of glyphosate acid in the atmosphere with hydroxyl radicals has been estimated using the method of Atkinson.

For the calculation, the Atmospheric Oxidation Program AOPWIN, version 1.92 was used. This is a computer programme that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It also estimates the gas-phase reaction between ozone and olefinic/acetylinic compounds. The rate constants estimated by the program are used to calculate an atmospheric half-life for the organic compound based upon average atmospheric concentrations of hydroxyl radicals and ozone.

II. RESULTS AND DISCUSSION

Results for the different glyphosate salts are presented in the table below.

Table 8.3.1.1-1: Results of Atkinson calculation

Compound	Half-life (hours)1	Half-life (days)	Overall Rate Constant (cm3/molecule/sec)
Glyphosate Free Acid2	1.6	0.135	79.0 × 10-12
Glyphosate Isopropylamine Salt	1.380	0.115	93.0 × 10-12
Glyphosate Potassium Salt	1.719	0.143	74.7 × 10-12
Glyphosate Ammonium Salt	1.719	0.143	74.7 × 10-12
Glyphosate Dimethyamine Salt	1.663	0.139	77.2 × 10-12

1 Tropospheric half-life is based on OH concentrations of 1.5 × 106 OH radicals/cm3 and 12 hours day.
2 Values from the Glyphosate Monograph list of endpoints as stipulated in SANCO 651l/VI/99-final.

III. CONCLUSIONS

The computer program AOPWIN has estimated the overall rate constant for the gas-phase reaction between hydroxyl radicals (OH) and three different salts of glyphosate to range from 74.7 x 10-12 to 93.0 x 10-12 cm3/molecule/sec. The atmospheric half-life of glyphosate salts is estimated to be in the range of 1.380 to 1.719 hours (0.115 to 0.143 days) as a result of gas-phase reactions between glyphosate salt and photochemically produced atmospheric hydroxyl radicals. Since glyphosate contains no olefinic or acetylenic sites, no reaction with atmospheric ozone was estimated.

Assessment and conclusion by applicant:

The photochemical oxidative decomposition of glyphosate in the atmosphere has been assessed via the method described by Atkinson, resulting in half-lives in a range of 1.380 to 1.719 hours for several glyphosate salts. The assessment is considered supportive.

Assessment and conclusion by RMS:

The study is considered acceptable.

B.8.3.1.2. Volatilisation from soil and plants

The vapour pressure of glyphosate is 1.31 x 10-5 Pa (25 °C). Based on EVA 3.2, this is equivalent to a vapour pressure of 6.81 x 10-6 Pa at 20°C. According to FOCUS Air criteria, glyphosate can be classified as not volatile from soil and plants.

The following experimental studies investigate volatilisation of glyphosate from soil and plant surfaces.

[redacted], *1997*

Data point:	CA 7.3.1/003
Report author	[redacted]
Report year	1997
Report title	Determination of the rate of volatilization of glyphosate from soil and plant surface (french beans)
Report No	191071
Guidelines followed in study	BBA Guideline Part IV, 6-1
Deviations from current test guideline	No current guideline in force
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Acceptable for plant experiment only

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled

Identification: [14C]glyphosate (NOTOX substance 63711)
Lot No.: 25A
Specific activity: 11.07 MBq/mg
Radiochemical purity: 98.3 %

Unlabelled
Identification: formulation glyphosate 360 g/L (NOTOX substance 68679)
Lot No.: 907116
Composition: 360 g/L
Spraying solutions of different concentrations were prepared by mixing [14C]glyphosate with non-radiolabelled formulation glyphosate 360 g/L.

2. Soil:

The study was performed with LUFA Speyer 2.1 standard soil. After receipt, the soil was stored at NOTOX in the open air, in open containers. Before use, the soil was sieved through a 2 mm analytical sieve. Before the start of the test, the soil was adjusted to approximately 60 % of the maximum water capacity. Soil properties are given in the table below.

Table 8.3.1.2-1: Soil properties of Speyer 2.1 (slight humic sand)

Location	RLP, RheinzabernTeufelskanzel
Horizon (cm)	20
Charge number	F12095
Organic carbon content (%)	0.62
Organic matter content (%) 1	1.07
Particles < 20 µm (%)	6.5
pH	5.9
Maximum water capacity (%)	30.6

1 Calculated from organic carbon according to OM = OC / 0.58

3. Plant:

French beans were cultivated in soil at approximately 22 °C and 16 hours simulated daylight each day. Leaves with an area of at least 30 cm² are used for the volatility test.

B. STUDY DESIGN

Several tests with soil and one test with leaves were carried out, an overview is given in the table below.

Table 8.3.1.2-2: Overview of test conditions

	Test 1	Test 2	Test 3	Test 4
Matrix	Soil	Soil	Soil	Leaves
Concentration in spraying solution (g/L)	21.6	21.6 1.1 348	3.66	21.6
Application rate (kg a.s./ha)	4.32	0.2 4 70	not indicated	4.32
Temperature (°C)	19.5 ± 1.3	20.1 ± 1.3	not indicated	20.8 ± 3.0
min/max Temperature (°C)	16.8 21.6	15.0 22.0	not indicated	14.8 24.9
rel. Humidity (%)	35.2 ± 5.0	20.0 ± 3.9	not indicated	42.8 ± 2.8
min/max rel. humidity (%)	25.7 42.5	16.2 34.4	not indicated	31.2 51.3
Air flow (m/s)	1.2-1.3	1.2	not indicated	1.1-1.2

1. Experimental conditions

The tests were conducted in a rectangular box. This was filled with either the soil samples or the leaves and a constant air flow was set. Samples (except for t=0) were kept in this apparatus allowing an air flow of 1.1-1.3 m/s to pass over the test soil or plants. The soil or plant samples, with the exception of the t=0 samples, were transferred into the experimental set up.

2. Sampling

Samples were taken 1 hour, 3 hours, 6 hours and 24 hours after application. Samples t=0 were extracted immediately after application and temperature, humidity and air flow were logged.

3. Analytical procedure

Soil samples were extracted and subsequently submitted to LSC in order to determine non-extractable residues or directly combusted and analysed via LSC. Plant leaf samples were not extracted but analysed directly via LSC. For direct LSC analysis, triplicate soil subsamples of 2 g or whole plant leaves were combusted using an oxidiser. The resulting 14C-CO2 was trapped and analysed using LSC.

The results of the first test showed that the amount of glyphosate in the soil was > 80 % relative to t=0 at each time point. The amount of glyphosate relative to the applied amount, however, was slightly below 80 % (74 %) at t=24 hours. For this reason, the volatilisation over 24 hours was further investigated at different application rates in the second test. Samples were either extracted prior to combustion or combusted directly without extraction.

Based on the results of test 1, it was suspected that the combustion is not completely quantitative for glyphosate. In order to confirm this, the third test was carried out with all samples combusted directly.

The radiochemical purity was determined before start and after finalisation of the experiments by HPLC method as 96.7 % and 97.1 %, respectively. LOD and LOQ were not given.

II. RESULTS AND DISCUSSION

A. DATA

Results of the determination of recovery on soils and plants are presented below.

Table 8.3.1.2-3: Recovery from soil, first test

t (h)	Recovery after extraction (% AR)	Recovery after combustion (% AR)	Total recovery (% AR)	Average recovery ± SD (% AR)	Average recovery ± SD relative to t=0
0	67.4	20.1	87.5	89 ± 4.5	100
0	62.7	21.8	84.5		
0	77.8	17.3	95.1		
0	72.5	17.1	89.6		
1	74.0	17.7	91.7	91 ± 0.5	102 ± 0.6
1	74.0	17.0	91.0		
3	72.5	14.6	87.1	87 ± 0.5	97 ± 0.5
3	73.4	13.0	86.4		
6	73.6	22.4	96.0	94 ± 3.3	105 ± 3.6
6	72.5	18.9	91.4		
24	61.7	10.6	72.3	74 ± 5.4	82 ± 6.0
24	59.6	8.8	68.4		
24	58.2	14.1	72.3		
24	64.1	17.0/16.61	81.1		

1 In order to check if the low recovery was due to non-reproducible combustion, the combustion of triplicate subsamples of this sample was repeated. The result was almost the same as the first combustion, confirming a reproducible combustion.

Table 8.3.1.2-4: Recovery from soil, second test

t (h)	Application rate (kg a.s./ha)	Recovery after extraction (% AR)	Recovery after combustion (% AR)
0	0.2	78.1	-
0		77.7	-
24		-	85.3
24		-	86.8
24		-	81.2
24		-	81.3
0	4	87.7	-
0		86.4	-
24			89.9
24			65.9/72.11
0	70	96.6	-
0		94.3	-
24		-	135.0
24		-	127.0
24		-	153.0
24		-	128.0

1 In order to check if the low recovery was due to inhomogeneity of the sample, the entire sample was combusted in portions of ca. 2 g. The result is similar the first combustion, indicating that the sample was homogeneous.

Table 8.3.1.2-5: Results volatility from soil, third test

t (h)	Recovery of test substance (% AR)	Recovery of test substance (% AR)
0	95.5	96.8
0	96.4	
0	96.7	
0	98.4	
72	86.3	80.2
72	89.5	
72	69.7	
72	75.1	

Table 8.3.1.2-6: Recovery from plant leaves

t (h)	Recovery after combustion (% AR)	Average recovery ± SD (% AR)	Recovery ± SD relative to t=0
0	90.7	92 ± 2.5	100
0	94.6		
0	89.6		
0	94.1		
1	89.2	91 ± 2.3	98 ± 2.5
1	92.4		
3	90.7	92 ± 1.7	100 ± 1.8
3	93.1		
6	91.0	92 ± 0.8	99 ± 0.8
6	92.1		
24	94.2	95 ± 0.9	103 ± 0.9
24	96.1		
24	95.3		
24	95.9		

B. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

At the first soil test after 24 hours, 82 ± 6 % relative to t=0 was recovered from the soil samples. The amount of test substance in the soil samples relative to applied varied from 68 to 96 %. The relatively low recovery, even at t=0 hours can be explained by the fact that the samples were first extracted, then stored for one day, and then combusted.

The results of the second test confirm the results of the first test. At both 0.2 and 4 kg a.s./ha application rate, the amount recovered after 24 hours relative to applied is around or slightly below 80 %. At the application rate of 70 kg a.s./ha recoveries of 127-153 % are found after 24 hours. Apparently, a mistake was made during preparation or application of this formulation (which is rather viscous due to the high concentration). Therefore, these results were not taken into account. The relatively low recovery, at t=0 h can be explained by the fact that the t=0 hours samples were only extracted and not combusted. Apparently, the recovery of the extraction is not quantitative. This is also supported by the data of the first soil test. In general, combustion leads to better recovery of the test item than extraction which shows these two methods are not directly comparable.

In the third soil test, recoveries decreased from 96.8 to 80.2 % after 3 days. From these results it can be concluded that glyphosate can be recovered from soil by combustion almost quantitatively directly after application However, the recovery after a period of three days was only 80 %. A possible explanation for the incomplete combustion after storage is a very strong binding of glyphosate to soil, even under the combustion conditions.

After 24 hours, 103 ± 1 % relative to t=0 was recovered from the plant samples. The amount of test substance on the plant samples relative to applied varied from 89 to 96 %. Based on these results, it is concluded that less than 2 % of the test substance evaporates from the plant leaves within 24 hours under the conditions of the test.

III. CONCLUSIONS

After 24 hours, 82 ± 6 % of the glyphosate relative to the amount determined at t=0 was recovered from the soil samples. The amount of test substance in the soil samples relative to applied varied from 68 to 96 %. The recovery of the [14C]glyphosate after combustion of the soil samples varies and is better than following extraction. Directly after application recoveries of 97 % were found. However, if the soil sample is combusted after storage, the recovery is not quantitative anymore. In a third experiment, the recovery after a storage period of three days was only 80 %. A possible explanation for the incomplete combustion after storage is very strong binding of glyphosate to soil, even under the combustion conditions. Overall, based on these results, it is concluded that less than 20 % of the test substance evaporates from the soil samples within 24 hours under the conditions of the test.

After 24 hours, 103 ± 1 % of the glyphosate relative to the amount determined at t=0 was recovered from the plant surface. The amount of test substance on the plant samples relative to applied (nominal value) varied from 89 to 96 %. Based on these results it is concluded that less than 20 % of the test substance evaporates from the plant leaves within 24 hours under the conditions of the test.

Assessment and conclusion by applicant:

The study was conducted in accordance with the guideline relevant at that time.

The methodology is to measure the loss of glyphosate from soil and plants indirectly through extraction/combustion. For the two types of experiments, only the recovery relative to time zero is considered applicable as the recoveries relative to nominal application rate were already below 100% at time zero for all relevant experiments.

For the plant experiment, after 24 hours complete recovery was achieved, which allows to conclude that volatilisation of glyphosate from plants was negligible. For the soil experiment, recovery after 24 hours was 82 %, but because the report indicated that recovery from extraction and combustion was not quantitative, and no measurements of volatiles were conducted, it could not be demonstrated that the difference in recovery was caused by volatilised glyphosate.

In conclusion, the results from the plant experiment are considered supporting information while the results from the soil experiment are considered invalid.

Assessment and conclusion by RMS:

For the soil experiment, recoveries of glyphosate in soil after 24h are low. These low recoveries can be explained as mentioned in the summary by no quantitative extraction and combustion. Therefore, the results reported for this experiment cannot be considered acceptable.

For the plant experiment, the results can be considered acceptable.

[redacted], *1993*

Data point:	CA 7.3.1/006
Report author	[redacted]
Report year	1993
Report title	Determination of the volatilization of Glyphosate 360 SL from soil and plants
Report No	BE-EA-149-92-01-VOL-1
Guidelines followed in study	Richtlinie Teil IV, 6-1 Biologische Bundesanstalt fur Land- und Forstwirtschaft der Bundesrepublik Deutschland, "Prufung des Verfluchtigungsverhaltens und des Verbleibs von Pflanzenschutzmitteln in der Luft",
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability / Reliability	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Glyphosate 360 SL

Active substance	Glyphosate (360 g/L) corresponding to Glyphosate Isopropylamine Salt (480 g/L)
Batch No.	229-JaK-24-l/F
Chemical purity	Not reported

^{14}C-glyphosate

Batch No.	CFA745C6
Specific activity	12.3 MBq/mg
Radiochemical purity	98.9 % (>97.7 % determined at test facility)
Chemical purity	Not reported

For the soil experiment, 0.24 mL of Glyphosate 360 SL and 0.345 mL of ^{14}C-glyphosate were diluted with demineralised water to a total volume of 10 mL. For the plant experiment, 0.607 mL of Glyphosate 360 SL and 0.709 mL of ^{14}C-glyphosate were diluted with demineralised water to a total volume of 25 mL.

2. Test Soil

The soil was sieved to 2 mm and stored at 2 to 8°C for less than three months. The water content before starting the test was determined as 6.8 %.

Table 8.3.1.2-7: Physico-chemical properties of test soil

Parameter	Results
Soil	Speyer 2.1
Horizon (cm)	Not reported
Geographic location	Speyer, Germany
Textural Class[1]	Sand
Sand (%)	87.4
Silt (%)	9.1
Clay (%)	3.5
pH[2]	5.9
Organic Carbon (%)	0.7
Organic Matter (%)	1.2
Maximum water holding capacity (%)	29.2

[1] Classification system not reported
[2] Medium not reported
[3] Calculated as OC x 1.724

2. Test Plants

The seeds of the French beans were cultivated in vermiculite substrate for 25 days (two leaf stage, leaf surface 30 cm^2 per plant) before use in the test.

French Beans

Species	*Phaseolus vulgaris var. nanus*
Variety	Nerina
Supplier	Joh. Fuchs, Bad Homburg, Germany

B. STUDY DESIGN

1. Experimental Conditions

The study consisted of two tests, one with soil and one with plants.

The tests were conducted in a flow-through test chamber in which the test vessels were placed. The test vessels were immersed into a temperature controlled water bath (20 ± 2 °C). An air-stream of more than 1 m/sec was generated across the soil or leaf surfaces. The air velocity in the chamber was high enough

to avoid water vapour saturation above the plant and soil surface. The relative air humidity was between 30 and 50 %.

The test solution was applied onto the soil or plant surface at a rate of 3.6 kg glyphosate/ha in 400 L water/ha by using a reproducible, semi-automatic method that delivers a homogeneous distribution pattern. The exact amount of test chemical delivered was determined 5 times each for the soil and plant test system.

For the soil experiment, 150 g of soil (dry weight) were weighed into each test vessel and adjusted to 60 % of the maximum water capacity using demineralized water. The soil surface per test vessel was 35 cm^2. The test item was applied at an amount of 1263 µg glyphosate per test vessel. Demineralised water was supplied to the test vessels via one capillary per test vessel by using a peristaltic pump to keep the soil moisture constant throughout the test period. The average soil moisture during the test was 58.0% (55.7 to 59.8%). The soil temperature was 19.9 to 20.2 °C.

For the plant experiment, plants were transplanted into the test vessels (one plant per test vessel) after reaching the two leaf stage. The substrate for the plants was vermiculite. The leaf surface of one plant was estimated to be on average 30 cm^2 (15 cm^2 per leaf). The test item was applied at an amount of 1082.1 µg glyphosate per test vessel.

Soil and plants were exposed independently from each other to the air stream in the test chamber at 20 ± 3 °C for a test period of 24 hours.

2. Analytical Procedures

Samples from the soil and plants were taken immediately before and after application and after 1, 3, 6 and 24 hours of exposure. For the sampling time point immediately after treatment and after 24 hours three replicates were sampled while duplicates were analysed for the other samplings. After sampling, the soil test vessels were weighed, wrapped with aluminium foil and frozen. Sampled plants were removed from the test vessels, the roots were cleaned before from the substrate. The plants were sliced manually into small pieces, transferred into 100 mL glass bottles, and 50 mL demineralised water was added, before the samples were frozen. All soil and plant samples were stored frozen for less than two months at -20° ± 10 °C.

The soil samples were extracted four times with 150 mL 0.5 N sodium hydroxide for 10 minutes each followed by centrifugation. The extracts were combined and added with 0.5 N NaOH to a total volume of 650 mL. Afterwards, 50 mL of 0.5 M Na_2HPO_4-buffer (adjusted with H_3PO_4 to pH = 7.3) were added, and aliquots of the solution were analysed by LSC to determine the extractable radioactivity in soil. Non-extractable radioactivity in soil was determined after extraction by combustion/ LSC.

After thawing, plant material and water were separated. The water was directly analysed by LSC to determine the washable radioactivity in plants. The plant samples were directly combusted (without any further drying or extraction step). For each plant 3 samples were obtained, consisting of the roots and two portions of leaves and stems. The evolved ^{14}C-CO_2 was trapped and analysed by LSC to determine the non-washable radioactivity in plants.

II. RESULTS AND DISCUSSION

The percentage of radioactivity determined in soil and plants during the volatilisation experiment of 24 h is given in the table below. The standard deviation between all values determined was <2.5 %.

Table 8.3.1.2-8: Measured ^{14}C-glyphosate-equivalents in soil and plants during 24 h volatilization from soil and plants (values are given in % of applied radioactivity)

Experiment	Replicate	Hours after treatment				
		0	**1**	**3**	**6**	**24**
Soil[1]	1	92.16	100.25	97.29	92.29	95.02
	2	96.40	95.99	92.10	99.06	94.36
	3	97.29	N/A	N/A	N/A	96.96
	Mean	**95.28**	**98.12**	**94.70**	**95.68**	**95.44**
Plant[2]	1	102.35	97.28	98.07	96.14	102.22
	2	90.71	99.10	97.54	96.92	103.93
	3	102.71	N/A	N/A	N/A	101.47

	Mean	**98.59**	**98.19**	**97.81**	**96.53**	**102.54**

N/A: not applicable
1 Values represent the sum of extractable and non-extractable radioactivity
2 Values represent the sum of washable and non-washable radioactivity

No significant differences in the total radioactivity were seen for both the soil and plants when measured at the beginning and after 24 hours. Thus, no volatilisation from soil and plants was observed at room temperature within 24 hours.

Assessment and conclusion by applicant:

The study was conducted in accordance with the guideline relevant at that time. Methods and results are sufficiently described and conclusive. The results obtained in the study show that glyphosate does not volatilise from soil or leaf surfaces to any significant extent. The study is considered as supportive.

Assessment and conclusion by RMS:

The study is considered acceptable.

[redacted], *1992*

Data point:	CA 7.3.1/007
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Volatilization from soil and leaf surfaces
Report No	RJ1237B
Guidelines followed in study	None
Deviations from current test guideline	No current guideline in force
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Not mentioned in RAR 2015 or DAR 1998
Acceptability/Reliability:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled
Identification: [14C]glyphosate-trimesium (labelled in the anionic position) (PMG)
Lot No.: 91-J19
Specific activity: 2.070 GBq/mmol
Radiochemical purity: 98.3 % determined before start

Identification: [14C]glyphosate-trimesium (labelled in the cationic position) (TMS)
Lot No.: 91-70
Specific activity: 2.020 GBq/mmol
Radiochemical purity: 92.2 % determined before start

Unlabelled
Identification: N-phosphonomethylglycine trimethylsulphonium salt
Lot No.: ICIA0224

2. Soil:

The soil was received from LUFA (type Speyer 2.1) on 22 May 1995. After receipt, the soil was stored in the designated plots. Before use, the soil was sieved through a 2 mm analytical sieve. Before the start of the test, the soil was adjusted to approximately 60 % of the maximum water capacity. Soil properties are not indicated.

3. Plant:
Dwarf French bean leaves from plants at the flowering/first fruit stage were used, obtained from ICI Agrochemicals.

B. STUDY DESIGN
1. Experimental conditions
Soil Experiment
Ten treated soil pots were placed at the edge of a fume cupboard. The sash was adjusted such that the air flow over the soil surfaces was > 1 m/s. To maintain the moisture content of the soil, deionised water was pumped continuously into the soil pots using a peristaltic pump. Variations in the moisture content observed at each sampling interval were counteracted by changing the pumping rate. The moisture content of the soil samples was determined at each sampling interval. At sampling the soil pots were weighed. This weight was then compared with the initial weight (at 60 % MWHC) and the moisture content was recalculated as a percentage of its MWHC.

Plant Experiment
Ten to twelve leaves, from plants in the same pot, were treated as above. The remainder of the leaves were removed and discarded. The plants were then transferred to a glasshouse where they were placed in front of an electric fan, the position of which (relative to the plants) was adjusted to deliver a wind speed > 2 m/s around the plants.
The final application rates for the glyphosate labelled [14C]glyphosate-trimesium were 3626 g a.s./ha for the soil study and 2836 g a.s./ha for the leaf study.

2. Sampling

Duplicate soil pots or individual leaves were removed at 0, 1, 3, 5.5 and 24 hours from the initiation of the air flow, for quantification. Samples t=0 were taken before the air flow was applied and temperature, relative humidity and air flow were logged.

3. Analytical procedure

The soil was quantitatively transferred into glass jars. The soil was then ultrasonicated with ca. 150 mL of acetonitrile for ca. 20 minutes. The extract was then separated from the debris by filtration under vacuum.

The individual leaves were macerated in the presence of ca. 50 mL of acetonitrile. The extract was separated by filtration under vacuum.

The amounts of radioactivity contained in extract and debris were measured using liquid scintillating counting (LSC and sample oxidation/LSC, respectively. LOD and LOQ were not indicated.

II. RESULTS AND DISCUSSION

A. DATA

Results of the determination of the volatility from soils and plants are presented in the following tables.

Table 8.3.1.2-9: Test concentrations and radioactivity measurements for the anion labelled soil volatility study

Time Interval (h)	Air Speed (m/s)	MWHC (%)	Activity Extracted (Bq)	Activity bound (Bq)	Total activity (Bq)	Mean activity (Bq)	% of 0 h samples
0 0	-1	-2	0.0 0.0	5440.3 5288.03	5440.3 5288.0	5364.2	100.0
	1.2					5289.4	98.6

1 1		58.9 58.9	0.0 0.0	5214.9 5363.9	5214.9 5363.9		
1 1	1.3	57.9 58.0	0.0 0.0	4730.3 5058.5	4730.3 5058.5	4894.4	91.2
5.5 5.5	1.3	56.9 57.3	0.0 0.0	5022.8 5506.6	5022.8 5506.6	5264.7	98.1
24 24	1.3	56.1 56.2	0.24 0.0	4873.1 5227.6	4873.3 5227.6	5050.5	94.2

1 The 0 h samples were taken before the air flow was applied to the soil pots.
[2] The 0 h moisture content was taken to be 60 % MWHC, as prepared.
[3] This figure represents half of the activity recovered as this soil pot was treated twice.
4 Value was corrected by the sponsor

Table 8.3.1.2-10: Test concentrations and radioactivity measurements for the anion labelled leaves volatility study

Time Interval (h)	Air Speed (m/s)	Activity Extracted (Bq)	Activity bound (Bq)	Total activity (Bq)	Mean activity (Bq)	% of 0 h samples
0 0	-1	0.0 0.0	4217.9 4196.0	4217.9 4196.0	4207	100.0
1 1	2.3	0.0 0.0	3926.4 4493.3	3926.4 4493.3	4210.1	100.1
1 1	2.6	0.0 0.0	4588.7 3651.9	4588.7 3651.9	4120.3	97.9
5.5 5.5	2.4	0.0 0.0	3911.1 4282.1	3911.1 4282.1	4096.6	97.4
24 24	2.2	0.0 0.0	4167.1 4589.0	4167.1 4589.0	4378.1	104.1

1 The 0 h samples were taken before the air flow was applied to the leaves.

B. EXTRACTABLE AND NON_EXTRACTABLE RESIDUES

After 24 hours, 94.2 % relative to t=0 were recovered from the soils treated with glyphosate labelled test material. After 24 hours 104.1 % relative to t=0 were recovered from leaves treated with test material labelled in the glyphosate part. Based on these results, it is concluded that no significant amounts of the test substance evaporate from the soil and plant leaves within 24 hours under the conditions of the test.

III. CONCLUSIONS

The results obtained in the study show that glyphosate does not volatilise from soil or leaf surfaces to any significant extent (i.e. < 10 % volatilisation after 24 hours). The indirect method and variability in recoveries does not allow to exactly quantify volatilisation.

For the glyphosate soil and leaf studies, the final radioactive recoveries were 94.2 % and 104.1 % of the applied radioactivity after 24 hours, respectively.

Assessment and conclusion by applicant:

Although no specific guideline is followed in the study, methods and results are sufficiently described and conclusive. Therefore, the study is considered as supportive information. The results obtained in the study show that glyphosate does not volatilise from soil or leaf surfaces to any significant extent (i.e. < 10 % volatilisation after 24 hours).

Assessment and conclusion by RMS:

This study is considered acceptable.

[redacted], *1996 &* [redacted], *1995*

Data point:	CA 7.3.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Determination of volatilisation - Field study
Report No	PR94/032
Guidelines followed in study	BBA Guideline, part IV, 6-1 "Analysenmethoden zur Bestimmung von Pflanzenschutzmittelrückständen in der Luft"; Nachrichtenblatt Deutscher Pflanzenschutzdienst, 46, 1994, 60-61
Deviations from current test guideline	No current guideline in force
GLP/Officially recognised testing facilities	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability:	Supportive

Data point:	CA 7.3.1/005
Report author	[redacted]
Report year	1995
Report title	Final report - About testing volatilization behaviour of TAIFUN forte in bush beans under field conditions
Report No	AGR/RV-95/FSG
Guidelines followed in study	Guidelines on Producing Pesticides Residue Data from Supervised Trials FAO Rome IVA-Guidelines for residue tests, Section IA and IB, 2nd edition BBA Guidelines Section IV/3-3 BBA Guidelines Section IV/6-1
Deviations from current test guideline	No current guideline in force
Previous evaluation	Yes, accepted in DAR 1998
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	TAIFUN forte
Lot No.:	0395040
Nominal concentration:	360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The test field site was located in Germany. The test substance Taifun forte was applied once only at growth stage ES 75-77 of the bush beans. The field size was 1600 m2. The test substance was applied to bush beans which had a height of approximately 0.5 m. The bush beans did cover the field tightly. Weather data were logged by a mobile weather station directly placed in the field. Characteristics of the trial location are summarised in the table below.

Table 8.3.1.2-11: Trial location

Location	47574 Goch, Germany
Soil type	Sandy loam
pH	6.5
OC (%)	1.9
OM (%)1	3.3.
Depth of topsoil (m)	0.3

1 Calculated from organic carbon according to OM = OC / 0.58

2. Application

Application was performed on 9 August 1995 with an application rate of 5.0 L/ha corresponding to 1794.58 g a.i./ha with a hardy trailed sprayer.

3. Sampling

Residue plant specimens were taken from treated plots before application, directly after treatment and at the time intervals 1, 2, 4, 8 and 24 hours after application. Air samples were collected in the middle of the field in two heights of 0.5 and 1.5 m above the plants.

4. Specimen handling and preparation

The specimens were frozen within 15 min after taking at a temperature ≤ -20 °C and transported in thermos containers to the test facility. Before analysis the samples were crushed and homogenised.

5. Analytical methods

Air samples:

The air sample was sucked through a gas washing bottle which is filled with 50 mL water serving as adsorbent. After the enrichment, internal standard was added. Furthermore the sample was acidified with phosphoric acid evaporated to dryness. Then derivatisation was performed using trifluoroacetic anhydrid and trifluoroethanol. The sample was cleaned using HPLC-clean-up. The HPLC fraction was diluted with water and concentrated using a RP 18-cartridge. Finally the sample was eluted from the cartridge using acetic ester. The determination of the substance was performed using GC-MS.

For method validation recovery tests were performed. Mean recovery of all performed recovery experiments was 89 % (± 13.6 %). The limit of quantification (LOQ) was set at 20 ng/sample which corresponds to about double the limit of detection (LOD) of 10 ng/sample.

Plant samples:

The plant specimens were extracted with water under addition of hydrochloric acid. The extract was filtered and brought to a defined volume. Isotope marked standards were added to an aliquot of the extract. The extract first was cleaned by means of charcoal, following a clean up step using an anion exchanger. The eluate of the ion exchanger was derivatised with trifluoroacetic anhydrid and trifluoroethanol. Finally the derivatised sample was cleaned by liquid / liquid partition. Quantitative determination was performed by GC-ECD.

The analytical method was validated by suitable fortification experiments. The fortification experiments performed at levels of 10 mg/kg and 200 mg/kg and the overall mean recovery of glyphosate was found to be 117 % (± 9 6 %). The limit of determination was set at 10 mg/kg corresponding to the limit of detection (LOD) of 0.1 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Results of air and plant samples are summarised below.

Table 8.3.1.2-12: Results of air analysis

Sample (h) / height (m)	determined value (ng/sample)
0 / 0.5	< 10
0 / 1.5	< 10
sample 11 / 0.5	< 10
sample 11 / 1.5	< 10
24 / 0.5	< 10
24 / 0.5	< 10
24 / 1.5	< 10
24 / 1.5	< 10

1 First samples taken after application

Table 8.3.1.2-13: Results of plant analysis

Sample (h)	determined value mg/kg
-1	< 1
0	363
1	351
2	329
4	348
8	272
24	174

B. CHARACTERISATION OF RESIDUES

No glyphosate was measurable in the air samples after the application of Taifun forte to the bush beans. Neither in the first 2 hour samples nor in the cumulative 24 hour samples glyphosate was determined.

The concentration in the measured plant samples is constant within the first 4 hours after application. Then the concentration in the plants decreases rapidly. Obviously this decrease is due to uptake and / or metabolism in the plants, in the case of glyphosate no conclusion can be drawn from the plant measurements (indirect method), because glyphosate in plants is not stable within the time scale of the test. Only the measurements of air samples (direct method) can be taken to receive results on volatilisation effects.

III. CONCLUSIONS

No volatilisation of glyphosate was observed after the application of Taifun forte in field conditions.

Assessment and conclusion by applicant:

The study is considered valid to conclude on no volatilisation of glyphosate in the field.

Assessment and conclusion by RMS:

The concentrations of glyphosate in plant samples decrease after 4h after application of the active substance. It cannot be stated if these losses of substance are due to volatilization or degradation of the active substance in the plant via metabolism. In air samples collected in the field, no glyphosate was detected. However, no detailed data are reported to ensure that the volatile traps in field are settled correctly to trap volatized glyphosate from plants (position of the traps, wind power and wind direction, etc.).

Contrary to the applicant's conclusion, based on the available data, no clear conclusion on volatilisation can be established from this study.

The study is considered as supportive.

Relevant articles from literature search

In the scientific literature review for glyphosate (2010-2020), one article was identified to provide further information relevant to the data point (Bento et al., 2017, CA 7.3.1/008).

Table 8.3.1.2-14: Behaviour in air – relevant articles from literature search

Annex point	Study	Study type	Status in RAR 2021
CA 7.3.1/008	Bento et al., 2017	Wind tunnel experiment	Reliable with restrictions

Bento et al., 2017

Data point:	CA 7.3.1/008
Report author	Bento, C.P.M. et al.
Report year	2017
Report title	Glyphosate and AMPA distribution in wind-eroded sediment derived from loess soil
Report No	DOI 10.1016/j.envpol.2016.11.033 E-ISSN 1873-6424
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

Materials & methods

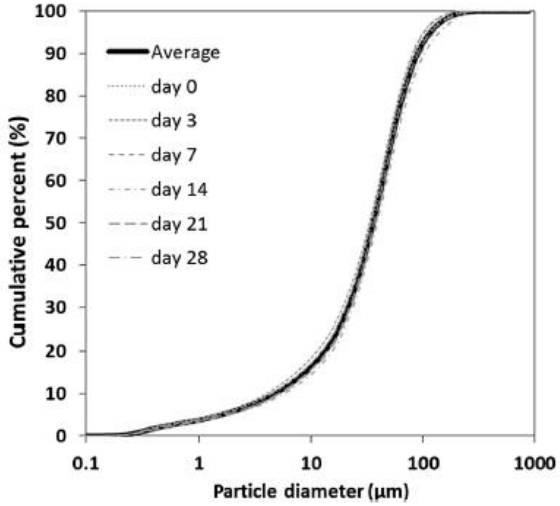
Soil

We used the topsoil of a silty loam loess soil from Huldenberg, Belgium. The soil was air-dried and then sieved through a 1-mm sieve. It was tested for glyphosate and AMPA residues and found free of glyphosate and AMPA. The main soil properties of the sieved soil are shown in the table below. Table 8.3.1.2-16 shows the grain size distribution of the soil after disintegration of all aggregates.

Table 8.3.1.2-15: Soil properties of the loess soil used in this study

Parameters	Value
Particle size distribution:	
<2 μm (clay) (%)	10
2–50 μm (silt) (%)	79
>50 μm (sand) (%)	11
pH $CaCl_2$	5.8
Organic matter (OM) (%)	3.2
Particle density (g cm^{-3})	2.5
N total (g kg^{-1})	1.7
P available (mg kg^{-1})	0.4
K available (mg kg^{-1})	209
Mg available (mg kg^{-1})	121
Na available (mg kg^{-1})	10
C/N ratio	9

Table 8.3.1.2-16: Particle size distribution of the start soil after disintegration of all aggregates



Glyphosate preparation and application in the soil

Preparation of glyphosate solution

Glyphosate solution was prepared by diluting 980 mL of CLINIC®, a glyphosate-based herbicide that contains 360 g/L of glyphosate, in Millipore water to achieve a final stock solution of 0.42 g/L. A concentration of glyphosate in soil of 8.4 mg/kg was used in this study, which corresponds to an application rate of 1.26 kg a.i./ha (typically applied in agricultural fields), assuming a soil depth of 1 cm and a bulk density of 1.5 g/cm3.

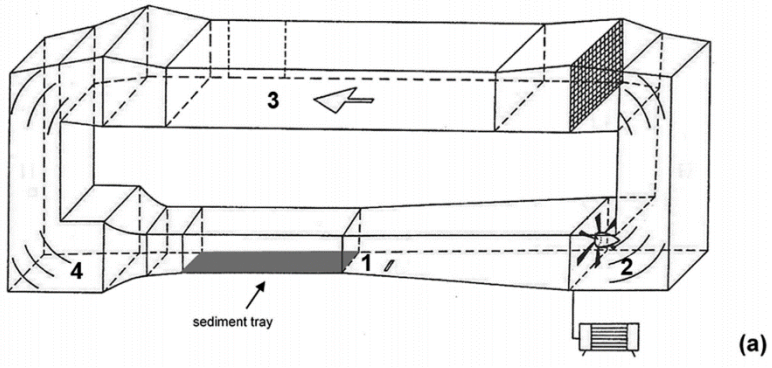
Application in soil

A plastic sheet was put on the ground and an approximately 5-cm thin layer of the air-dried and sieved soil (42 kg) was spread on it. The soil was then sprayed with the prepared glyphosate solution. During the application, the soil was thoroughly mixed with a rake. The soil was then stored in a plastic bag at room temperature (22 ºC) and dark conditions. A small portion of the soil was collected after glyphosate application and oven-dried (105 ºC) for 24 h to determine the initial soil moisture content, which was found to be 5.4 % (w/w).

Facilities and instrumentation

The experiment was carried out in the facilities of the Geography and Tourism Research Group of the Katholieke Universiteit Leuven, Belgium. A closed-return wind tunnel was used. The tunnel has two test sections, both of which were used in this study. The dimensions of the large test section are 760 cm (length) x 120 cm (width) x 60 cm (height), and those of the small test section are 150 cm (length) x 35 cm (width) x 30 cm (height). A detailed description of the wind tunnel can be found in the technical report by Goossens and Offer (1988).

Figure 8.3.1.2-1 :Leuven wind tunnel, with locations of the sampling sites 1 to 4



Apart from the wind tunnel, a modified version of the Soil Fine Particle Extractor developed in a previous study by Goossens (2012) was used. This instrument draws up the sediment, previously spread on a table, with a plastic hose attached to a BASE 440 three-engine vacuum cleaner connected to a cyclone dust separator (RIBO, Villanova, Italy). The hose is 300 cm long and 4 cm in diameter; the separator is 70 cm high and 40 cm in diameter. Coarse particles settle in the separator and are thus removed from the sample. Separation is accomplished by the circular motion of the particles and enhanced by selective gravitational settling. Some of the smallest particles remain suspended in the separator. After initial separation in the separator, the dust enters a tube 139 cm long and 16 cm in diameter, which operates as an elutriator. Dust is then accelerated through a small pipe 36 cm long and 7.6 cm in diameter and hits an impactor (diameter: 8.7 cm) installed near the bottom of a settling chamber. Only the finest particles will suspend in the chamber. These particles then enter a 200-cm long plastic tube. Further granulometrical separation is performed in this tube, which operates as a second elutriator. Particles then enter the vacuum cleaner and settle in a 50-L deposition chamber, where they can be collected. Three 1200-W engines that generate a suction rate up to 510 m3/h and create an under pressure of 2200-mm H2O power the instrument. For this study, only one engine (170 m3/h) was used.

Experimental design

To perform each experimental run, a total of 8 kg of pre-treated soil (enough to fill the sediment tray in the wind tunnel) was taken one day before each experimental run. The soil was then oven-dried at 37.5°C for 24 h to ensure a soil moisture 2 % (the highest soil moisture allowed to guarantee wind erosion; see Nourzadeh et al. (2013)). Soil samples (in duplicate) were always taken before and after the drying process to control for any effect on glyphosate decay and AMPA formation/decay. The oven-dried soil was then subjected to wind erosion in the wind tunnel. In the small test section, a tray 150 cm long x 35 cm wide x 2 cm deep was installed. The upwind 75 cm were filled with a piece of wood; the downwind 75 cm were covered with a thin sheet of plastic (to avoid direct contact between the glyphosate-treated soil and the metal of the tray). The oven-dried soil was then put into the tray. Its surface was carefully flattened using a slat. The wind tunnel was then closed and turned on to allow the soil sediment to erode until the entire tray was empty. We used a free-stream wind speed of 10.0 m/s, which was well above the deflation threshold of the sediment used (6.5 m/s according to visual observations made before the test). It took approximately 1 h until the tray was empty. After each run, sediment samples (in triplicate) were collected (≥2 g for most of the samples; and always ≥1 g) at four different places in the wind tunnel using a clean brush. The distances from the trailing edge of the tray were as follows: sample 1: 10 cm; sample 2: 480 cm, sample 3: 1290 cm, and sample 4: 1865 cm. Due to aeolian selection, the samples become finer as they are taken further from the source. Because of the restricted length of the wind tunnel, sample 4 was the finest sample that could be obtained with the wind tunnel technique. To collect even finer samples, the Soil Fine Particle Extractor was used and three more samples were collected. After each wind tunnel run, the tunnel was first thoroughly cleaned with the vacuum cleaner. A sample (sample 7) was then taken from the deposition chamber of the vacuum cleaner, which at this stage was directly connected to the cyclone separator. The sediment in the separator was then mixed with the remaining dust in the deposition chamber and put on a clean table. After assembling the entire Soil Fine Particle Extractor, the sediment on the table was sucked up and samples 5 and 6 were collected just downwind from the cyclone separator (sample 5) and in the

deposition chamber of the vacuum cleaner (sample 6). All experimental runs (wind tunnel + Soil Fine Particle Extractor) and collection of samples were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. All samples were stored in plastic tubes and frozen at -18°C until glyphosate and AMPA analysis.

Particle size distribution and organic matter content

To analyse the particle size distribution of samples 2 to 7, a Malvern Mastersizer S laser particle size analyser (Malvern Ltd, Malvern, UK) was used. Sample 1, which exclusively consisted of large aggregates, was analysed optically with a microscope. For the latter sample, a subsample from the main sample and measured the nominal diameter of all aggregates was collected. Using these data, the aggregate size distribution of the sample could be determined. To get an idea of the internal particle size distribution of the large aggregates themselves, also several of these aggregates were collected, carefully crushed and dispersed, and then analysed with the Mastersizer instrument. The OM content was estimated by oxidation at 600ºC and detected by close infra-red using a SC-144DR equipment (LECO Corporation, St Joseph, MI, USA). When there was insufficient sample for analysis, the triplicates were mixed together.

Glyphosate and AMPA content

Glyphosate and AMPA contents in the samples were analysed as described by Bento et al. (2016). Briefly, glyphosate and AMPA were extracted from 1 g of soil or wind-eroded sediment with 5 mL of 0.6 M KOH (potassium hydroxide, p.a. 85 %). After shaking and centrifuging the samples, 1 mL of the supernatant was transferred to a 10-mL plastic tube. Isotopically labelled glyphosate and AMPA were added at this point and then a derivatisation step was carried out with FMOC to improve retention and MS/MS detection as described by Bento et al. (2016). Solvent standards with isotopically labelled internal standards were prepared together with all the samples for each batch of samples, and derivatized the same way. Glyphosate and AMPA contents were then determined by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using an XBridge™ Shield RP C18 column 100 mm x 2.1 mm i.d. (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrople MS, Waters, The Netherlands). Chemicals used, mobile phases and instrumentation conditions of the HPLC-MS/MS were as described by Yang et al. (2015b) and Bento et al. (2016). With each batch of samples, two blank soil samples of the loess soil used in this study were fortified at 0.5 mg/kg and added as quality control (QC) samples. To ensure the quality of the analysis when processing real samples, the fortified samples were analysed twice, at the beginning and at the end of each batch. The quantification of the sample batch was considered satisfactory when the QC recoveries were between 70 and 120 %. A detailed description of the method validation and quality control can be found in Bento et al. (2016).

Statistical analysis

All statistical analyses were performed in SPSS 22, and the graphs in Figure 8.3.1.2-4 were produced in SigmaPlot 10.0. A one-way ANOVA to ln-transformed data followed by Dunett T3 post-hoc tests was performed to test for significant ($p < 0.05$) differences in clay, silt or organic matter (OM) content between extracted size fractions of the wind-eroded sediment. Besides, a power function was applied to the non-aggregated samples (sample 3-7) to test the correlation between the clay or OM content and the particle size of the samples. To test for significant differences of glyphosate or AMPA residues between extracted size fractions of the wind-eroded sediment, an analysis of covariance (ANCOVA) to ln-transformed data followed by Bonferroni tests was performed ($p < 0.05$). The assumption of homogeneity of regression slopes was not violated. Moreover, a categorical principal components analysis (non-linear PCA) was performed to determine the relationship between sediment properties (clay, silt, OM) and glyphosate or AMPA content in the wind-eroded sediment. The loading of a given variable was considered meaningful if its absolute value was ≥ 0.40 for a given component. Besides, a Pearson correlation was computed to assess the relationship between glyphosate or AMPA contents and clay, silt or OM. A reconstruction of the distribution of glyphosate in the original soil in the sediment tray before the start of each wind tunnel experiment was also performed. This was done by considering the glyphosate content for a large number of narrow grain size classes, which could be estimated by applying an exponential regression analysis to the data (only the samples without aggregates, i.e., samples 3-7).

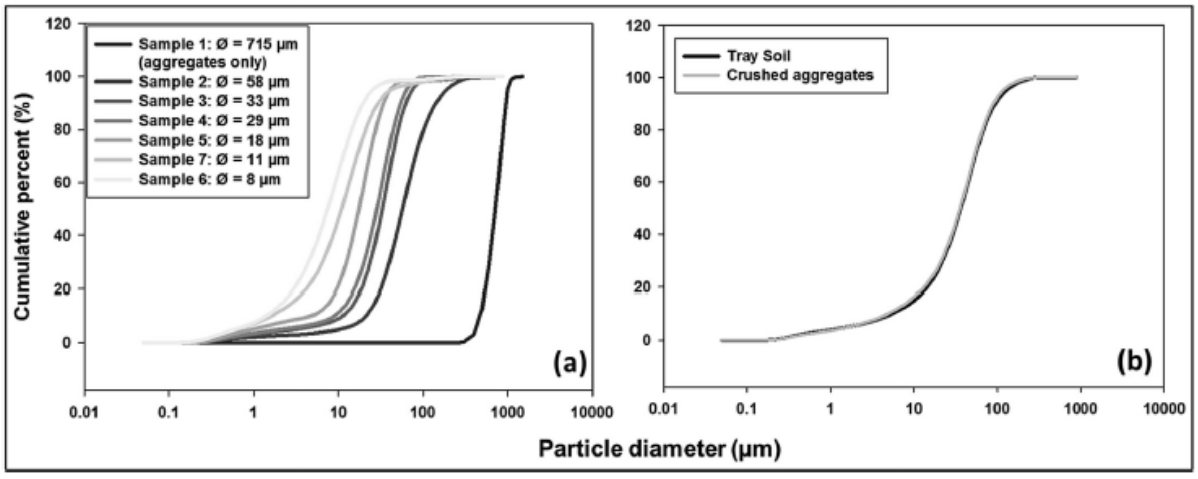
Results & Discussion

Physico-chemical composition of the wind-eroded sediment

Particle size distribution

The particle size distribution of the different extracted fractions of the wind-eroded sediment is shown in Figure 8.3.1.2-2. Sample 1 was composed of large, macroscopic aggregates only. Sample 2 consisted of individual grains and micro-aggregates, mixed with a few macroscopic aggregates. Samples 3-7 only contained individual grains with some small micro-aggregates (as verified under the microscope) and were mostly composed of particles ≤100 µm in diameter. More than 96 % of the particles of samples 5-7 were ≤50 µm in diameter. The median diameters of the samples were: 715 ± 69 µm (sample 1), 58 ± 2 µm (sample 2), 33 ± 1 µm (sample 3), 29 ± 1 µm (sample 4), 18 ± 1 µm (sample 5), 8 ± 1 µm (sample 6) and 11 ± 3 µm (sample 7). These median diameters are further used as reference codes in the data analysis presented here.

Figure 8.3.1.2-2: Particle size distribution of (a) the different extracted fractions of wind-eroded sediment; (b) the crushed aggregates and the original sediment in the sample tray. Ø = median diameter

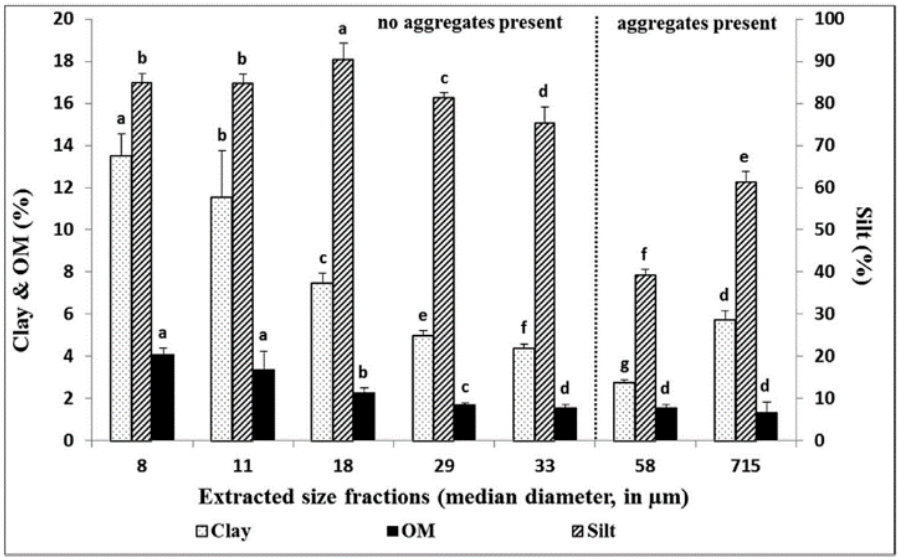


Crushing of the macroscopic aggregates (sample 1) and analysing their grain size distribution showed that the aggregates are perfect compositions of the original tray sediment (Figure 8.3.1.2-2), with a median particle diameter of 36 ± 2 µm for both the aggregates and the original tray soil.

Clay, silt and OM content

The clay (<2 µm), silt (2-50 µm) and OM content of the different extracted fractions of the wind-eroded sediment are shown in Figure 8.3.1.2-3. The clay content was significantly higher for the finest extracted size fraction (median diameter of 8 µm) and lowered significantly with increasing particle size (Figure 8.3.1.2-3), except for the samples with a 715-µm median diameter which consisted exclusively of macroscopic aggregates. A strong negative correlation was also observed between the clay content and the particle size of the non-aggregated samples (median diameters between 8 and 33 µm; Clay (%) = 67.7 MDES-0.78, R2 = 0.99; MDES = median diameter of the extracted sample). Likewise, the OM content was highest for the finest extracted fractions (samples with median diameter of 8 and 11 µm) and lowered significantly with increasing particle size (Figure 8.3.1.2-3). Nevertheless, this decrease in OM was no longer significant after a particle size ≥33 µm. A strong negative correlation was also observed between the OM content and the particle size of the non-aggregated samples (OM (%) = 13.1 MDES-0.61, R2 = 0.90). All samples were mostly composed of silt (Figure 8.3.1.2-3). The silt content decreased as the samples became coarser, but to a lower extent compared to clay and OM. In the aggregated samples (median diameters of 58 and 715 µm), the silt content was significantly lower than in the non-aggregated samples.

Figure 8.3.1.2-3: Clay, silt and organic matter (OM) content of the extracted size fractions. The 715-µm samples consist exclusively of large aggregates. Different lowercase letters within the same type of bars mean significant differences in silt or clay or OM between extracted size fractions (p <0.05)



Glyphosate and AMPA content in the wind-eroded sediment

Relationship between glyphosate or AMPA and particle size

Glyphosate content (Figure 8.3.1.2-4) varied between 5.5 and 16 µg/g, with a significantly higher content in the finest extracted fractions (median diameters from 8 to 18 µm). AMPA content, on the other hand, was rather low, varying between 0.07 and 0.7 µg/g. Here too, AMPA content was significantly higher in the finest extracted fractions. In Figure 8.3.1.2-5, the relationship between glyphosate (or AMPA) content and particle size of the wind-eroded sediment is better shown. Here, it is clearly visible that glyphosate and AMPA contents were highest in the finest samples (median diameter: 8 µm) and became lower with increasing particle size until ≈33 µm (Figure 8.3.1.2-5). Note that this does not necessarily mean that the highest amounts of glyphosate and AMPA in a sample occur in the finest fractions of that sample: the mass of coarse grains is much higher than that of fine grains, so even when the concentration is higher in the fine fractions it is possible that the coarse fractions contain more glyphosate and AMPA in weight. A larger spread was observed for AMPA than for glyphosate (Figure 8.3.1.2-5). However, this larger spread is not meaningful since it just reflects the increase of AMPA content in the course of time (see Figure 8.3.1.2-4). For the individual days, the lower AMPA content with increasing particle size became better visible. It also became stronger over time. The effect of the presence of macroscopic aggregates in a sample was also very prominent (Figure 8.3.1.2-5). Once macroscopic aggregates were present (samples with median diameters of 58 and 715 µm), glyphosate and AMPA contents remained constant regardless of how numerous or how large the aggregates were. This seems to be related with the fact that the aggregates are perfect compositions of the original soil in the sediment tray (Figure 8.3.1.2-2) regardless of their size. Because, in an aggregate, the largest mass is represented by the coarsest grains, glyphosate and AMPA contents will be rather low, approaching the concentration in the coarsest individual grains, albeit a little higher because of the presence of a higher percentage of fine particles in the aggregates. When comparing the glyphosate content in the different sediment fractions with its content in the parent soil, it was, on average, 1.4 times higher in the finest fractions of the wind-eroded sediment (median diameters between 8 and 18 µm) than in the parent soil. In contrast, the coarsest fractions (median diameters between 29 and 58 µm) had glyphosate contents that were, on average, 1.2 times lower than that in the parent soil. Only the samples entirely composed of macroscopic aggregates (median diameter of 715 µm) matched the glyphosate content of the parent soil, confirming once again that the large aggregates are perfect compositions of the original soil in the sediment tray. Clymo et al. (2005) also reported a much higher concentration of the herbicide pendimethalin in the PM2.5 fraction when compared to their field soil, but not for the herbicide metolachlor. According to these authors, pendimethalin is less volatile than

metolachlor and therefore, the former has a higher affinity to the particle phase while the latter has a higher affinity to the gas phase. Glyphosate is also non-volatile and tends to strongly adsorb to soil particles; therefore its preference to the particle phase is also expected.

Figure 8.3.1.2-4: Glyphosate (a) and AMPA (b) content in the different extracted size fractions of the wind-eroded sediment during the 28 days after glyphosate application, and respective trend lines. Note the different vertical scales between (a) and (b). To the right of the legends, different lowercase letters mean significant differences in glyphosate (a) and AMPA (b) content between extracted size fractions, using an ANCOVA followed by Bonferroni tests (p <0.05)

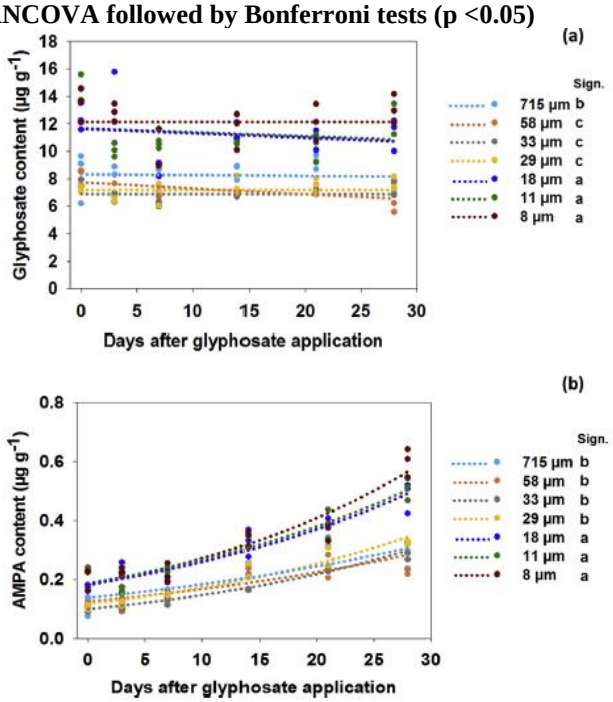
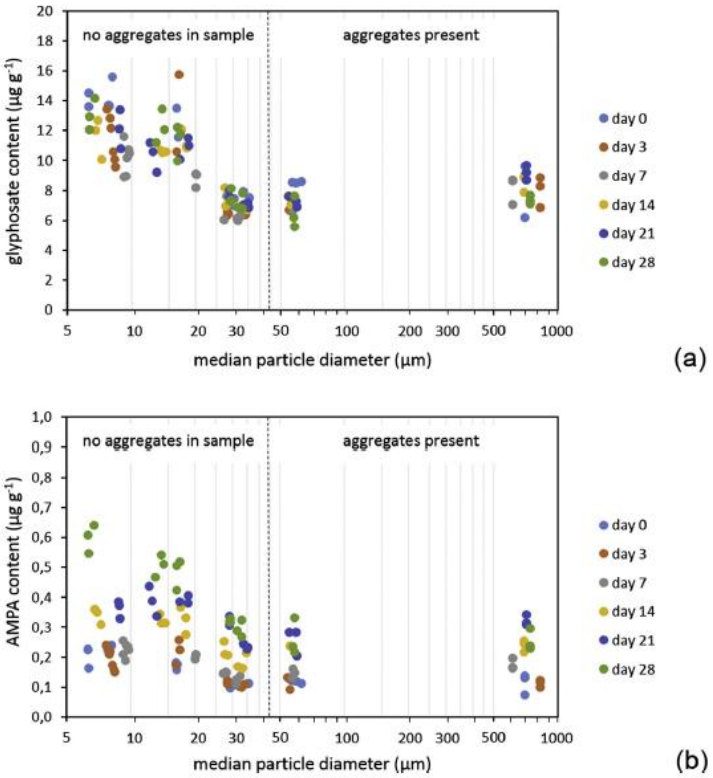


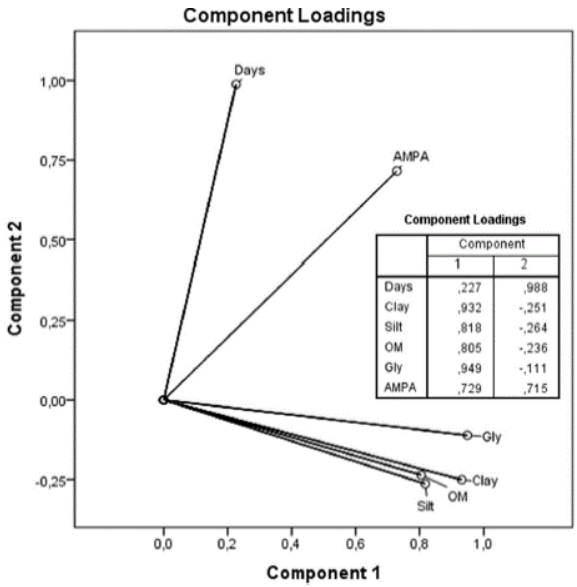
Figure 8.3.1.2-5: Relationship between (a) glyphosate content and particle size, (b) AMPA content and particle size



Relationship between glyphosate or AMPA and clay, silt and OM

Figure 8.3.1.2-6 shows the results of the categorical principal components analysis performed to determine the relationship between the studied sediment properties (clay, silt and OM) and glyphosate and AMPA content. The proportion of variance-accounted-for by the first component is 61.1 %, whereas the second component accounts for 28.1 %. Thus, the two components together account for a considerable proportion (89.2 %) of the variance. All sediment properties analysed in this study loaded in the first component together with glyphosate and AMPA, whereas only the duration of the experiment (days) loaded in the second component together with AMPA (Figure 8.3.1.2-6). The studied sediment properties do, therefore, play a major role in adsorbing glyphosate and AMPA. The duration of the experiment, on the other hand, was only meaningful for AMPA.

Figure 8.3.1.2-6: Categorical principal components analysis (non-linear PCA).
Gly = glyphosate; OM = organic matter



Component Loadings

	Component	
	1	2
Days	,227	,988
Clay	,932	-,251
Silt	,818	-,264
OM	,805	-,236
Gly	,949	-,111
AMPA	,729	,715

The order to which glyphosate and AMPA contents in the wind-eroded sediment are influenced by the studied sediment properties is as follows: clay >OM >silt (Figure 8.3.1.2-6). Glyphosate content correlates significantly and positively to the clay content ($R2 = 0.63$, $p < 0.01$). For coarser soil fractions, such as silt, the relationship with glyphosate content is considerably less expressed ($R2 = 0.27$) but still significant ($p < 0.01$). Significantly positive correlations were also observed between AMPA content and clay ($R2 = 0.16$, $p < 0.01$), and AMPA content and silt ($R2 = 0.10$, $p < 0.01$). Organic matter also appears as a strong factor influencing glyphosate adsorption to wind-eroded sediment: glyphosate content correlates significantly and positively to the OM content ($R2 = 0.49$, $p < 0.01$). However, one should realize that a positive correlation between glyphosate content and OM would be observed anyway because both are a function of particle size (both are higher for smaller particles, see Figure 8.3.1.2-3). Therefore, the effect of OM on glyphosate adsorption cannot be confirmed with certainty. In summary, these results show that the highest concentrations of glyphosate and AMPA in the finest fractions are related to the higher clay and OM content in these same fractions, although the role of silt cannot be ignored. Sprankle et al. (1975) also reported that glyphosate was readily adsorbed to clay and OM, and that less glyphosate was adsorbed by a sandy loam soil than by a clayey loam soil.

Glyphosate and AMPA content through time and consequences for their airborne off-site transport with dust

The fact that glyphosate and AMPA contents are highest in the fine fractions of the soil has important consequences for the airborne off-site transport of these compounds, because particles <20 µm have the capacity of being transported in long-term suspension. This can easily be shown by calculating the aeolian threshold for long-term suspension, which, according to the model of Pye and Tsoar (1990), is $u\infty/u^* < 0.1$, where $u\infty$ is the terminal fall velocity and u^* the friction velocity. Using this criterion, 20-µm particles are already transported in long-term suspension when $u^* < 0.3$ m/s. Assuming a roughness length z0 of 3-10 cm (typical value for agricultural areas, depending on the type of crop, see Ramli et al. (2009)), this corresponds to a 10-m height wind speed of 3.5-4.4 m/s, which are very typical values for many inland agricultural areas. For 10-µm particles, the critical wind speed is much lower: only 1.2-1.4 m/s (at 10 m height). At these wind speeds, particles are able to travel tens to even several hundreds of km before they settle back to the Earth's surface. During the 4-week experiment, nearly no glyphosate decay took place (Figure 8.3.1.2-4). Consequently, the formation of AMPA was very slow and remained low during the experimental period. Glyphosate and AMPA decay mostly by microbial activity (Bento et al., 2016; Gimsing et al., 2004; Nomura and Hilton, 1977), and for the latter a minimum soil moisture is required (Bento et al., 2016; Schroll et al., 2006). In our study, the soil moisture content during storage, after applying glyphosate but before the 24-h drying process prior to each wind tunnel test, was 5.4 %. This soil moisture content revealed to be very low to allow for soil microbial activity and consequent glyphosate decay. Very important in this context is that wind erosion

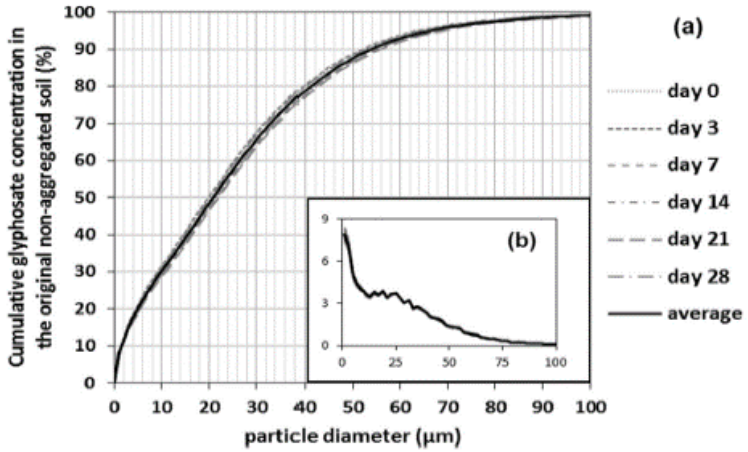
of fine, dusty particles only occurs when the topsoil (and, therefore, also the particles themselves) is sufficiently dry. Nourzadeh et al. (2013) tested several types of loamy soils using a field wind tunnel and found that the maximum moisture content to allow wind erosion of these soils was only 2 %, well below the limit for a substantial decay of glyphosate. Besides wind erosion, for many silty soils tillage erosion is a second (and in many cases even more important) mechanism for emission of fine particulates.

For tillage-emitted particles the probability for off-site transport is also highest when the particles are dry. Since the decay of glyphosate in our study occurred already extremely slowly for a soil moisture content of 5.4 %, its decay would be nearly inexistent for such dry wind-eroded sediment. Therefore, if glyphosate is applied during a dry period and emission of fine particles happens thereafter (either by wind erosion if the soil cover is still small, or by tillage activities if there is already some cover), then the potential for airborne glyphosate transport to off-site areas is considerable.

Potential contribution of glyphosate and/or AMPA contaminated airborne dust to human exposure

Figure 8.3.1.2-7 shows the reconstruction of the distribution of glyphosate in the original non-aggregated soil in the sediment tray before the start of each wind tunnel experiment. As expected, the glyphosate distribution was nearly identical for the six experimental runs, and it was predominantly concentrated in the finest fractions. On average for the six experimental runs, 13 % of the glyphosate in the original soil was concentrated in the PM2.5 fraction (particles <2.5 µm), 15 % in the PM4 fraction, and 28 % in the PM10 fraction. It is currently unknown whether the distribution of glyphosate in Figure 8.3.1.2-7 also applies to the macroscopic aggregates, but because the aggregates are almost perfect compositions of the original soil in the sediment tray (see Figure 8.3.1.2-2) the distribution of glyphosate within the aggregates is probably not far off from that shown in Figure 8.3.1.2-7. For AMPA, 14 % was concentrated in the PM2.5 fraction, 15 % in the PM4 fraction, and 29 % in the PM10 fraction. These results reconfirm that glyphosate and AMPA are considerably susceptible to be transported with airborne dust. After having accomplished their airborne transport trajectory, the glyphosate and/or AMPA containing soil particles will settle to the ground, thereby contaminating the deposition area. When the deposition is induced by rainfall and the particles and the soil become wet, glyphosate and/or AMPA will most probably further decay. When dry deposition occurs and the conditions remain dry for a while, glyphosate may remain in the deposited sediment until the soil becomes wet and the soil microorganisms active.

Figure 8.3.1.2-7: Calculated cumulative (a) and non-cumulative (b) distribution of glyphosate in the original soil (after destruction of the aggregates) for the six experimental days.



Conclusion

The study indicates that glyphosate and AMPA contents are highest in sediment particles <10 µm (PM10), and that their content diminishes with increasing particle size. The risk of off-site airborne transport of glyphosate and AMPA with dust is, therefore, very high. Because glyphosate and AMPA hardly decay under dry conditions of the soil, this risk is intensified if glyphosate is applied in arid and

semi-arid areas or during long periods of draught. If glyphosate and AMPA contaminated PM10 fractions of soil are emitted to the atmosphere, they may be inhaled by humans and animals. This contributes to the risk of human and animal exposure and, therefore, more attention should be paid to this route of exposure in environmental and human health risk assessment studies. Moreover, glyphosate applications during dry periods in regions susceptible to wind erosion should be avoided.

Assessment and conclusion by applicant:

The article describes the glyphosate and AMPA distribution in wind-eroded sediment derived from a laboratory wind tunnel experiment with loess soil. The distribution of the substances in different particle size fractions is evaluated. Correlations to different soil parameters are presented. Methods and results are sufficiently described.

The article was seen as reliable.

Assessment and conclusion by RMS:

The article provides supportive information on the potential transport of glyphosate and AMPA in wind-eroded sediment but no reliable endpoints can be derived for use in risk assessment.

The potential transport of glyphosate in wind-eroded sediment could be a potential explanation for glyphosate quantification in air in a french exploratory pesticide campaign (please refer to section 8.5 for more details) but further data would be needed to confirm this hypothesis.

B.8.3.1.3. Summary of route and rate of degradation in air

The vapour pressure of glyphosate is 1.31 x 10^{-5} Pa (25 °C). Based on EVA 3.2, this is equivalent to a vapour pressure of 6.81 x 10^{-6} Pa at 20°C. According to FOCUS Air criteria, glyphosate can be classified as not volatile from soil and plants.

No significant volatilisation of glyphosate from plants and soil was observed after the application of glyphosate in laboratory experiments.

Glyphosate degrades very rapidly in air with an estimated half-life of 0.135 days (1.625 hours), indicating that long-range transport is not expected.

Due to no significant UV-absorption, direct photolysis in air is not relevant. In case reaching the atmosphere, glyphosate should be rapidly removed by photochemical oxidative degradation.

Based on glyphosate properties, the active substance is not considered volatile and has no potential for long range transport according to FOCUS guidance Air (2008). However, it should be noted that glyphosate is quantified in a national exploratory pesticide campaign in air in France. Please refer to section B.8.5 for more details.

B.8.3.2. Transport via air

Based vapour pressure of 6.81 x 10^{-6} Pa at 20°C, glyphosate is not expected to volatilise in significant amounts.

Any glyphosate that might enter the atmosphere would not be subject to gas phase transport over large distances, due to rapid indirect photochemical degradation; DT_{50}air =1.6 hours for hydroxyl radical reaction.

B.8.3.3. Local and global effects

Due to the low volatilisation potential and the fast degradation of glyphosate in air, no significant local and global effects from atmospheric transport of glyphosate are expected.

B.8.4. RESIDUE DEFINITION

The following residue definition for risk assessment in environmental compartments is proposed:

Soil: Glyphosate and AMPA

Groundwater: Glyphosate and AMPA

Surface water: Glyphosate, AMPA and HMPA

Sediment: Glyphosate, AMPA and 1-oxo-AMPA

Air: Glyphosate

B.8.5. MONITORING DATA CONCERNING FATE AND BEHAVIOUR OF THE ACTIVE SUBSTANCE, METABOLITES, DEGRADATION AND REACTION PRODUCTS

Considering the length and weight of this current document and the length and weight of monitoring data, this part B5 is presented in a separated document.

B.8.6. LITERATURE REVIEW

Article 8(5) of Regulation (EC) No 1107/2009 requires applicants submitting dossiers for approval of active substances to provide relevant scientific peer reviewed open literature.

The literature review as performed and reported by the applicant is presented below. The assessment by RMS is presented in B 8.6.6.

B.8.6.1. Summary

This summary of scientific peer reviewed open literature conforms to EFSA guidance "Submission of scientific peer-reviewed open literature under Regulation (EC) No 1107/2009, EFSA Journal 2011; 9(2):2092".

Peer reviewed open literature containing data and analysis dealing with the side effects on health, environment, and non-target species for common name, and its relevant metabolites should be provided. The data published within the last ten years before the date of the submission of glyphosate (renewal) dossier were reviewed. This initial search was reported in the Literature Review Report of May 2020.

Upon request of RMS, an additional literature search covering the period from January to June 2020 has been conducted. This additional search was reported in the Literature Review Report of October 2020.

A total of 1147 references were identified for glyphosate and its metabolites for the fate and behaviour in environment and evaluated for potential relevance.

842 references were determined to be not relevant based on rapid assessment. 126 were determined to be not relevant based on detailed assessment. 179 studies were considered as relevant for inclusion in the dossier, with 98 relevant articles and 79 articles considered as relevant but supplementary.

B.8.6.2. Search strategy

The applicant has performed the search via the online service provider STN (www.stn-international.de).

The following databases have been used in order to cover the requirements of the EFSA Guidance Document: AGRICOLA, BIOSIS, CABA, CAPLUS, EMBASE, ESBIOBASE, MEDLINE, TOXCENTER, FSTA, PQSCITECH, and SCISEARCH.

Due to a large amount of public literature available for the active substance glyphosate, the search has been divided into six parts. As the number of records returned by a "single concept search" was extremely large for the searches Part 0, Part 1, Part 2, Part 3 and Part 5b, a "focused search for grouped data requirements" have been performed (a combination of a substance search and "search filters" defined for the four relevant sections – ecotoxicology, toxicology, environmental fate, and residues).

A "single concept search" was used for the searches Part 4, Part 5a and Part 6.

In October 2020, upon request of RMS, an additional literature search covering the period from January to June 2020 has been conducted.

The table below summarises the seven search parts that cover the period from January 2010 to June 2020.

Overview of the searches conducted for glyphosate and its metabolites

Search	Performed for	Covering publication period	Conducted on
Part 0	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2010 – Dec 2011	28th Oct 2019
Part 1	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2012 – Dec 2017	08th Jun 2018.
Part 2a	glyphosate, AMPA, N-acetyl-AMPA	Jan 2018 – Dec 2018	04th Jul 2019
Part 2b	and N-acetyl-glyphosate	Jan 2019 – Jun 2019	10th Jul 2019
Part 3	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jul 2019 – Dec 2019	7th Jan 2020
Part 4	HMPA	Jan 2010 – Feb 2020	24th Feb 2020
Part 5a	N-methyl-AMPA, N-glyceryl-AMPA, N-malonyl-AMPA	Jan 2010 – Feb 2020	27th Feb 2020
Part 5b	methylphosphonic acid	Jan 2010 – Feb 2020	27th Feb 2020
Part 6	N-methylglyphosate	Jan 2010 – April 2020	04th May 2020
Additional search upon RMS request	Glyphosate AMPA N-acetyl-AMPA N-acetyl-glyphosate HMPA N-methyl-AMPA N-glyceryl-AMPA N-malonyl-AMPA methylphosphonic acid N-methylglyphosate	January 2020 – June 2020 (incl. June 2020)	02-July 2020

AMPA = (aminomethyl)phosphonic acid
HMPA = (hydroxymethyl)phosphonic acid

Bibliographic Databases used in the literature review

Databases	Frequency of updates
AGRICOLA	Monthly
BIOSIS	Weekly
CABA	Weekly
CAPLUS	Daily updates bibliographic data; weekly updates indexing data
EMBASE	Daily
ESBIOBASE	Weekly
MEDLINE	Six times each week, with an annual reload
TOXCENTER	Weekly
FSTA	Weekly
PQSCITECH	Monthly
SCISEARCH	Weekly

B.8.6.2.1. Input parameters for literature search

Substance name	Glyphosate Salts: isopropylamine, potassium, ammonium, methylmethanamine
IUPAC name	2-(phosphonomethylamino)acetic acid
CAS number	1071-83-6

	Salts: 38641-94-0, 70901-12-1, 39600-42-5, 69200-57-3, 34494-04-7, 114370-14-8, 40465-66-5, 69254-40-6

metabolite	AMPA
IUPAC name	(aminomethyl)phosphonic acid
CAS number	1066-51-9

metabolite	N-acetyl glyphosate
IUPAC name	N-acetyl-N-(phosphonomethyl)glycine
CAS number	129660-96-4

metabolite	N-acetyl AMPA
IUPAC name	[(acetylamino)methyl]phosphonic acid
CAS number	57637-97-5

metabolite	HMPA
IUPAC name	(hydroxymethyl)phosphonic acid
CAS number	2617-47-2

metabolite	N-methyl AMPA
IUPAC name	[(methylamino)methyl]phosphonic acid
CAS number	35404-71-8

metabolite	N-glyceryl AMPA
IUPAC name	(2,3-dihydroxypropanoylamino)methylphosphonic acid
CAS number	No data

metabolite	N-malonyl AMPA
IUPAC name	3-oxo-3-(phosphonomethylamino)propanoic acid
CAS number	no data

metabolite	methylphosphonic acid
IUPAC name	methylphosphonic acid
CAS number	993-13-5

metabolite	N-methylglyphosate
IUPAC name	2-[methyl(phosphonomethyl)amino]acetic acid
CAS number	24569-83-3

B.8.6.2.2. Endpoint-specific search terms

The approach used for the searches was either the “single concept search” (in searches Part 4, 5a and 6 of Literature Review report of May 2020) or the “focused search for grouped data requirements” (in searches Part 0, 1, 2, 3, 5b of Literature Review report of May 2020, and searches from literature review Report of October 2020), which combines the active substance / metabolites keywords with the search filters used in the technical sections.

Environmental fate [Gly1] OR [Gly2] OR [Gly3] OR [Gly4] OR [Gly5] AND the following search filters

soil OR water OR sediment OR degradat? OR photo? OR soil residues OR soil accumulat? OR soil contaminat? OR mobility OR sorption OR column leaching OR aged residue OR leach? OR lysimeter OR groundwater OR contaminat? OR microb? OR exudation OR rhizosphere OR dissipation OR saturated zone OR hydrolysis OR drift OR run-off OR runoff OR drainage OR volat? OR atmosphere OR long-range transport OR short-range transport OR transport OR micronutrient OR phosphate OR iron OR manganese OR half-life OR halflife OR half-lives OR halflives OR DT50 OR kinetics OR off-site movement OR removal OR drinking water OR water treatment processes OR atmospheric deposition OR tile-drains OR surface water OR monitoring data OR disinfectant OR ozone OR tillage OR infiltration OR hard surface OR rainwater OR rain water OR chelat? OR complex? OR mineralization OR persistence OR ligand

Keywords used for the active substance glyphosate and its metabolites

Gly1: Glyphosate and AMPA	glyphosat? OR glifosat? OR glyfosat? OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR aminomethyl phosphonic OR aminomethylphosphonic OR 1066-51-9
Gly2: N-acetyl glyphosate and N-acetyl AMPA	2 acetyl phosphonomethyl amino acetic acid OR n acetyl glyphosate OR n acetylglyphosate OR n acetyl n phosphonomethyl glycine OR 129660-96-4 OR n acetyl ampa OR acetylamino methyl phosphonic acid OR acetylaminomethyl phosphonic acid OR 57637-97-5
Gly 3: HMPA	2617-47-2 OR hydroxymethanephosphonic acid OR hydroxymethyl phosphonate OR hydroxymethylphosphonate OR hydroxymethyl phosphonic acid OR hydroxymethylphosphonic acid OR methanehydroxyphosphonic acid OR phosphonic acid(1w)hydroxymethyl OR phosphonomethanol
Gly 4: N-methyl AMPA	35404-71-8 OR methylamino methyl phosphonic acid OR methylaminomethyl phosphonic acid OR methylaminomethylphosphonic acid OR n methyl ampa OR nsc 244826 OR phosphonic acid methylamino methyl OR phosphonic acid p methylamino methyl
Gly 4: N-glyceryl AMPA	2 3 dihydroxy 1 oxopropyl aminomethyl phosphonic acid OR 2 3 dihydroxy 1 oxopropyl aminomethylphosphonic acid OR n glyceryl ampa
Gly 4: N-malonyl AMPA	3 oxo 3 phosphonomethyl amino propanoic acid OR 3 oxo 3 phosphonomethyl aminopropanoic acid OR n malonyl ampa
Gly 4: methylphosphonic acid	993-13-5 OR dihydrogen methylphosphonate OR methanephosphonic acid OR methyl phosphonic acid OR methylphosphonic acid OR nsc 119358 OR phosphonic acid methyl OR phosphonic acid p methyl
Gly 5: N-methylglyphosate (NMG)	24569-83-3 OR 2 methyl phosphonomethyl amino acetic acid OR 2 methyl phosphonomethyl aminoacetic acid OR acetic acid 2 n methyl n phosphonatomethyl amino OR glycine n methyl n phosphonomethyl OR glyphosate n methyl OR methyl glyphosate OR methyl phosphonomethyl amino acetic acid OR methyl phosphonomethyl aminoacetic acid OR n methyl n phosphonomethyl glycine OR n methylglyphosate OR n phosphonomethyl n methyl glycine OR n phosphonomethyl n methylglycine

(1w) = proximity operator (this order, up to 1 word between)
AND / OR / NOT = boolean search operators
? = any character(s)

B.8.6.3. Relevance assessment

After combination of all six searches and removal of duplicates, the remaining articles were assessed for their relevance at title / abstract level (so-called rapid assessment). Articles that were identified as "non-relevant" in the rapid assessment were excluded from further evaluation. For articles that were not excluded in the rapid assessment, full-text documents were reviewed (detailed assessment).

B.8.6.3.1. Criteria for relevance assessment at "title / abstract" level

Articles identified as "non-relevant" in the rapid assessment belong to one of the following categories. These articles were excluded from further evaluation.

- Publications related to efficacy (resistance related articles, new uses of control of pest/crops) or to agricultural / biological research (crop science, breeding, fertilization, tillage, fundamental plant physiology / micro / molecular biology).
- Publications dealing with analytical methods / development.
- Publications describing new methods of synthesis (discovery / developments) or other aspects of basic (organic / inorganic) chemistry.
- Patents.
- Wastewater treatment.
- Abstracts referring to a conference contribution that does not contain sufficient data / information for risk assessment.
- Publications focusing on genetically modified organisms / transgenic crops; no data directly relevant to glyphosate evaluation (e.g. crop compositional analysis, gene flow, protein characterization).
- Publications where glyphosate or a relevant metabolite were not the focus of the paper.
- Secondary information including scientific and regulatory reviews (Reviews have been partly evaluated on full text level as well – case by case decision).
- Articles dealing with political / socio / economic analysis.
- Observations caused by mixture of compounds / potentially causal factors and thus not attributable to a substance of concern (*e.g.* mixture toxicity).
- Study design, test system, species tested, exposure routes etc. are not relevant for the European regulatory purposes.
- Findings related to ecotoxicology, toxicology, metabolism, environmental fate.
- Publications not dealing with EU representative uses / conditions (*e.g.* field locations, soil properties, non-EU monitoring etc.).

B.8.6.3.2. Criteria for relevance assessment at "full text" level

Articles that have been identified as "non-relevant" in the detailed assessment belong to one of the following categories:

- Publications dealing with a Roundup formulation that is not the representative formulation for the AIR5 dossier in Europe.
- Publications dealing with general pesticide exposures (not glyphosate specific).
- The presented endpoints are not relatable to the EU level risk assessment.
- Opinion articles where no new data is provided that can be used for risk assessment.
- Findings based on cellular and molecular level that cannot be related to the risk assessment.
- Criteria outlined in Section 2.4.1.1, that needed the full text document to determine.

B.8.6.3.1. Categorisation of "relevant" articles at "full text" level

Articles that have been identified as "relevant" in the rapid assessment have been categorized as recommended in the EFSA GD 2011; 9(2):2092, Point 5.4.1:

- Category (A) Studies that provide data for establishing or refining risk assessment parameters. These studies should be summarised in detail following the subsequent steps of the OECD Guidance documents (OECD, 2005; 2006) and should be considered for reliability.
- Category (B) Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters. After expert judgement, essential reliability parameters affect the full reliability of the study. A justification for such a decision should be provided.
- Category (C) Studies for which relevance cannot be clearly determined. For each of these studies the applicants should provide an explanation of why the relevance of such studies could not be definitively determined.

B.8.6.4. Reliability assessment

For articles, which have been identified as category A, under the Point 5.4.1 of the EFSA GD document, a reliability assessment has been performed. The reliability criteria for each technical section are summarized below.

For articles (category A) that have been identified as reliable or reliable with restrictions, summaries have been compiled. These summaries are presented in the MCA / MCP parts of the respective dossier section.

Articles of category A which have been identified as non-reliable were downgraded to articles of category B (relevant but supplementary).

Table 8.6.3.1-1: Reliability criteria

Applied for	Reliability criteria
Ecotoxicology, Environmental Fate, Residues	For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines are met.
Ecotoxicology, Environmental Fate, Residues	(No) previous exposure to other chemicals is documented (where relevant).
Ecotoxicology	For aquatic studies, the test substance is dissolved in water or where a carrier is required, it is appropriate (non-toxic) and a carrier control / positive control is considered in the test design.
Environmental Fate, Residues	The test substance is dissolved in water or non-toxic solvent.
Ecotoxicology, Environmental Fate, Residues	Test item is sufficiently documented, and reported (i.e. purity, source, content, storage conditions).
Ecotoxicology	For tests including vertebrates, compliance of the batches used in toxicity studies compared to the technical specification.
Ecotoxicology	Species used in the experiment are clearly reported, including source, experimental conditions (where relevant): strain, adequate age/life stage, body weight, acclimatization, temperature, pH, oxygen (dissolved oxygen for aquatic tests) content, housing, light conditions, humidity (terrestrial species) incubation conditions, feeding.
Ecotoxicology	The validity criteria from relevant test guidelines can be extrapolated across different species but not necessarily across different test designs. If different, then the nature of the difference and impact should ideally be discussed.
Ecotoxicology, Environmental Fate, Residues	Only glyphosate or its metabolites is the test substance (excluding mixture), and information on application of the test substance is described.
Ecotoxicology, Environmental Fate, Residues	The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite).
Ecotoxicology, Environmental Fate, Residues	Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc.), dilution/mixture of test item (solvent, vehicle) where relevant.
Ecotoxicology, Environmental Fate, Residues	Analytical verifications performed in test media (concentration) / collected samples, stability of the test substance in test medium should be documented.
Ecotoxicology	The test has been performed in several dose levels (at least 3) including a positive / negative control where relevant.
Ecotoxicology	Suitable exposure throughout the whole exposure period was demonstrated and reported.
Ecotoxicology	A clear concentration response relationship is reported – in studies where the dose response test design is employed.
Ecotoxicology	A sufficient number of animals per group to facilitate statistical analysis reported: mortality in control groups reported, observations/findings in positive/negative control clearly reported (where relevant).
Ecotoxicology, Environmental Fate, Residues	Assessment of the statistical power of the assay is possible with reported data.
Ecotoxicology, Environmental Fate, Residues	Statistical methodology is reported (e.g., checking the plots and confidence intervals).

Applied for	Reliability criteria
Ecotoxicology	Description of the observations (including time-points), examinations, and analyses performed, with (where relevant) dissections being well documented.
Ecotoxicology	For terrestrial ecotoxicological studies in the laboratory or the field, the substrates used should be adequately described e.g. nature of substrate i.e. species of leaf or soil type.
Ecotoxicology, Environmental Fate, Residues	Field locations relevant / comparable to European conditions.
Ecotoxicology, Environmental Fate, Residues	Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5%), bulk density, water retention, microbial biomass (~1% of organic carbon).
Ecotoxicology, Environmental Fate	Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass.
Ecotoxicology, Environmental Fate, Residues	For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year.
Ecotoxicology, Environmental Fate	For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max 3 months at 4 +/- 2°C).
Ecotoxicology, Environmental Fate, Residues	Data on precipitation is recorded.
Environmental Fate	The temperature was in the range between 20-25°C and the moisture was reported.
Environmental Fate	The presence of glyphosate identified in samples were collected from European groundwater, soil, surface waters, sediments or air.
Ecotoxicology	For lab terrestrial studies, the temperature was appropriate to the species being tested and generally should fall within the range between 20-25°C and soil moisture / relative humidity was reported.
Ecotoxicology	For bee studies, temperature of the study should be appropriate to species.
Ecotoxicology	For lab aquatic studies:
	The source and / or composition of the media used should be described.
	The temperature of the water should be appropriate to the species being tested and generally fall within the 15-25°C.
Ecotoxicology, Residues	The residue data can be linked to a clearly described GAP table, appropriate in the context of the renewal of approval of glyphosate (crop, application method, doses, intervals, PHI).
Ecotoxicology, Environmental Fate, Residues	Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate, and where relevant its metabolites.
Ecotoxicology, Environmental Fate, Residues	Analytical methods are clearly described; and adequate statement of specificity and sensitivity of the analytical methods is included.
Ecotoxicology	Assessment of the ECX for the width of the confidence interval around the median value; and the certainty on the level of protection offered by the median ECX is reported.
Environmental Fate	Radiolabel characterization: purity, specific activity, location of label is reported.
Environmental Fate	If degradation kinetics are included: data tables / model description / statistical parameters for kinetic fit to be provided.
Environmental Fate, Residues	Monitoring data: description of matrix analysed, and analytical methods to be fully described.
Environmental Fate	Clear description of application rate and relevance to approved uses.
Overall assessment: Reliable / Reliable with restrictions / Not reliable	

B.8.6.5. Search results

The table below summarised the number of published papers resulting from the search based on the criteria described above.

Table 8.6.3.1-1: Summary of the literature review

Section	Number of articles found	Rapid assessment (title/abstract level)		Detailed assessment (full-text level)	
		non-relevant articles	potentially relevant / unclear relevance	non-relevant articles	relevant articles (category A+B+C)
Fate and behaviour (initial search)	1062	759	303	132	171

Additional search[a]	85	83	2	0	2
Total	1147	842	305	132	173

[a] number of published papers identified upon removal of duplicates within the additional search (January 2020 – June 2020) and articles found already in the initial search.

The categorisation of relevant articles after full-text assessment is provided in the table below.

Table 8.6.3.1-2: Relevant articles by full text level – according to the EFSA GD, Point 5.4.1

	Relevant articles by full-text (EFSA GD, Point 5.4.1)*		
Section	Category A*	Category B*	Category C*
Fate and behaviour	97+1[a]	73	0
Additional search	2	0	0
Total	99+1	73	0

*Category A = relevant articles, Category B = relevant but supplementary articles, Category C = articles of unclear relevance.

[a] One e-fate entry (+1) is an erratum to the respective e-fate article

B.8.6.6. RMS evaluation

The literature search was performed by the applicant according to the EFSA Guidance 2011.

From the initial check of the literature search submitted in June 2020, RMS has checked the literature search, the lists of studies and Excel sheet for studies that were not submitted by the GRG. For the 759 articles that were deemed "non-relevant after rapid assessment", this check was based on the title only. For the 303 articles considered "potentially relevant/unclear relevance", the justification provided by the applicant was checked. When justification was not convincing, the abstract was also checked. When justification pointed major drawbacks these were not considered further by RMS and no summary is required.

Following this initial check, the applicant was requested to:

- Provide further justifications on the studies seemingly excluded because they were non-EU studies
- Take into account the publications that were conducted with other formulations. RMS estimated that they should be considered as relevant as they may provide useful information that can be considered in a weight of evidence approach.
- Provide study summaries for 6 articles excluded after detailed assessment by the applicant (from table 38 of initial Literature Review Report, May 2020) (reports then provided, see table above).

When assessing the literature review, RMS has considered in particular that:

- Studies that were conducted with other formulations may provide useful supportive information.
- Endpoints not relatable to the EU risk assessment might provide relevant supportive information. RMS then does not consider it should be used as a criteria for "non-relevance".
- Regarding the reliability criteria: The criteria used by the applicant to state on the reliability of each study are considered too restrictive for literature data. Indeed the reliability assessment is close to the one used for studies conducted according to test guidelines such OECD/ISO standards (for example, withdraw of studies with no analytics, not GLP…). However, this may exclude many studies bringing potentially supportive information.

The tables below listed the outcome of RMS analysis of the literature search (from the applicant Literature Review Report of May 2020 and October 2020).

Table 8.6.6-1: Results of the article selection process for fate and behaviour

Summary of the review	Number	Justification
Total number of summary records retrieved from search	-	
Total number of summary records retrieved after removing duplicates from all database searches	1158[a]	
Number of summary records excluded after rapid assessment for relevance (by title/abstract)	842	
Number of studies excluded from the risk assessment after detailed assessment of full-text documents, based on relevance criteria (i.e. not relevant)	132	See Studies excluded from the RAR (studies not relevant) – sorted by author Table
Number of studies excluded from the risk assessment after detailed assessment of full-text documents, based on reliability criteria (Cat B studies)	65	See Table 8.6.6-3
Number of studies included in the RAR as supporting information (Cat A and C studies)	**119 (+4[b])**	See Table 8.6.6-4 and Table 8.6.6-5

[a] 11 studies not included by the applicant in the literature search but still included in the RAR
[b] English translations of 4 publications

A total of 119 publications are considered relevant by RMS and are therefore summarised and assessed in the RAR. Compared to the 99+1 publications listed by the applicant, the following were added by RMS:

- 6 articles considered as Cat B by the applicant were summarized in the RAR after RMS request;
- 2 articles considered as Cat B by the applicant were summarized in the RAR after further analysis of the article by RMS (Boye, 2019 and Skeff, 2015);
- 7 articles from the previous literature review (AIR 2) did not appear in the 2010-2020 literature search as they were published earlier, but were still summarised by the applicant and included in the RAR (corresponding to table footnote [a]);
- 2 additional articles (Rosenbom 2019 & 2020) were provided after the initial literature search and did not then appear in the applicant table (corresponding to table footnote [a]);
- 2 articles were not provided by the applicant and not mentioned in the literature search but were summarized by the applicant in the RAR and considered as relevant by RMS (Van der Hoek, 2014 and Gillefalk, 2018) (corresponding to table footnote [a]);

Within the 119 studies, 2 studies were considered as reliable by the applicant but not by RMS. They are still presented and assessed in the RAR for transparency.
Please note that for 4 publications, English translations were also provided and are listed in Table 8.6.6-4 and Table 8.6.6-5.

RMS considers that no article considered as non-relevant after a rapid assessment by the applicant (from Excel file provided with the additional Literature Review Report, October 2020, filtered in 'final section' on fate and behaviour in environement) need further consideration.

The following table provides the list of publications excluded after detailed assessment of full-text. RMS agrees with the applicant's analysis.

Studies excluded from the RAR (studies not relevant) – sorted by author

Table 8.6.6-2: Publications excluded from the risk assessment after detailed assessment of full-text documents – Based on relevance criteria

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance criteria)
Adelowo F. E. et al.	2014	Biodegradation of Glyphosate by Fungi Species	Advances in Bioscience and Bioengineering (2014), Vol. 2, No. 1, pp. 104	Degradation of glyphosate by fungal isolates from Nigerian soil not relevant to EU risk assessment.
Ahmed S. et al.	2011	Influence of parameters on the heterogeneous photocatalytic degradation of pesticides and phenolic contaminants in wastewater: A short review.	Journal of Environmental Management (2011), Vol. 92, No. 3, pp. 311-330	This paper is a literature review with no experimental data provided. Investigation of specific methods of wastewater treatment are also not relevant to the data requirements
Allinson G. et al.	2016	Pesticide and trace metals in surface waters and sediments of rivers entering the Corner Inlet Marine National Park, Victoria, Australia.	Environmental science and pollution research international (2016), Vol. 23, No. 6, pp. 5881- 91	No glyphosate analysis included in paper.
Alza-Camacho W. R. et al.	2016	Voltammetric quantification of Paraquat and glyphosate in surface waters. Determinacion voltametrica de paraquat y glifosato en aguas superficiales.	Revista Corpoica - Ciencia y Tecnologia Agropecuarias (2016), Vol. 17, No. 3, pp. 331-345	Primarily a methods paper. Includes analysis of 10 water samples from Colombia but only minimal details on collection of samples provided.
Anon.	2016	Reply to: Comments on the paper: Re- evaluation of groundwater monitoring data for glyphosate and bentazone by taking detection limits into account	Science of the Total Environment (2016), Vol. 557-558, pp. 916	No new data presented, just discussion of statistical methods for re-evaluation.
Aparicio V. C. et al.	2013	Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins.	Chemosphere (2013), Vol. 93, No. 9, pp. 1866- 73	Analysis of soil and surface water samples related to cultivation of transgenic crops in Argentina are not representative for European agricultural practice.
Arroyave J. M. et al.	2017	Desorption rate of glyphosate from goethite as affected by different entering ligands: hints on the desorption mechanism.	Environmental Chemistry (2017), Vol. 14, No. 5, pp. 288-294	Desorption of glyphosate from geothite is studied relative to other competing ligands. Provides useful information on glyphosate desorption factors but not relevant to risk assessment.
Babic S. et al.	2018	Assessment of river sediment toxicity: Combining empirical zebrafish embryotoxicity testing with in silico toxicity characterization.	The Science of the total environment (2018), Vol. 643, pp. 435-450	Toxicity of sediments containing mixtures of chemicals are discussed.
Baez M. E. et al.	2015	Sorption-desorption behavior of pesticides and their degradation products in volcanic and nonvolcanic soils: interpretation of interactions through two-way principal component analysis	Environmental science and pollution research international (2015), Vol. 22, No. 11, pp. 8576- 85	Adsorption/ desorption studies done on a mixture of glyphosate and AMPA. Not relevant for EU risk assessment.
Baez M. E. et al.	2014	Determination of glyphosate and aminomethylphosphonic acid in aqueous soil matrices: a critical analysis of the 9-fluorenylmethyl chloroformate derivatization reaction and application to adsorption studies.	Journal of separation science (2014), Vol. 37, No. 21, pp. 3125-32	Adsorption/ desorption studies done on a mixture of glyphosate and AMPA. Not relevant for EU risk assessment.
Bandana B. et al	2015	Dissipation kinetics of glyphosate in tea and tea-field under northwestern mid-hill conditions of India	Journal of Pesticide Science (2015), Vol. 40, No. 3, pp. 82-86	Not relevant by full text: The article concerns crop/country not representative for the glyphosate EU renewal.
Battaglin W. A. et al.	2014	Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. Special Issue: Contaminants of emerging concern II.	Journal of the American Water Resources Association (2014), Vol. 50, No. 2, pp. 275-290	Analysis of soil, groundwater, surface water and sediment samples from USA are not representative for European agricultrual practice.

Bento C. P. M. et al.	2018	Spatial glyphosate and AMPA redistribution on the soil surface driven by sediment transport processes - A flume experiment	Environmental pollution (2018), Vol. 234, pp. 1011-1020	Artificial run-off situation not relevant for risk assessment.
Berzins A. et al.	2019	Modeling the mobility of glyphosate from two contrasting agricultural soils in laboratory column experiments	Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes (2019), Vol. 54, No. 7, pp. 539-548	Study of glyphosate degradation in Latvian sandy and loamy sand soils +/- augmentation with endophytic bacteria and fungi isolated from oilseed rape and barley followed by column leaching of same soils. Method not relevant for EU risk assessment.
Bois P. et al.	2013	Herbicide mitigation in microcosms simulating stormwater basins subject to polluted water inputs.	Water research (2013), Vol. 47, No. 3, pp. 1123-35	Glyphosate concentrations in the microcosm system were 1000- fold higher than typical concentrations. Glyphosate degradation results not relevant for risk assessment.
Bois P. et al.	2011	Herbicide degradation and copper complexation by bacterial mixed cultures from a vineyard stormwater basin.	Journal of Soils and Sediments (2011), Vol. 11, No. 5, pp. 860-873	Cultivation and analysis of bacterial communities as well as analysis of glyphosate in the respective culture supernatants are not relevant to the data requirements.
Bonansea R. I. et al.	2018	The Fate of Glyphosate and AMPA in a Freshwater Endorheic Basin: An Ecotoxicological Risk Assessment.	Toxics (2017), Vol. 6, No. 3, pp. 1	Paper reports concentrations of glyphosate & AMPA in water, sediment and suspended particulate matter in a river in Argentina. No information on product use provided. Not relevant to EU risk assessment.
Bonfleur E. J. et al.	2011	Mineralization and degradation of glyphosate and atrazine applied in combination in a Brazilian Oxisol.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2011), Vol. 46, No. 1, pp. 69-75	Laboratory soil degradation experiments with Brazilian soils are not representative for European conditions.
Botero-Coy A. M. et al.	2013	Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry.	Journal of chromatography. A (2013), Vol. 1292, pp. 132-41	Primarily a methods paper. Includes analysis of 26 soil samples from Colombia and Argentina but no details on source or collection of samples provided.
Boz B. et al.	2015	Analysis of suspended solids and Glyphosate and efficacy of the cross- compliance standard 5.2 'buffer strips' in the protection of superficial water from suspended solids in runoff conveyed through a vineyard. Special Issue: Cross compliance. Results of t	Italian Journal of Agronomy (2015), Vol. 10, No. s1, 701 p	The effectiveness of vegetated buffer to prevent glyphosate run- off events was investigated but the concentration of glyphosate from the vineyard runoff were all below the detection limit therefore it was not possible to evaluate the efficiency of the buffer zone in removing glyphosate.
Bradley P. M. et al.	2018	Reconnaissance of Mixed Organic and Inorganic Chemicals in Private and Public Supply Tapwaters at Selected Residential and Workplace Sites in the United States.	Environmental Science and Technology (2018), Vol. 52, No. 23, pp. 13972-13985	Paper describes analysis of glyphosate and AMPA in tapwater from multiple sampling sites in the U.S. Glyphosate and AMPA were not reported to have been found in any samples. Not relevant to EU risk assessment.
Caceres-Jensen L. et al.	2019	Electrochemical method to study the environmental behavior of Glyphosate on volcanic soils: Proposal of adsorption-desorption and transport mechanisms.	Journal of hazardous materials (2019), Vol. 379, pp. 120746	Adsorption /Desorption studies did not follow OECD guideline. Solutions did not contain CaCl2.
Cao L. et al.	2014	Determination of Herbicides and Its Metabolite in Soil and Water Samples by Capillary Electrophoresis-laser Induced Fluorescence Detection Using	Analytical Sciences (2014), Vol. 30, No. 7, pp. 759	Analytical method paper, testing fortified environmental samples only to demonstrate method.

		Microwave-assisted Derivatization		
Choubert J. M. et al.	2011	Limiting the emissions of micro-pollutants- what efficiency can we expect from wastewater treatment plants?	Water Science and Technology (2011), Vol. 63, No. 1, pp. 57-65	No specific analysis results for glyphosate or AMPA reported. Investigation of the removal efficiencies of different treatment processes of wastewater treatment plants are not relevant to the data requirements.
Chretien F. et al.	2017	Surface runoff and subsurface tile drain losses of neonicotinoids and companion herbicides at edge-of-field.	Environmental pollution (2017), Vol. 224, pp. 255-264	Concentration measurements in run-off and drainage water from fields cultivated with corn and soybean in Canada are not representative for European agricultural practice.
Clua A. et al.	2012	The effects of glyphosate on the growth of birdsfoot trefoil (Lotus corniculatus) and its interaction with different phosphorus contents in soil.	Journal of Agricultural Science (2012), Vol. 4, No. 7, pp. 208-218	No analysis of glyphosate or its metabolites. Outdoor study conducted in Argentina, hence conditions are not representative for Europe.
Danial R. et al.	2019	FTIR, CHNS and XRD analyses define mechanism of glyphosate herbicide removal by electrocoagulation.	Chemosphere (2019), Vol. 233, pp. 559-569	Theoretical beaker scale test for removing glyphosate from water. Natural water was not used. Not relevant for EU risk assessment.
Daouk S. et al.	2015	Fluorescence spectroscopy to study dissolved organic matter interactions with agrochemicals applied in Swiss vineyards.	Environmental science and pollution research international (2015), Vol. 22, No. 12, pp. 9284- 92	No new data on glyphosate are presented. The article focuses on analysis of dissolved organic matter in soil water samples and correlates them with glyphosate concentrations determined in another study (Daouk, 2013).
Degenhardt D. et al.	2012	Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2012), Vol. 47, No. 7, pp. 631-9	Field trials in Canadian prairie wetlands are not representative for European agricultural practice.
Delmonico E. L. et al.	2014	Determination of glyphosate and aminomethylphosphonic acid for assessing the quality tap water using SPE and HPLC.	Acta Scientiarum Technology (2014), Vol. 36, No. 3, pp. 513-519	Development of glyphosate analytical method and demonstration of the method through analysis of public water supply samples from Brazil. Not relevant to EU risk assessment.
dos Santos S. C. et al.	2014	Development of electroanalytical methodology for determination of pesticide glyphosate in environmental samples	Revista Virtual de Quimica (2014), Vol. 6, No. 4, pp. 866-883	Mainly analytical method. Only one natural sample collected and analyzed to demonstrate method.
Erban T. et al.	2018	The different behaviors of glyphosate and AMPA in compost-amended soil	Chemosphere (2018), Vol. 207, pp. 78-83	Effect of compost amendment on dissipation of glyphosate and AMPA in Czech soil after multiple glyphosate applications. Not relevant to EU risk assessment.
Ermakova I. T. et al.	2010	Bioremediation of glyphosate-contaminated soils.	Applied microbiology and biotechnology (2010), Vol. 88, No. 2, pp. 585-94	Biodegradation by selected bacterial strains in open microcosms and field plots in Russia are not relevant to the data requirement and not representative for European conditions
Farenhorst A. et al.	2015	Bulk deposition of pesticides in a Canadian city: Part 1. Glyphosate and other agricultural pesticides.	Water, Air, and Soil Pollution (2015), Vol. 226, No. 3, 47 p	Analysis of urban dust deposition samples from agricultural areas in Winnipag, Canada are not representative for European agricultural practice
Faria R. R. et al.	2019	Parameters for glyphosate in OPLS- AA force field	Molecular Simulation (2019), 45(1), 80-85	Mechanism of action study not relevant to EU risk assessment. Use of molecular dynamics (MD) simulations to provide an atomistic detail in the description of such a

				system. Herein, partial atomic charges and dihedral angles were obtained quantum mechanism for glyphosate molecule. Parameters for MD simulation were implemented in the OPLS-AA force field to better understand the herbicide mechanism action. Results showed that atomic charges were consistent with the database of the force field. Additionally., potential energy curves for the dihedrals were consistent and could be used to run MD simulations. Therefore, the parameterisation reported for this molecule can be useful to explain studies involving its interaction with many enzymes and proteins such as 5-enolpyruvylshikimate 3-phosphate synthase enzyme (EPSP). Furthermore, considering these new data in OPLS-AA, numerous simulations can be proposed to unveil the effects of the glyphosate as an environment contaminant.
Ferrario C. et al.	2017	Legacy and emerging contaminants in meltwater of three Alpine glaciers	Science of the Total Environment (2017), Vol. 574, pp. 350-357	The paper is about contaminants in meltwater of Alpine glaciers, no glyphosate or AMPA were measured.
Gasperi J. et al.	2010	Occurrence and removal of priority pollutants by lamella clarification and biofiltration	Water Research (2010), Vol. 44, No. 10, pp. 3065-3076	Experiments on wastewater treatment are not relevant to EU data requirements.
Giaccio G. C. M. et al.	2019	Glyphosate and nutrient retention in preferential flow pathways	Ecologia Austral (2019), Vol. 29, No. 3, pp. 329-338	Study of vegetative strips in Argentina, not relevant to EU.
Ginebreda A. et al.	2018	Reconciling monitoring and modeling: An appraisal of river monitoring networks based on a spatial autocorrelation approach - emerging pollutants in the Danube River as a case study	Science of the Total Environment (2018), Vol. 618, pp. 323-335	Relevant for Water Framework Directive but not pesticide registration. The results of this study show how auto-correlation models can aid water managers to improve the design of river monitoring networks. Not relevant for EU Risk Assessment.
Gloria O. N. et al.	2010	In vitro effects of four heavy metals on glyphosate utilization by some bacteria isolated from rice fields.	African Journal of Microbiology Research (2010), Vol. 4, No. 16, pp. 1775-1783	Experiments on the influence of heavy metals on the growth of isolated bacteria in the presence of glyphosate are not relevant to the data requirements.
Gomes M. P. et al.	2015	Consequences of phosphate application on glyphosate uptake by roots: Impacts for environmental management practices.	The Science of the total environment (2015), Vol. 537, pp. 115-9	Analysis of glyphosate in roots and leaves of hydroponically cultivated willow plants are not relevant to the data requirements.
Gurson A. P. et al.	2019	Mobility of 2,4-Dichlorophenoxyacetic Acid, Glyphosate, and Metribuzine Herbicides in Terra Rossa-Amended Soil: Multiple Approaches with Experimental and Mathematical Modeling Studies.	Water Air and Soil Pollution (2019), Vol. 230, No. 9, pp. Article No.: 220	Soil used for A/D and mobility testing is not a natural soil but rather a soil mixture. Not relevant for EU risk assessment.
Gustavsson M. et al.	2017	Pesticide mixtures in the Swedish streams: Environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation	Science of the Total Environment (2017), Vol. 598, pp. 973-983	No glyphosate data presented.

Hansen C. T. et al.	2015	Re-evaluation of groundwater monitoring data for glyphosate and bentazone by taking detection limits into account.	The Science of the total environment (2015), Vol. 536, pp. 68-71	No new data presented, only discussion of statistical methods to re-evaluation.
Hedegaard M. J. et al.	2017	Microbial pesticide removal in rapid sand filters for drinking water treatment - Potential and kinetics (vol 48, pg 71, 2014).	Water Research (2017), Vol. 122, pp. 708-713	Erratum to Hedegaard et al. 2014; does not contain any data for glyphosate.
Henault-Ethier L. et al.	2017	Herbaceous or Salix miyabeana 'SX64' narrow buffer strips as a means to minimize glyphosate and aminomethylphosphonic acid leaching from row crop fields.	Science of the total environment (2017), pp. 1177-1186	Field trials in Canada with glyphosate resistant crops and Salix miyabeana buffer strips are not relevant to the data requirement and not representative to European agricultural practice
Herath G. A. D. et al.	2019	Statistical optimization of glyphosate adsorption by biochar and activated carbon with response surface methodology.	Chemosphere (2019), Vol. 227, pp. 533-540	Test tube optimization of glyphosate adsorption using biochar and activated carbon. Not relevant for commercial application. Not relevant for EU risk assessment.
Herrman K. S. et al.	2012	Nutrient Loss Following Phragmites australis Removal in Controlled Soil Mesocosms	Water, air and soil pollution (2012), Vol. 223, No. 6, pp. 3333-3344	No analysis of glyphosate or its metabolites.
Hosseini N. et al.	2019	Removal of 2,4-D, glyphosate, trifluralin, and butachlor herbicides from water by polysulfone membranes mixed by graphene oxide/TiO2 nanocomposite: study of filtration and batch adsorption	JOURNAL OF ENVIRONMENTAL HEALTH SCIENCE AND ENGINEERING (2019), Vol. 17, No. 1, pp. 247-258	Testing of new synthetic membranes for glyphosate adsorption/ rejection at lab scale not relevant for EU risk assessment.
Hu Y. S. et al.	2011	Removal of glyphosate from aqueous environment by adsorption using water industrial residual	Desalination (2011), Vol. 271, No. 1-3, pp. 150- 156	Experiments on glyphosate adsorption to residual alum sludge from water treatment plants are not relevant to the data requirements.
Jarvis N.	2018	Meta-analysis of pesticide sorption in subsoil	Environmental Toxicology and Chemistry (2018), Vol. 37, No. 3, pp. 755-761	Comparison of the Koc model vs the power law model to characterize adsorption in sub-soils. While glyphosate existing data is considered, the approach is not relevant to EU risk assessment.
Johnsen A. R. et al.	2016	Comments on the article: Re-evaluation of groundwater monitoring data for glyphosate and bentazone by taking detection limits into account	Science of the Total Environment (2016), Vol. 557-558, pp. 914-915	No new data presented, only disussion of statistical methods for re-evaluation.
Junges C. M. et al.	2013	Effectiveness evaluation of glyphosate oxidation employing the H(2)O(2)/UVC process: toxicity assays with Vibrio fischeri and Rhinella arenarum tadpoles.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2013), Vol. 48, No. 3, pp. 163-70	No relevant information on environmental fate included. Paper is about removal of glyphosate from wastewater polluted by commercial formulations of glyphosate
Kaur S. et al.	2017	Pesticides Curbing Soil Fertility: Effect of Complexation of Free Metal Ions	FRONTIERS IN CHEMISTRY (2017), Vol. 5, Article 43, pp. 1	Experiments on reaction of pesticides with isolated metal salts are not relevant to EU data requirements.
Keesstra S. D. et al.	2019	Straw mulch as a sustainable solution to decrease runoff and erosion in glyphosate-treated clementine plantations in Eastern Spain. An assessment using rainfall simulation experiments	Catena (2019), Vol. 174, pp. 95-103	No measurement of glyphosate in this article. Glyphosate only used for weed control.
Knerr H. et al.	2015	Micropollutants from WWTPs in Rheinland-Palatinate	Wasser und Abfall (2015), Vol. 17, No. 1/2, pp. 23-28	Does not present any numerical measurement data. Discusses evaluation of occurrence and levels of micropollutants at waste water treatment plants of rural and urban geopgraphies.

Lashermes G. et al.	2010	Sorption and mineralization of organic pollutants during different stages of composting.	Chemosphere (2010), Vol. 79, No. 4, pp. 455- 62	Sorption and mineralization in artificial lab compost mixtures are not relevant to the data requirement.
Linklater N. et al.	2013	Real-Time and Near Real-Time Monitoring Options for Water Quality.	Ahuja, S. (2013) pp. 189-225, Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation, Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation, Publisher: ELSEVIER SCIENCE BV, ISBN: 978-0-444-59395-5(H), 978-0-444-59404-4(P)	No specific monitoring data for glyphosate or AMPA is reported (a book chapter).
Lucadamo L. et al.	2018	Evaluation of glyphosate drift and anthropogenic atmospheric trace elements contamination by means of lichen transplants in a southern Italian agricultural district.	Air Quality Atmosphere and Health (2018), Vol. 11, No. 3, pp. 325-339	Atmospheric contamination due to glyphosate and trace elements were monitored in a southern Italian agricultural district by means of transplanted thalli of the lichen Pseudevernia furfuracea. An unusual technique which provides information on atmospheric dispersion of glyphosate but not relevant to risk assessment.
Lupi L. et al.	2015	Occurrence of glyphosate and AMPA in an agricultural watershed from the southeastern region of Argentina.	The Science of the total environment (2015), Vol. 536, pp. 687-694	Analysis of soil, water and sediment samples from agricultural areas in Argentina cultivated with soybean are not representative for European agricultural practice.
Lupi L. et al.	2019	Glyphosate runoff and its occurrence in rainwater and subsurface soil in the nearby area of agricultural fields in Argentina.	Chemosphere (2019), Vol. 225, pp. 906-914	Glyphosate measurements in rainfall in Brazil not relevant for EU risk assessment. Soil column leaching experiment on an Argentinian soil in which the control also contains glyphosate, and is not relevant to EU risk assessment.
Magga Z. et al.	2012	Combining experimental techniques with non-linear numerical models to assess the sorption of pesticides on soils.	Journal of contaminant hydrology (2012), Vol. 129-130, pp. 62-9	The article describes batch experiments to derive equilibrium and non–equilibrium sorption parameters for glyphosate. However, the current guideline (OECD 106) for those experiments was not followed (use of triple distilled water instead of CaCl2, only liquid phase analyzed but stability of test item not shown, test concentrations not reported). Further, continuous flow soil column experiments were conducted with synthetic groundwater. This experiment is not relevant according to the data requirements.
Majewski M. S. et al.	2014	Pesticides in Mississippi air and rain: a comparison between 1995 and 2007.	Environmental toxicology and chemistry (2014), Vol. 33, No. 6, pp. 1283-93	Analysis of air and rainfall samples from agricultural areas in Mississippi (USA) cultivated with soybean are not representative for European agricultural practice.
Malviya B. J. et al.	2015	Bioremediation of Glyphosate by Bacteria Isolated from Glyphosate Contaminated Soil.	Journal of Pure and Applied Microbiology (2015), Vol. 9, No. 4, pp. 3315-3319	Study of bacterial isolates from area of glyphosate production plant in India for ability to degrade glyphosate as a sole carbon source. Not relevant to EU risk assessment.
Mamy L. et al.	2010	Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops.	Environmental pollution (2010), Vol. 158, No. 10, pp. 3172-8	Modelling approach on balances and overall toxicity potential; no new environmental fate data generated.

Mamy L. et al.	2016	Glyphosate fate in soils when arriving in plant residues.	Chemosphere (2016), Vol. 154, pp. 425-433	Laboratory experiment on oilseed rape plant residues treated with glypohosate and placed on/mixed with soil samples are not relevant to the data requirement.
Mardiana-Jansar K. et al.	2014	Residue determination and levels of glyphosate in surface waters, sediments and soils associated with oil palm plantation in Tasik Chini, Pahang, Malaysia	AIP Conference Proceedings (2014), 1614 (1, 2014 UKM FST Postgraduate Colloquium), pp. 795-802	Field trials in oil palm plantation in Malaysia are not representative for European agricultural practice.
Mattos R. et al.	2017	Quantitation and Adsorption of Glyphosate Using Various Treated Clay	Zeitschrift fuer Physikalische Chemie (2017), Vol. 231, No. 11-12, pp. 1815-1829	Adsorption studies for glyphosate conducted with clay chemically modified with metals. Not relevant to natural soils.
Mauffrey F. et al.	2017	Bacterial Community Composition and Genes for Herbicide Degradation in a Stormwater Wetland Collecting Herbicide Runoff	Water, air, and soil pollution (2017), Vol. 228, No. 12, 452 p	Investigation of bacterial community composition and genetic analyses not relevant to EU data requirements.
Mazzei P. et al.	2012	Quantitative evaluation of noncovalent interactions between glyphosate and dissolved humic substances by NMR spectroscopy.	Environmental science & technology (2012), Vol. 46, No. 11, pp. 5939-46	Experiments on reaction of glyphosate with isolated humic and fulvic acids are not related to the data requirements.
McMurry S. T. et al.	2016	Land use effects on pesticides in sediments of prairie pothole wetlands in North and South Dakota.	The Science of the total environment (2016), Vol. 565, pp. 682-689	Analysis of wetland sediment samples from prairie pothole wetlands in North and South Dakota (USA) are not representative for European conditions and agricultural practice.
Mendez M. J. et al.	2017	Glyphosate and Aminomethylphosphonic acid (AMPA) contents in the respirable dust emitted by an agricultural soil of the central semiarid region of Argentina	AEOLIAN RESEARCH (2017), Vol. 29, pp. 23-29	Analysis of artificially generated dust from Argentinian field locations are not relevant to the data requirements and not representative for European agricultural practice.
Mercurio P. et al.	2014	Glyphosate persistence in seawater.	Marine pollution bulletin (2014), Vol. 85, No. 2, pp. 385-90	Experiments on glyphosate degradation in seawater samples from the Great Barrier Reef (Australia) are not representative to European conditions.
Metzger S. et al.	2014	Trace substance removal in wastewater treatment plants-Experiences in Baden-Wuerttemberg	Gewaesserschutz, Wasser, Abwasser (2014), 234, 57/1-57/19	Main focus of the paper is use of activated carbon to remove contaminants. No glyphosate data presented. AMPA data presented in only one figure. Text indicates AMPA not effectively removed by amounts of activated carbon being studied. Since the AMPA is derived from other sources, relevance to glyphosate degradation cannot be established.
Minh H. D. et al.	2015	Molecularly imprinted polymer-based electrochemical sensor for the sensitive detection of glyphosate herbicide.	International Journal of Environmental Analytical Chemistry (2015), Vol. 95, No. 15, pp. 1489-1501	Analytical method. Fortified tap water samples used to demonstrate method; no real world samples analyzed.
Moneke A. N. et al.	2010	Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields.	African Journal of Biotechnology (2010), Vol. 9, No. 26, pp. 4067-4074	Experiments on in-vitro biodegradation with isolated bacteria strains are not relevant to the data requirement.
Moraes P. V. D. et al.	2010	Environmental behaviour of glyphosate. Comportamento ambiental do glifosato.	Scientia Agraria Paranaensis (2010), Vol. 9, No. 3, pp. 22-35	Literature review, secondary source of information.
Mueller T. C. et al.	2015	Methods Related to Herbicide Dissipation or Degradation under Field or Laboratory Conditions.	Weed Science (2015), Vol. 63, No. Sp. Iss. 1, pp. 133-139	No measurement of glyphosate or AMPA. Glyphosate was used for weed control.

Nourouzi M. M. et al.	2012	Application of ferric chloride for removal of Glyphosate: modeling of axial and radial flow impellers using artificial neural networks.	Journal of Environmental Engineering (2012), Vol. 138, No. 11, pp. 1157-1164	Investigation of formation of insoluble ferric chloride complex is not relevant to the data requirement.
Ocenaskova V. et al.	2012	Occurrence of pesticides not regularly monitored in the hydrosphere of the Czech Republic	Vodohospodarske Technicko-Ekonomicke Informace (2012), Vol. 54, No. 6, pp. 13S-16S	No glyphosate data presented. AMPA data in only one figure. Since the AMPA is derived from other sources, relevance to glyphosate degradation cannot be established.
Oliveira Pereira E. A. et al.	2019	Determination of glyphosate and aminomethylphosphonic acid by sequential-injection reversed-phase chromatography: method improvements and application in adsorption studies.	Analytical and bioanalytical chemistry (2019), Vol. 411, No. 11, pp. 2317-2326	Analytical method and adsorption testing using a glyphosate formulation: Roundup® Original DI.
Ololade O. O. et al.	2019	Influence of electrolyte composition and pH on glyphosate sorption by cow-dung amended soil	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2019), Vol. 54, No. 9, pp. 758-769	Nigerian soil, no textural characterization of soil, non-guideline CaCl2 concentration and no basis for comparison to guideline studies.
Ololade O. O. et al.	2019	Influence of cow-dung amendment on glyphosate mobility in soil	Toxicological & Environmental Chemistry (2019), Vol. 101, No. 3-6, pp. 265-280	Adsorption/desorption of Nigerian soil and cow dung from grazing cows. Not relevant to EU risk assessment.
Orcelli T. et al.	2018	Study of Interaction Between Glyphosate and Goethite Using Several Methodologies: an Environmental Perspective	Water, air, and soil pollution (2018), Vol. 229, No. 5, 150 p	Information regarding adsorption of glyphosate onto goethite under varying pH. Not relevant to EU risk assessment.
Otalvaro J. O. et al.	2018	Interaction of pesticides with natural and synthetic solids. Evaluation in dynamic and equilibrium conditions.	Environmental science and pollution research international (2018), Vol. 25, No. 7, pp. 6707- 6719	Paper includes study of binding of glyphosate to Humic acid and effect of binding on dissolution of humic acid. Not relevant since binding to soil components were studied separately and not in soil.
Padilla J. T. et al.	2018	Glyphosate transport in two Louisiana agricultural soils: miscible displacement studies and numerical modeling	Soil Systems (2018), Vol. 2, No. 3, pp. 53	Does not follow OECD column leaching or adsorption / desorption guidelines.
Padilla J. T. et al.	2019	Time-dependent sorption and desorption of glyphosate in soils: multi-reaction modeling	Vadose Zone Journal (2019), Vol. 18, No. 1, pp.	Experiments on batch adsorption and time-dependent sorption are not in line with OECD 106 guideline or guidance on aged sorption, thus not relevant to the data requirement.
Padilla-Sanchez J. A. et al.	2012	Innovative determination of polar organophosphonate pesticides based on high-resolution Orbitrap mass spectrometry.	Journal of mass spectrometry (2012), Vol. 47, No. 11, pp. 1458-65	Development and performance of a multi-component analytical method. For the analysis of agricultural soil samples, no experimental details are reported.
Penders E. J. M. et al.	2012	Genotoxic effects in the Eastern mudminnow (Umbra pygmaea) after prolonged exposure to River Rhine water, as assessed by use of the in vivo SCE and Comet assays	Environmental and Molecular Mutagenesis (2012), Vol. 53, No. 4, pp. 304-310	Toxicity of river water. No environmental data on glyphosate presented.
Pereira E. A. O. et al.	2019	Adsorption of glyphosate on Brazilian subtropical soils rich in iron and aluminum oxides	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2019), Vol. 54, No. 11, pp. 906-914	Does not follow guideline OECD adsorption/desorption method.
Pinto E. et al.	2018	Quantitative analysis of glyphosate, glufosinate and AMPA in irrigation water by in situ derivatization- dispersive liquid-liquid microextraction combined with UPLC-MSMS	Analytical methods (2018), Vol. 10, No. 5, pp. 554-561	Analytical method for detection of glyphosate, AMPA, glufosinate in water. Real water samples analyzed by locations not identified.

Polyakova N. N. et al.	2018	Effect of Herbicides Application on the Soil Biological Activity in the Tree Nursery.	Agrokhimiya (2018), No. 12, pp. 35-41	Non-EU studies not relevant to EU. Study of cellulose degradation in glyphosate treated soil conducted in Russia.
Prasanthi Y. et al.	2012	Glyphosate levels in soil, water and air before and after application on agricultural farms	Organohalogen Compounds (2012), Vol. 74, pp. 316-319, 4 pp.	Non-EU study. Measurement of glyphosate concentrations in soil and runoff water from university site in Kentucky, USA. Not relevant for EU risk assessment.
Qin J. et al.	2013	Can rainwater induce Fenton-driven degradation of herbicides in natural waters?.	Chemosphere (2013), Vol. 92, No. 8, pp. 1048- 52	Study not conducted in natural system. No direct relevance to risk assessment.
Ratola N. et al.	2014	Biomonitoring of pesticides by pine needles - Chemical scoring, risk of exposure, levels and trends	Science of the Total Environment (2014), Vol. 476-477, pp. 114-124	No monitoring of glyphosate in the study.
Rendon-von Osten J. et al.	2017	Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchen, Campeche, Mexico.	International journal of environmental research and public health (2017), Vol. 14, No. 6, pp. E595	Analysis of groundwater, and drinking water samples from intensive agricultural areas in Mexico are not representative for European agricultural practice. Analysis of urine samples is not relevant to the data requirements.
Richards B. K. et al.	2012	Surveying upstate NY well water for pesticide contamination: Cayuga and Orange counties	Ground Water Monitoring & Remediation (2012), Vol. 32, No. 1, pp. 73-82	Analysis of pesticides in groundwater wells in the US not relevant to EU risk assessment. No glyphosate or AMPA measurements reported.
Romano-Armada N. et al.	2019	Construction of a combined soil quality indicator to assess the effect of glyphosate application	Science of the Total Environment (2019), Vol. 682, pp. 639-649	Soil quality assessment conducted based on Argentinian soils. Not relevant fo EU risk assessment.
Ronco A. E. et al.	2016	Water quality of the main tributaries of the Parana Basin: glyphosate and AMPA in surface water and bottom sediments	Environmental Monitoring and Assessment (2016), Vol. 188, No. 8, pp. 458	Analyses of glyphosate and AMPA water and sediment samples from Argentinian agricultural areas planted with genetically modified glyphosate-resistant crops are not representative for European agricultural practice
Rott E. et al.	2017	Removal of phosphorus from phosphonate-loaded industrial wastewaters via precipitation/flocculation	JOURNAL OF WATER PROCESS ENGINEERING (2017), Vol. 17, pp. 188-196	No data on glyphosate or AMPA presented. Only references to journal articles before 2010.
Rudolph W.	2015	Greening conditions, glyphosate skepticism and groundwater protection: Three G-Core issues. Greeningauflagen, Glyphosatskepsis und Grundwasserschutz: Drei G-Themen im Fokus	Agrarmanager (2015), No. 8, pp. 58-61	There is no environmental fate data related to glyphosate. The article talks about farm machinery.
Sandy E. H. et al.	2013	Oxygen isotope signature of UV degradation of glyphosate and phosphonoacetate: tracing sources and cycling of phosphonates.	Journal of hazardous materials (2013), Vol. 260, pp. 947-54	Experiments on the reaction mechanism of molecule cleavage uder UV radiation at pH 2.5 are not relevant to the data requirements.
Schulte-Oehlmann U. et al.	2011	Before the curtain falls: endocrine- active pesticides - a German contamination legacy.	Reviews of environmental contamination and toxicology (2011), Vol. 213, pp. 137-59	Literature review on pesticide occurrence in Germany. Neither experimental data nor specific results for glyphosate are reported.
Sebiomo A. et al.	2012	The impact of four herbicides on soil minerals	Research Journal of Environmental and Earth Sciences (2012), Vol. 4, No. 6, pp. 617-624	Soils tested originate from region not representative for Europe (Nigeria) no analysis of glyphosate residues, only mineral ions (calcium, sodium, potassium, magnesium, zinc and iron)
Sen K. et al.	2017	Statistical optimization study of adsorption parameters for the removal of glyphosate on forest	Environmental earth sciences (2017), Vol. 76, No. 1, pp. 22	Experiments on glyphosate removal by Indian forest soils are not relevant to the data requirements.

		soil using the response surface methodology		
Shanmugam S. R. et al.	2019	Adsorption and desorption behavior of herbicide using bio-based materials	Transactions of the ASABE (2019), Vol. 62, No. 6, pp. 1435-1445	Adsorption of glyphosate to activated carbon and biochar was measured as a potential soil amendment to bind glyphosate. Not relevant to EU risk assessment.
Shimako A. H. et al.	2017	Operational integration of time dependent toxicity impact category in dynamic LCA	Science of the Total Environment (2017), Vol. 599-600, pp. 806-819	Life-cycle assessment. No specific glyphosate end-points that can be used in EU assessment.
Shipitalo M. J. et al.	2010	Impact of grassed waterways and compost filter socks on the quality of surface runoff from corn fields.	Journal of environmental quality (2010), Vol. 39, No. 3, pp. 1009-18	Field experiments performed in the US on concentration of glyphosate in run-off from experimental watersheds cropped with glyphosate-tolerant corn collected in grassed artificial waterways and removal by artificial compost filter socks. These are not relevant to the data requirements and not representative for EU agricultural practice.
Shushkova T. et al.	2010	Glyphosate bioavailability in soil.	Biodegradation (2010), Vol. 21, No. 3, pp. 403- 10	Experiments on soil degradation and adsorption in soil columns amended with mineral salts and introduced bacteria strains are not relevant to the data requirement
Si Y-B. et al.	2013	Complex Interaction and Adsorption of Glyphosate and Lead in Soil	Soil & sediment contamination (2013), Vol. 22, No. 1, pp. 72-84	Experiments on the influence of Pb on glyphosate adsorption in NaNO3 solution are not relevant to the data requirements
Silva J. T. B. et al.	2018	Glyphosate and turbidity removal in water conditions by clarification with Tanfloc. Remocao de glifosato e turbidez em meio aquoso por meio da clarificacao com Tanfloc	Periodico Tche Quimica (2018), Vol. 15, No. 30, pp. 489-496	Demonstrates glyphosate removal from raw water at pH 5.0 - 5.5 using natural tannin flocculant, but glyphosate concentration tested (8 mg/L) is not a relevant concentration for water treatment.
Sjerps R. M. A. et al.	2017	Projected impact of climate change and chemical emissions on the water quality of the European rivers Rhine and Meuse: A drinking water perspective	Science of the Total Environment (2017), Vol. 601-602, pp. 1682-1694	No new data are presented. Modeling of future surface water quality (year 2050) based on assumptions on climate change and future emission scenarios are not relevant to the data requirements.
Smith D. R. et al.	2015	What is causing the harmful algal blooms in Lake Erie?	Journal of soil and water conservation (2015), Vol. 70, No. 2, p. 27A-29A	Paper is a general review with no new data about reasons for increased soluble P loading to Lake Erie.
Sonne A. T. et al.	2017	Assessing the chemical contamination dynamics in a mixed land use stream system	Water Research (2017), Vol. 125, pp. 141-151	No glyphosate measurements reported from water monitoring. Some AMPA monitoring but source of AMPA unknown hence not relevant for risk assessment.
Struger J. et al.	2015	Sources of aminomethylphosphonic acid (AMPA) in urban and rural catchments in Ontario, Canada: Glyphosate or phosphonates in wastewater?.	Environmental pollution (2015), Vol. 204, pp. 289-97	Results of concentration measurements in Canadian urban and rural catchments are not representative for European agricultural practice.
Styczen M. et al.	2011	Macroscopic Evidence of Sources of Particles for Facilitated Transport during Intensive Rain	Vadose zone journal (2011), pp. 1151-1161	No new experimental data generated, only review & conclusions on results of data from literature.
Sviridov A. V. et al.	2011	New approaches to identification and activity estimation of glyphosate degradation enzymes.	Biochemistry. Biokhimiia (2011), Vol. 76, No. 6, pp. 720-5	Experiments on isolated and cultivated bacteria are not relevant to EU data requirement.
Sviridov A. V. et al.	2012	Distribution of glyphosate and methylphosphonate catabolism systems in soil bacteria	Applied Microbiology and Biotechnology (2012), Vol. 93, pp. 787-796	Experiments on isolated and cultivated bacteria are not relevant to the EU data requirements.

		Ochrobactrum anthropi and Achromobacter sp.		
Tang X. et al.	2012	A review of rapid transport of pesticides from sloping farmland to surface waters: Processes and mitigation strategies.	JOURNAL OF ENVIRONMENTAL SCIENCES (2012), Vol. 24, No. 3, pp. 351-361	The paper is a review of pesticide transport from sloping farmland to surface water. Glyphosate is not explicitly mentioned.
Tzaskos D. F. et al.	2012	Development of sampling for quantification of glyphosate in natural waters.	Ciencia e Agrotecnologia (2012), Vol. 36, No. 4, pp. 399-405	Development of an analytical method for water analysis in Brazil is not relevant to the data requirements for environmental fate. The analyses of Brazilian stream water samples from an area with transgenic soy plantations are not representative for European agricultural practice.
Van Stempvoort D. R. et al.	2016	Glyphosate residues in rural groundwater, Nottawasaga River Watershed, Ontario, Canada.	Pest management science (2016), Vol. 72, No. 10, pp. 1862-72	Results of concentration measurements in Canadian shallow rural groundwater are not representative for European agricultural practice.
Virginia A. et al.	2018	Industrial agriculture and agroecological transition systems: A comparative analysis of productivity results, organic matter and glyphosate in soil	Agricultural systems (2018), pp. 103-112	Economic and ecological study performed in Argentina. Comparison of industrial agriculture with agro-ecological system. Soil organic matter and glyphosate / AMPA concentrations in soil measured in addition to economic measures.
Vrain T. C.	2016	The nutritional status of GMOs	Acta horticulturae (2016), No. 1124, pp. 97-100	Limited review and commentary on glyphosate properties in relation to GMO nutritional status.
Waiman C. V. et al.	2013	A real time in situ ATR-FTIR spectroscopic study of glyphosate desorption from goethite as induced by phosphate adsorption: effect of surface coverage.	Journal of colloid and interface science (2013), Vol. 394, pp. 485-9	Adsorption experiments with isolate minerals (geothite and magnetite) are not relevant to the data requirements.
Wang K. et al.	2018	Application of least-squares support vector machines for quantitative evaluation of known contaminant in water distribution system using onlinewater quality parameters	Sensors (2018), Vol. 18, No. 4, pp. 938/1- 938/19	No reference to glyphosate, AMPA, HMPA
Welch H. L.	2015	Occurrence of pesticides in groundwater underlying areas of high- density row-crop production in Alabama, 2009-2013	Scientific Investigations Report (2015), 2015- 5014, 1-44	Groundwater monitoring data from areas of high density row- crop production in the US are not representative for European agricultural practice.
Wu X. et al.	2011	Degradation characteristics of organophosphate-degradation microorganism BR13.	Environmental Science & Technology (2011), Vol. 34, No. 11, pp. 54-58	Experiments on degradation of glyphosate by individual micro-organisms isolated from activated sludge are not relevant to the data requirements.
Xiao G. et al.	2020	D151 resin preloaded with Fe(3+) as a salt resistant adsorbent for glyphosate from water in the presence 16% NaCl	Ecotoxicology and environmental safety (2020), Vol. 190, pp. 110140	Experimental investigation of resins for removal of glyphosate from water. Not relevant for EU risk assessment.
Yadav V. et al.	2017	Effect of light conditions and chemical characteristics of water on dissipation of glyphosate in aqueous medium.	Environmental monitoring and assessment (2017), Vol. 189, No. 12, pp. 613	Non-EU study (India). Used glyphosate formulation to study degradation of glyphosate in distilled water and local water. Not relevant for EU risk assessment.
Yang X. et al.	2015	Short-term transport of glyphosate with erosion in Chinese loess soil - a flume experiment.	The Science of the total environment (2015), Vol. 512-513, pp. 406-414	Laboratory experiments on run-off with hydraulic flumes are not relevant to EU data requirements.

Zhang X. et al.	2019	Photomineralization of Effluent Organic Phosphorus to Orthophosphate under Simulated Light Illumination	Environmental Science & Technology (2019), Vol. 53, No. 9, pp. 4997-5004	Study of photomineralization of Effluent Organic Phosphorus (including glyphosate) to Orthophosphate under Simulated Light Illumination. Not relevant to EU risk assessment.
Zhao Y. Q. et al.	2013	Current status of pesticides application and their residue in the water environment in Ireland	International journal of environmental studies (2013), Vol. 70, No. 1, pp. 59-72	Glyphosate use data are the basis on suggesting potential water pollution without presenting any water monitoring data.

Studies excluded from the RAR: relevant but provide only supplementary information (Cat B studies) – sorted by author

Table 8.6.6-3: Publications excluded from the risk assessment after detailed assessment of full-text documents – Based on reliability criteria

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on reliability criteria)
Ahmed A. A. et al.	2018	Unravelling the nature of glyphosate binding to goethite surfaces by ab initio molecular dynamics simulations.	Physical chemistry chemical physics (2018), Vol. 20, No. 3, pp. 1531	5.4.1 case b) Relevant but supplementary information: Explores possible binding mechanisms for glyphosate with three goethite surface planes (010, 001, and 100) in the presence of water. Supplementary and not directly relevant to EU risk assessment.
Alexa E. et al.	2010	Research on the weed control degree and glyphosate soil biodegradation in apple plantations (Pioneer variety).	Analele Universitatii din Oradea, Fascicula Biologie (2010), Vol. 17, No. 1, pp. 5	5.4.1 case b) Relevant but supplementary information: Only glyphosate mineralization analyzed (measurement of 14CO2), no details on soil characteristics or experimental set-up reported.
Armbruster D. et al.	2019	Characterization of phosphonate-based antiscalants used in drinking water treatment plants by anion-exchange chromatography coupled to electrospray ionization time-of-flight mass spectrometry and inductively coupled plasma mass spectrometry.	Journal of chromatography A (2019), Vol. 1601, pp. 189	5.4.1 case b) Relevant but supplementary information: Article is primarily about identification of impurities in anti-scaling products used in drinking water treatment. AMPA is identified as being present in some antiscalants at concentrations from 1.9 to 157 mg/L after 10,000 fold dilution of the commercial antiscalants. Information may be used qualitatively but not directly for EU risk assessments.
Arroyave J. M. et al.	2016	Effect of humic acid on the adsorption/desorption behavior of glyphosate on goethite. Isotherms and kinetics.	Chemosphere (2016), Vol. 145, pp. 34	5.4.1 case b) Relevant but supplementary information: Study of effects of humic acid (HA) on the adsorption/desorption of glyphosate (glyphosate) on goethite. Not related to efate guideline, but supplemental information on glyphosate sorption.
Ascolani Y. J. et al.	2014	Abiotic degradation of glyphosate into aminomethylphosphonic acid in the presence of metals.	Journal of agricultural and food chemistry (2014), Vol. 62, No. 40, pp. 9651	5.4.1 case b) Relevant but supplementary information: The paper is about abiotic degradation of glyphosate into AMPA in the presence of metals but it does not change the risk assessment.

Aslam S. et al.	2018	Mulch of plant residues at the soil surface impact the leaching and persistence of pesticides: A modelling study from soil columns.	Journal of contaminant hydrology (2018), Vol. 214, pp. 54	5.4.1 case b) Relevant but supplementary information: Model developed to predict glyphosate degradation / movement in presence of mulch. Not an EU validated model. Experimental data used to test the model were from a previous paper.
Aslam S. et al.	2015	Effect of rainfall regimes and mulch decomposition on the dissipation and leaching of S-metolachlor and glyphosate: a soil column experiment.	Pest management science (2015), Vol. 71, No. 2, pp. 278	5.4.1 case b) Relevant but supplementary information: The study describes a soil column leaching tests with glyphosate in French soils. Glyphosate recovery from the soil column at Day 0 was only 52%. This recovery in not acceptable to draw further conclusions from the study. This publication is considered unreliable.
Braun C. et al.	2013	The load from rail wastewater. Emissions of micropollutants from rail traffic into the watershed	Aqua & Gas (2013), Vol. 93, No. 7/8, pp. 40	5.4.1 case b) Relevant but supplementary information: No new glyphosate water concentrations are presented. Using worst-case measured values, glyphosate concentrations are predicted in various size flowing water bodies.
Brock A. L. et al.	2019	Microbial Turnover of Glyphosate to Biomass: Utilization as Nutrient Source and Formation of AMPA and Biogenic NER in an OECD 308 Test.	Environmental science & technology (2019), Vol. 53, No. 10, pp. 5838	5.4.1 case b) Relevant but supplementary information: Uses data from another study (Wang, 2016) to test model to predict glyphosate mineralisation, degradation, and incorporation into non-extractable residues. Not directly relevant to EU risk assessment.
Carles L. et al.	2019	Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms.	Environment international (2019), Vol. 124, pp. 284	5.4.1 case b) Relevant but supplementary information: High phosphorus concentrations in surface water can reduce complete glyphosate degradation by biofilms and favour the accumulation of AMPA in river water.
Carretta L. et al.	2019	A new rapid procedure for simultaneous determination of glyphosate and AMPA in water at sub µg/L level.	Journal of chromatography. A (2019), Vol. 1600, pp. 65	5.4.1 case b) Relevant but supplementary information: Analytical method. Analyzed runoff samples from the Po River Valley in Italy. Only ranges of values provided not individual values. Indicates glyphosate concentrations are lower in the presence of a buffer strip than without buffer strip.
De Geronimo E. et al.	2018	Glyphosate sorption to soils of Argentina. Estimation of affinity coefficient by pedotransfer function	Geoderma (2018), Vol. 322, pp. 140	5.4.1 case b) Relevant but supplementary information: Reports most important parameters for glyphosate adsorption. Provides equation to predict Freundlich constant Kf. Useful qualitative information but not directly relevant for risk assessment.
di Guardo A. et al.	2016	A case study on monitoring glyphosate in water. Monitoraggio delle acque: il caso studio glifosate.	Informatore Agrario (2016), Vol. 72, No. 23, pp. 55	5.4.1 case b) Relevant but supplementary information: No new data presented. Describes a method for evaluating areas around monitoring stations in Lombardi region of Italy where the concentrations of glyphosate exceed the drinking water standard.

Dollinger J. et al.	2016	Variability of glyphosate and diuron sorption capacities of ditch beds determined using new indicator-based methods.	The Science of the total environment (2016), Vol. 573, pp. 716	5.4.1 case b) Relevant but supplementary information: Supplementary information of glyphosate sorption. Sorption properties of glyphosate to the ditch-bed materials
Dollinger J. et al.	2017	Using fluorescent dyes as proxies to study herbicide removal by sorption in buffer zones.	Environmental science and pollution research international (2017), Vol. 24, No. 12, pp. 11752	5.4.1 case b) Relevant but supplementary information: Soil adsoption data for glyphosate are reported but they are well within the numbers reported in the dossier. Adsorption compared to that of sulforhodamine B flourescent dye.
Exterkoetter R. et al.	2019	Potential of terracing to reduce glyphosate and AMPA surface runoff on Latosol	Journal of soils and sediments (2019), Vol. 19, No. 5, pp. 2240	5.4.1 case b) Relevant but supplementary information: Study in Brazil. Demonstrates effectiveness of terrace in reducing total mass loss of glyphosate and AMPA by reducing run-off volume. Did not reduce concentrations of glyphosate in run-off water. Potentially useful information but not directly relevant to EU risk assessment.
Geng C. et al.	2015	Modeling the release of organic contaminants during compost decomposition in soil.	Chemosphere (2015), Vol. 119, pp. 423	5.4.1 case b) Relevant but supplementary information: The paper is about degradation and adsorption of glyphosate on compost and soils and the data is consistent with endpoints reported in the dosier it does not change the risk assessment.
Ghafoor A. et al.	2013	Modelling pesticide sorption in the surface and subsurface soils of an agricultural catchment.	Pest management science (2013), Vol. 69, No. 8, pp. 919	5.4.1 case b) Relevant but supplementary information: Sorption of glyphosate was measured in surface and subsurface soils to test an 'extended' partitioning model that also accounts for inorganic sorbents and pH as well as organic sorbents.
Grandcoin A. et al.	2017	AminoMethylPhosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate.	Water research (2017), Vol. 117, pp. 187	5.4.1 case b) Relevant but supplementary information: Review paper, paper does not report experimental results but it is a comprehensive review on the sources of AMPA in the environment.
Gros P. et al.	2017	Glyphosate binding in soil as revealed by sorption experiments and quantum- chemical modeling.	The Science of the total environment (2017), Vol. 586, pp. 527	5.4.1 case b) Relevant but supplementary information: A multitude of binding mechanisms to clay minerals and organic colloids studied make the occurrence of free glyphosate rather unlikely but a leaching of glyphosate complexes via preferential flow path through soil and transfer to waterways rather likely.
Hagner M. et al.	2013	The effects of biochar, wood vinegar and plants on glyphosate leaching and degradation	European journal of soil biology (2013), Vol. 58, pp. 1	5.4.1 case b) Relevant but supplementary information: The paper investigated addition of biochar, plants, and wood vinegar to the soil in pots and reported that biochar decreased the leaching of glyphosate, it is only relevant for mechanism of sorption but not for risk assessment.

Jiang Y. et al.	2016	The role of Fe(III) on phosphate released during the photo-decomposition of organic phosphorus in deionized and natural waters.	Chemosphere (2016), Vol. 164, pp. 208	5.4.1 case b) Relevant but supplementary information: Study of the role of Fe3+ in photodegradation of glyphosate in natural water.
Karasali H. et al.	2019	Investigation of the presence of glyphosate and its major metabolite AMPA in Greek soils.	Environmental science and pollution research international (2019), Vol. 26, No. 36, pp. 36308	5.4.1 case b) Relevant but supplementary information: Paper provides data on glyphosate & AMPA concentrations in Greek soils, but there is no correlating information on glyphosate rates applied or any information on soil characterization.
Kepler R. M. et al.	2019	Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate.	Applied and environmental microbiology (2020), Vol. 18, No. 86	5.4.1 case b) Relevant but supplementary information: Not relevant to existing endpoint but provide support that glyphosate does not have a negative impact on soil microorganisms.
Kjaer J. et al.	2011	Reply to Comments on "Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils".	Chemosphere (2011), Vol. 85, No. 9, pp. 1539	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Reply to Comments on by Petersen et al_2011, Chemosphere (2011), Vol. 84. No. 4, pp. 471-479.
Klatyik S. et al.	2017	Dissipation of the herbicide active ingredient glyphosate in natural water samples in the presence of biofilms	International journal of environmental analytical chemistry (2017), Vol. 97, No. 10, pp. 901	5.4.1 case b) Relevant but supplementary information: The article reports glyphosate dissipation in irradiated natural water samples from European surface waters under laboratory conditions. The water was only characterised for pH and conductivity. No dark control experiments were conducted. Average results of concentration measurements are only presented as graphical plots and not discussed in detail (focus on effect of biofilms). This publication is considered unreliable.
Kuhn R. et al.	2017	Identification of the Complete Photodegradation Pathway of Ethylenediaminetetra(methylenephosphonic acid) in Aqueous Solution	Clean: Soil, Air, Water (2017), Vol. 45, No. 5, pp. 1	5.4.1 case b) Relevant but supplementary information: Paper describes another source of AMPA other than glyphosate - supplemental information.
Kylin H.	2013	Time-integrated sampling of glyphosate in natural waters.	Chemosphere (2013), Vol. 90, No. 6, pp. 1821	5.4.1 case b) Relevant but supplementary information: Provides information on storage stability of surface water samples that can be used to evaluate results from other surface water monitoring studies.
la Cecilia D. et al.	2018	Analysis of glyphosate degradation in a soil microcosm	Environmental pollution (2018), Vol. 233, pp. 201	5.4.1 case b) Relevant but supplementary information: Factors affecting chemical and microbial degradation of glyphosate.
la Cecilia D. et al.	2018	Glyphosate dispersion, degradation, and aquifer contamination in vineyards and wheat fields in the Po Valley, Italy.	Water research (2018), Vol. 146, pp. 37	5.4.1 case b) Relevant but supplementary information: Numeric model used to predict glyphosate degradation in soil layers and concentrations of glyphosate and AMPA in shallow acquifer from use of glyphosate in vineyards and wheat fields in PoValley, Italy. See Conclusions for results of interest. Since model, not directly relevant to risk assessment, supplementary only.

Li H. et al.	2016	Degradation and Isotope Source Tracking of Glyphosate and Aminomethylphosphonic Acid.	Journal of agricultural and food chemistry (2016), Vol. 64, No. 3, pp. 529	5.4.1 case b) Relevant but supplementary information: Provides information on the molecular mechanism of glyphosate degradation. No information relevant for route of degradation.
Maillard E. et al.	2012	Removal of dissolved pesticide mixtures by a stormwater wetland receiving runoff from a vineyard catchment: an inter-annual comparison	International journal of environmental analytical chemistry (2012), Vol. 92, No. 8, pp. 979	5.4.1 case b) Relevant but supplementary information: Confirmatory data showing storm water wetlands removed glyphosate/AMPA from agricultural runoff.
Mailler R. et al.	2014	Biofiltration vs conventional activated sludge plants: what about priority and emerging pollutants removal?	Environmental Science and Pollution Research (2014), Vol. 21, No. 8, pp. 5379	5.4.1 case b) Relevant but supplementary information: Paper compares glyphosate removal in waste water treatment by two primary and two biological treatments.
Mandiki S. N. M. et al.	2014	Effect of land use on pollution status and risk of fish endocrine disruption in small farmland ponds	Hydrobiologia (2014), Vol. 723, No. 1, pp. 103	5.4.1 case b) Relevant but supplementary information: Provides glyphosate concentrations in 15 Belgian ponds in different seasons and different land uses. End-points cannot be used directly in the risk assessment for the renewal of glyphosate at EU level. Only summary glyphosate concentrations available.
Munz N. et al.	2012	Pesticide measurements in watercourses	Aqua & Gas (2012), Vol. 92, No. 11, pp. 32	5.4.1 case b) Relevant but supplementary information: Describes evaluation of concentrations of glyphosate and other PPP's and biocides from flowing water bodies of different sizes in Switzerland. Total 545 sites (32 sites for glyphosate). Only data presented is Maximum and Mean concentrations across all sites.
Mutzner L. et al.	2016	Model-based screening for critical wet- weather discharges related to micropollutants from urban areas.	Water research (2016), Vol. 104, pp. 547	5.4.1 case b) Relevant but supplementary information: Model to predict glyphosate concentration from storm water outlets and combined sewer overflows. Glyphosate does not exceed EQS based on conservative modeling. Not directly relevant for risk assessment but useful information.
Nguyen N. K. et al.	2018	Large variation in glyphosate mineralization in 21 different agricultural soils explained by soil properties.	The Science of the total environment (2018), Vol. 627, pp. 544	5.4.1 case b) Relevant but supplementary information: Study of 21 European soils to determine factors influencing glyphosate mineralization. Exchangeable acidity identified as only univariate factor with negative correlation. NaOH extractable residues have strong negative correlation with glyphosate mineralizaiton. Doesn't fit risk assessment directly but provides useful information.
Okada E. et al.	2016	Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage.	Geoderma (2016), Vol. 263, pp. 78	5.4.1 case b) Relevant but supplementary information: Soil adsorption data for glyphosate are reported but they are well within the numbers provided in the dossier.

Ololade I. A. et al.	2014	Sorption of Glyphosate on Soil Components: The Roles of Metal Oxides and Organic Materials	Soil & sediment contamination (2014), Vol. 23, No. 5, pp. 571	5.4.1 case b) Relevant but supplementary information: No new data presented, therefore supplementary. This publication is also considered unreliable.
Ozbay B. et al.	2018	Sorption and desorption behaviours of 2,4- D and glyphosate in calcareous soil from Antalya, Turkey	Water and environment journal (2018), Vol. 32, No. 1, pp. 141	5.4.1 case b) Relevant but supplementary information: Test soil was selected to be representative for the region of Antalya, Turkey. The use of oven-dried soil is considered not appropriate for the risk assessment.
Padilla J. T. et al.	2019	Interactions among Glyphosate and Phosphate in Soils: Laboratory Retention and Transport Studies.	Journal of environmental quality (2019), Vol. 48, No. 1, pp. 156	5.4.1 case b) Relevant but supplementary information: Study conducted with U.S. soils but shows that Kf values of glyphosate are lower in the presence of phosphate. Addition of phosphate also impacts glyphosate movement in soil columns. Kf values are in range of previously reported.
Pandey P. et al.	2019	Assessing Glyphosate and Fluridone Concentrations in Water Column and Sediment Leachate.	Frontiers in Environmental Science (2019), Vol. 7, pp. Article No.: 22	5.4.1 case b) Relevant but supplementary information: This U.S. study was aimed to improve the existing understanding of the deposition of herbicides from water column to bed sediment and leachate of herbicides from bed sediment to water column. The study was prompted by herbicide treatment of water for aquatic weeds. Results may provide useful information although not directly relevant for EU risk assessment.
Paudel P. et al.	2015	Birnessite-Catalyzed Degradation of Glyphosate: A Mechanistic Study Aided by Kinetics Batch Studies and NMR Spectroscopy.	Soil Science Society of America Journal (2015), Vol. 79, No. 3, pp. 815	5.4.1 case b) Relevant but supplementary information: No relevant information on environmental fate included but a new abiotic (birnessite) degradation of glyphosate is discussed.
Petersen C. T. et al.	2011	Comments on "Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils".	Chemosphere (2011), Vol. 85, No. 9, pp. 1538	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, comment on Kjaer et al_2011, Chemosphere (2011), Vol. 84, No. 4, pp. 471-479.
Qin J. et al.	2017	Potential effects of rainwater-borne H2O2 on competitive degradation of herbicides and in the presence of humic acid.	Chemosphere (2017), Vol. 170, pp. 146	5.4.1 case b) Relevant but supplementary information: Provides information on degradation of glyphosate in the presence of hydrogen peroxide, Fe2+, and humic acid and the presence of another pesticide simulating conditions found in natural waters.
Quaglia G. et al.	2019	A spatial approach to identify priority areas for pesticide pollution mitigation	JOURNAL OF ENVIRONMENTAL MANAGEMENT (2019), Vol. 246, pp. 5833	5.4.1 case b) Relevant but supplementary information: This paper describes a modeling approach to assess potential risk of glyphosate loads in waterbodies but does not utilize or report measured glyphosate concentrations. Provides supplemental information but not directly relevant for glyphosate EU risk assessment.
Reding M.-A.	2012	Letter to the editor regarding "Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by	Analytical and bioanalytical chemistry	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, comments

		on-line solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry".	(2012), Vol. 404, No. 2, pp. 613	on Sanchis et al_2011, Analytical and bioanalytical chemistry (2012), Vol. 402, No. 7, pp. 2335-45.
Richards B. K. et al.	2018	Antecedent and Post-Application Rain Events Trigger Glyphosate Transport from Runoff-Prone Soils	Environmental science & technology letters (2018), Vol. 5, No. 5, pp. 249	5.4.1 case b) Relevant but supplementary information: Run-off study in New York State, USA. The proposed soil hydrologic condition in 7 days pre-spraying is important in determining degree of runoff. Conclusion from study of interest even though data not appropriate for EU risk assessment.
Sagliker H. A.	2018	Carbon mineralisation in orange grove soils treated with different doses of glyphosate- amine salt	Journal of Environmental Protection and Ecology (2018), Vol. 19, No. 3, pp. 1102	5.4.1 case b) Relevant but supplementary information: Study demonstrates that glyphosate application at up to 4x recommended rates does not decrease carbon mineralisation in soil and in some cases increases carbon mineralisation. Data is supplementary of previously reported work.
Silva V. et al.	2019	Pesticide residues in European agricultural soils - A hidden reality unfolded	Science of the total environment (2019), Vol. 653, pp. 1532	5.4.1 case b) Relevant but supplementary information: Analysis for glyphosate & AMPA and other pesticides in 317 soil samples from 11 EU countries. Provides inidcation of residues but no use history.
Singh B. et al.	2014	Soil characteristics and herbicide sorption coefficients in 140 soil profiles of two irregular undulating to hummocky terrains of western Canada	Geoderma (2014), Vol. 232- 234, pp. 107	5.4.1 case b) Relevant but supplementary information: Soil adsoption data for glyphosate are reported but they are well within the numbers reported in the dossier.
Slomberg D. L. et al.	2017	Insights into natural organic matter and pesticide characterisation and distribution in the Rhone River.	Environmental Chemistry (2017), Vol. 14, No. 1, pp. 64	5.4.1 case b) Relevant but supplementary information: Supplementary information on glyphosate detection in surface water.
Staufer P. et al.	2012	Diffuse inflow from settlements	Aqua & Gas (2012), Vol. 92, No. 11, pp. 42	5.4.1 case b) Relevant but supplementary information: Describes modeling to predict contamination of 4 chemicals (one of which is glyphosate) in rainfall runoff and stormwater overflow discharge from WWTP outflow. Evaluates results at both the local and the Rhein River scale.
Suleman M. et al.	2019	Laboratory simulation studies of leaching of the priority pesticides and their transformation products in soils	Journal of Animal and Plant Sciences (2019), Vol. 29, No. 4, pp. 1112	5.4.1 case b) Relevant but supplementary information: It does not follow the OECD Column Leaching Guideline (OECD 312). Rather than applying artificial rain continuously for 48 hrs as per guideline, an unspecified amount of artificial rain is applied at the end of the day to achieve 35-40 mL of leachate the following morning.
Swartjes F. A. et al.	2020	Measures to reduce pesticides leaching into groundwater-based drinking water resources: An appeal to national and local governments, water boards and farmers	The Science of the total environment (2020), Vol. 699, pp. 134186	5.4.1 case b) Relevant but supplementary information: Does not provide new data but summarizes exceedances of >75% of 0.1 ug/L for GW abstractions used for Drinking Water. Also proposes measures to reduce pesticide concentrations in GW.

Tang T. et al.	2017	Hysteresis and parent-metabolite analyses unravel characteristic pesticide transport mechanisms in a mixed land use catchment.	Water Research (2017), Vol. 124, pp. 663	5.4.1 case b) Relevant but supplementary information: Use of adapted hysteresis modeling to improve understanding on pesticide metabolite transport behaviours in catchments with diverse pesticide sources and complex transport mechanisms and provide a basis for effective management strategies. Provides information on other sources of AMPA (besides glyphosate degradation).
Tauchnitz N. et al.	2017	Quantification of pesticide input into surface waters in a small catchment area (Querne/Weida). Quantifizierung von Pflanzenschutzmittel(PSM)-Eintraegen in Oberflaechengewaesser in einem Kleineinzugsgebiet (Querne/Weida).	Lysimeter Forschung-Moeglichkeiten und Grenzen Lysimeter research - options and limits, 9-10 May 2017, Raumberg-Gumpenstein, Austria (2017), pp. 11	5.4.1 case b) Relevant but supplementary information: Provides information on surface water sampling in Germany, but no concentrations of glyphosate reported.
Todorovic G. R. et al.	2010	Dispersion of glyphosate in soils through erosion. Environmental Quality 4	Air, water, and soil pollution (2010), Vol. 4, pp. 15	5.4.1 case b) Relevant but supplementary information: Analysis of runoff samples from small vegetative field plots following glyphosate application and subsequent artificial rain is not expected to provide additional relevant data. Furthermore, no details of analytical methods is reported.
Waiman C. V. et al.	2016	The simultaneous presence of glyphosate and phosphate at the goethite surface as seen by XPS, ATR-FTIR and competitive adsorption isotherms	Colloids and Surfaces A: Physicochemical and Engineering Aspects (2016), Vol. 498, pp. 121	5.4.1 case b) Relevant but supplementary information: The study does not investigate soil adsorption but mineral. The study does not include an endpoint relevant for the risk assessment.
Wang M. et al.	2019	Montmorillonites Can Tightly Bind Glyphosate and Paraquat Reducing Toxin Exposures and Toxicity	ACS omega (2019), Vol. 4, No. 18, pp. 17702	5.4.1 case b) Relevant but supplementary information: Article provides binding properties of glyphosate to calcium and sodium montmorillonite clay. Supplementary information as clay is a soil component, not a soil.
Yan W. et al.	2018	Molecular Insights into Glyphosate Adsorption to Goethite Gained from ATR- FTIR, Two-Dimensional Correlation Spectroscopy, and DFT Study.	Environmental science & technology (2018), Vol. 52, No. 4, pp. 1946	5.4.1 case b) Relevant but supplementary information: Study of molecular-level interfacial configurations and reaction mechanisms of glyphosate with iron (hydr)oxides. The influence of phosphate is also described.
Yang Y. et al.	2018	Comparative study of glyphosate removal on goethite and magnetite: Adsorption and photo-degradation.	Chemical Engineering Journal (2018), Vol. 352, pp. 581	5.4.1 case b) Relevant but supplementary information: Study of photodegradation of glyphosate in environment by goethite and magnetite.
Zhang K. et al.	2019	Can we use a simple modelling tool to validate stormwater biofilters for herbicides treatment?	Urban Water Journal (2019), Vol. 16, pp. 412	5.4.1 case b) Relevant but supplementary information: Biofilter validation model. Field validation work performed in Australia. Model may be of interest even though field data not directly relevant to the EU.
Zhang W. et al.	2019	A method for determining glyphosate and its metabolite aminomethyl phosphonic acid by gas chromatography-flame photometric detection.	Journal of chromatography. A (2019), Vol. 1589, pp. 116	5.4.1 case b) Relevant but supplementary information: Primarily an analytical methods paper with examples of hydrolysis and column leaching data provided. Insufficient

				methodology information provided for risk assessment.
Zhao Y. et al.	2015	Use of Fe/Al drinking water treatment residuals as amendments for enhancing the retention capacity of glyphosate in agricultural soils.	Journal of environmental sciences (2015), Vol. 34, pp. 133	5.4.1 case b) Relevant but supplementary information: Use of Fe/Al drinking water treatment residuals (WTRs) as a soil amendment to increase glyphosate sorption and decrease desorption in soils. Supplementary information not directly related to efate guideline studies.

Studies included in the RAR – sorted by data requirement

Table 8.6.6-4: Relevant studies included in Assessment Report after detailed assessment of full-text documents for relevance: sorted by data requirement(s)

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 7.1.1.1/012	Sun et al.	2019	Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil water system	Water Research 163 (2019) 114840
CA 7.1.1.1, CA 7.1.2.1.1	Muskus A. M. *et al.*	2019	Effect of temperature, pH and total organic carbon variations on microbial turnover of $^{13}C_3{}^{15}N$-glyphosate in agricultural soil	Science of the Total Environment 658 (2019) 697-707
CA 7.1.2.1.1/010, CA 7.1.3.1.1/020	Zhelezova, A. et al.	2017	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides	Water Air Soil Pollut (2017) 228: 216
CA 7.1.2.1.1/011, CA 7.1.3.1.1/021	Cassigneul, A. et al.	2016	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study	Science of the Total Environment 545–546 (2016) 582–590
CA 7.1.2.1.1/012	Norgaard, T. et al.	2015	Can Simple Soil Parameters Explain Field-Scale Variations in Glyphosate-, Bromoxyniloctanoate-, Diflufenican-, and Bentazone Mineralization?	Water Air Soil Pollut (2015) 226: 262
CA 7.1.2.1.1/013, CA 7.1.2.1.3/002, CA 7.1.3.1.1/025	Kanissery, R. G. et al.	2015	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate	Journal of environmental quality 44:137–144 (2015)
CA 7.1.2.1.1/014, CA 7.1.3.1.1/028	Rampoldi, E. et al.	2014	Carbon-14-Glyphosate Behavior in Relationship to Pedoclimatic Conditions and Crop Sequence	Journal of environmental quality 43:558–567 (2014)
CA 7.1.2.1.1/015, CA 7.1.4.2/002	Al-Rajab, A. et al.	2014	Behavior of the non-selective herbicide glyphosate in agricultural soil	American Journal of Environmental Science 10 (2): 94-101, 2014
CA 7.1.2.1.1/016	Nghia, N.K. et al.	2013	Soil properties governing biodegradation of the herbicide glyphosate in agricultural soils	Proc. 24th Asian Pacific Weed Science Society conference, October 22-25, 2013, Bandung, Indonesia
CA 7.1.2.1.1/017, CA 7.1.3.1.1/029, CA 7.1.4.2/003	Bergström, L. et al.	2011	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil	Journal of environmental quality 40:98–108 (2011)

CA 7.1.2.1.1/018	Ghafoor, A. et al.	2011	Measurements and modelling of pesticide persistence in soil at the catchment scale	Science of the Total Environment 409 (2011) 1900–1908
CA 7.1.2.1.1/019	Alexa, E. et al.	2010	Studies on the biodegradation capacity of 14C–labelled glyphosate in vine plantation soils	Journal of Food, Agriculture & Environment Vol.8 (3&4): 1193-1198. 2010
CA 7.1.2.1.1/020	Al–Rajab, A., Schiavon, M.	2010	Degradation of 14C–glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils	Journal of Environmental Sciences 2010, 22(9) 1374–1380
CA 7.1.2.1.1, CA 7.1.2.1.4	Bento C. P. M. *et al.*	2016	Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness	Science of the Total Environment, (2016) Vol. 572, pp. 301-311
CA 7.1.2.2.1/026	Passeport, E., *et al.*	2013	Dynamics and mitigation of six pesticides in a "Wet" forest buffer zone	Environ Sci Pollut Res (2014) 21:4883–4894
CA 7.1.1.3, CA 7.1.3.1.1/	Tush D. *et al.*	2018	Dissipation of polyoxyethylene tallow amine (POEA) and glyphosate in an agricultural field and their co-occurrence on streambed sediments	Science of the total environment (2018), Vol. 636, pp. 212-219
CA 7.1.2.2.1/027	Todorovic, Rampazzo ., *et al*	2014	Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils	Int. Agrophys., 2014, 28, 93-100
CA 7.1.2.2.1/028	Rampazzo ., *et al*	2013	Adsorption of glyphosate and aminomethylphosphonic acid in soils	International Agrophysics, (2013) Vol. 27, pp. 203-209
CA 7.1.3.1.1/014	Albers, C. et al.	2018	Soil Domain and Liquid Manure Affect Pesticide Sorption in Macroporous Clay Till	Journal of Environmental Quality 48:147–155 (2019)
CA 7.1.3.1.1/015	Dollinger, J. et al.	2018	Contrasting soil property patterns between ditch bed and neighbouring field profiles evidence the need of specific approaches when assessing water and pesticide fate in farmed landscapes	Geoderma 309 (2018) 50–59
CA 7.1.3.1.1/016, CA 7.1.3.1.2/007	Skeff, W. et al.	2018	Adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid, and 2-aminoethylphosphonic acid on three typical Baltic Sea sediments	Marine Chemistry 198 (2018) 1–9
CA 7.1.3.1.1/017	Gómez Ortiz, A.M. et al.	2017	Sorption and desorption of glyphosate in mollisols and ultisols soils of Argentina	Environmental Toxicology and Chemistry, Vol. 36, No. 10, pp. 2587–2592, 2017
CA 7.1.3.1.1/018	Munira, S., Farenhorst, A.	2017	Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate	Journal of environmental science and health, Part B 2017, VOL. 0, NO. 0, 1–9
CA 7.1.3.1.1/019	Munira, S. et al	2017	Phosphate and glyphosate sorption in soils following long-term phosphate applications	Geoderma 313 (2018) 146–153
CA 7.1.3.1.1/022	Munira, S. et al.	2016	Phosphate fertilizer impacts on glyphosate sorption by soil	Chemosphere 153 (2016) 471-477
CA 7.1.3.1.1/023, CA 7.1.3.1.2/008	Sidoli, P. et al.	2016	Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules	Environ Sci Pollut Res (2016) 23:5733–5742
CA 7.1.3.1.1/024	Dollinger, J. et al.	2015	Glyphosate sorption to soils and sediments predicted by pedotransfer functions	Environ Chem Lett (2015) 13:293-307
CA 7.1.3.1.1/026	Tévez, H., dos Santos, A.M	2015	pH dependence of Glyphosate adsorption on soil horizons	Boletin de la Sociedad Geologica Mexicana, Volumen 67, num 3, 2015, P 509-516

CA 7.1.3.1.1/027	Jodeh S. et al.	2014	Fate and Mobility of Glyphosate Leachate in Palestinian Soil Using Soil Column	J. Mater. Environ. Sci. 5 (6) (2014) 2008-2016
CA 7.1.3.1.1/028, CA 7.2.1.3/001	Maqueda C. *et al.*	2017	Behaviour of glyphosate in a reservoir and the surrounding agricultural soils	Science of the Total Environment, (2017) Vol. 593-594, pp. 787-795
CA 7.1.3.1.1/029	Paradelo M. *et al.*	2015	Prediction of the glyphosate sorption coefficient across two loamy agricultural fields	Geoderma, (2015) Vol. 259-260, pp. 224-232
CA 7.1.4.1.1/008	Gjettermann, B. et al.	2010	Kinetics of Glyphosate Desorption from Mobilized Soil Particles	Soil Sci. Soc. Am. J. 75:434–443
CA 7.1.4.1.1/009	Gjettermann, B. et al.	2011	Evaluation of Sampling Strategies for Pesticides in a Macroporous Sandy Loam Soil	Soil and Sediment Contamination, 20:986–994, 2011
CA 7.1.4.2/001	Napoli, M. et al	2015	Leaching of Glyphosate and Aminomethylphosphonic Acid through Silty Clay Soil Columns under Outdoor Conditions	J. Environ. Qual. 44:1667–1673 (2015)
CA 7.1.4.2/004	Gros, P. *et al.*	2020	Leaching and degradation of $^{13}C_2$-^{15}N-glyphosate in field lysimeters	Environ Monit Assess (2020) 192: 127
CA 7.1.4.3/001	Ulen, B.M. *et al.*	2014	Spatial variation in herbicide leaching from a marine clay soil via subsurface drains	Pest Management Science 2014; 70: 405–414
CA 7.1.4.3/002	Ulen, B.M. *et al*	2012	Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains	Acta Agriculturae Scandinavica Section B _ Soil and Plant Science, 2012; 62: Supplement 2, 241_251
CA 7.1.4.3/003	Aronsson, H. *et al.*	2010	Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop	Soil Use and Management, March 2011, 27, 54–68
CA 7.1.4.3/004	Kjaer, J. *et al*	2011	Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils	Chemosphere 84 (2011) 471–479
CA 7.1.4.3/005	Candela, L. *et al.*	2010	Glyphosate transport through weathered granite soils under irrigated and non–irrigated conditions–Barcelona, Spain	Science of the Total Environment 408 (2010) 2509–2516
CP 9.2.4	Rasmussen, S., et al.	2015	Effects of Single Rainfall Events on Leaching of Glyphosate and Bentazone on Two Different Soil Types, using the DAISY Model	Vadose Zone Journal; Advancing Critical Zone Science; Published November 13, 2015
CA 7.2.2.3/023	Wang, S. *et al*	2016	(Bio)degradation of glyphosate in water-sediment microcosms - A stable isotope co-labeling approach	Water Research 99 (2016) 91e100
CA 7.3.1/008	Bento, C.P.M. *et al*	2016	Glyphosate and AMPA distribution in wind-eroded sediment derived from loess soil	Environmental Pollution 220 (2017) 1079e1089
KCA 7.5/003	Karanasios E. et al	2018	Monitoring of glyphosate and AMPA in soil samples from two olive cultivation areas in Greece: aspects related to spray operators activities	Environmental Monitoring and Assessment (2018), Vol. 190, No. 6, pp. 1
CA 7.5/004	Silva, V. et al.	2018	Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union	The Science of the total Environment (2018) 621:1352-1359
CA 7.5/005	Napoli, M. et al.	2016	Transport of glyphosate and aminomethylphosphonic acid under two soil management practices in an Italian vineyard.	Journal of Environmental Quality (2016) 45:1713-1721

CA 7.5/006	Szekacs, A. et al.	2014	Monitoring and biological evaluation of surface water and soil micropollutants in Hungary	Carpathian Journal of Earth and Environmental Sciences (2014) 9:47-60
CA 7.5/007	Daouk, S. et al.	2013	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part II: the role of infiltration and surface runoff.	Journal of Environmental Science and Health. Part B (2013) 48:725-736
CA 7.5/016	Rosenbom, A.E. et al.	2019	The Danish Pesticide Leaching Assessment Programme	Geological Survey of Denmark and Greenland
CA 7.5/017	Poiger, T. et al.	2017	Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS.	Environmental Science and Pollution Research (2017) 24:1588-1596
CA 7.5/018	Di Guardo, A., Finizio, A.	2016	A moni-modeling approach to manage groundwater risk to pesticide leaching at regional scale	The Science of the total Environment (2016) 545-546:200-209
CA 7.5/019	Rosenbom, A.E. et al.	2015	Pesticide leaching through sandy and loamy fields - Long-term lessons learnt from the Danish Pesticide Leaching Assessment Programme	Environmental Pollution 201 (2015) 75-90
CA 7.5/020	McManus, S.L. et al.	2014	Pesticide occurrence in groundwater and the physical characteristics in association with these detections in Ireland	Environmental Monitoring and Assessment (2014) 186:7819-7836
CA 7.5/021	Norgaard, T. et al.	2014	Leaching of glyphosate and aminomethylphosphonic acid from an agricultural field over a twelve-year period	Vadose Zone Journal (2014) 13:18
CA 7.5/022	Martin, J. et al.	2013	Sugarcane, herbicides and water pollution in Reunion Island: achievements and perspectives after ten years of monitoring. - ORIGINAL	22e Conference du COLUMA journées internationale sur la lutte contre les mauvaises herbes 10-12 Dec 2013 (2013) 641-651
CA 7.5/023	Martin, J. et al.	2013	Sugarcane, herbicides and water pollution in Reunion Island: achievements and perspectives after ten years of monitoring. - ENGLISH	
CA 7.5/024	Mörtl, M. et al.	2013	Determination of glyphosate residues in Hungarian water samples by immunoassay	Microchemical Journal (2013) 107:143-151
CA 7.5/025	Sanchís, J. et al.	2012	Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry.	Analytical and Bioanalytical Chemistry (2012) 402:2335-2345
CA 7.5/026	Sanchís, J. et al.	2012	Erratum to: Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry.	Analytical and Bioanalytical Chemistry (2012) 404:617
CA 7.5/027	Bruchet A. *et al*.	2011	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge	Eur. j. water qual. 42 (2011) 123–133

CA 7.5/036	Di Guardo, A., Finizio, A.	2018	A new methodology to identify surface water bodies at risk by using pesticide monitoring data: The glyphosate case study in Lombardy Region (Italy)	The Science of the total Environment (2018) 610-611:421-429
CA 7.5/037	Huntscha, S. *et al*.	2018	Seasonal dynamics of glyphosate and AMPA in Lake Greifensee: rapid microbial degradation in the epilimnion during summer.	Environmental Science and Technology (2018) 52:4641-4649
CA 7.5/038	Masiol, M. *et al*.	2018	Herbicides in river water across the northeastern Italy: occurrence and spatial patterns of glyphosate, aminomethylphosphonic acid, and glufosinate ammonium.	Environmental Science and Pollution Research (2018) 25:24368-24378
CA 7.5/039	Dairon, R. *et al*.	2017	Long-term impact of reduced tillage on water and pesticide flow in a drained context	Environmental Science and Pollution Research (2017) 24:6866-6877
CA 7.5/040	Lefrancq, M. *et al*.	2017	High frequency monitoring of pesticides in runoff water to improve understanding of their transport and environmental impacts.	The Science of the total Environment (2017) 587-588:75-86
CA 7.5/041	Lerch, R.N. *et al*.	2017	Vegetative buffer strips for reducing herbicide transport in runoff: effects of buffer width, vegetation, and season.	Journal of the American Water Resources Association (2017) 53:667-683
CA 7.5/042	Mottes, C. *et al*.	2017	Relationships between past and present pesticide applications and pollution at a watershed outlet: The case of a horticultural catchment in Martinique, French West Indies.	Chemosphere (2017) 184:762-773
CA 7.5/043	Reoyo-Prats, B. *et al*.	2017	Multicontamination phenomena occur more often than expected in Mediterranean coastal watercourses: Study case of the Tet River (France)	The Science of the total Environment (2017) 579:10-21
CA 7.5/044	Desmet, N. *et al*.	2016	A hybrid monitoring and modelling approach to assess the contribution of sources of glyphosate and AMPA in large river catchments.	The Science of the total Environment (2016) 573:1580-1588
CA 7.5/045	Larsbo, M. *et al*.	2016	Surface runoff of pesticides from a clay loam field in Sweden.	Journal of Environmental Quality (2016) 45:1367-1374
CA 7.5/046	Schreiner, V.C. et al.	2016	Pesticide mixtures in streams of several European countries and the USA	The Science of the total Environment (2016) 573:680-689
CA 7.5/047	Stenrod, M.	2015	Long-term trends of pesticides in Norwegian agricultural streams and potential future challenges in northern climate	Acta Agriculturae Scandinavica Section B - Soil and Plant Science (2015) 65:199-216
CA 7.5/048	Szekacs, A. et al.	2015	Monitoring pesticide residues in surface and ground water in Hungary: surveys in 1990-2015	Journal of Chemistry (2015) Article ID 717948
CA 7.5/049	Tang, T. et al.	2015	Quantification and characterization of glyphosate use and loss in a residential area.	The Science of the total Environment (2015) 517:207-214
CA 7.5/050	Gasperi, J. et al.	2014	Micropollutants in urban stormwater: occurrence, concentrations, and atmospheric contributions for a wide range of contaminants in three French catchments	Environmental Science and Pollution Research (2014) 21:5267-5281
CA 7.5/051	Maillard, E., Imfeld, G.	2014	Pesticide mass budget in a stormwater wetland.	Environmental Science and Technology (2014) 48:8603-8611

CA 7.5/052	Ramwell, C.T. et al.	2014	Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains.	Pest Management Science (2014) 70:1823-1830
CA 7.5/053	Daouk S. et al.	2013	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, Western Switzerland: proof of widespread export to surface waters. Part I: method validation in different water matrices.	Journal of Environmental Science and Health. Part B (2013) 48:717-724
CA 7.5/054	Houtman, C.J. et al.	2013	A multicomponent snapshot of pharmaceuticals and pesticides in the river Meuse basin	Environmental Toxicology and Chemistry (2013) 32:2449-2459
CA 7.5/055	Imfeld G. et al.	2013	Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland.	Chemosphere (2013) 90:1333-1339
CA 7.5/056	Vialle, C. et al.	2013	Pesticides in roof runoff: study of a rural site and a suburban site.	Journal of Environmental Management (2013) 120:48-54
CA 7.5/057	Botta, F. et al.	2012	Phyt'Eaux Cites: application and validation of a programme to reduce surface water contamination with urban pesticides.	Chemosphere (2012) 86:166-176
CA 7.5/058	Coupe R.H. et al.	2012	Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins.	Pest Management Science (2012) 68:16-30
CA 7.5/059	Petersen, J. et al.	2012	Sampling of herbicides in streams during flood events.	Journal of Environmental Monitoring (2012) 14:3284-3294
CA 7.5/060	Zgheib, S. et al.	2012	Priority pollutants in urban stormwater: Part 1 - Case of separate storm sewers	Water Research (2012) 46:6683-6692
CA 7.5/061	Birch, H. et al.	2011	Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark.	Water Science and Technology (2011) 64:485-493
CA 7.5/062	Lamprea, K., Ruban, V.	2011	Pollutant concentrations and fluxes in both stormwater and wastewater at the outlet of two urban watersheds in Nantes (France)	Urban Water Journal (2011) 8:219-231
CA 7.5/063	Litz, N.T. *et al*.	2011	Comparative studies on retardation and reduction during subsurface passage	Water research 45 (2011) 3047-3054
CA 7.5/064	Maillard, E. et al.	2011	Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment.	The Science of the total Environment (2011) 409:2317-2324
CA 7.5/065	Meyer, B. et al.	2011	Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg	Environmental Monitoring and Assessment (2011) 180:127-146
CA 7.5/066	Busetto, M., Frattini, V.	2010	Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province - ORIGINAL ITALIAN	Bollettino - Unione Italiana degli Esperti Ambientali (2010) 61:46-57
CA 7.5/067	Busetto, M., Frattini, V.	2010	Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province - ENGLISH	
CA 7.5/068	Gregoire, C. et al.	2010	Use and fate of 17 pesticides applied on a vineyard catchment.	International Journal of Environmental Analytical Chemistry (2010) 90:406-420
CA 7.5/069	Hanke, I. et al.	2010	Relevance of urban glyphosate use for surface water quality.	Chemosphere (2010) 81:422-429

CA 7.5/070	Botta, F. et al.	2009	Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems	Chemosphere 77(1): 133-139
CA 7.5/071[a]	Ghanem, A. et al.	2007	Concentrations and specific loads of glyphosate, diuron, atrazine, nonylphenol and metabolites thereof in French urban sewage sludge	Chemosphere Volume 69 Issue 9, 1368 - 1373
CA 7.5/072	Peschka, M. *et al.*	2006	Trends in pesticide transport into the River Rhine	Hdb Env Chem Vol. 5, Part L (2006): 155–175
CA 7.5/073	Augustin, B.	2003	Urban areas - sources of pesticide-contamination of surface water?	Presentation at: Second International Symposium Plant Health in Urban Horticulture, Berlin, August 27-29
CA 7.5/077	Malaguerra, F. et al.	2012	Pesticides in water supply wells in Zealand, Denmark: A statistical analysis.	The Science of the total Environment (2012) 414:433-444
CA 7.5/078	Sabatier, P. et al.	2014	Long-term relationships among pesticide applications, mobility, and soil erosion in a vineyard watershed.	Proceedings of the National Academy of Sciences of the United States of America (2014) 111:15647-15652
CA 7.5/079	Ravier, S. et al.	2019	Monitoring of glyphosate, glufosinate-ammonium, and (aminomethyl) phosphonic acid in ambient air of Provence-Alpes-Cote-d'Azur Region, France.	Atmospheric Environment (2019) 204:102-109
CA 7.5/082	Hamann, E. *et al.*	2016	The fate of organic micropollutants during long-term/long-distance river bank filtration	Science of the Total Environment 545–546 (2016) 629–640
CA 7.5/083	Hedegaard, M., Albrechtsen, H.	2014	Microbial pesticide removal in rapid sand filters for drinking water treatment – Potential and kinetics	Water research 48 (2014) 71-81
CA 7.5/084	Jönsson J. *et al.*	2013	Removal and degradation of glyphosate in water treatment	Journal of Water Supply: Research and Technology—AQUA, 62.7, 2013
CA 7.5/085[b]	Malaguerra, F. et al.	2013	Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques	Journal of Hydrology 476 (2013) 321–331
CA 7.5/086	Ruel, S. *et al.*	2012	Occurrence and fate of relevant substances in wastewater treatment plants	Water Science & Technology | 65.7 | 2012
CA 7.5/087	Ruel, S. *et al.*	2011	On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications	Water Science & Technology | 63.11 | 2011
CA 7.5/088	Schoonenberg Kegel, F.S. *et al.*	2010	Reverse osmosis followed by activated carbon filtration for efficient removal of organic micropollutants from river bank filtrate	Water Science & Technology—WST | 61.10 | 2010
CA 7.5/089	Shen, Y. *et al.*	2011	Ozonation of Herbicide Glyphosate	Acta Scientiae Circumstantiae, 31(8):1647-1652
CA 7.5/090	Shen, Y. *et al.*	2011	Translation of CA 7.5/089	
CA 7.5/091	Assalin, M. *et al.*	2010	Degradation by several oxidative chemical processes	Journal of Environmental Science and Health Part B (2010) 45, 89–94
CA 7.5/092	Boucherie, C. *et al.*	2010	Ozone and GAC filtration synergy	Water Science & Technology: Water Supply—WSTWS | 10.5 | 2010
CA 7.5/093	Manassero, A. *et al.*	2010	Glyphosate degradation in water employing the H_2O_2/UVC process	Water research 44 (2010) 3875-3882
CA 7.5/094	Brosillon, S. *et al.*	2006	Chlorination kinetics of glyphosate and its by-products	Water research 40 (2006) 2113-2124

CA 7.5/095	Mehrsheikh, A. *et al.*,	2006	Investigation of the mechanism of chlorination of glyphosate	Water research 40 (2006) 3003-3014
CA 7.5/096	Klinger, J. *et al.*	2008	Formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra	Ozone Science & Engineering, Vol 20, pp 99-110
CA 7.5/097	Gillefalk *et al.*	2018	Potential Impacts of Induced Bank Filtration on Surface Water Quality: A Conceptual Framework for Future Research	Water 2018, 10, 1240
CA 7.5-098	Van der Hoek *et al*	2014	Practical Paper Drinking water treatment technologies in Europe: state of the art – challenges – research needs	Journal of Water Supply: Research and Technology—AQUA | 63.2 | 2014
CA 7.5-099	Rosenbom, A.E. et al.	2020	The Danish Pesticide Leaching Assessment Programme, Monitoring results May 1999–June 2018	Geological Survey of Denmark and Greenland
CA 7.5/100	De Polo, A. et al.	2020	From the traces in the wells of the urban aqueduct network to the subsequent prohibition of the use of glyphosate: the case of an area of high-intensity wine production in the province of Treviso, Veneto – English	Igiene e sanita pubblica, (2019) Vol. 75, No. 6, pp. 451-460
CA 7.5/101	De Polo, A. et al.	2020	Dai residui nei pozzi della rete acquedottistica urbana al successivo divieto di utilizzo del glifosate: il caso di un'area ad alta intensità vitivinicola in provincia di Treviso, Veneto - Original	
CA 7.5	Skeff, W. et al.	2015	Glyphosate and AMPA in the estuaries of the Baltic Sea method optimization and field study.	Marine Pollution Bulletin 100 (2015) 577–585
CA 7.5	Boye K. et al.	2019	Long-term data from the swedish national environmental monitoring program of pesticides in surface waters	Journal of Environemental Quality 48:1109–1119 (2019)

[a] This study was considered as reliable with restrictions by the applicant for data point "Monitoring in surface water". RMS considers that it is not relevant, but the study summary is still presented in the dossier for transparency

[b] This study was considered as reliable with restrictions by the applicant for data point "impact of water treatment processes". RMS considers that it is not relevant, but the study summary is still presented in the dossier for transparency.

Studies included in the RAR – sorted by author

Table 8.6.6-5: Relevant studies included in Assessment Report after detailed assessment of full-text documents for relevance: sorted by author(s)

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Albers, C. et al.	CA 7.1.3.1.1/014	2018	Soil Domain and Liquid Manure Affect Pesticide Sorption in Macroporous Clay Till	Journal of Environmental Quality 48:147–155 (2019)
Alexa, E. et al.	CA 7.1.2.1.1/019	2010	Studies on the biodegradation capacity of 14C–labelled glyphosate in vine plantation soils	Journal of Food, Agriculture & Environment Vol.8 (3&4): 1193-1198. 2010
Al-Rajab, A. et al.	CA 7.1.2.1.1/015, CA 7.1.4.2/002	2014	Behavior of the non-selective herbicide glyphosate in agricultural soil	American Journal of Environmental Science 10 (2): 94-101, 2014

Al–Rajab, A., Schiavon, M.	CA 7.1.2.1.1/020	2010	Degradation of 14C–glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils	Journal of Environmental Sciences 2010, 22(9) 1374–1380
Aronsson, H. *et al.*	CA 7.1.4.3/003	2010	Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop	Soil Use and Management, March 2011, 27, 54–68
Assalin, M. *et al.*	CA 7.5/091	2010	Degradation by several oxidative chemical processes	Journal of Environmental Science and Health Part B (2010) 45, 89–94
Augustin, B.	CA 7.5/073	2003	Urban areas - sources of pesticide-contamination of surface water?	Presentation at: Second International Symposium Plant Health in Urban Horticulture, Berlin, August 27-29
Bento C. P. M. *et al.*	CA 7.1.2.1.1, CA 7.1.2.1.4	2016	Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness	Science of the Total Environment, (2016) Vol. 572, pp. 301-311
Bento, C.P.M. *et al*	CA 7.3.1/008	2016	Glyphosate and AMPA distribution in wind-eroded sediment derived from loess soil	Environmental Pollution 220 (2017) 1079e1089
Bergström, L. et al.	CA 7.1.2.1.1/017, CA 7.1.3.1.1/029, CA 7.1.4.2/003	2011	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil	Journal of environmental quality 40:98–108 (2011)
Birch, H. et al.	CA 7.5/061	2011	Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark.	Water Science and Technology (2011) 64:485-493
Botta, F. et al.	CA 7.5/057	2012	Phyt'Eaux Cites: application and validation of a programme to reduce surface water contamination with urban pesticides.	Chemosphere (2012) 86:166-176
Botta, F. et al.	CA 7.5/070	2009	Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems	Chemosphere 77(1): 133-139
Boucherie, C. *et al.*	CA 7.5/092	2010	Ozone and GAC filtration synergy	Water Science & Technology: Water Supply—WSTWS | 10.5 | 2010
Boye K. et al.	CA 7.5	2019	Long-term data from the swedish national environmental monitoring program of pesticides in surface waters	Journal of Environemental Quality 48:1109–1119 (2019)
Brosillon, S. *et al.*	CA 7.5/094	2006	Chlorination kinetics of glyphosate and its by-products	Water research 40 (2006) 2113-2124
Bruchet A. *et al.*	CA 7.5/027	2011	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge	Eur. j. water qual. 42 (2011) 123–133
Busetto, M., Frattini, V.	CA 7.5/066	2010	Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province - ORIGINAL ITALIAN	Bollettino - Unione Italiana degli Esperti Ambientali (2010) 61:46-57
Busetto, M., Frattini, V.	CA 7.5/067	2010	Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province - ENGLISH	Bollettino - Unione Italiana degli Esperti Ambientali (2010) 61:46-57
Candela, L. *et al.*	CA 7.1.4.3/005	2010	Glyphosate transport through weathered granite soils under irrigated and non–irrigated conditions–Barcelona, Spain	Science of the Total Environment 408 (2010) 2509–2516
Cassigneul, A. et al.	CA 7.1.2.1.1/011, CA 7.1.3.1.1/021	2016	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study	Science of the Total Environment 545–546 (2016) 582–590
Coupe R.H. et al.	CA 7.5/058	2012	Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins.	Pest Management Science (2012) 68:16-30

Dairon, R. *et al.*	CA 7.5/039	2017	Long-term impact of reduced tillage on water and pesticide flow in a drained context	Environmental Science and Pollution Research (2017) 24:6866-6877
Daouk S. et al.	CA 7.5/053	2013	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, Western Switzerland: proof of widespread export to surface waters. Part I: method validation in different water matrices.	Journal of Environmental Science and Health. Part B (2013) 48:717-724
Daouk, S. et al.	CA 7.5/007	2013	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part II: the role of infiltration and surface runoff.	Journal of Environmental Science and Health. Part B (2013) 48:725-736
De Polo, A. et al.	CA 7.5/100	2020	From the traces in the wells of the urban aqueduct network to the subsequent prohibition of the use of glyphosate: the case of an area of high-intensity wine production in the province of Treviso, Veneto – English	Igiene e sanita pubblica, (2019) Vol. 75, No. 6, pp. 451-460
De Polo, A. et al.	CA 7.5/101	2020	Dai residui nei pozzi della rete acquedottistica urbana al successivo divieto di utilizzo del glifosate: il caso di un'area ad alta intensità vitivinicola in provincia di Treviso, Veneto - Original	Igiene e sanita pubblica, (2019) Vol. 75, No. 6, pp. 451-460
Desmet, N. *et al.*	CA 7.5/044	2016	A hybrid monitoring and modelling approach to assess the contribution of sources of glyphosate and AMPA in large river catchments.	The Science of the total Environment (2016) 573:1580-1588
Di Guardo, A., Finizio, A.	CA 7.5/018	2016	A moni-modeling approach to manage groundwater risk to pesticide leaching at regional scale	The Science of the total Environment (2016) 545-546:200-209
Di Guardo, A., Finizio, A.	CA 7.5/036	2018	A new methodology to identify surface water bodies at risk by using pesticide monitoring data: The glyphosate case study in Lombardy Region (Italy)	The Science of the total Environment (2018) 610-611:421-429
Dollinger, J. et al.	CA 7.1.3.1.1/015	2018	Contrasting soil property patterns between ditch bed and neighbouring field profiles evidence the need of specific approaches when assessing water and pesticide fate in farmed landscapes	Geoderma 309 (2018) 50–59
Dollinger, J. et al.	CA 7.1.3.1.1/024	2015	Glyphosate sorption to soils and sediments predicted by pedotransfer functions	Environ Chem Lett (2015) 13:293-307
Gasperi, J. et al.	CA 7.5/050	2014	Micropollutants in urban stormwater: occurrence, concentrations, and atmospheric contributions for a wide range of contaminants in three French catchments	Environmental Science and Pollution Research (2014) 21:5267-5281
Ghafoor, A. et al.	CA 7.1.2.1.1/018	2011	Measurements and modelling of pesticide persistence in soil at the catchment scale	Science of the Total Environment 409 (2011) 1900–1908
Ghanem, A. et al.	CA 7.5/071[a]	2007	Concentrations and specific loads of glyphosate, diuron, atrazine, nonylphenol and metabolites thereof in French urban sewage sludge	Chemosphere Volume 69 Issue 9, 1368 - 1373
Gillefalk *et al.*	CA 7.5/097	2018	Potential Impacts of Induced Bank Filtration on Surface Water Quality: A Conceptual Framework for Future Research	Water 2018, 10, 1240
Gjettermann, B. et al.	CA 7.1.4.1.1/008	2010	Kinetics of Glyphosate Desorption from Mobilized Soil Particles	Soil Sci. Soc. Am. J. 75:434–443
Gjettermann, B. et al.	CA 7.1.4.1.1/009	2011	Evaluation of Sampling Strategies for Pesticides in a Macroporous Sandy Loam Soil	Soil and Sediment Contamination, 20:986–994, 2011

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Gros, P. *et al.*	CA 7.1.4.2/004	2020	Leaching and degradation of $^{13}C_2$-^{15}N-glyphosate in field lysimeters	Environ Monit Assess (2020) 192: 127
Hamann, E. *et al.*	CA 7.5/082	2016	The fate of organic micropollutants during long-term/long-distance river bank filtration	Science of the Total Environment 545–546 (2016) 629–640
Hanke, I. et al.	CA 7.5/069	2010	Relevance of urban glyphosate use for surface water quality.	Chemosphere (2010) 81:422-429
Hedegaard, M., Albrechtsen, H.	CA 7.5/083	2014	Microbial pesticide removal in rapid sand filters for drinking water treatment – Potential and kinetics	Water research 48 (2014) 71-81
Houtman, C.J. et al.	CA 7.5/054	2013	A multicomponent snapshot of pharmaceuticals and pesticides in the river Meuse basin	Environmental Toxicology and Chemistry (2013) 32:2449-2459
Huntscha, S. *et al.*	CA 7.5/037	2018	Seasonal dynamics of glyphosate and AMPA in Lake Greifensee: rapid microbial degradation in the epilimnion during summer.	Environmental Science and Technology (2018) 52:4641-4649
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Jönsson J. *et al.*	CA 7.5/084	2013	Removal and degradation of glyphosate in water treatment	Journal of Water Supply: Research and Technology—AQUA, 62.7, 2013
Kanissery, R. G. et al.	CA 7.1.2.1.1/013, CA 7.1.2.1.3/002, CA 7.1.3.1.1/025	2015	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate	Journal of environmental quality 44:137–144 (2015)
Karanasios E. et al	KCA 7.5/003	2018	Monitoring of glyphosate and AMPA in soil samples from two olive cultivation areas in Greece: aspects related to spray operators activities	Environmental Monitoring and Assessment (2018), Vol. 190, No. 6, pp. 1
Kjaer, J. *et al*	CA 7.1.4.3/004	2011	Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils	Chemosphere 84 (2011) 471–479
Klinger, J. *et al.*	CA 7.5/096	2008	Formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra	Ozone Science & Engineering, Vol 20, pp 99-110
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Larsbo, M. *et al.*	CA 7.5/045	2016	Surface runoff of pesticides from a clay loam field in Sweden.	Journal of Environmental Quality (2016) 45:1367-1374
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Manassero, A. *et al.*	CA 7.5/093	2010	Glyphosate degradation in water employing the H_2O_2/UVC process	Water research 44 (2010) 3875-3882
Maqueda C. *et al.*	CA 7.1.3.1.1/028, CA 7.2.1.3/001	2017	Behaviour of glyphosate in a reservoir and the surrounding agricultural soils	Science of the Total Environment, (2017) Vol. 593-594, pp. 787-795
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Meyer, B. et al.	CA 7.5/065	2011	Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg	Environmental Monitoring and Assessment (2011) 180:127-146
Mörtl, M. et al.	CA 7.5/024	2013	Determination of glyphosate residues in Hungarian water samples by immunoassay	Microchemical Journal (2013) 107:143-151
Mottes, C. *et al.*	CA 7.5/042	2017	Relationships between past and present pesticide applications and pollution at a watershed outlet: The case of a horticultural catchment in Martinique, French West Indies.	Chemosphere (2017) 184:762-773
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Munira, S. et al.	CA 7.1.3.1.1/022	2016	Phosphate fertilizer impacts on glyphosate sorption by soil	Chemosphere 153 (2016) 471-477
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Muskus A. M. *et al.*	CA 7.1.1.1, CA 7.1.2.1.1	2019	Effect of temperature, pH and total organic carbon variations on microbial turnover of $^{13}C_3{}^{15}N$-glyphosate in agricultural soil	Science of the Total Environment 658 (2019) 697-707
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Nghia, N.K. et al.	CA 7.1.2.1.1/016	2013	Soil properties governing biodegradation of the herbicide glyphosate in agricultural soils	Proc. 24th Asian Pacific Weed Science Society conference, October 22-25, 2013, Bandung, Indonesia
Norgaard, T. et al.	CA 7.1.2.1.1/012	2015	Can Simple Soil Parameters Explain Field-Scale Variations in Glyphosate-, Bromoxyniloctanoate-, Diflufenican-, and Bentazone Mineralization?	Water Air Soil Pollut (2015) 226: 262
Norgaard, T. et al.	CA 7.5/021	2014	Leaching of glyphosate and aminomethylphosphonic acid from an agricultural field over a twelve-year period	Vadose Zone Journal (2014) 13:18
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Passeport, E., *et al.*	CA 7.1.2.2.1/026	2013	Dynamics and mitigation of six pesticides in a "Wet" forest buffer zone	Environ Sci Pollut Res (2014) 21:4883–4894
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Poiger, T. et al.	CA 7.5/017	2017	Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS.	Environmental Science and Pollution Research (2017) 24:1588-1596
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Rampoldi, E. et al.	CA 7.1.2.1.1/014, CA 7.1.3.1.1/028	2014	Carbon-14-Glyphosate Behavior in Relationship to Pedoclimatic Conditions and Crop Sequence	Journal of environmental quality 43:558–567 (2014)
Ramwell, C.T. et al.	CA 7.5/052	2014	Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains.	Pest Management Science (2014) 70:1823-1830
Rasmussen, S., et al.	CP 9.2.4	2015	Effects of Single Rainfall Events on Leaching of Glyphosate and Bentazone on Two Different Soil Types, using the DAISY Model	Vadose Zone Journal; Advancing Critical Zone Science; Published November 13, 2015
Ravier, S. et al.	CA 7.5/079	2019	Monitoring of glyphosate, glufosinate-ammonium, and (aminomethyl) phosphonic acid in ambient air of Provence-Alpes-Cote-d'Azur Region, France.	Atmospheric Environment (2019) 204:102-109
Reoyo-Prats, B. *et al*.	CA 7.5/043	2017	Multicontamination phenomena occur more often than expected in Mediterranean coastal watercourses: Study case of the Tet River (France)	The Science of the total Environment (2017) 579:10-21
Rosenbom, A.E. et al.	CA 7.5/016	2019	The Danish Pesticide Leaching Assessment Programme	Geological Survey of Denmark and Greenland
Rosenbom, A.E. et al.	CA 7.5/019	2015	Pesticide leaching through sandy and loamy fields - Long-term lessons learnt from the Danish Pesticide Leaching Assessment Programme	Environmental Pollution 201 (2015) 75-90
Rosenbom, A.E. et al.	CA 7.5-099	2020	The Danish Pesticide Leaching Assessment Programme, Monitoring results May 1999–June 2018	Geological Survey of Denmark and Greenland

Ruel, S. *et al.*	CA 7.5/086	2012	Occurrence and fate of relevant substances in wastewater treatment plants	Water Science & Technology | 65.7 | 2012
Ruel, S. *et al.*	CA 7.5/087	2011	On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications	Water Science & Technology | 63.11 | 2011
Sabatier, P. et al.	CA 7.5/078	2014	Long-term relationships among pesticide applications, mobility, and soil erosion in a vineyard watershed.	Proceedings of the National Academy of Sciences of the United States of America (2014) 111:15647-15652
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Sanchís, J. et al.	CA 7.5/026	2012	Erratum to: Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry.	Analytical and Bioanalytical Chemistry (2012) 404:617
Schoonenberg Kegel, F.S. *et al.*	CA 7.5/088	2010	Reverse osmosis followed by activated carbon filtration for efficient removal of organic micropollutants from river bank filtrate	Water Science & Technology—WST | 61.10 | 2010
Schreiner, V.C. et al.	CA 7.5/046	2016	Pesticide mixtures in streams of several European countries and the USA	The Science of the total Environment (2016) 573:680-689
Shen, Y. *et al.*	CA 7.5/089	2011	Ozonation of Herbicide Glyphosate	Acta Scientiae Circumstantiae, 31(8):1647-1652
Shen, Y. *et al.*	CA 7.5/090	2011	Translation of CA 7.5/089	
Sidoli, P. et al.	CA 7.1.3.1.1/023, CA 7.1.3.1.2/008	2016	Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules	Environ Sci Pollut Res (2016) 23:5733–5742
Silva, V. et al.	CA 7.5/004	2018	Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union	The Science of the total Environment (2018) 621:1352-1359
Skeff, W. et al.	CA 7.5	2015	Glyphosate and AMPA in the estuaries of the Baltic Sea method optimization and field study.	Marine Pollution Bulletin 100 (2015) 577–585
Skeff, W. et al.	CA 7.1.3.1.1/016, CA 7.1.3.1.2/007	2018	Adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid, and 2-aminoethylphosphonic acid on three typical Baltic Sea sediments	Marine Chemistry 198 (2018) 1–9
Stenrod, M.	CA 7.5/047	2015	Long-term trends of pesticides in Norwegian agricultural streams and potential future challenges in northern climate	Acta Agriculturae Scandinavica Section B - Soil and Plant Science (2015) 65:199-216
Sun et al.	CA 7.1.1.1/012	2019	Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil water system	Water Research 163 (2019) 114840
Szekacs, A. et al.	CA 7.5/006	2014	Monitoring and biological evaluation of surface water and soil micropollutants in Hungary	Carpathian Journal of Earth and Environmental Sciences (2014) 9:47-60
Szekacs, A. et al.	CA 7.5/048	2015	Monitoring pesticide residues in surface and ground water in Hungary: surveys in 1990-2015	Journal of Chemistry (2015) Article ID 717948
Tang, T. et al.	CA 7.5/049	2015	Quantification and characterization of glyphosate use and loss in a residential area.	The Science of the total Environment (2015) 517:207-214

Tévez, H., dos Santos, A.M	CA 7.1.3.1.1/026	2015	pH dependence of Glyphosate adsorption on soil horizons	Boletin de la Sociedad Geologica Mexicana, Volumen 67, num 3, 2015, P 509-516
Todorovic, Rampazzo ., *et al*	CA 7.1.2.2.1/027	2014	Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils	Int. Agrophys., 2014, 28, 93-100
Tush D. *et al.*	CA 7.1.1.3, CA 7.1.3.1.1/	2018	Dissipation of polyoxyethylene tallow amine (POEA) and glyphosate in an agricultural field and their co-occurrence on streambed sediments	Science of the total environment (2018), Vol. 636, pp. 212-219
Ulen, B.M. *et al*	CA 7.1.4.3/002	2012	Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains	Acta Agriculturae Scandinavica Section B _ Soil and Plant Science, 2012; 62: Supplement 2, 241 251
Ulen, B.M. *et al.*	CA 7.1.4.3/001	2014	Spatial variation in herbicide leaching from a marine clay soil via subsurface drains	Pest Management Science 2014; 70: 405–414
Van der Hoek *et al*	CA 7.5-098	2014	Practical Paper Drinking water treatment technologies in Europe: state of the art – challenges – research needs	Journal of Water Supply: Research and Technology—AQUA | 63.2 | 2014
Vialle, C. et al.	CA 7.5/056	2013	Pesticides in roof runoff: study of a rural site and a suburban site.	Journal of Environmental Management (2013) 120:48-54
Wang, S. *et al*	CA 7.2.2.3/023	2016	(Bio)degradation of glyphosate in water-sediment microcosms - A stable isotope co-labeling approach	Water Research 99 (2016) 91e100
Zgheib, S. et al.	CA 7.5/060	2012	Priority pollutants in urban stormwater: Part 1 - Case of separate storm sewers	Water Research (2012) 46:6683-6692
Zhelezova, A. et al.	CA 7.1.2.1.1/010, CA 7.1.3.1.1/020	2017	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides	Water Air Soil Pollut (2017) 228: 216

[a] This study was considered as reliable with restrictions by the applicant for data point "Monitoring in surface water". RMS considers that it is not relevant, but the study summary is still presented in the dossier for transparency

[b] This study was considered as reliable with restrictions by the applicant for data point "impact of water treatment processes". RMS considers that it is not relevant, but the study summary is still presented in the dossier for transparency.

All these studies were fully summarised in the relevant sections of the RAR. The nomenclature proposed by the applicant was kept, and therefore after assessment by RMS, the study were qualified as:

- Reliable: the study is sufficiently robust and can be used;
- Reliable with restrictions: some deviations are identified, but the study provides supportive information;
- Not reliable: major drawbacks are identified, or the level of information is not sufficient to fully assess the reliability;
- Not relevant: after complete review, RMS considers that the study finally does not address any data requirement.

B.8.7. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company)	Vertebrate study Y/N	Data protection claimed	Justification if data protectio	Owner	Previously used[1] Y/N If yes, for which

			GLP/ Officially recognised testing facilities[2,3] Published or not		Y/N	n is claimed		data point?
KCA 7.1.1.1 -001	[redacted]	201 0	Rate and route of degradation of [14C]-glyphosate in one soil incubated under aerobic conditions Report No.: 1923W-1 Document No.: MSL0023070 PTRL West, Inc. GLP/GEP: Y Published: N	N	N	--	GTF	Y RAR 2017: IIA 7.1.1/01 Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.1.1 -003	[redacted]	199 6	(14C)-Glyphosate: aerobic soil metabolism Report No.: 1413/1-1015 Document No.: - Corning Hazleton (Europe) GLP/GEP: Y Published: N	N	N	--	NUF	Y RAR 2017: IIA 7.1.1/02 Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.1.1 -005	[redacted]	199 5	HR-001: Aerobic soil metabolism and route of degradation Report No.: SNY-333/951445 Document No.: - Huntingdon Life Sciences Ltd. GLP/GEP: Y Published: N	N	N	--	ARY	Y RAR 2017: IIA 7.1.1/05 Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.1.1 -006	[redacted]	199 3	Degradation and metabolism of 14C-Glyphosate in soil incubated under aerobic conditions Report No.: 246486 Document No.: 151 GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.1.1 Monograph 1998: EG:AIIA-7.1.1.1 Monograph Trimesium: --
KCA 7.1.1.2 -003	[redacted]	200 3	The degradation of [14C]-Glyphosate in soil under anaerobic conditions Report No.: 22581 Document No.: MSL-18018 Inveresk Research GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.1.2/01 Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.1.3 -001	[redacted]	202 0	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from a soil photolysis study Report No.: 112148-007 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	--	GRG	N

KCA 7.1.1.3 -003	[redacted]	199 3	Photodegradation study of 14C-Glyphosate on soil Report No.: 315764 Document No.: - RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.1.3 Monograph 1998: EG:AIIA-7.1.1.1.2 Monograph Trimesium: --
KCA 7.1.2.1 .1-001 KCA 7.1.2.1 .2-001	[redacted]	202 0a	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from aerobic laboratory soil degradation studies Report No.: 112148-001 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	--	GRG	N
KCA 7.1.2.1 .1-002	[redacted]	201 0	Rate of degradation of [14C]glyphosate in three soils incubated under aerobic conditions Report No.: 1946W-1 Document No.: MSL0023071 PTRL West, Inc. GLP/GEP: Y Published: N	N	N	--	GTF	Y RAR 2017: IIA 7.2.1/01 Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.2.1 .1-003	[redacted]	199 3	Degradation of 14C-glyphosate in three soils incubated under aerobic conditions Report No.: 271618 Document No.: 157-Gly RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.2.1 Monograph 1998: EG:AIIA-7.1.1.2.1 Monograph Trimesium: --
KCA 7.1.2.1 .1-004	[redacted]	200 2	First amendment (addendum) to report - Degradation of 14C-glyphosate in three soils incubated under aerobic conditions Report No.: 271618 Document No.: 157-GLY amdt-1 RCC Ltd. GLP/GEP: Y Published: N	N	N	--	FMC	Y RAR 2017: IIA 7.2.1/02 Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.2.1 .1-005	[redacted]	199 2	Glyphosate-Trimesium: Soil dissipation study (incl. addendum to final report) Report No.: 7043-38/165 Document No.: - Hazleton UK GLP/GEP: Y Published: N	N	N	--	SYN	Y RAR 2017: IIA 7.2.1 Monograph 1998: -- Monograph Trimesium:

								EG:AIIA-7.1.1.1.1
KCA 7.1.2.1.2-002	[redacted]	2020	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil Report No.: 3202599 Document No.: - Smithers ERS Limited GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.2.1.2-003	[redacted]	2017	Aminomethylphosphonic Acid (AMPA): Rate of Degradation of AMPA in one Acidic Soil Incubated under Aerobic Conditions Report No.: S16-04460 Document No.: EPS-2016-0309 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.2.1.2-004	[redacted]	2020	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil - Report Addendum Report No.: 3202599 Document No.: - Smithers ERS Limited GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.2.1.3-001	[redacted]	2020	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from an anaerobic laboratory soil degradation study Report No.: 112148-004 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	--	GRG	N
KCA 7.1.2.2.1-001	[redacted]	2020	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from terrestrial field dissipation studies in Europe Report No.: 112148-003 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	--	GRG	N
KCA 7.1.2.2.1-002	[redacted]	2020	Glyphosate: Ecoregion Crosswalk for Nineteen Terrestrial Field Dissipation Study Locations in North America Report No.: 112148-005 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	--	GRG	N
KCA 7.1.2.2.1-003	[redacted]	2020b	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from terrestrial field dissipation studies in the USA and Canada Report No.: 112148-006 Document No.: -	N	N	--	GRG	N

			knoell Germany GmbH GLP/GEP: N Published: N					
KCA 7.1.2.2 .1-005	[redacted]	199 3	The terrestrial field dissipation of Glyphosate in Canadian soil Report No.: MSL-12605 Document No.: - Monsanto Company GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: EG:AIIA-7.1.1.2.2 Monograph Trimesium: --
KCA 7.1.2.2 .1-006	[redacted]	199 3a	The terrestrial field dissipation of glyphosate: Final Report Report No.: MSL-12651 Document No.: - Monsanto Company GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: EG:AIIA-7.1.1.2.2 Monograph Trimesium: --
KCA 7.1.2.2 .1-007	[redacted]	199 3b	Storage stability of Glyphosate and AMPA in Soil and Stream sediment Report No.: MSL-12682 Document No.: - Monsanto Company GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: EG:AIIA-7.2.1.3.2 Monograph Trimesium: --
KCA 7.1.2.2 .1-009	[redacted]	199 2b	Field soil dissipation rate determination of Glyphosate 360 (Egerkingen, Switzerland) Report No.: 280416 Document No.: 108-GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.3.1 Monograph 1998: EG:AIIA-7.1.1.2.2 Monograph Trimesium: --
KCA 7.1.2.2 .1-010	[redacted]	199 2c	Field soil dissipation rate determination of Glyphosate 360 (Bad Krozingen, Germany) Report No.: 280427 Document No.: 95-GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.3.1 Monograph 1998: EG:AIIA-7.1.1.2.2 Monograph Trimesium: --
KCA 7.1.2.2 .1-011	[redacted]	199 2d	Field soil dissipation rate determination of Glyphosate 360 (Menslage, Germany) Report No.: 280438 Document No.: 109-GLY	N	N	--	BCS	Y RAR 2017: IIA 7.3.1 Monograp

			RCC Umweltchemie AG GLP/GEP: Y Published: N					h 1998: EG:AIIA-7.1.1.2.2 Monograph Trimesium: --
KCA 7.1.2.2.1-012	[redacted]	1995	Storage Stability of Glyphosate and AMPA in Soil Report No.: 303625 Document No.: 326-GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	FMC	Y RAR 2017: Not accepted in RAR (2015) Monograph 1998: -- Monograph Trimesium: --
KCA 7.1.3.1.1-001	[redacted]	2020	Glyphosate – Adsorption/Desorption of [14C]Glyphosate in Ten Soils, Experiments Supporting IES Study20190441 - Final Report Report No.: 20190441 Document No.: - Innovative Environmental Services (IES) Ltd. GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.3.1.1-002	[redacted]	2020	Glyphosate – Adsorption/Desorption of [14C]Glyphosate in Ten Soils - First Amendment to Final Report - Report No.: 20190441 Document No.: - Innovative Environmental Services (IES) Ltd. GLP/GEP: N Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.3.1.1-030	[redacted]	2020	Glyphosate – Adsorption/Desorption of [14C]Glyphosate in Ten Soils, Experiments Supporting IES Study 20190441 - Final Report - Report No.: 20200276 Document No.: - Innovative Environmental Services (IES) Ltd. GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.3.1.2-001	[redacted]	2020	Adsorption/Desorption of 14C-AMPA in Six Soils Report No.: S19-23618 Document No.: - Eurofins Agrosciences EcoChem GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.3.1.2-006	[redacted]	1993	Aminomethylphosphonic acid – Determination of the sorption and desorption properties Report No.: 92-8-4390 Document No.: MSL-12703 Springborn Laboratories, Inc.	N	N	--	BCS	Y RAR 2017: IIA 7.4.2 Monograph 1998:

			GLP/GEP: Y Published: N					EG:AIIA-7.1.2 Monograph Trimesium: --
KCA 7.1.3.1.2-009	[redacted]	2020	Adsorption/Desorption of 14C-AMPA in Six Soils - Amendment Report No.: S19-23618 Document No.: - Eurofins Agrosciences EcoChem GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 7.1.4.1.1-001	[redacted]	1996	[14C]-Glyphosate: Determination of the mobility of aged residues in one soil Report No.: 96-121-1020 Document No.: - Springborn Laboratories (Europe) AG GLP/GEP: Y Published: N	N	N	--	SIN	Y RAR 2017: IIA 7.4.5/01 Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.1.1-004	[redacted]	1993	Glyphosate isopropylamine salt. Hydrolysis in water at 3 different pH-values Report No.: PR93/009 Document No.: - Dr. G. Krebs Analytik GLP/GEP: Y Published: N	N	N	--	ADM	Y RAR 2017: -- Monograph 1998: EG:AIIA-7.2.1 Monograph Trimesium: --
KCA 7.2.1.1-005	[redacted]	1992	MON-8722: Determination of hydrolysis as a function of pH Report No.: 91-MON024-1207 Document No.: - Life Science Research Limited GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: EG:AIIA-2.9 Monograph Trimesium: --
KCA 7.2.1.1-007	[redacted]	1990	Hydrolysis determination of 14C-glyphosate (PMG) at different pH values Report No.: 238500 Document No.: - RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.5 Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.1.2-002	[redacted]	2005	Degradation Study: Photodegradation of [14C]glyphosate in Sterilized Pure Water and Natural Water by Artificial Light Report No.: 1318W-2 Document No.: MSL-19384 PTRL West, Inc.	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: -- AIR2 Doc L: IIA 2.9.2.1/05

			GLP/GEP: Y Published: N					Monograph Trimesium: --
KCA 7.2.1.2-005	[redacted]	1990	Degradation Study: Photodegradation of [14C]glyphosate in Buffered Aqueous Solution at pH 5, 7 and 9 by Natural Sunlight Report No.: 233W-1 Document No.: MSL-10575 Pharmacology and Toxicology Research Laboratory, Inc. GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: EG:AIIA-7.2.1.1 Monograph Trimesium: --
KCA 7.2.1.2-006	[redacted]	1992	Photodegradation study of [14C]glyphosate in water at pH 5, 7 and 9 Report No.: 250751 Document No.: - RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.6 Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.2.1-001	[redacted]	2009	Ready biodegradability of Glyphosate in a manometric respirometry test Report No.: 53981163 Document No.: - Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP: Y Published: N	N	N	--	NUF	Y RAR 2017: IIA 7.7/ 01 - new study (not submitted) Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.2.1-002	[redacted]	1991	A study to evaluate Ready Biodegradability of Glyphosate Technical Report No.: RB-09 Document No.: - Institute of Freshwater Ecology GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.7 Monograph 1998: EG:AIIA-7.2.1.3.1 Monograph Trimesium: --
KCA 7.2.2.1-003	[redacted]	1990	Glyphosate technical: inherent biodegradability: “modified Zahn-Wellens Test” Report No.: 271653 Document No.: - RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.7 Monograph 1998: EG:AIIA-7.2.1.3.1 Monograph Trimesium: --
KCA 7.2.2.2-001	[redacted]	2020	Glyphosate – Aerobic Mineralisation of [14C]Glyphosate in Surface	N	Y	First submission in EU	GRG	N

	[redacted]		Water Report No.: 815731 Document No.: TR0000198 Charles River Laboratories Edinburgh Ltd. GLP/GEP: Y Published: N					
KCA 7.2.2.3 -001	[redacted]	2020	Estimation of kinetic endpoints for glyphosate and its metabolites AMPA and HMPA from laboratory water-sediment studies Report No.: 112148-002 knoell Germany GmbH GLP/GEP: N Published: N	N	N	--	GRG	N
KCA 7.2.2.3 -002	[redacted]	1999	Glyphosate-Trimesium: Degradation of 14C-PMG Labelled Compound in Natural Water-Sediment Systems Under Laboratory Conditions Report No.: RR99-039B Document No.: VV-320975 Zeneca Agrochemicals GLP/GEP: Y Published: N	N	N	--	SYN	Y RAR 2017: IIA 7.8.3 Monograph 1998: AIIA-7.2.1.3.2 AIR2 Doc M: IIA 7.8 Monograph Trimesium: --
KCA 7.2.2.3 -005	[redacted]	1993	Determination of the degradability and persistence of 14C-Glyphosate in the water/sediment-system Report No.: ET01SE01 Document No.: 161GLY Battelle Institut e.V. GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.8.3 Monograph 1998: EG:AIIA-7.2.1.3.2 Monograph Trimesium: --
KCA 7.2.2.3 -006	[redacted]	1995	Amendment to the final report - Determination of the Degradability and Persistence of 14C-Glyphosate in the Water/Sediment-System - Report on the additional metabolite identification Report No.: ET01SE01 Document No.: 161GLY amdt-1 Battelle Memorial Institute, BCO GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.8.3 Monograph 1998: EG:AIIA-7.2.1.3.2 Monograph Trimesium: --
KCA 7.2.2.3 -018	[redacted]	2004	[14C]-AMPA: Degradation and fate in water/sediment systems Report No.: SNN/03 Document No.: - BioDynamics Research Ltd. GLP/GEP: Y Published: N	N	N	--	SIN	Y RAR 2017: IIA 7.8.3/03 Monograph 1998: -- Monograph Trimesium: --

KCA 7.2.2.3 -019	[redacted]	200 3	Aerobic aquatic degradation of aminomethylphosphonic acid according to SETAC, part 1.8.2 (March 1995) Report No.: IF-02/00005222 Document No.: - Institute Fresenius, Chemische und Biologische Laboratorien AG GLP/GEP: Y Published: N	N	N	--	ARY	Y RAR 2017: IIA 7.8.3/02 Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.2.3 -020	[redacted]	200 2	Aminomethylphosphonic acid: fate and behaviour in water-sediment Report No.: A&M01-106 Document No.: - A & M Labor für Analytik und Metabolismusforschung Service GmbH GLP/GEP: Y Published: N	N	N	--	AD M	Y RAR 2017: IIA 7.8.3/01 Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.2.3 -021	[redacted]	199 9	Aminomethylphosphonic acid: Water/Sediment Metabolism Report No.: IF-98/14727-00 Document No.: MSL-19217 Institute Fresenius, Chemische und Biologische Laboratorien AG GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: IIA 7.8.3 Monograph 1998: -- Monograph Trimesium: --
KCA 7.2.2.3 -022	[redacted]	198 9	Storage Stability of Glyphosate in Environmental Water Report No.: MSL-8626 Document No.: - Monsanto Agricultural Company GLP/GEP: Y Published: N	N	N	--	BCS	Y RAR 2017: -- Monograph 1998: EG:AIIA-7.2.1.3.2 Monograph Trimesium: --
KCA 7.3.1-001	[redacted]	202 0	Glyphosate: Calculation of the Chemical Half-Life in the Troposphere Report No.: 110054-016 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 7.3.1-002	[redacted]	201 2	Atmospheric Oxidation of Glyphosate Salts - Atkinson Calculation Report No.: MSL0024050 Document No.: MSL0024050 Environmental Science Technology Center, Monsanto Company GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: KIIA 2.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 7.3.1-003	[redacted]	199 7	Determination of the rate of volatilization of glyphosate from soil and plant surface (french beans) Report No.: 191071 Document No.: -	N	N	-	ARY	Y RAR 2017: IIA 7.4.9 Monograph 1998: -

			NOTOX B.V. GLP/GEP: Y Published: N					Monograph Trimesium: -
KCA 7.3.1-006	[redacted]	1993	Determination of the volatilization of Glyphosate 360 SL from soil and plants Report No.: BE-EA-149-92-01-VOL-1 Document No.: - - GLP/GEP: N Published:	N	N	-	BCS	Y RAR 2017: IIA 7.4.9 Monograph 1998: EG:AIIA-7.2.2 Monograph Trimesium: -
KCA 7.3.1-007	[redacted]	1992	Glyphosate-trimesium: Volatilization from soil and leaf surfaces Report No.: RJ1237B Document No.: VV-322306 ICI Agrochemicals GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: - Monograph 1998: - Monograph Trimesium: EG:AIIA-7.2.2
KCA 7.5-001	[redacted]	2020	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA Report No.: 110057-1 Document No.: EnSa-20-0244 knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 7.5-002	[redacted]	2020	Glyphosate (GLY) and the primary metabolites Aminomethyl Phosphonic Acid (AMPA) and Hydroxymethyl Phosphonic Acid (HMPA): Public monitoring data assessment and interpretation Report No.: EnSa-20-0322 Document No.: CEA.2162 Bayer Agriculture BV GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 7.5-005	Napoli, M. *et al.*	2016	Transport of glyphosate and aminomethylphosphonic acid under two soil management practices in an Italian vineyard Report No.: DOI 10.2134/jeq2016.02.0061; E-ISSN 1537-2537 Document No.: - Journal of Environmental Quality (2016) 45:1713-1721 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-008	[redacted]	2019	Phase 1: Traitements et analyses statistiques sur les données soes UIPP 2008-2014 - Analyse des données de suivi de glyphosate et de l'AMPA dans les eaux de France Période 2008 -2014	N	N	-	GRG	N

			Report No.: REA-DOC-026 Document No.: - envilys GLP/GEP: N Published: N					
KCA 7.5-009	[redacted]	2016	Analyse des données de suivi du glyphosate et de l'AMPA dans les eaux de France Période 1997-2013 Report No.: - Document No.: Rapport_AMPA_Glyphosate_1997-2013(V3) Monsanto France GLP/GEP: N Published: N	N	N	-	BCS	N
KCA 7.5-010	[redacted]	2016	Survey of glyphosate and AMPA in ground waters and surface waters in Europe - 2015/16 update review – final report Report No.: MSL0027535 Document No.: - Helene Horth, Adviser, Water Quality and European Policy & Legislation GLP/GEP: N Published: N	N	N	-	GTF	N
KCA 7.5-014	[redacted]	2006	Clarification of well-related findings of glyphosate and AMPA in groundwater Report No.: IF-06/00603024 Document No.: BVL No. 2310282 SGS INSTITUT FRESENIUS GmbH GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: IIA 7.12/02 Monograph 1998: - Monograph Trimesium: -
KCA 7.5-016	Rosenbom, A.E. *et al.*	2019	The Danish Pesticide Leaching Assessment Programm Report No.: ISSN (print): 2446-4228 ISSN (online): 2446-4236 ISBN (print) 978-87-7871-492-3 ISBN (online) 978-87-7871-491-6 Document No.: - Environmental Pollution 201 (2015) 75e90 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-017	Poiger, T. *et al.*	2017	Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS Report No.: DOI 10.1007/s11356-016-7835-2; E-ISSN 1614-7499 Document No.: - Environmental Science and Pollution Research (2017) 24:1588-1596 GLP/GEP: N Published: Y	N	N	-	LIT	N

KCA 7.5-019	Rosenbom, A.E. *et al.*	2015	Pesticide leaching through sandy and loamy fields - Long-term lessons learnt from the Danish Pesticide Leaching Assessment Programme Report No.: DOI 10.1016/j.envpol.2015.03.002; E-ISSN 1873-6424 Document No.: - Environmental Pollution (2015) 201:75-90 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-025	Sanchís, J. *et al.*	2012	Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry Report No.: DOI 10.1007/s00216-011-5541-y; E-ISSN 1618-2650 Document No.: - Analytical and Bioanalytical Chemistry (2012) 402:2335-2345 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-026	Sanchís, J. *et al.*	2012	Erratum to:Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry Report No.: DOI 10.1007/s00216-012-5992-9 E-ISSN 1618-2650 Document No.: - Analytical and Bioanalytical Chemistry (2012) 404:617 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: IIA 7.12 Monograph 1998: - Monograph Trimesium: -
KCA 7.5-028	[redacted]	2011	Investigation of the potential glyphosate groundwater contamination in Lombardia region (North Italy) Report No.: BVL No. 2310280 Document No.: - OPERA Research Centre, Università Cattolica del Sacro Cuore GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: IIA 7.12/01 Monograph 1998: - Monograph Trimesium: -
KCA 7.5-029	[redacted]	2010	Evaluatie van metingen van glyfosaat en AMPA in grondwater in Nederland (Evaluation of glyphosate and AMPA measurements in groundwater in The Netherlands) - ORIGINAL DUTCH Report No.: 354 Document No.: - Plant Research International,	N	N	-	GTF	Y RAR 2017: IIA 7.12/03 Monograph 1998: - Monograph Trimesium: -

			onderdeel van Wageningen UR, Business Unit Agrosysteemkunde GLP/GEP: N Published: N					
KCA 7.5-030	[redacted]	2010	Evaluation of glyphosate and AMPA measurements in groundwater in the Netherlands Report No.: 354 Document No.: - Plant Research International, onderdeel van Wageningen UR, Business Unit Agrosysteemkunde GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: IIA 7.12/03 Monograph 1998: - Monograph Trimesium: -
KCA 7.5-031	[redacted]	2019	Mitigating glyphosate levels in surface waters: Pilot catchment details and monitoring results Report No.: - Document No.: - Vito NV GLP/GEP: N Published: N	N	N	-	BCS	N
KCA 7.5-032	[redacted]	2019	phase 3 et 4:traitements et analyses statistiques sur les données SOES UIPP 2008–2014 analyses des données de surveillances sur 6 territoires témoins synthèse des données sur l'ensemble des territoires viticoles - Analyse des données de suivi de glyphosate et de l'AMPA dans les eaux de France Période 2008 -2014 Report No.: - Document No.: REA-DOC-026 envilys GLP/GEP: N Published: N	N	N	-	BCS	N
KCA 7.5-033	[redacted]	2018	Etude environnementale du Glyphosate et de l'AMPA à l'échelle des 10 points de surveillance les plus préoccupants pour le Glyphosate et pour l'AMPA Analyse des suivis du Glyphosate et de l'AMPA en lien avec les bassins versants drainés par les stations de mesures et l'occupation des sols - Etudes des stations sur l'AMPA Report No.: Envilys Report Version 1 (2018) Document No.: REA-DOC-026 envilys GLP/GEP: N Published: N	N	N	-	BCS	N
KCA 7.5-034	[redacted]	2018	Etude environnementale du Glyphosate et de l'AMPA à l'échelle des 10 points de surveillance les plus préoccupants pour le Glyphosate et pour l'AMPA Analyse des suivis du Glyphosate et de l'AMPA en lien avec les bassins versants drainés par les stations	N	N	-	BCS	N

			de mesures et l'occupation des sols - Etudes des stations sur l'AMPA Report No.: Envilys Report Version 1 (2018) Document No.: REA-DOC-026 envilys GLP/GEP: N Published: N					
KCA 7.5-037	Huntscha, S. *et al.*	2018	Seasonal dynamics of glyphosate and AMPA in Lake Greifensee: rapid microbial degradation in the epilimnion during summer Report No.: DOI 10.1021/acs.est.8b00314; E-ISSN 1520-5851 Document No.: - Environmental Science and Technology (2018) 52:4641-4649 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-038	Masiol, M. *et al.*	2018	Herbicides in river water across the northeastern Italy: occurrence and spatial patterns of glyphosate, aminomethylphosphonic acid, and glufosinate ammonium Report No.: DOI 10.1007/s11356-018-2511-3; E-ISSN 1614-7499 Document No.: - Environmental Science and Pollution Research (2018) 25:24368-24378 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-039	Dairon, R. *et al.*	2017	Long-term impact of reduced tillage on water and pesticide flow in a drained context Report No.: DOI 10.1007/s11356-016-8123-x; E-ISSN 1614-7499 Document No.: - Environmental Science and Pollution Research (2017) 24:6866-6877 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-040	Lefrancq, M. *et al.*	2017	High frequency monitoring of pesticides in runoff water to improve understanding of their transport and environmental impacts Report No.: DOI 10.1016/j.scitotenv.2017.02.022; E-ISSN: 1879-1026 Document No.: - The Science of the total Environment (2017) 587-588:75-86 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-042	Mottes, C. *et al.*	2017	Relationships between past and present pesticide applications and pollution at a watershed	N	N	-	LIT	N

			outlet: The case of a horticultural catchment in Martinique, French West Indies Report No.: DOI 10.1016/j.chemosphere.2017.06.061; E-ISSN 1879-1298 Document No.: - Chemosphere (2017) 184:762-773 GLP/GEP: N Published: Y					
KCA 7.5-045	Larsbo, M. *et al.*	2016	Surface runoff of pesticides from a clay loam field in Sweden Report No.: DOI 10.2134/jeq2015.10.0528; E-ISSN 1537-2537 Document No.: - Journal of Environmental Quality (2016) 45:1367-1374 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-046	Schreiner, V.C. *et al.*	2016	Pesticide mixtures in streams of several European countries and the USA Report No.: DOI 10.1016/j.scitotenv.2016.08.163; E-ISSN: 1879-1026 Document No.: - The Science of the total Environment (2016) 573:680-689 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-049	Tang, T. *et al.*	2015	Quantification and characterization of glyphosate use and loss in a residential area Report No.: DOI 10.1016/j.scitotenv.2015.02.040; E-ISSN 1879-1026 Document No.: - The Science of the total Environment (2015) 517:207-214 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-052	Ramwell, C.T. *et al.*	2014	Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains Report No.: DOI 10.1002/ps.3274; E-ISSN 1526-4998 Document No.: - Pest Management Science (2014) 70:1823-1830 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data Off-side movement Monograph Trimesium: -
KCA 7.5-055	Imfeld, G. *et al.*	2013	Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland Report No.: DOI 10.1016/j.chemosphere.2012.04.	N	N	-	LIT	Old RAR 2017: - Monograph 1998: -

			054; E-ISSN 1879-1298 Document No.: - Chemosphere (2013) 90:1333-1339 GLP/GEP: N Published: Y					RAR 2015: B.8.11.6.3 Monitoring data Off-side movement Monograph Trimesium: -
KCA 7.5-059	Petersen, J. *et al.*	2012	Sampling of herbicides in streams during flood events. Report No.: DOI 10.1039/c2em30771e; E-ISSN 1464-0333 Document No.: - Journal of Environmental Monitoring (2012) 14:3284-3294 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-063	Litz, N.T. *et al.*	2011	Comparative studies on the retardation and reduction of glyphosate during subsurface passage Report No.: DOI 10.1016/j.watres.2011.02.015; E-ISSN 1879-2448 Document No.: - Water Research (2011) 45:3047-3054 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.3.2 Mobility in soil Lysimeter studies or field leaching studies Monograph Trimesium: -
KCA 7.5-064	Maillard, E. *et al.*	2011	Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment Report No.: DOI 10.1016/j.scitotenv.2011.01.057; E-ISSN 1879-1026 Document No.: - The Science of the total Environment (2011) 409:2317-2324 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data Off-side movement Monograph Trimesium: -
KCA 7.5-065	Meyer, B. *et al.*	2011	Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg Report No.: DOI 10.1007/s10661-010-1777-9; ISSN 0167-6369 Document No.: - Environmental Monitoring and Assessment (2011) 180:127-146	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data

			GLP/GEP: N Published: Y					Surface water monitoring Monograph Trimesium: -
KCA 7.5-066	Busetto, M., Frattini, V.	2010	Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province - ORIGINAL ITALIAN Report No.: ISSN 0393-2796 Document No.: - Bollettino - Unione Italiana degli Esperti Ambientali (2010) 61:46-57 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data Surface water monitoring Monograph Trimesium: -
KCA 7.5-067	Busetto, M., Frattini, V.	2010	Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province - ENGLISH Report No.: - Document No.: - Bollettino - Unione Italiana degli Esperti Ambientali (2010) 61:46-57 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data Surface water monitoring Monograph Trimesium: -
KCA 7.5-068	Gregoire, C. *et al.*	2010	Use and fate of 17 pesticides applied on a vineyard catchment Report No.: DOI 10.1080/03067310903131230; E-ISSN 1029-0397 Document No.: - International Journal of Environmental Analytical Chemistry (2010) 90:406-420 GLP/GEP: N Published: Y	N	N	-	LIT	Old RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data Off-side movement Monograph Trimesium: -
KCA 7.5-069	Hanke, I. *et al.*	2010	Relevance of urban glyphosate use for surface water quality Report No.: DOI 10.1016/j.chemosphere.2010.06.067; E-ISSN 1879-1298 Document No.: - Chemosphere (2010) 81:422-429	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.5 Impact on water

			GLP/GEP: N Published: Y					treatment procedures Monograph Trimesium: -
KCA 7.5-074	[redacted]	2015	Survey of glyphosate and AMPA in drinking water supplies in Europe - 2015 update report Report No.: - Document No.: - Helene Horth, Adviser, Water Quality and European Policy & Legislation GLP/GEP: N Published: N	N	N	-	GTF	N
KCA 7.5-075	[redacted]	2008	Review of glyphosate and AMPA in drinking water in selected European countries Report No.: UC7729.04 Document No.: BVL No. 2310278 WRc Swindon GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: IIA 7.12/07 Monograph 1998: - Monograph Trimesium: -
KCA 7.5-078	Sabatier, P. *et al.*	2014	Long-term relationships among pesticide applications, mobility, and soil erosion in a vineyard watershed Report No.: DOI 10.1073/pnas.1411512111; E-ISSN 1091-6490 Document No.: - Proceedings of the National Academy of Sciences of the United States of America (2014) 111:15647-15652 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-079	Ravier, S. *et al.*	2019	Monitoring of glyphosate, glufosinate-ammonium, and (aminomethyl) phosphonic acid in ambient air of Provence-Alpes-Cote-d'Azur Region, France Report No.: DOI 10.1016/j.atmosenv.2019.02.023 ; E-ISSN 1873-2844 Document No.: - Atmospheric Environment (2019) 204:102-109 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-080	[redacted]	2012	Review of sustainable water treatment Report No.: UC8408v2 Document No.: BVL No. 2316001 WRc Swindon GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: KIIIA1 9.6.4/01 Monograph 1998: - Monograph Trimesium: -

KCA 7.5-081	[redacted]	2010	Removal of glyphosate and AMPA by water treatment Report No.: UC8154v2 Document No.: BVL No. 2316003 WRc Swindon GLP/GEP: N Published: N	N	N	-	GTF	Y RAR 2017: KIIIA1 9.6.4/02 Monograph 1998: - Monograph Trimesium: -
KCA 7.5-084	Joensson, J. *et al.*	2013	Removal and degradation of glyphosate in water treatment: a review Report No.: DOI 10.2166/aqua.2013.080; E-ISSN 1365-2087 Document No.: - Journal of Water Supply Research and Technology (2013) 62:395-408 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.5 Impact on water treatment procedures Monograph Trimesium: -
KCA 7.5-087	Ruel, S.M. *et al.*	2011	On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications Report No.: DOI 10.2166/wst.2011.470; ISSN 0273-1223 Document No.: - Water Science and Technology (2011) 63:2486-2497 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.6.3 Monitoring data Off-side movement Monograph Trimesium: -
KCA 7.5-089	Shen, Y., *et al.*	2011	Ozonation of herbicide glyphosate CHINESE Report No.: ISSN 0253-2468 Document No.: - Acta Scientiae Circumstantiae (2011) 31:1647-1652 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - AIR2 Doc L: IIA 7.13 Monograph Trimesium: -
KCA 7.5-090	Shen, Y., *et al.*	2011	Ozonation of herbicide glyphosate **ENGLISH** Report No.: ISSN 0253-2468 Document No.: - Acta Scientiae Circumstantiae (2011) 31:1647-1652 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - AIR2 Doc L: IIA 7.13 Monograph Trimesium: -

KCA 7.5-091	Assalin, M.R. *et al.*	2010	Studies on degradation of glyphosate by several oxidative chemical processes: ozonation, photolysis and heterogeneous photocatalysis Report No.: DOI 10.1080/03601230903404598; E-ISSN 1532-4109 Document No.: - Journal of Environmental Science and Health. Part B (2010) 45:89-94 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.2.2 Rate of degradation, Laboratory studies Monograph Trimesium: -
KCA 7.5-093	Manasseo, A. *et al.*	2010	Glyphosate degradation in water employing the H2O2/UVC process Report No.: DOI 10.1016/j.watres.2010.05.004; E-ISSN 1879-2448 Document No.: - Water Research (2010) 44:3875-3882 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.4 Fate and behaviour in water; B.8.11.4.1 Photochemical degradation Monograph Trimesium: -
KCA 7.5-094	Brosillon, S. *et al.*	2006	Chlorination kinetics of glyphosate and its by-products: Modeling approach Report No.: doi:10.1016/j.watres.2006.03.028 Document No.: - Water Research 40, 2113 – 2124 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.5 Impact on water treatment procedures Monograph Trimesium: -
KCA 7.5-095	Mehrsheikh, A. *et al.*	2006	Investigation of the mechanism of chlorination of glyphosate and glycine in water Report No.: doi:10.1016/j.watres.2006.06.027 Document No.: - Water Research 40, 3003 – 3014 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: - Monograph 1998: - RAR 2015: B.8.11.5 Impact on water treatment procedures Monograph

								Trimesium: -
KCA 7.5-096	Klinger, J. *et al.*	2008	Formation of Glyphosate and AMPA During Ozonation of Waters Containing Ethylenediaminetetra(methylene phosphonic acid) Report No.: DOI: 10.1080/01919519808547279; ISSN: 0191-9512 Document No.: - OZONE SCIENCE & ENGINEERING, Vol. 20, pp. 99-110 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-097	Gillefalk, M. *et al.*	2018	Review: Potential Impacts of Induced Bank Filtration on Surface Water Quality: A Conceptual Framework for Future Research Report No.: doi:10.3390/w10091240 Document No.: - mdpi.com/journal/water GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-098	van der Hoek, J.P. *et al.*	2014	Drinking water treatment technologies in Europe: state of the art – challenges – research needs Report No.: doi: 10.2166/aqua.2013.007 Document No.: - Journal of Water Supply: Research and Technology—AQUA GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 7.5-099	Rosenbom, A.E. et al.	2020	The Danish Pesticide Leaching Assessment Programm Report No.: ISSN (print): 2446-4228 ISSN (online): 2446-4236 ISBN (print) 978-87-7871-517-3 ISBN (online) 978-87-7871-518-0 GLP/GEP: N	N	N	-	LIT	N

1 In order to facilitate the compilation of the final list of the tests and studies relied upon and the corresponding data protection, indicate whether the study was used in the previous DAR/RAR or, when the information is available, whether the study was already submitted in the framework of national authorisations.
2 See Art.3 of Annex of Regulation No 283/2013 and 284/2013
3 The RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

APPENDIX 1: LABORATORY DATA - KINETIC FITTINGS FROM [redacted] 2020 FOR SOILS NOT CONSIDERED AS RELIABLE BY RMS

[redacted], *1996*

Table 8.6.3.1-1: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1996)

Time (d)	Glyphosate (% AR)	AMPA (% AR)	Glyphosate (% AR)	AMPA (% AR)	Glyphosate (% AR)	AMPA (% AR)	Glyphosate (% AR)	AMPA (% AR)
	Speyer 2.1		**Speyer 2.2**		**Speyer 2.3, 20°C**		**Speyer 2.3, 10°C**	
0	97.7[1]	0.0[2]	103.8[1]	0.0[2]	99.1[1]	0.0[2]	99.3[1]	0.0[2]
1	84.8	12.1	96.1	4.3	76.2	13.0	87.3	8.7
2	74.3	12.9	84.2	7.9	63.9	27.0	80.0	9.2
4	59.2	25.1	77.1	12.9	34.2	25.7	62.2	19.3
7	53.9	27.3	71.8	15.7	18.4	32.0	54.9	22.1
15	38.2	27.5	60.3	21.0	13.3	25.3	35.9	25.8
29	21.0	37.9	41.7	34.5	0.05[3]	31.1	21.7	28.7
60	8.5	42.3	26.7	42.4	3.0	18.5	7.5	34.3
90	2.2	50.1	25.9	39.0				
120			19.0	40.9				

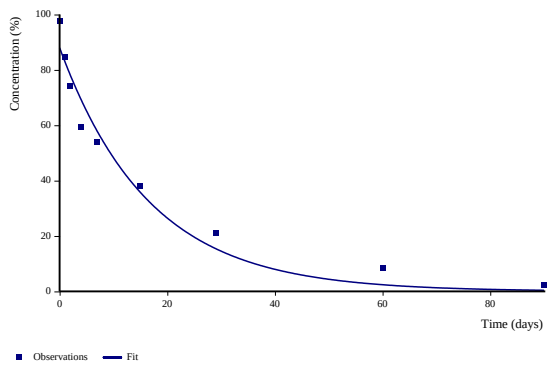
[1] Set to material balance
[2] Amounts of metabolites set to 0 at day 0
[3] Value below LOD (= 0.1 %AR) set to ½ LOD

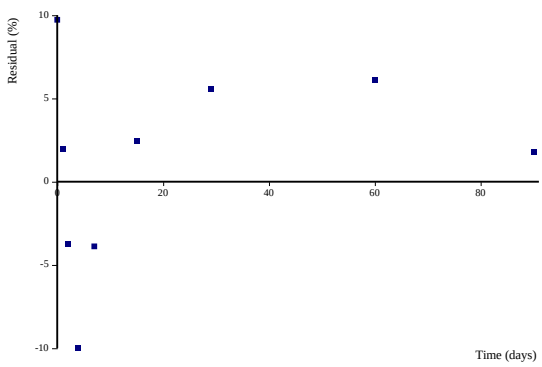
Speyer 2.1 soil

Table 8.6.3.1-2: Kinetic models and statistics for soil Speyer 2.1 of study [redacted] (1996) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	88.0	k: 0.0601	9.5	k: <0.001	k: 0.0385	k: 0.082	11.5	38.3
FOMC	Good	94.9	α: 1.13 β: 9.71	5.7	-[1]	β: -0.8596	β: 20.28	8.2	64.8
DFOP	Good	98.3	k_1: 0.474 k_2: 0.0371 g: 0.3278	2.5	k_1: 0.003 k_2: <0.001	k_1: 0.2054 k_2: 0.0288	k_1: 0.743 k_2: 0.046	8.3	51.3
Applicant's conclusion		Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides a better visual fit (M_0 as well as the residues at the last four sampling dates) and the lowest χ^2 error. Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints							

SFO





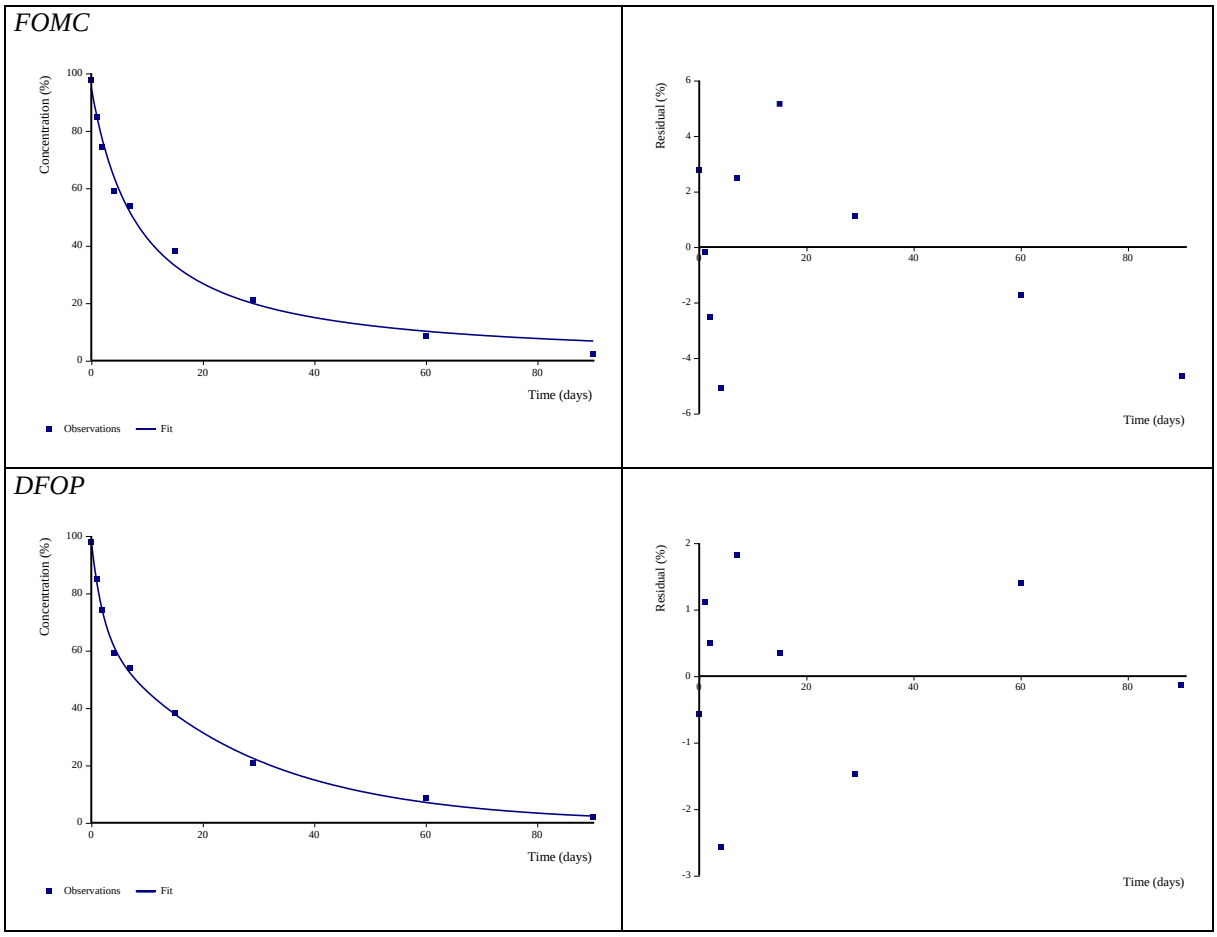
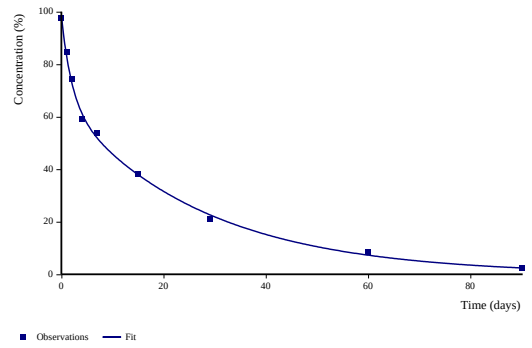
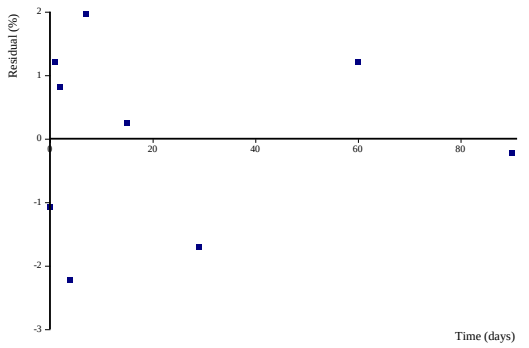


Table 8.6.3.1-3: Kinetic models and statistics for soil Speyer 2.1 of study [redacted] (1996) - Pathway fit (parent and metabolite)

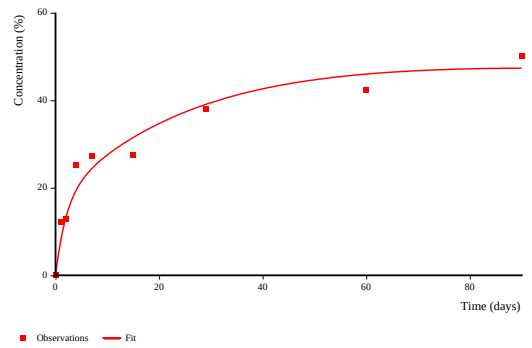
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	98.8	k_1: 0.4904 k_2: 0.0367 g: 0.3344	2.5	k_1: <0.001 k_2: <0.001	k_1: 0.2753 k_2: 0.0300	k_1: 0.705 k_2: 0.043	8.14	51.7	-
AMPA: SFO	Accep-table	-	k: 0.0008	9.4	k: 0.327	k: -0.0032	k: 0.005	829	2750	0.523 (±0.047)
Applicant's conclusion	The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable. Conclusion:Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate No endpoints can be derived for AMPA									

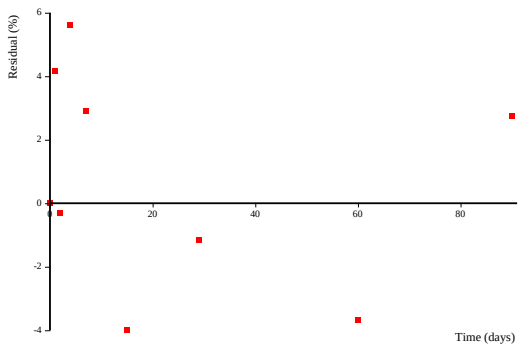
Glyphosate (DFOP)





AMPA (SFO)





Speyer 2.2 soil

Table 8.6.3.1-4: Kinetic models and statistics for soil Speyer 2.2 of study [redacted] (1996) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.3	k: 0.0201	11.0	k: <0.001	k: 0.0119	k: 0.028	34.5	115
FOMC	Good	101.3	α: 0.5744 β: 7.976	4.0	-[1]	β: 1.585	β: 14.37	18.7	431
DFOP	Good	100.7	k_1: 0.1338 k_2: 0.0098 g: 0.4358	5.0	k_1: 0.023 k_2: 0.005	k_1: 0.0040 k_2: 0.0034	k_1: 0.264 k_2: 0.016	19.3	176
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar reliable and visually acceptable results. As 10 % of the initial concentration was not reached within the experimental period, the DFOP model is selected as the best-fit model as well as for deriving modelling endpoints. Conclusion: DFOP to be used in pathway fits for trigger endpoints DFOP to be used in pathway fits for modelling endpoints								

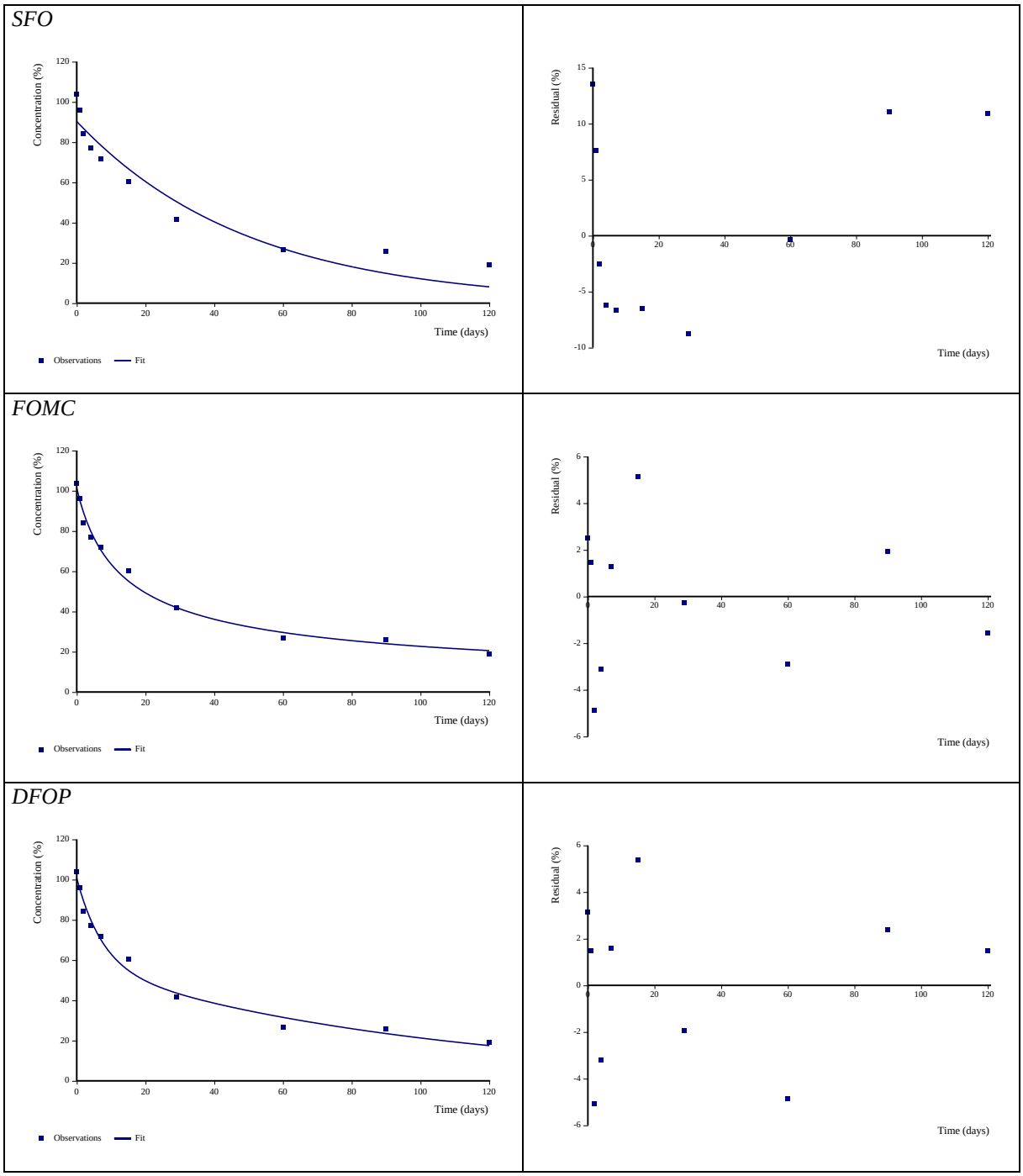
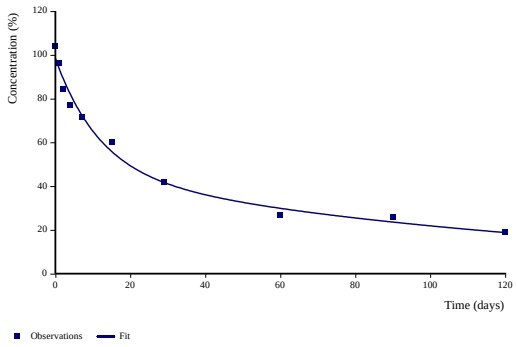


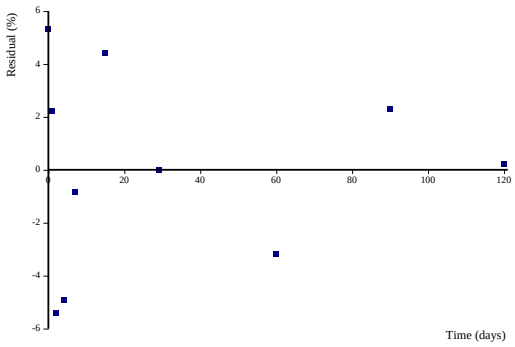
Table 8.6.3.1-5: Kinetic models and statistics for soil Speyer 2.2 of study [redacted] (1996) – Pathway fit (parent and metabolite)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting								
Glyphosate DFOP	Acceptable	98.5	k_1: 0.0858 k_2: 0.0076 g: 0.5275	5.1	k_1: 0.001 k_2: 0.016	20.1	205	-
AMPA: SFO	Good	-	k: 0.0019	8.1	k: 0.122	362	1200	0.618 (±0.071)

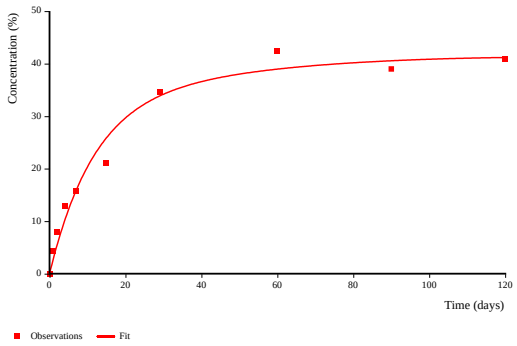
Applicant's conclusion	For glyphosate the visual fit is acceptable but M_0 is underestimated compared to the measured value. As the residue data of AMPA are well described, the fitting was repeated with initial parameter M_0 for parent fixed to the measured initial concentration (103.8 %).

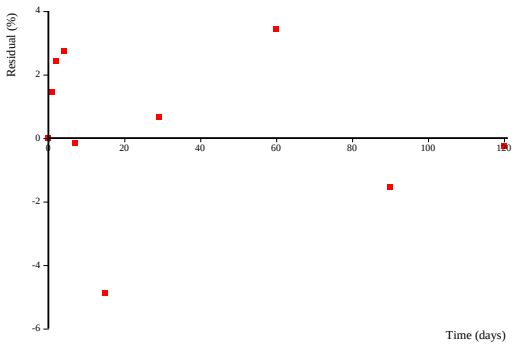
Initial fitting: Glyphosate (DFOP)





Initial fitting: AMPA (SFO)



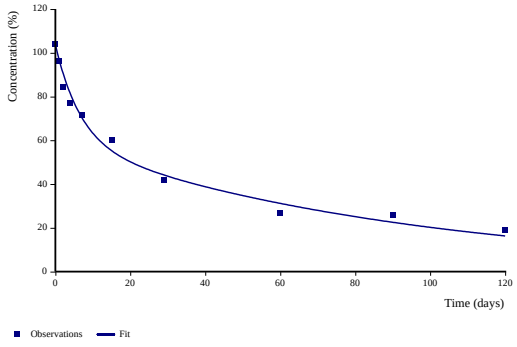


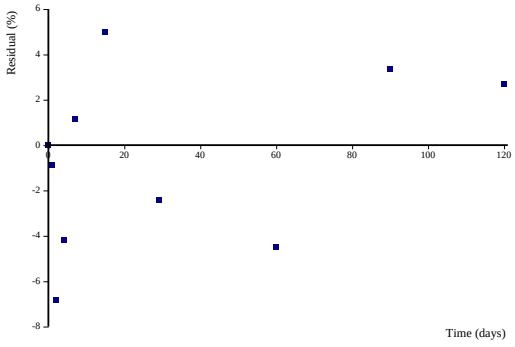
Repeated fitting 1: parent M_0 fixed to measured initial concentration

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 er-ror (%)	Prob > t (5 % level)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 103.8	k_1: 0.1507 k_2: 0.0108 g: 0.4259	5.9	k_1: 0.001 k_2: <0.001	18.1	162	-
AMPA: SFO	Acceptable	-	k: 0.0014	9.2	k: 0.191	497	>1000	0.548 (±0.052)

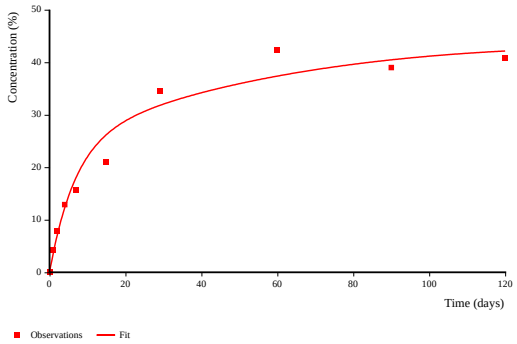
Applicant's conclusion	The visual fit of glyphosate improved. For AMPA, the parameter k is not significantly different from zero, but overall the formation of AMPA is well described and the estimated formation fraction is plausible with a low standard deviation. Therefore, the fitting was repeated again with additionally fixing ff for AMPA to the estimated value (0.548).

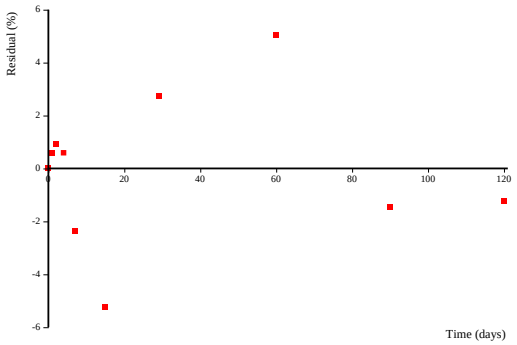
Repeated fitting 1: Glyphosate (DFOP) – fixed parent M_0





Repeated fitting 1: AMPA (SFO)



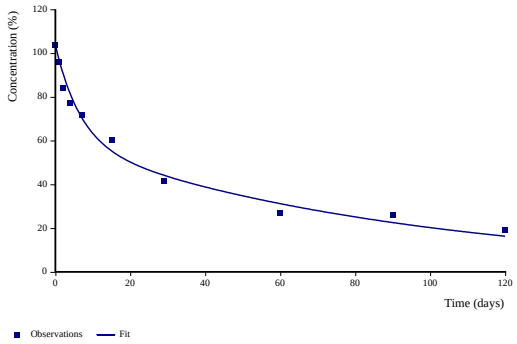


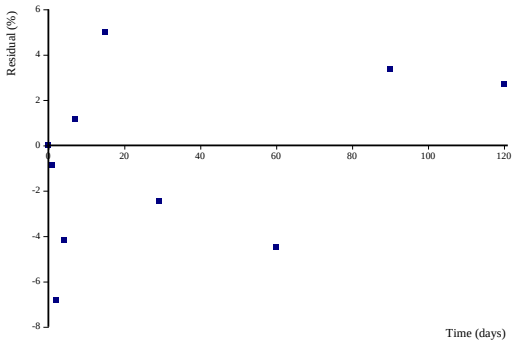
Repeated fitting 2: parent M_0 fixed to measured initial concentration, AMPA ff fixed to previously estimated value

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 er-ror (%)	Prob > t (5 % level)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: DFOP	Good	fixed to 103.8	k_1: 0.1507 k_2: 0.0108 g: 0.4259	5.9	k_1: <0.001 k_2: <0.001	18.1	162	-
AMPA: SFO	Acceptable	-	k: 0.0014	8.8	k: 0.043	497	>1000	fixed to 0.548

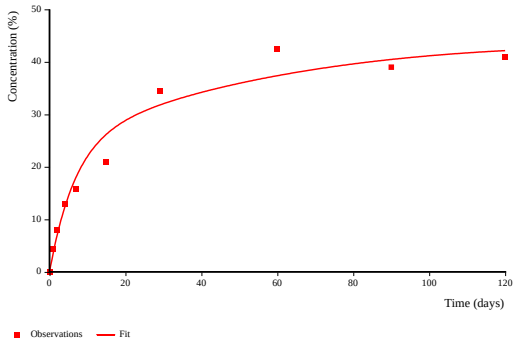
Applicant's conclusion	The estimated parameters for glyphosate and AMPA are statistically reliable. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

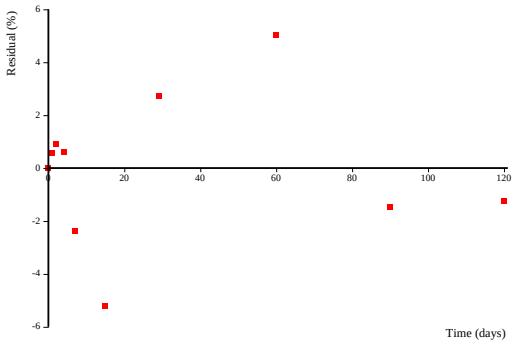
Repeated fitting 2: Glyphosate (DFOP) – fixed parent M_0 and metabolite ff





Repeated fitting 2: AMPA (SFO)



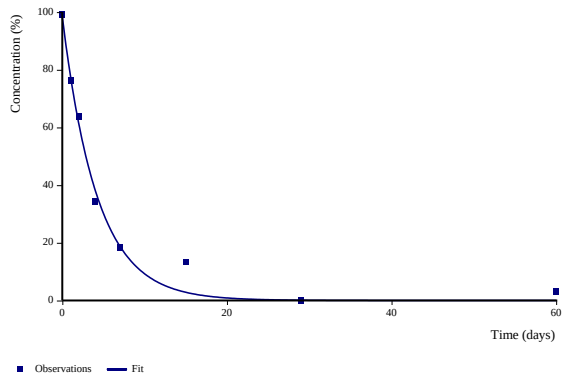


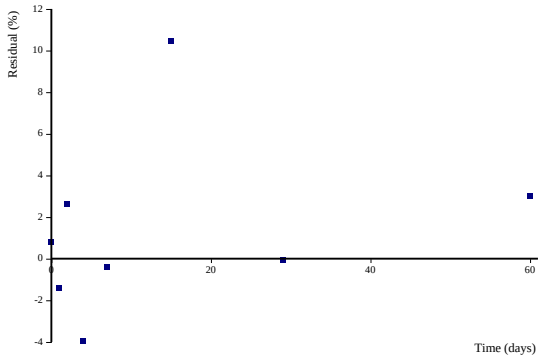
Speyer 2.3 soil, 20°C

Table 8.6.3.1-6: Kinetic models and statistics for soil Speyer 2.3, 20 °C, of study [redacted] (1996) - Parent-only fits

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	98.3	k: 0.2365	8.8	k: <0.001	k: 0.1771	k: 0.296	2.9	9.7
FOMC	Good	100.3	α: 2.691 β: 9.209	7.7	-[1]	β: -9.483	β: 27.9	2.7	12.5
DFOP	Good	100.1	k_1: 0.3169 k_2: 0.0497 g: 0.8345	7.5	k_1: 0.016 k_2: 0.252	k_1: 0.0433 k_2:-0.1388	k_1: 0.59 k_2: 0.238	2.7	13.0
Applicant's conclusion	The SFO model provides an acceptable visual and statistically reliable fit. The FOMC and DFOP bi-phasic models improve the visual fit. The DFOP model provides the best visual fit and the lowest χ^2 error. The parameter k_2 of the DFOP model is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.8345). Conclusion: DFOP to be used in pathway fit for trigger endpoints SFO to be used in pathway fit for modelling endpoints								

SFO





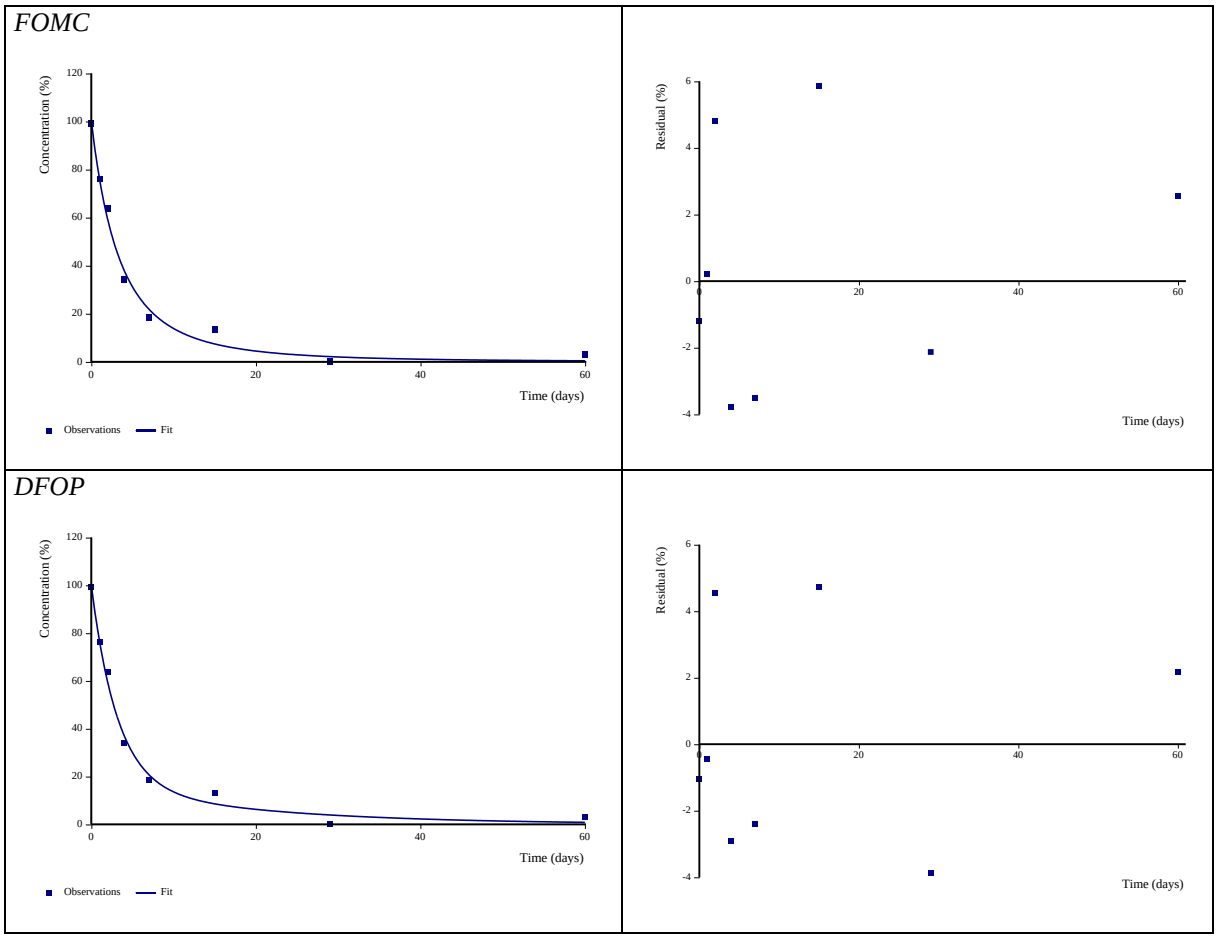
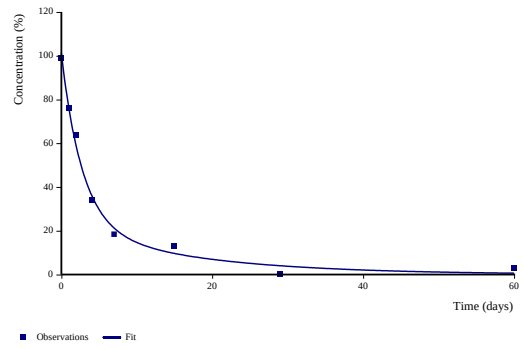
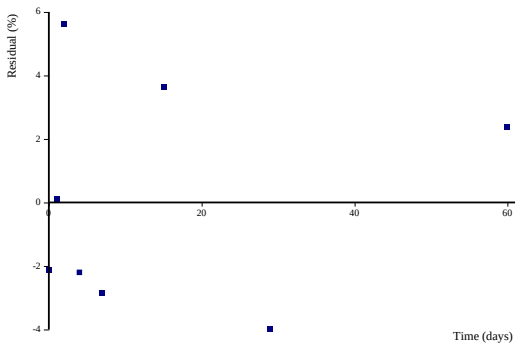


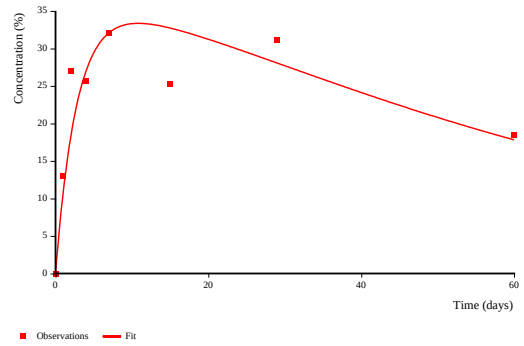
Table 8.6.3.1-7: Kinetic models and statistics for soil Speyer 2.3 (20 °C) of study [redacted] (1996) - Pathway fits (parent and metabolite) – Trigger endpoint

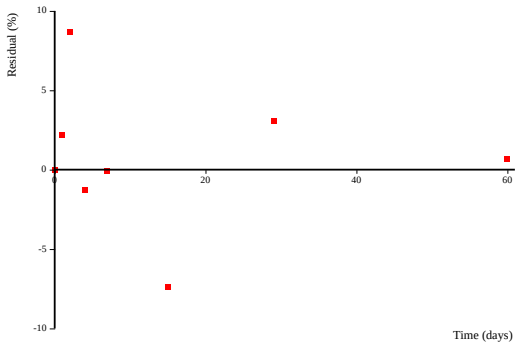
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Trigger endpoint - Initial fitting										
Glyphosate: DFOP	Good	101.2	k_1: 0.3621 k_2: 0.0599 g: 0.7742	7.7	k_1: 0.006 k_2: 0.162	k_1: 0.1025 k_2: -0.07	k_1: 0.622 k_2: 0.19	2.6	14.3	-
AMPA: SFO	Good	-	k: 0.0166	14.8	k: 0.015	k: 0.0021	k: 0.031	41.7	138	0.434 (±0.059)
Applicant's conclusion	The visual fits for glyphosate and AMPA are good, but for glyphosate the parameter k_2 is not significantly different from zero. As the residue data of AMPA are well described, the fitting was repeated with initial parameters for parent (M_0, k_1, k_2 and g) fixed to results from parent-only DFOP fit.									
Initial fitting: Glyphosate (DFOP)										





Initial fitting: AMPA (SFO)

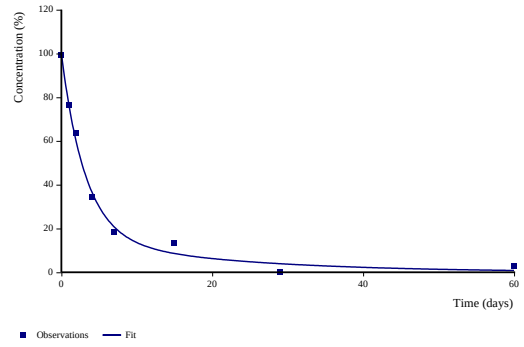


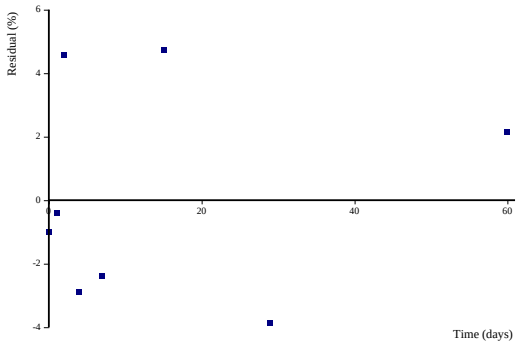


Trigger endpoint - Repeated fitting: initial parameters for parent fixed to results from parent-only fit

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 100.1	fixed to k_1: 0.3169 k_2: 0.0497 g: 0.8345	7.9	-[1]	-[1]	-[1]	2.7	13.0	-
AMPA: SFO	Good	-	k: 0.0168	15.8	k: 0.007	k: 0.0040	k: 0.03	41.4	137	0.440 (±0.048)
Applicant's conclusion	The repeated fitting resulted in reliable degradation parameters for glyphosate and AMPA.Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA									

Glyphosate (DFOP) – fixed input parameters from parent-only fit





AMPA (SFO)

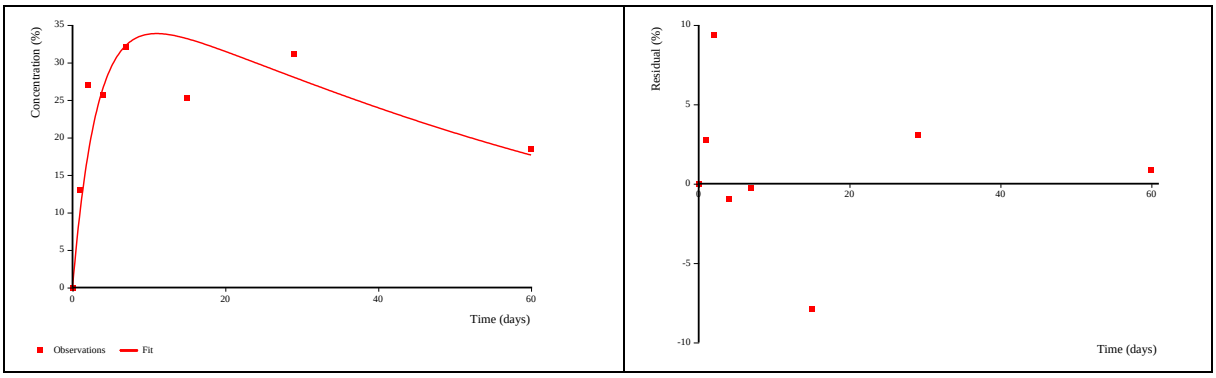
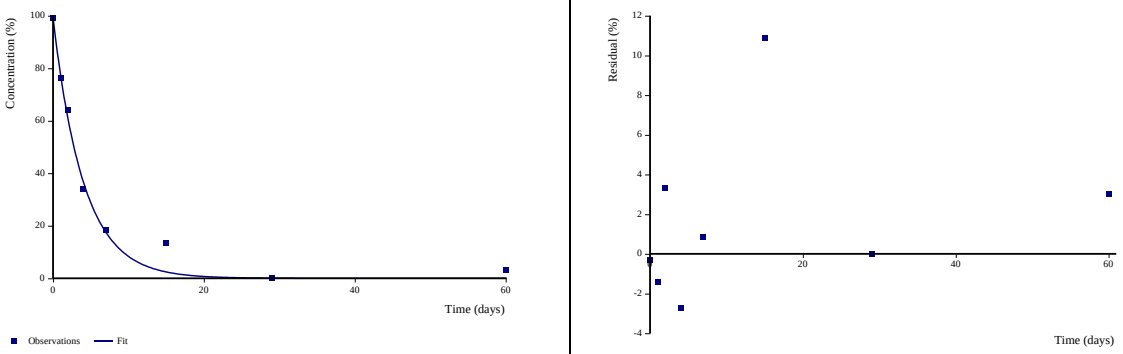


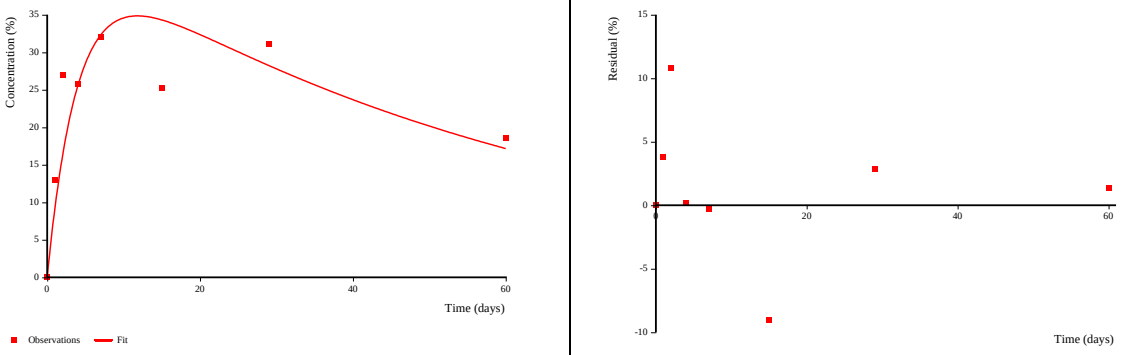
Table 8.6.3.1-8: Kinetic models and statistics for soil Speyer 2.3 (20 °C) of study [redacted] (1996) - Pathway fits (parent and metabolite) – Modelling endpoint

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Modelling endpoint										
Glypho-sate: SFO	Accep-table	99.4	k: 0.2476	8.9	k: <0.001	k: 0.1904	k: 0.305	2.8	9.3	-
AMPA: SFO	Accep-table	-	k: 0.0161	18.2	k: 0.022	k: <0.001	k: 0.032	43.1	143	0.424 (±0.065)
Applicant's conclusion	The degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA.									

Glyphosate (SFO)



AMPA (SFO)

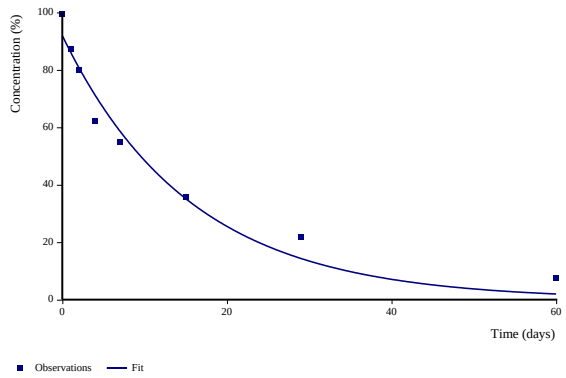


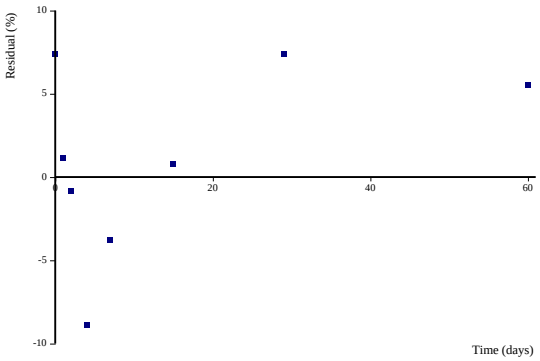
Speyer 2.3 soil, 10°C

Table 8.6.3.1-9: Kinetic models and statistics for soil Speyer 2.3, 10 °C, of study [redacted] (1996) - Parent-only fits

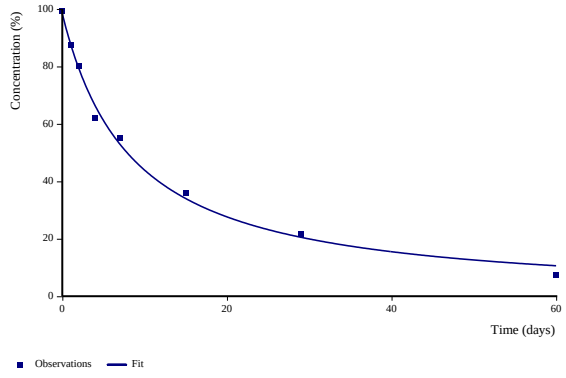
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	91.9	k: 0.0641	7.7	k. <0.001	k: 0.0424	k: 0.086	10.8	35.9
FOMC	Good	98.3	α: 1.115 β: 9.422	3.3	-[1]	β: 1.638	β: 17.21	8.1	64.9
DFOP	Good	99.5	k_1: 0.3001 k_2: 0.0361 g: 0.3756	2.3	k_1: 0.010 k_2: 0.001	k_1: 0.0807 k_2: 0.0221	k_1: 0.52 k_2: 0.05	8.1	50.8
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (the residues at the last three sampling dates) and the lowest χ^2 error. Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints								

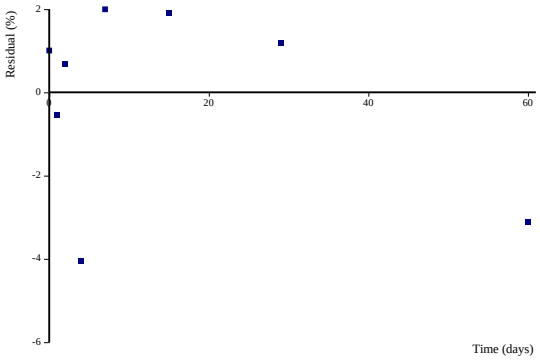
SFO



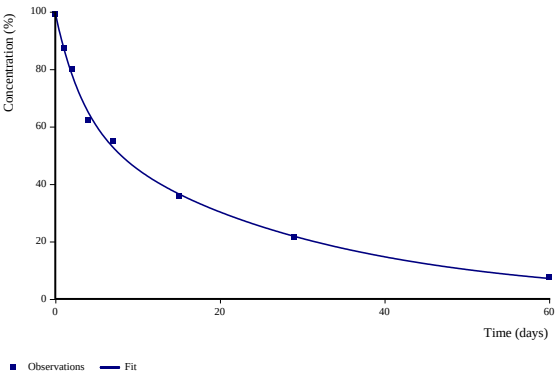


FOMC





DFOP



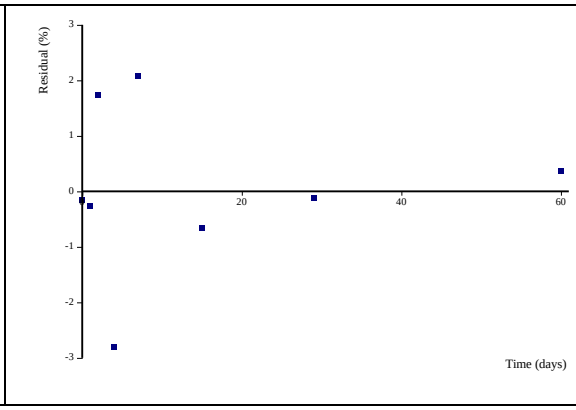
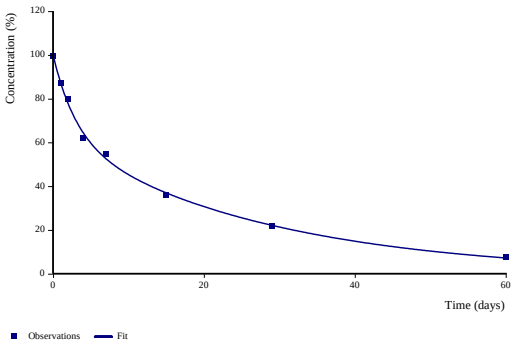
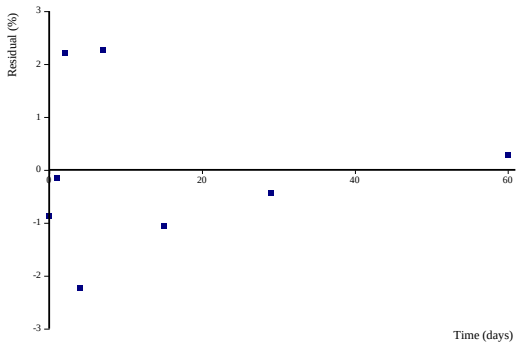


Table 8.6.3.1-10: Kinetic models and statistics for soil Speyer 2.3 (10 °C) of study [redacted] (1996) - Pathway fits

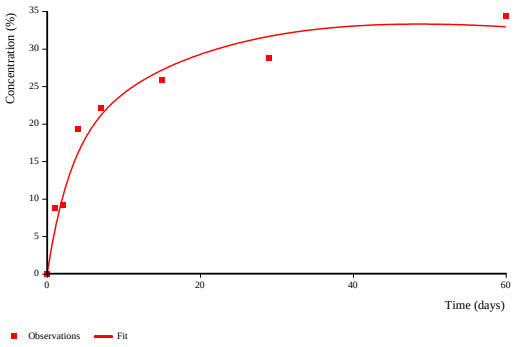
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	100.2	k_1: 0.3317 k_2: 0.0361 g: 0.37	2.4	k_1: 0.001 k_2: <0.001	k_1: 0.157 k_2: 0.0260	k_1: 0.506 k_2: 0.046	7.9	50.9	-
AMPA: SFO	Good	-	k: 0.0054	8.2	k: 0.047	k: -0.0011	k: 0.012	129	429	0.454 (±0.040)
Applicant's conclusion	Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									

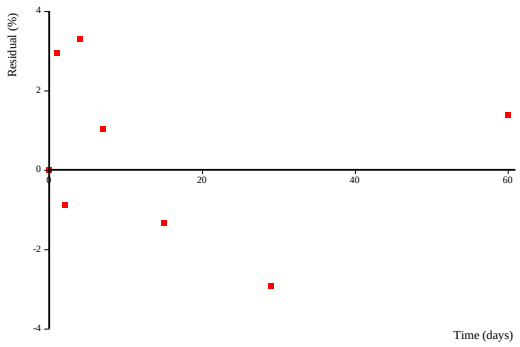
Glyphosate (DFOP)





AMPA (SFO)





[redacted] *(1993)*

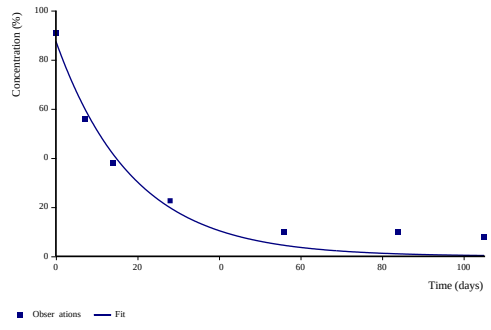
Time (d)	Glyphosate (% AR)	AMPA (% AR)
Speyer 2.1		
0	91.1	0.0[2]
7	56.0	21.7
14	38.1	41.2
28	22.6	32.6
56	9.7	40.0
84	9.7	38.7
105	8.0	23.5

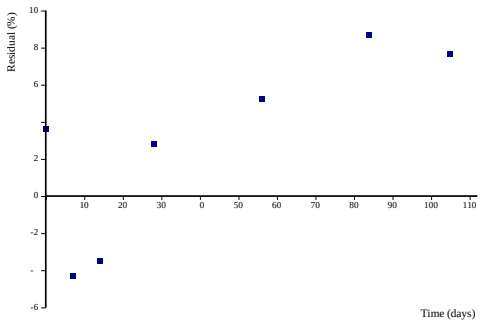
Speyer 2.1 soil

Table 8.6.3.1-11: Kinetic models and statistics for soil Speyer 2.1 of study [redacted] (1993) - Parent-only fits

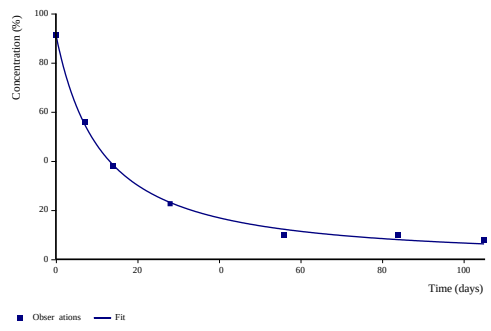
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.5	k: 0.0531	13.1	k: <0.001	k: 0.0316	k: 0.075	13.1	43.4
FOMC	Good	91.3	α: 1.255 β: 14.09	3.5	-[1]	β: 5.349	β: 22.83	10.4	74.2
DFOP	Good	90.7	k_1: 0.0848 k_2: 0.008 g: 0.8063	3.3	k_1: 0.002 k_2: 0.105	k_1: 0.0491 k_2:-0.0079	k_1: 0.121 k_2: 0.024	10.8	83.8
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (residues at the last sampling dates) and the lowest χ^2 error. The parameter k_2 is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.8063). Conclusion: DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints								

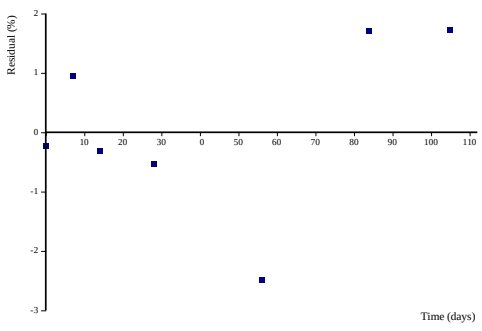
SFO



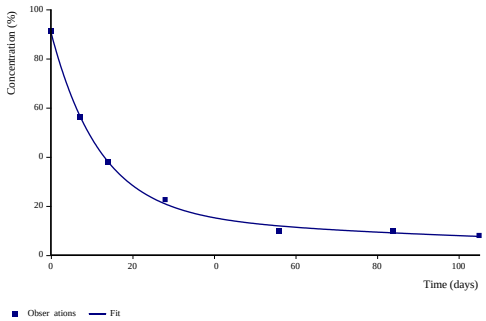


FOMC





DFOP



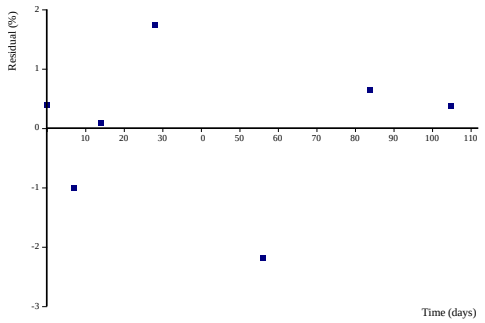
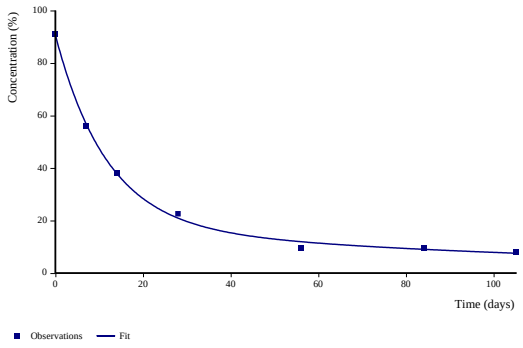
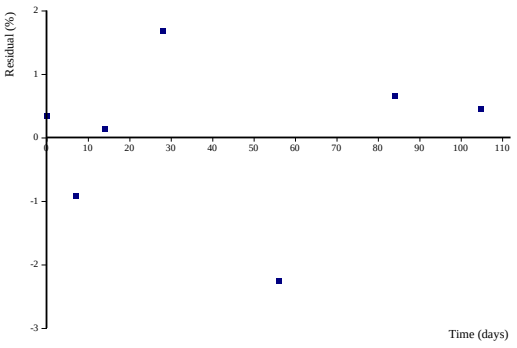


Table 8.6.3.1-12: Kinetic models and statistics for soil Speyer 2.1 of study [redacted] (1993) - Pathway fits (parent and metabolites)

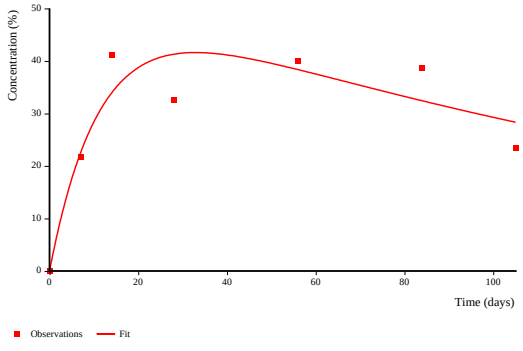
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	90.8	k_1: 0.0859 k_2: 0.0084 g: 0.7999	3.3	k_1: <0.001 k_2: 0.054	k_1: 0.0610 k_2:-0.0024	k_1: 0.111 k_2: 0.019	10.8	84	-
AMPA: SFO	Good	-	k: 0.0080	13.7	k: 0.025	k: 3.81 × 10^{-5}	k: 0.016	86.5	288	0.687 (±0.108)
Applicant's conclusion	The visual fits for glyphosate and AMPA are good and degradation parameters for AMPA are reliable. For glyphosate, the p-value of the t-test for parameter k_2 is still slightly above >0.05 (0.054) but this again can be accepted as the overall degradation of glyphosate is dominated by k_1 as indicated by a high value for parameter g (0.7999), and the modelling endpoint for glyphosate is derived from DT_{90}/3.32 as 10 % of the initial concentration was reached within the experimental period. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									

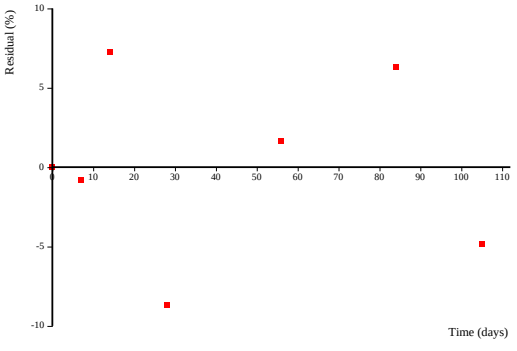
Glyphosate (DFOP)





AMPA (SFO)





[redacted] *(1992)*

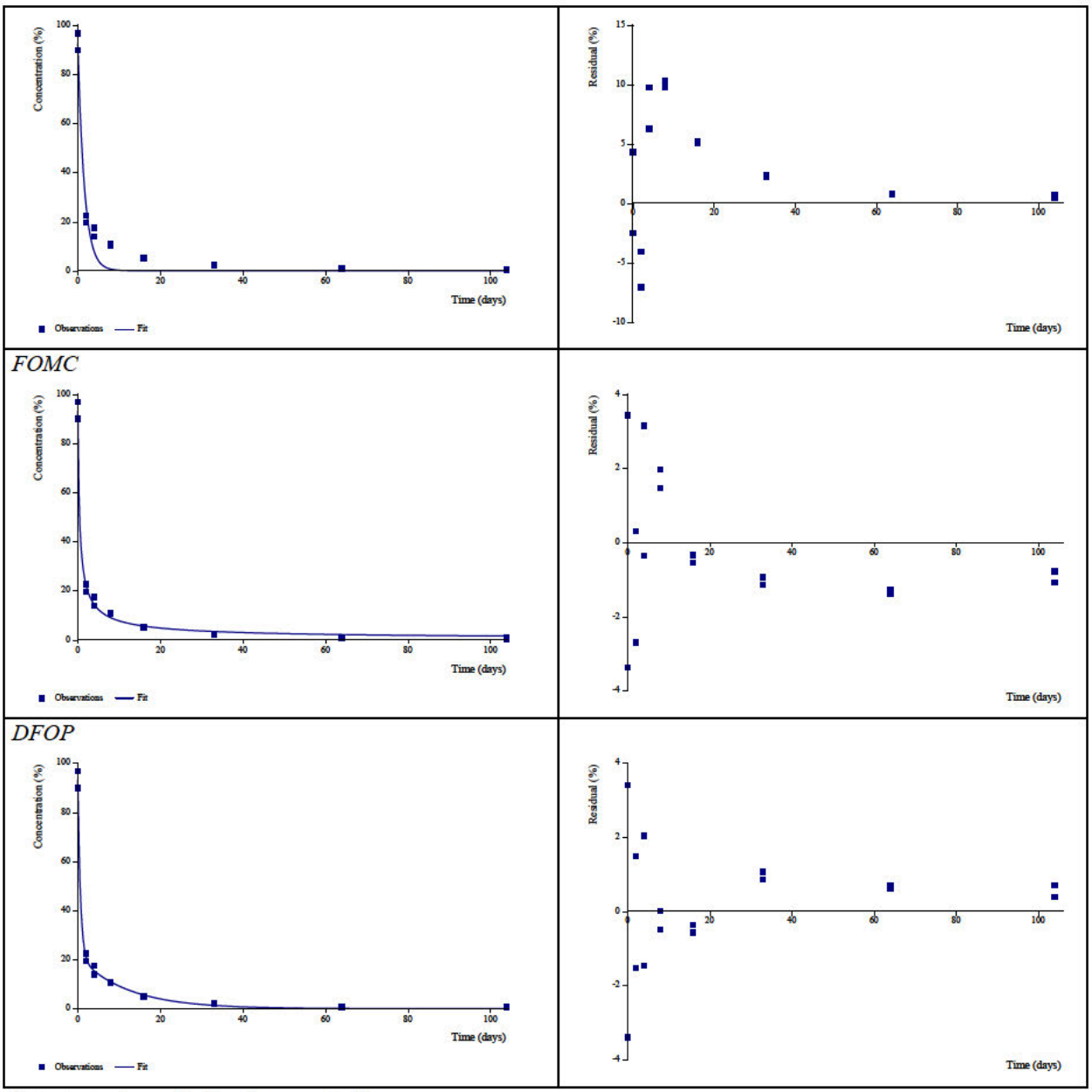
Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
	Beedon manor, dose group F 20 °C, 40 % MWHC, 4 mg/kg	
0	96.6[2]	0.0[3]
0	89.8[2]	0.0[3]
2	22.5	7.4
2	19.5	7.1
4	17.4	10.9
4	13.9	8.9
8	10.9	13.6
8	10.4	13.5
16	5.0	12.4
16	5.2	12.3
33[a]	2.4	12.7
33 [a]	2.2	12.2
64 [b]	0.8	6.9
64 [b]	0.7	6.9
104	0.4	3.4
104	0.7	3.5

[1] Residues are mean values of two solvent system (solvent system 1 and solvent system 5). As data in the two solvent systems are similar, mean values were calculated and used for kinetic analysis.
[2] Set to material balance
[3] Amounts of metabolites set to 0 at day 0

Beedon manor soil

Table 8.6.3.1-13: Kinetic models and statistics for soil Beedon manor, dose group F (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992) - Parent-only fits

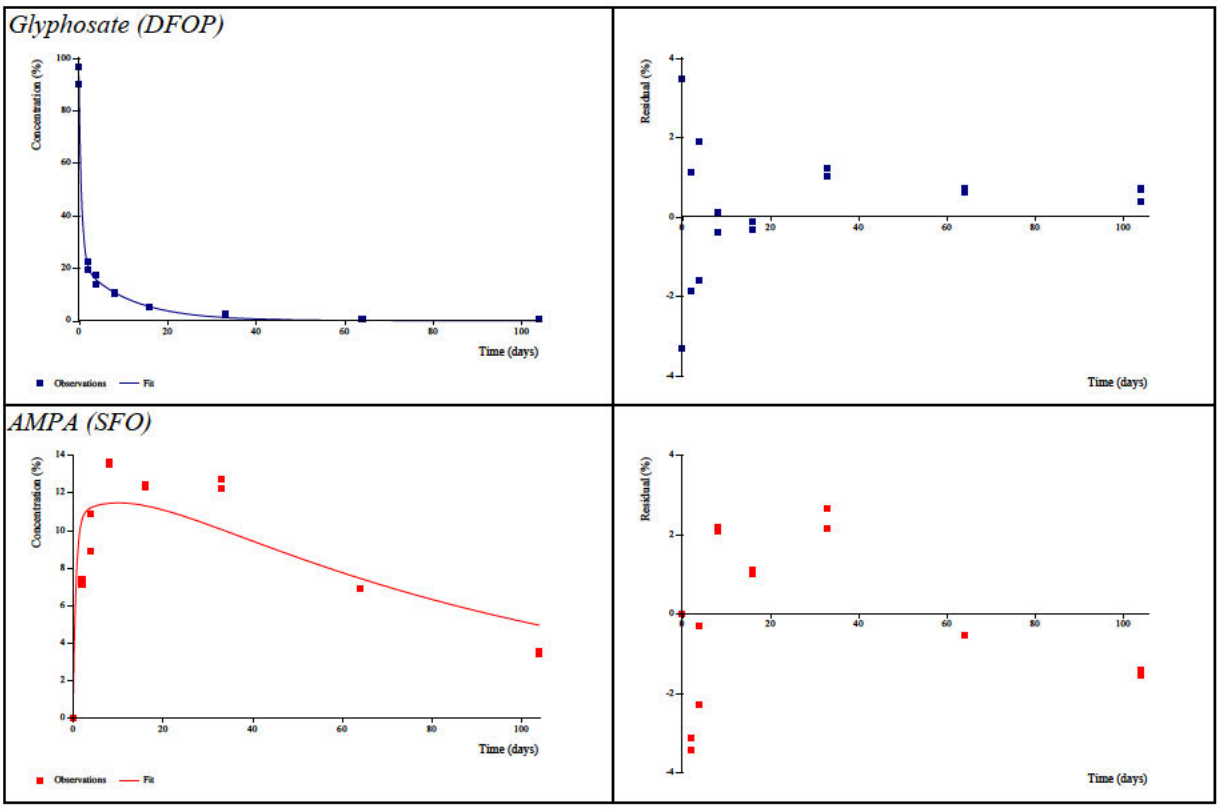
Kinetic model	**Visual assessment**	**M_0**	**Parameters**	**χ^2 error (%)**	**Prob > t (5 % level)**	**Lower CI (95 %)**	**Upper CI (95 %)**	**DT_{50} (d)**	**DT_{90} (d)**
SFO	Poor	92.3	k: 0.6228	22.8	k: <0.001	k: 0.4709	k: 0.7750	1.1	3.7
FOMC	Good	93.2	α: 0.7097 β: 0.3056	5.2	-[1]	β: 0.0640	β: 0.5470	0.5	7.5
DFOP	Good	93.2	k_1: 1.588 k_2: 0.0839 g: 0.7714	2.5	k_1: <0.001 k_2: <0.001	k_1: 0.7635 k_2: 0.0463	k_1: 2.412 k_2: 0.121	0.6	9.9
Applicant's conclusion	Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits but the DFOP model provides the lowest χ^2 error. **Conclusion:** DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints								
SFO									



[1] t-test not relevant for kinetic parameter β

Table 8.6.3.1-14: Kinetic models and statistics for soil Beedon manor, dose group F (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992) - Pathway fits (parent and metabolite)

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	93.1	k_1: 1.58 k_2: 0.0885 g: 0.7649	2.6	k_1: <0.001 k_2: <0.001	k_1: 0.7877 k_2: 0.0510	k_1: 2.3720 k_2: 0.1260	0.6	9.7	-
AMPA: SFO	Accep-table	-	k: 0.0103	16.4	k: <0.001	k: 0.0050	k: 0.016	67.3	224	0.149 (±0.011)
Applicant's conclusion	Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA									



APPENDIX 2: FIELD DATA - KINETIC FITTINGS FROM [redacted] 2020 AND [redacted] 2020B – SITES OR STUDIES NOT CONSIDERED AS RELIABLE BY RMS

Büchen

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)
0	0.0	-	100.00	0.00
7	2.0	0.0	96.94	10.61
14	4.9	2.9	84.73	15.51
28	11.8	9.8	66.71	17.37
61	27.5	25.5	34.42	22.26
91	46.6	44.6	27.45	35.83
121	67.7	65.7	12.12	14.93
182	103.7	101.7	13.59	28.24
240	120.8	118.8	9.39	23.09
322	131.4	129.4	8.04	14.76
475	198.8	196.8	7.89	23.81

Table 8.6.3.1-1: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

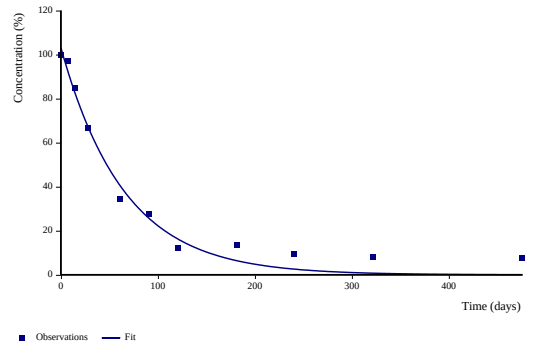
Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	103.0	k: 0.0153	10.1	k: <0.001	k: 0.0120	k: 0.0190	45.3	150
FOMC	Acceptable	105.8	α: 2.537 β: 127.7	8.8	-[1]	β: -78.58	β: 334	40.1	189
DFOP	Good	105.2	k1: 0.0190 k2: 3.38×10^{-12} g: 0.9264	6.6	k1: <0.001 k2: 0.5	k1: 0.0124 k2: -0.0061	k1: 0.0260 k2: 0.0060	40.7	187

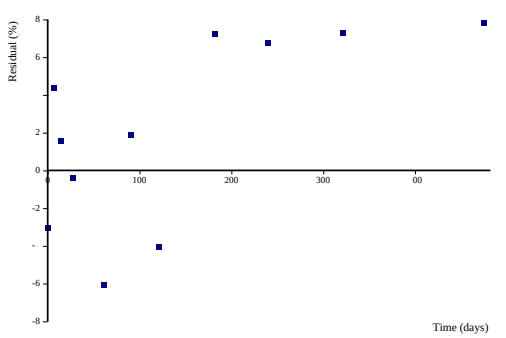
Applicant's conclusion

The SFO model fit is visually (residues well described until the DT_{90}) and statistically acceptable. FOMC and DFOP models were further tested, which led to better visual fit with smaller $\chi 2$ errors. The DFOP model provides the best visual fit and the lowest $\chi 2$ error. The parameter k2 of the DFOP model is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k1 as indicated by a high value for parameter g (0.9264).

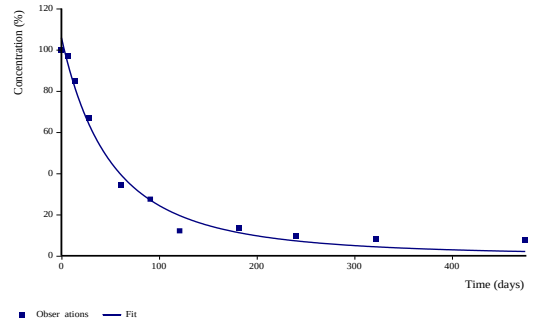
Conclusion: DFOP to be used in pathway fit for trigger endpoints

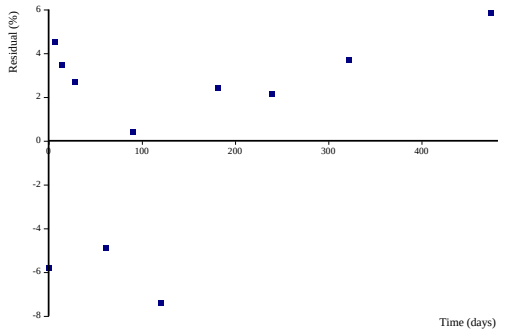
SFO



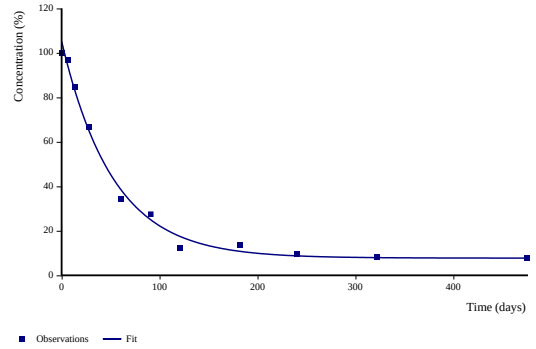


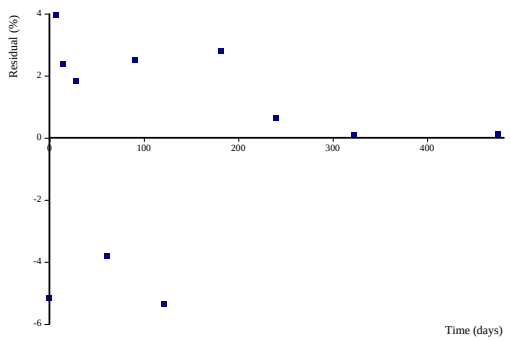
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

Table 8.6.3.1-2: Kinetic models and goodness-of-fit statistics of pathway fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	105.4	k1: 0.0193 k2: 3.93×10-9 g: 0.9245	6.6	k1: <0.05. k2: 0.5	k1: 0.0136 k2: -0.0051	k1: 0.0250 k2: 0.0050	40.3	188
AMPA: SFO	Poor	-	k: 0.0019	26.3	k: 0.0623	k: -0.0006	k: 0.0040	365	1213

Applicant's conclusion

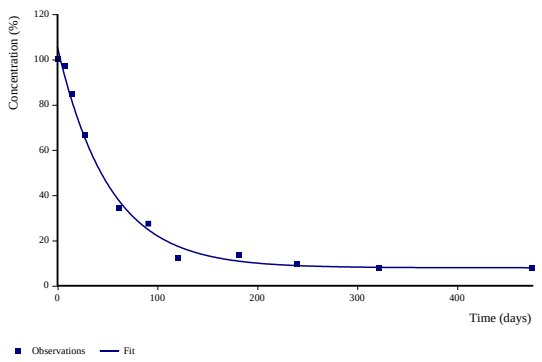
The dissipation of glyphosate is well described in the pathway fit with the DFOP model. For AMPA, due to the wide scatter of residue data, the SFO model does not adequately describe the formation and decline of the metabolite. A decline fit for AMPA was not performed, as there is no clear decline phase.

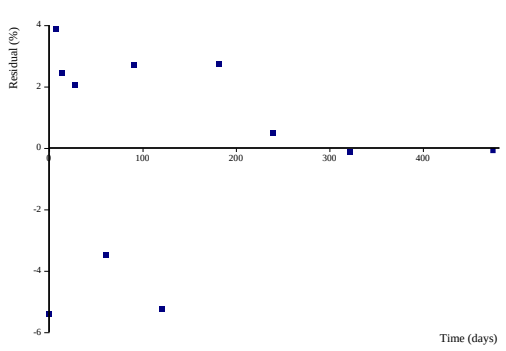
Conclusion:

Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate

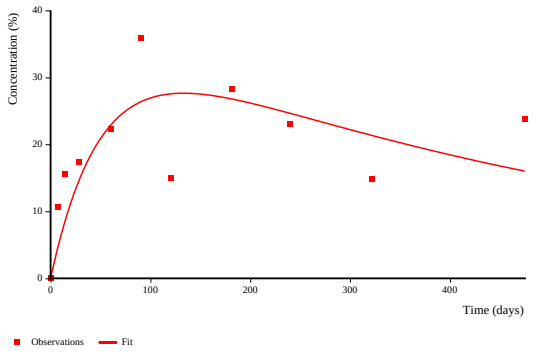
No reliable trigger endpoints for AMPA can be determined

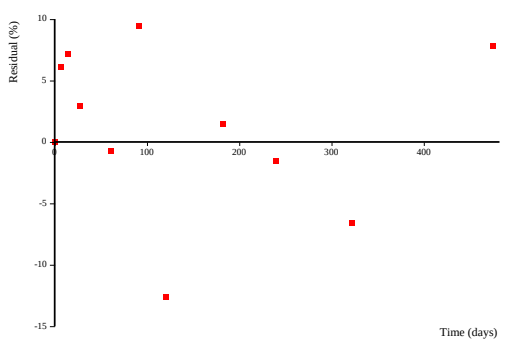
Glyphosate (DFOP)





AMPA (SFO)





Determination of modelling endpoints

Table 8.6.3.1-3: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	92.6	k: 0.0301	12.9	k: <0.05	k: 0.0213	k: 0.0390	23.0	76.5

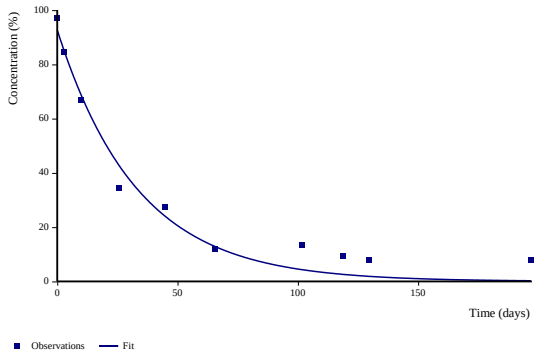
Table 8.6.3.1-3: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

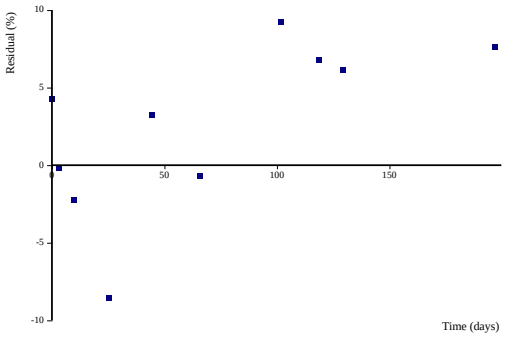
Applicant's comment

Visually, the SFO model describes the degradation of glyphosate well until the DT_{90}. Statistically the fit is acceptable (χ2 error <15 % and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO





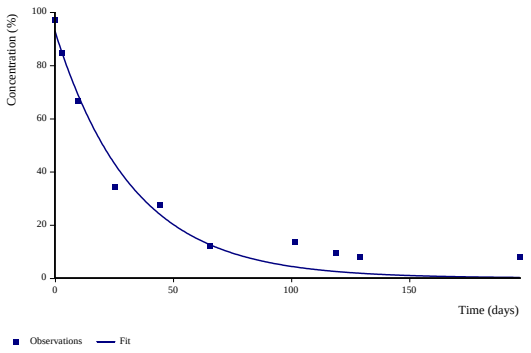
1 Representing $DegT_{50}$ matrix according to EFSA (2014)

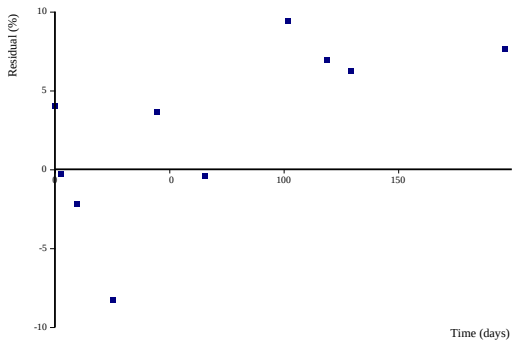
Table 8.6.3.1-4: Kinetic models and goodness-of-fit statistics of pathway fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic Model	Visual assess-ment	M0	Kinetic para-meters	χ2 error (%)	Prob > t (5 % level)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Accep-table	92.9	k: 0.0305	12.9	k: <0.001	22.7	75.5	-
AMPA: SFO	Poor	13.0	k: 0.0031	24.0	k: 0.1613	225	747	0.2085 (±0.125)

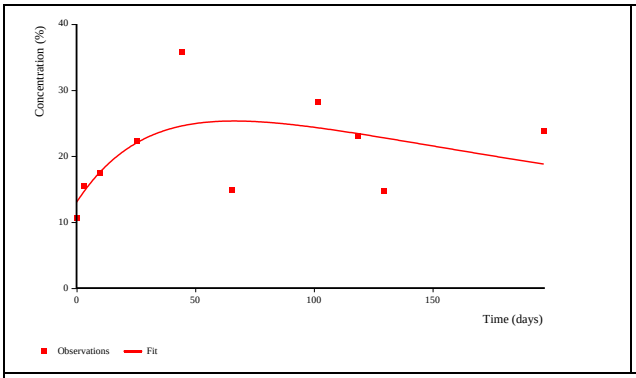
Visually, the SFO model in the pathway fit describes the degradation of glyphosate well until the DT_{90}. Statistically the fit is acceptable (χ2 error <15 % and the estimated degradation rate is reliable).
For AMPA, the SFO model does not describe the data well visually or statistically, mainly due to the large scatter of residue data and no clear decline phase.
Conclusion: Parent-only SFO fit to be used for deriving modelling endpoints for glyphosate
No reliable modelling endpoints for AMPA can be determined

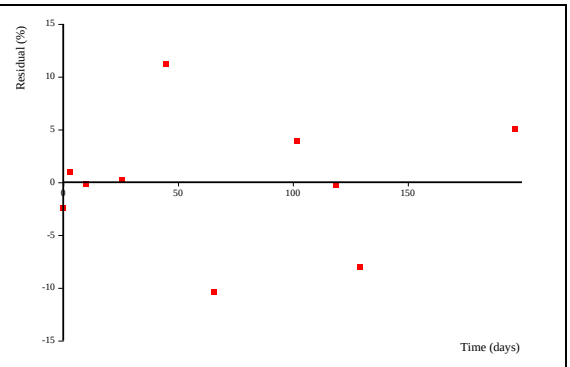
Glyphosate (SFO)





AMPA (SFO)





1 Representing DegT$_{50}$ matrix according to EFSA (2014)

Klein-Zecher

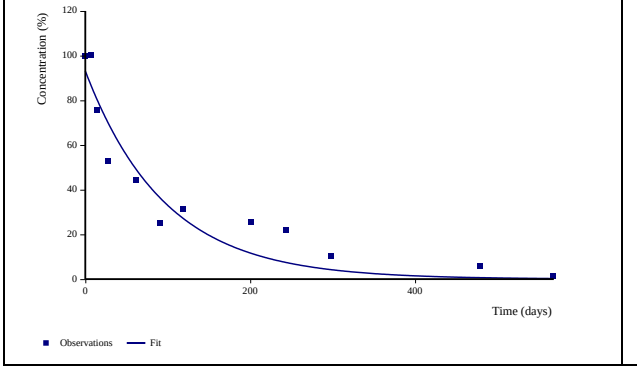
DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0.0	-	100.00	0.00
7	6.2	0.0	100.10	22.16
14	10.7	4.5	75.69	25.03
28	20.9	14.7	52.74	24.64
61	36.6	30.4	44.16	34.94
91	47.4	41.3	25.28	27.42
119	53.7	47.5	31.26	30.83
201	64.4	58.2	25.55	38.06
244	76.7	70.5	22.15	36.15
298	94.0	87.8	10.36	29.89
479	196.1	190.0	6.09	35.51
567	211.1	205.0	1.42	26.80

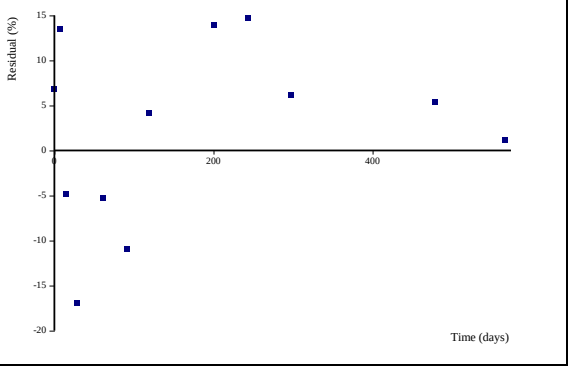
Table 8.6.3.1-5: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT$_{50}$ (d)	DT$_{90}$ (d)
SFO	Poor	93.1	k: 0.0104	19.5	k: <0.05	k: 0.0061	k: 0.0150	66.8	222
FOMC	Good	104.9	α: 0.912 β: 34.66	12.6	-1	β: -11.64	β: 80.97	39.5	398
DFOP	Good	105.8	k1: 0.0411 k2: 0.0038 g: 0.583	11.5	k1: <0.05 k2: <0.05	k1: 0.0038 k2: 0.0004	k1: 0.0780 k2: 0.0070	35.5	378

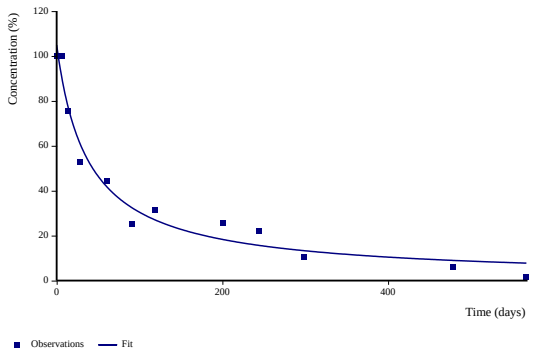
The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best fit for the whole study duration, with small residuals scattered randomly about zero.
Conclusion: DFOP to be used in pathway fit for trigger endpoints

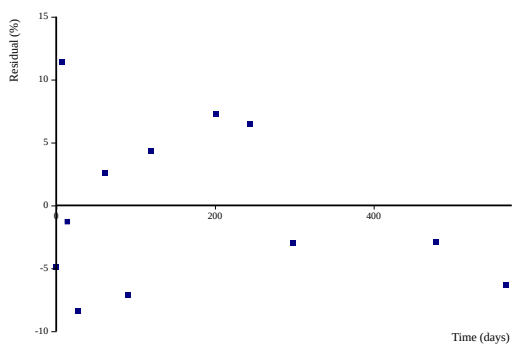
SFO



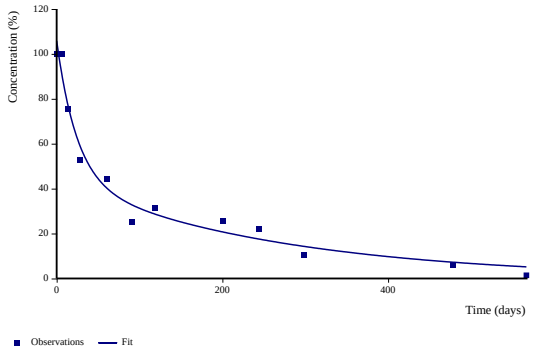


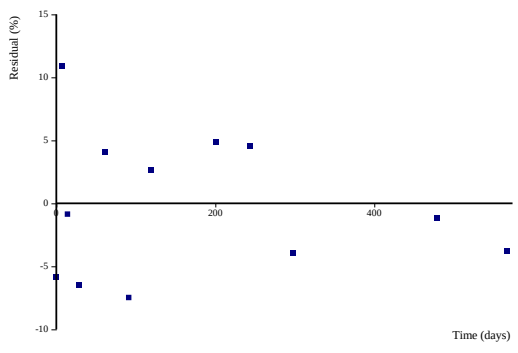
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

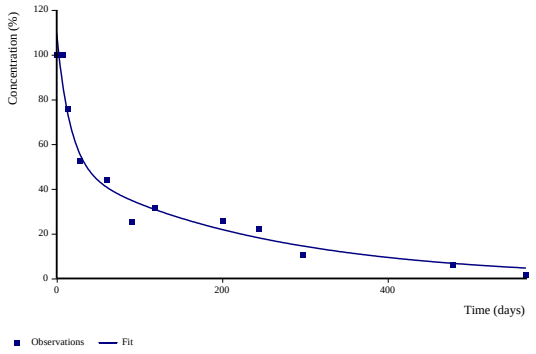
Table 8.6.3.1-6: Kinetic models and goodness-of-fit statistics of pathway fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

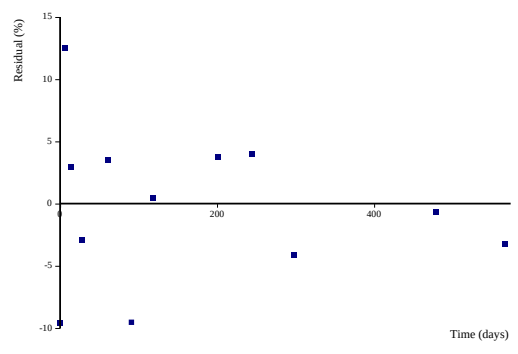
Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	109.6	k1: 0.0614 k2: 0.0042 g: 0.5368	12.7	k1<0.05 k2<0.05	k1: 0.0188 k2: 0.0015	k1: 0.1040 k2: 0.0070	29.1	364
AMPA: SFO	Accep-table	-	k: 0.0013	13.9	k<0.05	k: 0.0003	k: 0.0020	521	>1000

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The residuals are randomly scattered about zero and are relatively small.

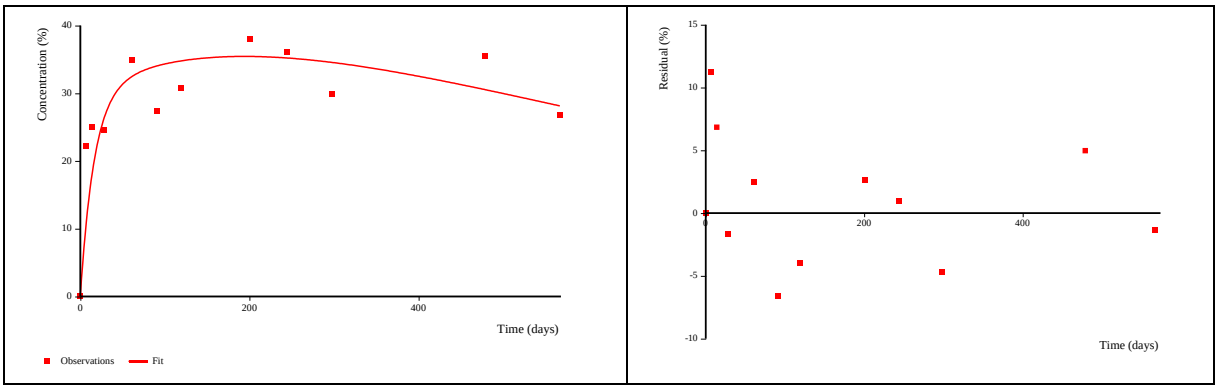
Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate (DFOP)



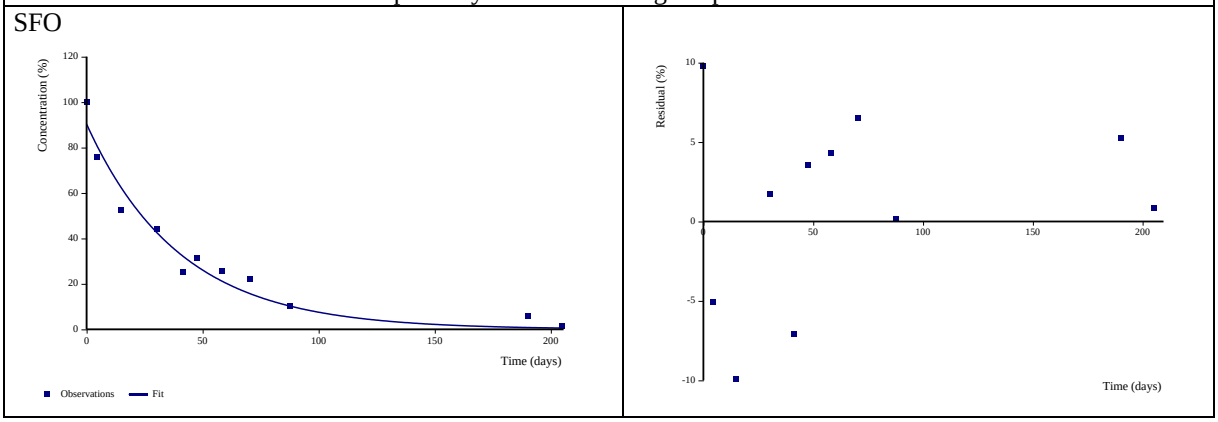


AMPA (SFO)



Determination of modelling endpoints

Table 8.6.3.1-7: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	90.3	k: 0.0249	13.1	k: <0.001	k: 0.0191	k: 0.0310	27.9	92.7
Visually, given the scatter in the residue data, the SFO model acceptably describes the degradation of glyphosate, with generally small residuals. Statistically the fit is also acceptable (χ2 error <15 % and the estimated degradation rate is reliable). Conclusion: SFO to be used in pathway fit for modelling endpoints									
SFO 									

1 Representing DegT_{50} matrix according to EFSA (2014)

Table 8.6.3.1-8: Kinetic models and goodness-of-fit statistics of pathway fits for soil Klein-Zecher of study [redacted] (1992) – modelling endpoints

Kinetic Model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting								
Glyphosate: SFO	Accep-table	90.3	k: 0.0248	13.1	k: <0.05	27.9	92.7	-
AMPA: SFO	Accep-table	22.2	k: 0.0015	9.6	k: 0.07	471	>1000	0.1984 (±0.070)
Repeated fitting: AMPA ff fixed to previously estimated value								
Glyphosate: SFO	Accep-table	90.3	k: 0.0248	13.1	k: <0.05	27.9	92.7	-
AMPA: SFO	Accep-table	22.2	k: 0.0015	9.2	k< 0.05	471	>1000	fixed to: 0.1984

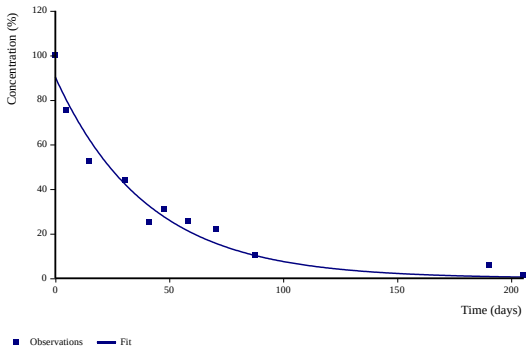
Initial fitting: The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. For AMPA, the t-test is not acceptable for parameter kAMPA. However, the estimated formation fraction is reliable with a low standard deviation. Therefore, the fitting was repeated with fixing ff for AMPA to the estimated value (0.1984).

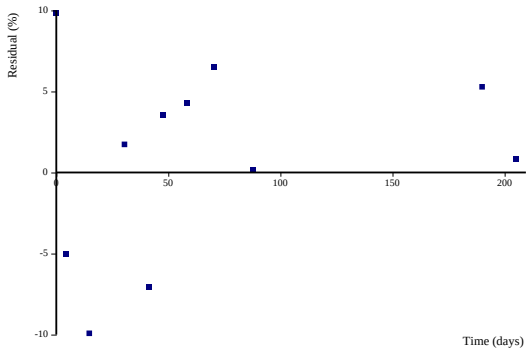
Repeated fitting: With the formation fraction fixed, the statistical fit is improved slightly for AMPA; the parameter is significantly different from zero.

Conclusion: SFO-SFO (repeated fitting) to be used for deriving modelling endpoints for glyphosate and AMPA

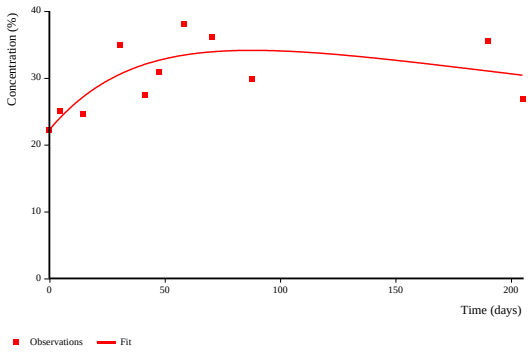
Repeated fitting: AMPA ff fixed to previously estimated value

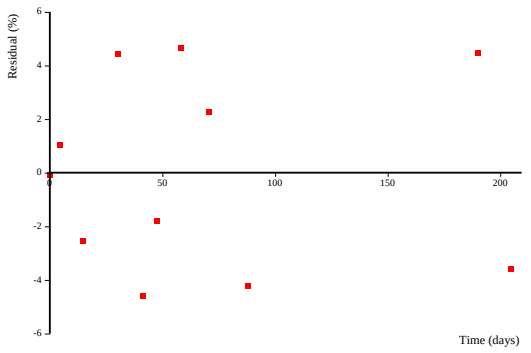
Glyphosate (SFO)





AMPA (SFO)





1 Representing DegT$_{50}$ matrix according to EFSA (2014)

Unzhurst

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0.0	-	100.00	0.00
7	4.5	0.0	74.61	10.63
13	8.0	3.5	71.12	13.72
27	16.5	12.0	55.36	11.91
57	38.6	34.2	20.52	23.92
90	68.4	64.0	15.22	26.91
117	95.4	91.0	9.98	23.63
187	132.9	128.5	7.22	23.04
251	146.7	142.2	7.05	26.98
314	155.8	151.3	6.30	24.21
418	201.7	197.3	4.26	18.74

Table 8.6.3.1-9: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT$_{50}$ (d)	DT$_{90}$ (d)
SFO	Poor	95.2	k: 0.0225	11.8	k: <0.05	k: 0.0170	k: 0.0280	30.8	102

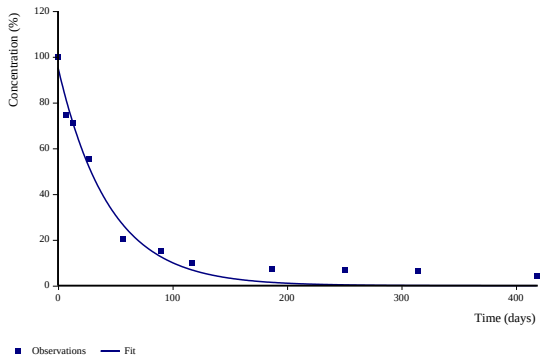
FOMC	Acceptable	99.0	α: 2.025 β: 63.96	9.8	-1	β: -19.43	β: 147.4	26.1	135
DFOP	Good	97.3	k1: 0.0281 k2: 0.0009 g: 0.9214	8.4	k1: <0.05 k2: 0.3958	k1: 0.0170 k2: -0.0069	k1: 0.0390 k2: 0.0090	27.7	122

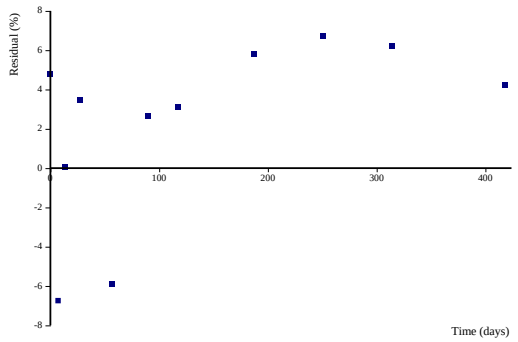
Applicant's conclusion:

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best fit for the whole study duration, with small residuals scattered randomly about zero. The parameter k2 of the DFOP model is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k1 as indicated by a high value for parameter g (0.9214).

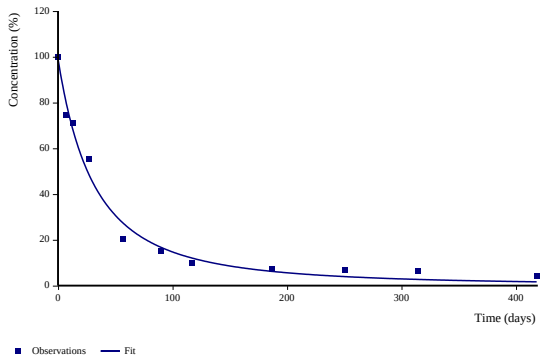
Conclusion: DFOP to be used in pathway fit for trigger endpoints

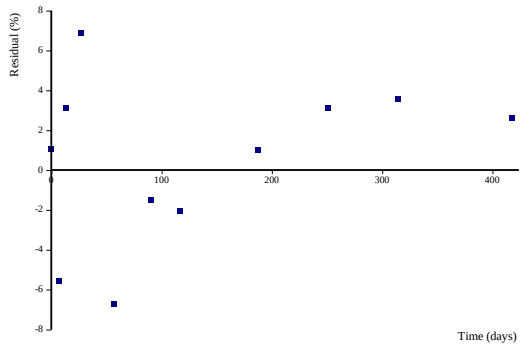
SFO



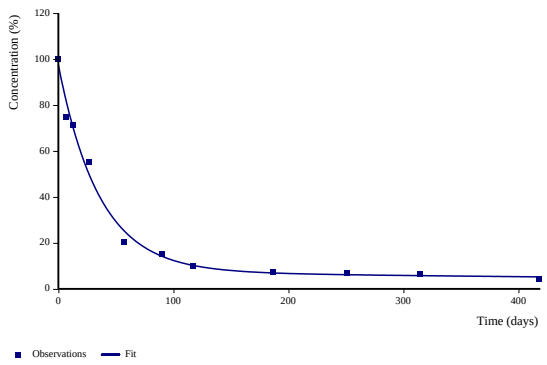


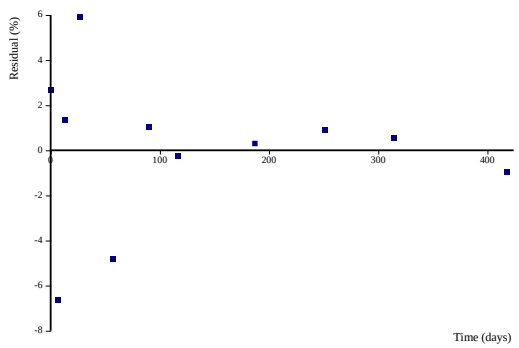
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

Table 8.6.3.1-10: Kinetic models and goodness-of-fit statistics of pathway fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

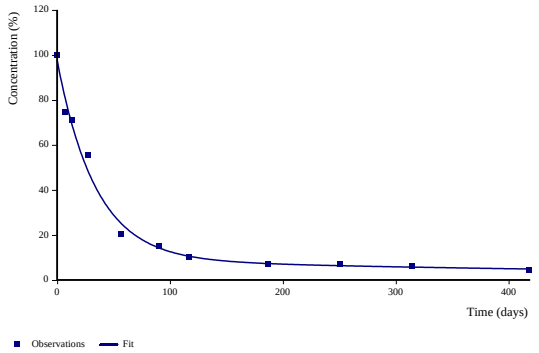
Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	97.8	k1: 0.0294 k2: 0.0015 g: 0.9061	8.5	k1: <0.05. k2: 0.3093	k1: 0.0197 k2: -0.0048	k1: 0.039 k2: 0.0080	27.0	126
AMPA: SFO	Good	-	k: 0.0011	11.9	k: <0.05	k: -2.6×10-6	k: 0.0020	634	>1000

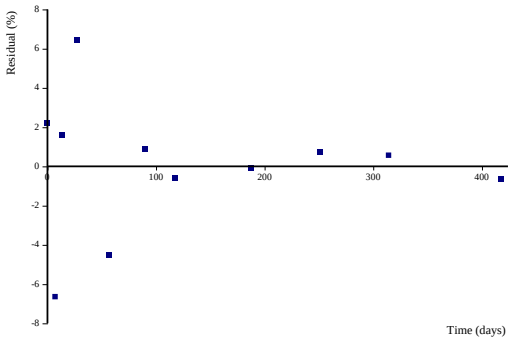
Applicant's conclusion

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. For AMPA, the SFO model provides an acceptable visual fit given the scatter in the data.

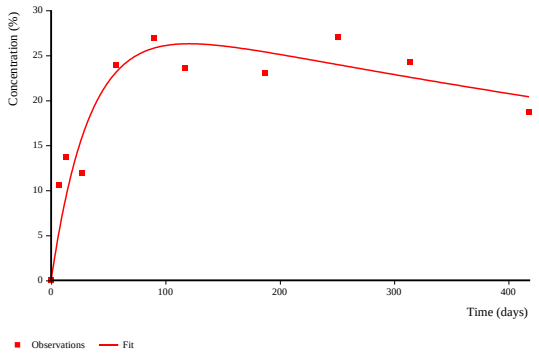
Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

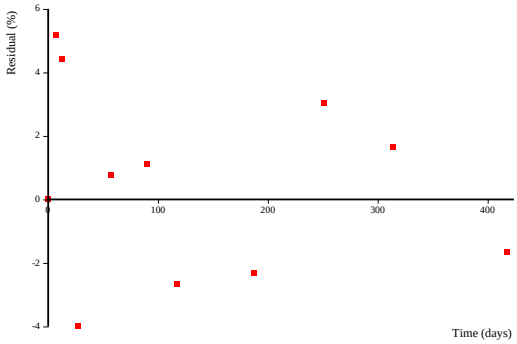
Glyphosate (DFOP)





AMPA (SFO)





Determination of modelling endpoints

Table 8.6.3.1-11: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	75.3	k: 0.0274	13.4	k: <0.001	k: 0.0192	k: 0.0360	25.3	84.1

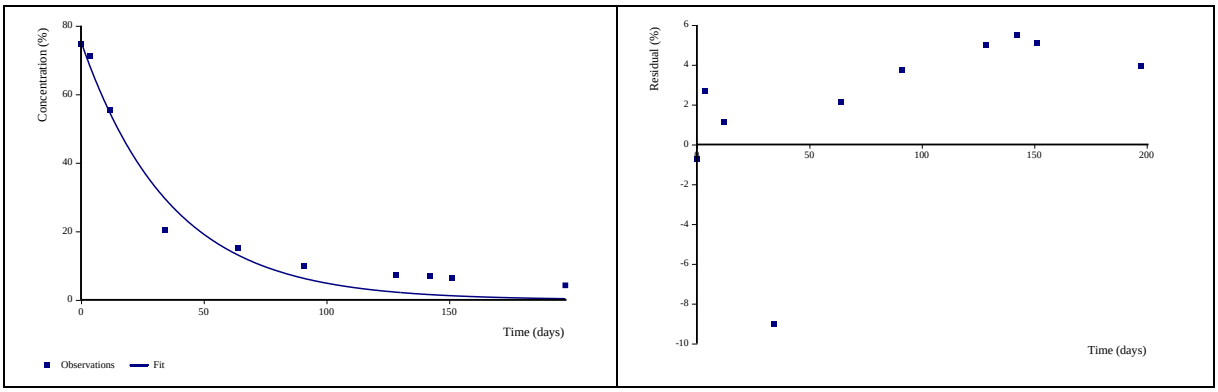
Applicant's conclusion

Visually, the SFO model acceptably describes the degradation of glyphosate. The model does overestimate degradation from 64 days, but the DT_{50} and M_0 are well represented and the residuals are small. Statistically the fit is also acceptable (χ2 error <15 % and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO	

Table 8.6.3.1-11: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints



1 Representing DegT_{50} matrix according to EFSA (2014)

Table 8.6.3.1-12: Kinetic models and goodness-of-fit statistics of pathway fits for soil Unzhurst of study [redacted] (1992) – modelling endpoints

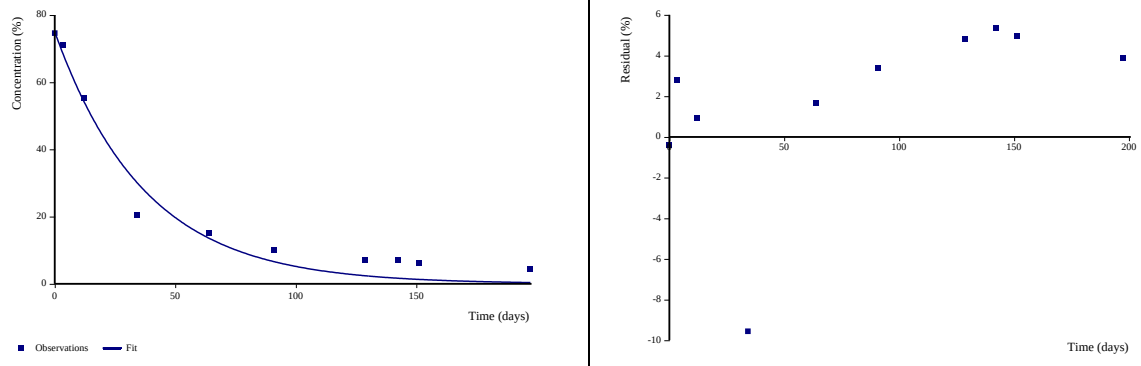
Kinetic Model	Visual assess-ment	M0	Kinetic para-meters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Accep-table	75.0	k: 0.0267	13.4	k: <0.001	k: 0.0193	k 0.0340	25.9	86.2	-
AMPA: SFO	Accep-table	9.9	k: 0.0029	8.9	k: 0.0107	k: 0.0005	k: 0.0050	238	789	0.3192 (±0.068)

Applicant's conclusion

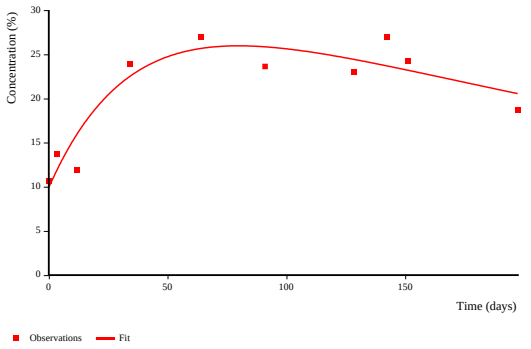
The SFO model acceptably describes the degradation of glyphosate. The model does overestimate degradation from 64 days, but the DT_{50} and M_0 are well represented and the residuals are generally small. Statistically, the χ2 error <15 % and the estimated degradation rate is reliable. For AMPA, the SFO model well describes the data visually and statistically.

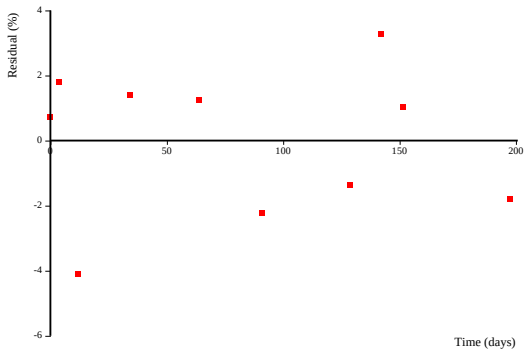
Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

Glyphosate (SFO)



AMPA (SFO)





[1] Representing $DegT_{50}$ matrix according to EFSA (2014)

Rohrbach

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0.0	-	100.00	0.00
7	8.0	-	117.47	22.36
14	15.4	-	89.68	30.53
28	26.8	-	59.67	29.28
56	45.8	0.0	19.20	43.65
85	60.8	15.0	8.66	41.86
231	88.8	43.0	1.53	37.58
282	105.4	59.6		34.41
418	204.0	158.2		18.60
582	246.0	200.2		15.61

Table 8.6.3.1-13: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

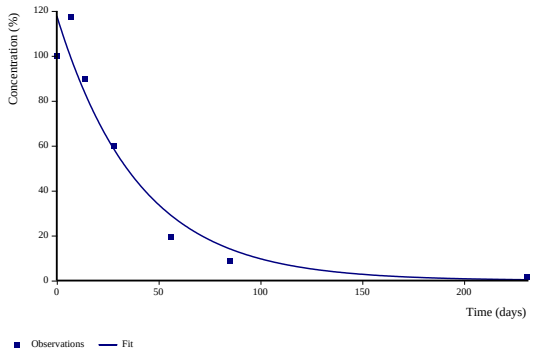
Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	117.8	k: 0.0250	15.4	k<0.05	k: 0.0109	k: 0.0390	27.8	92.2
FOMC	Acceptable	126.8	α: 5940 β: 169000	16.6	-1	β: 152000	β: 185000	19.7	65.3
DFOP	Not calculated								

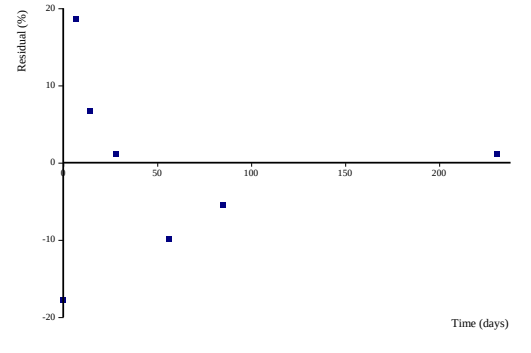
Applicant's conclusion

The SFO model provides an acceptable visual and statistical fit. The biphasic FOMC model does not improve the fit.

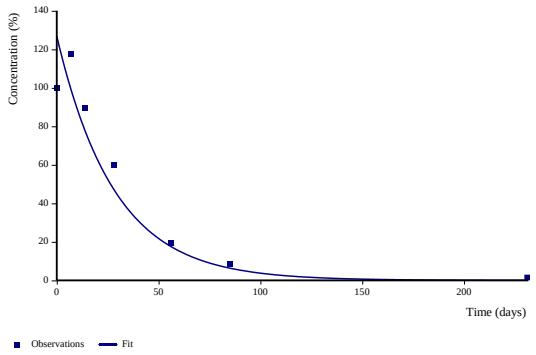
Conclusion: SFO to be used in pathway fit for trigger endpoints

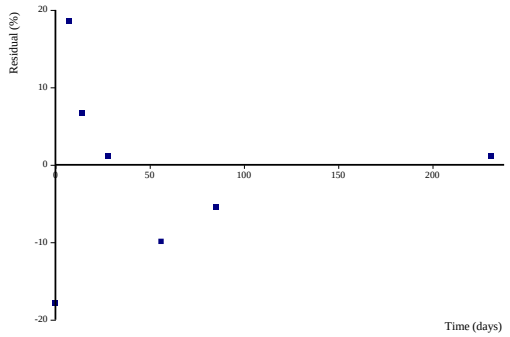
SFO





Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
FOMC									





1 t-test not relevant for kinetic parameter β

Table 8.6.3.1-14: Kinetic models and goodness-of-fit statistics of pathway fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

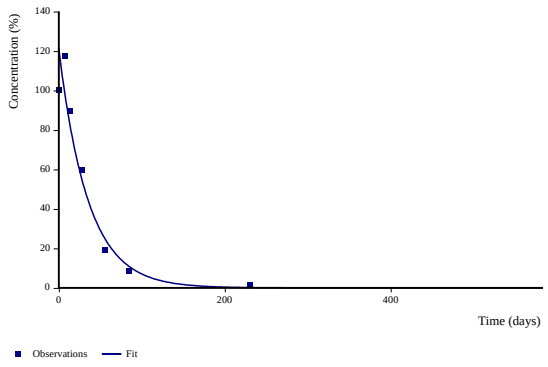
Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: SFO	Accep-table	121.3	k: 0.0284	16.0	k<0.05	k: 0.01632	k: 0.0410	24.4	81.0
AMPA: SFO	Accep-table	-	k: 0.0027	15.5	k<0.05	k: 0.0012	k: 0.0040	255	847

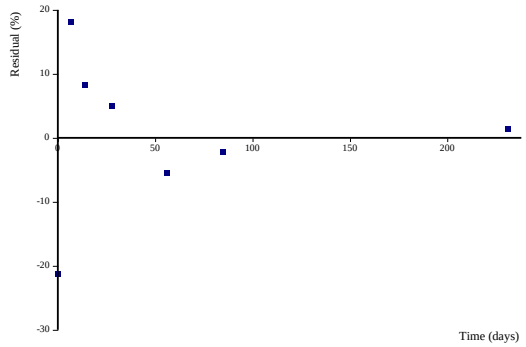
Applicant's conclusion

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The estimated parameters are statistically reliable.

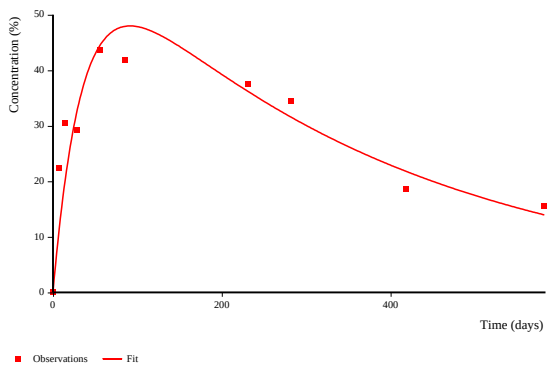
Conclusion: SFO-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

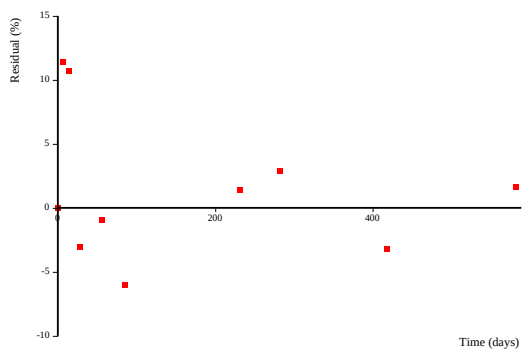
Glyphosate (SFO)





AMPA (SFO)





Determination of modelling endpoints

Table 8.6.3.1-15: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

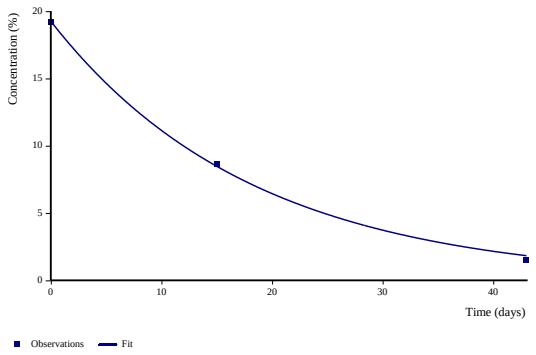
Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Good	19.3	k: 0.0547	1.9	k: 0.0151	k: 0.0217	k: 0.088	12.7	42.1

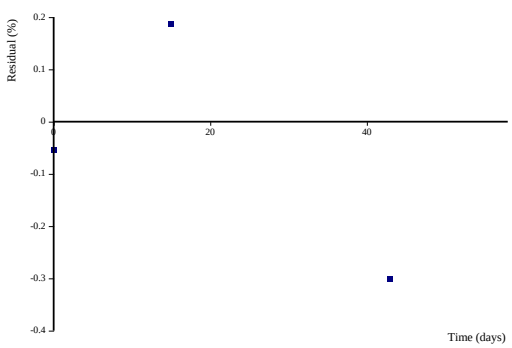
Applicant's conclusion

Visually, the SFO model describes the degradation of glyphosate very well for the remaining datapoints after those prior to 10 mm rainfall have been removed. Statistically the fit is also acceptable (χ2 error is low and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO





1 Representing DegT_{50} matrix according to EFSA (2014)

Table 8.6.3.1-16: Kinetic models and goodness-of-fit statistics of pathway fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic Model	Visual assess-ment	M0	Kinetic para-meters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Good	19.3	k: 0.0546	1.9	k: <0.001	k: 0.0484	k 0.0610	12.7	42.2	-
AMPA: SFO	Good	43.5	k: 0.0058	1.2	k: <0.001	k: 0.0052	k: 0.0060	119	394	0.2399 (±0.060)

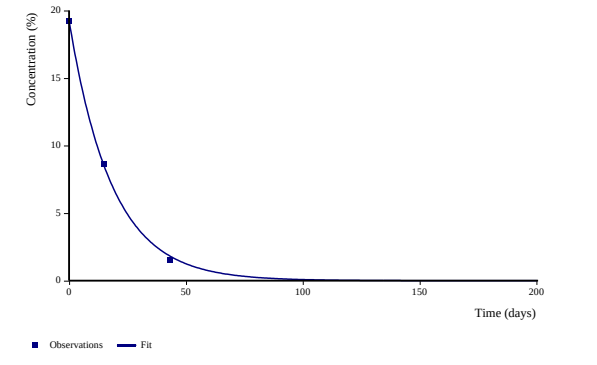
Applicant's conclusion

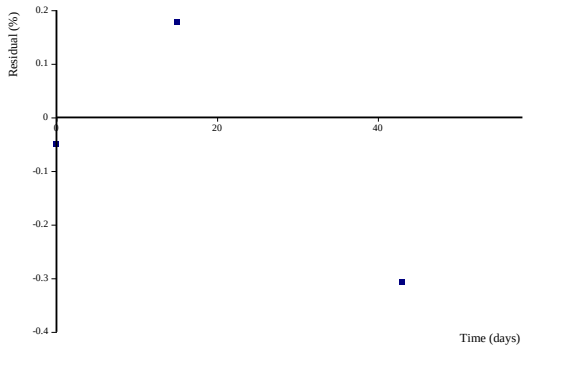
The degradation of glyphosate is well described by the SFO model for datapoints remaining after the 10 mm rain cutoff. The χ2 error value is very low and the estimated degradation rate is significantly different from zero.

For AMPA, the SFO model describes the data very well visually and statistically. Although the metabolite formation phase was not completely included, the estimated parameters are reliable as the metabolite decline occurred after the parent compound has mostly dissipated and, thus, the metabolite degradation rate was estimated independently.

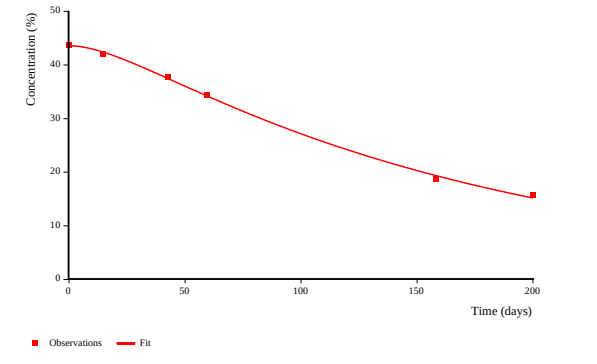
Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

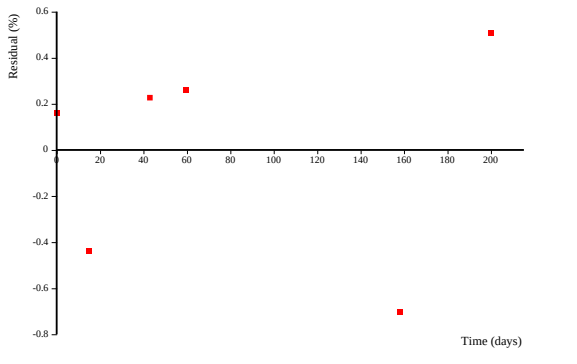
Glyphosate (SFO)





AMPA (SFO)





1 Representing $DegT_{50}$ matrix according to EFSA (2014)

Herrngiersdorf

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0.0	-	100.00	0.00
6	3.7	0.0	95.89	26.44
13	8.1	4.3	72.53	21.64
28	16.2	12.5	71.60	30.46
58	37.9	34.2	23.08	30.44
90	63.8	60.1	14.61	30.47
125	91.3	87.6	8.81	27.93
168	111.4	107.6	1.96	20.11
330	136.7	132.9		20.91
464	217.4	213.7		9.64

Table 8.6.3.1-17: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	103.5	k: 0.0206	10.6	k: <0.05	k: 0.0139	k: 0.0270	33.7	112
FOMC	Acceptable	103.5	α: 605.7 β: 29400	11.3	-1	β: 2700	β: 56100	33.7	112
DFOP	Not calculated								

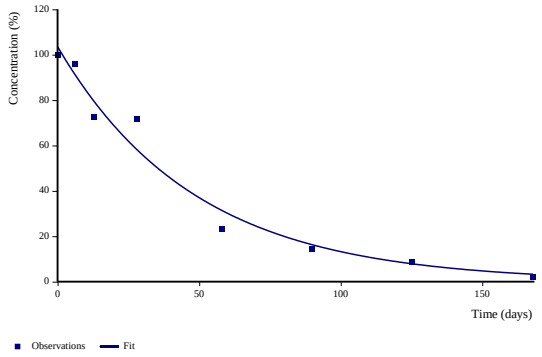
Applicant's conclusion

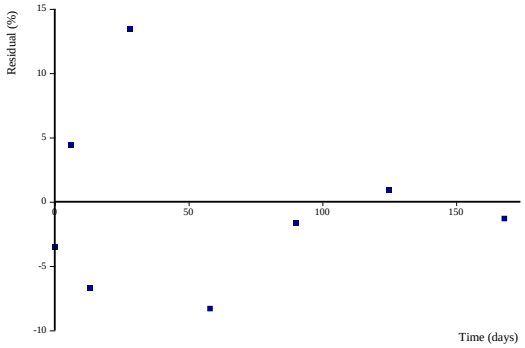
The SFO model provides an acceptable visual and statistical fit. The biphasic FOMC model does not improve the fit.

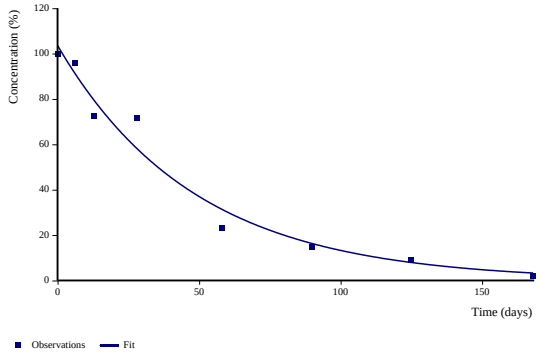
Conclusion: SFO to be used in pathway fit for trigger endpoints

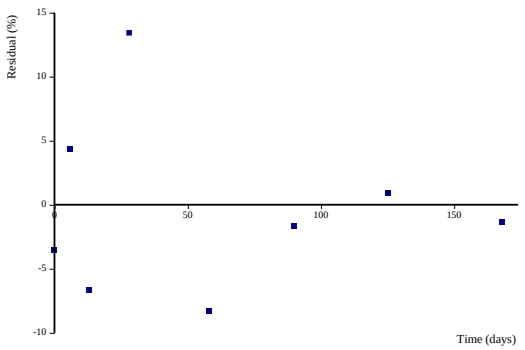
SFO	

Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
FOMC									









1 t-test not relevant for kinetic parameter β

Table 8.6.3.1-18: Kinetic models and goodness-of-fit statistics of pathway fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Pathway fit									
Glyphosate: SFO	Accep-table	104.4	k: 0.0214	10.6	k: <0.001	k: 0.0152	k: 0.0280	32.4	108
AMPA: SFO	Poor	-	k: 0.0060	29.4	k: 0.0268	k: -0.0001	k: 0.0120	115	381
Decline fit for AMPA									
AMPA: SFO	Accep-table	29.4	k: 0.0024	11.0	0.0251	k: -2.01×10-6	k: 0.0050	288	958
AMPA: FOMC	Accep-table	29.4	α: 33.39 β: 13700	12.6	-[1]	β: -3.47×104	β: 6.21×104	288	980

Applicant's conclusion

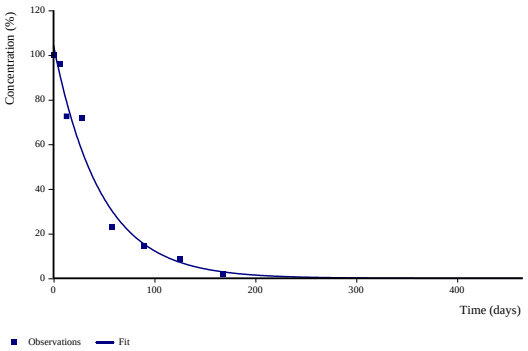
Pathway fit: The dissipation of glyphosate is well described by the SFO model in the pathway fit. For AMPA, the SFO model does not adequately fit the data due to the scatter in the data during the decline phase. Hence, a decline fit was performed for AMPA.

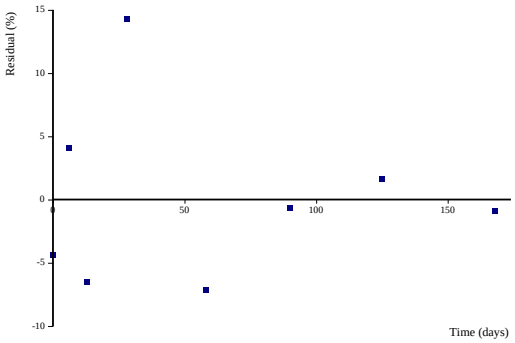
Decline fit for AMPA: The SFO model provides a visually and statistically acceptable fit. The $\chi 2$ error above 15 % is considered acceptable as it results from the scattering of the data. The FOMC model does not improve the fit.

Conclusion: Parent-only SFO fit to be used for deriving trigger endpoints for glyphosate
Decline fit (SFO) to be used for deriving trigger endpoints for AMPA

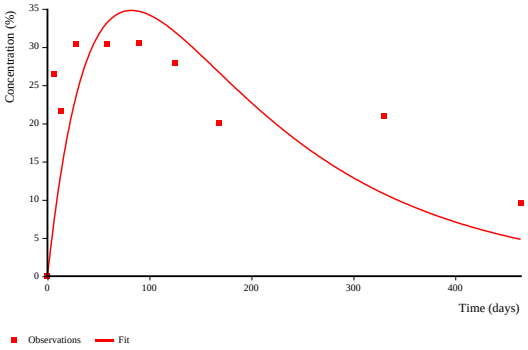
Pathway fit (SFO-SFO)

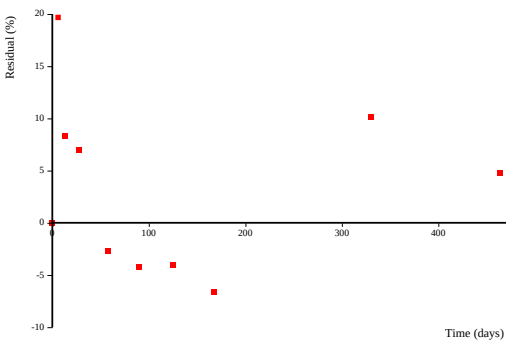
Glyphosate (SFO)





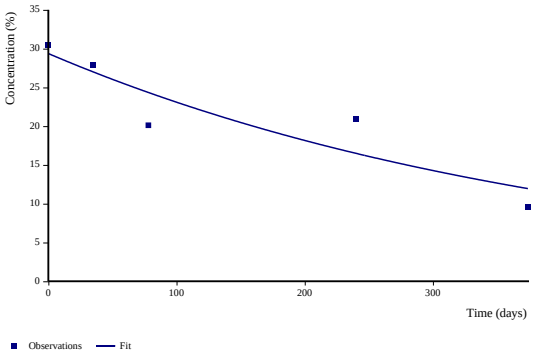
AMPA (SFO)

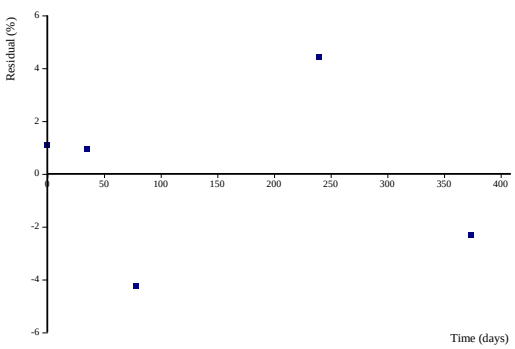




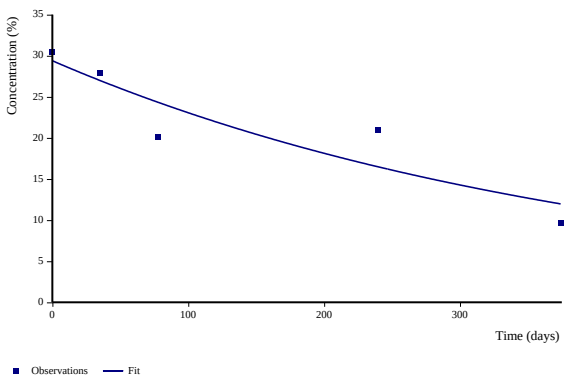
Decline fit for AMPA

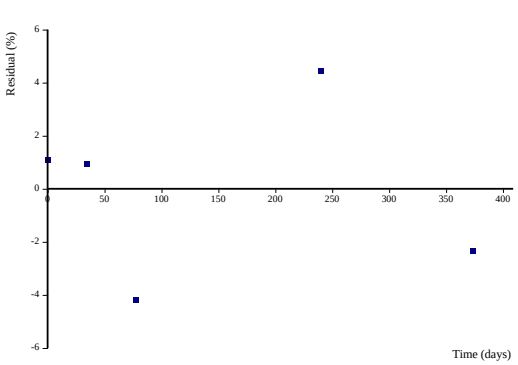
AMPA (SFO)





AMPA (FOMC)





1 t-test not relevant for kinetic parameter β

Determination of modelling endpoints

Table 8.6.3.1-19: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

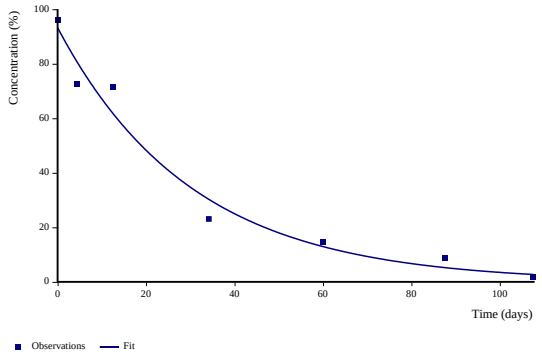
Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Good	93.2	k: 0.0329	11.3	k: <0.05	k: 0.0202	k: 0.0460	21.1	70.0

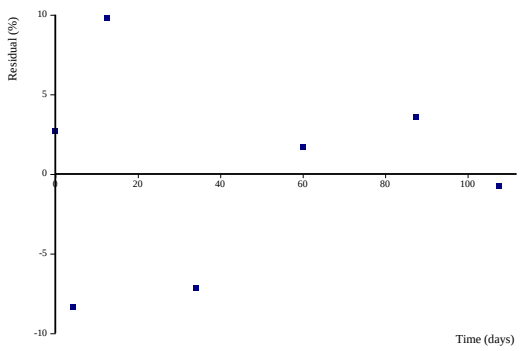
Applicant's conclusion

The SFO model describes the degradation of glyphosate well. M0 is well represented and residuals are generally small and randomly scattered about zero. Statistically the fit is also acceptable ($\chi 2$ error <15 % and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO





1 Representing $DegT_{50}$ matrix according to EFSA (2014)

Table 8.6.3.1-20: Kinetic models and goodness-of-fit statistics of pathway fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

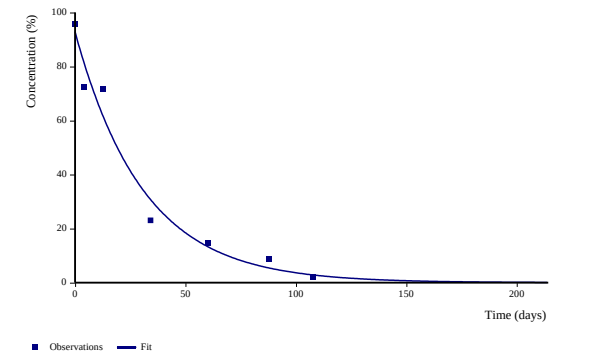
Kinetic Model	Visual assess-ment	M0	Kinetic para-meters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Good	92.9	k: 0.0323	11.4	k: <0.001	k: 0.0216	k 0.0430	21.5	71.2	-
AMPA: SFO	Good	23.6	k: 0.0076	7.8	k: <0.001	k: 0.0042	k: 0.0110	90.7	301	0.2508 (±0.072)

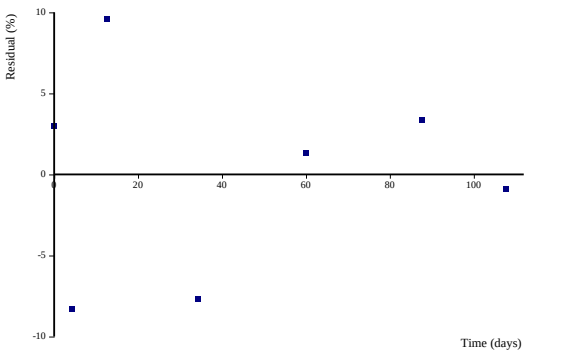
Applicant's conclusion

The degradation of glyphosate is described well both visually and statistically by the SFO model. Similarly, for AMPA, the SFO model describes the data very well visually and statistically. Although the metabolite formation phase was not completely included, the estimated parameters are reliable as the metabolite decline occurred after the parent compound has mostly dissipated and, thus, the metabolite degradation rate was estimated independently.

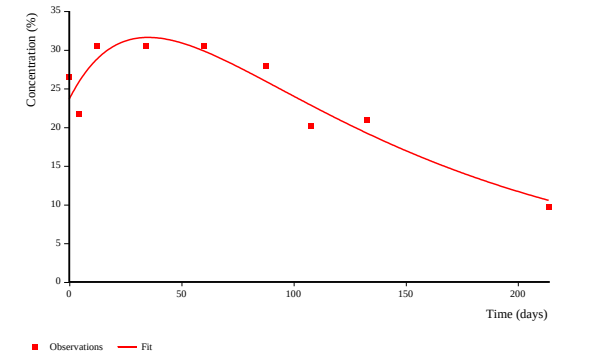
Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

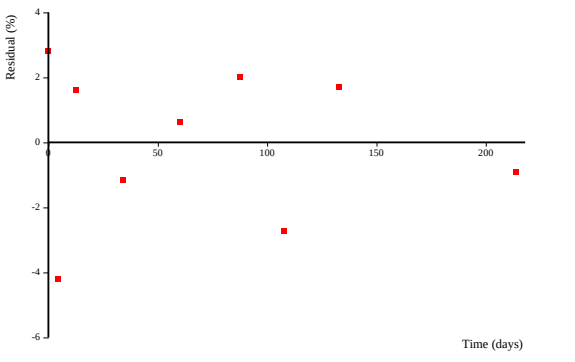
Glyphosate (SFO)	





AMPA (SFO)





1 Representing $DegT_{50}$ matrix according to EFSA (2014)

Wang-Inzkofen

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
0	0	-	100.00	0.00
7	4.3	0.0	71.03	29.03
15	9.8	5.5	48.50	33.71
29	20.2	15.9	46.22	41.12
58	44.0	39.7	23.10	32.67
94	61.9	57.6	13.60	33.39
114	70.3	66.0	12.24	33.96
275	93.3	89.0	7.93	30.06
414	173.2	168.9	4.88	23.55
549	216.2	211.9	1.32	17.82

Table 8.6.3.1-21: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

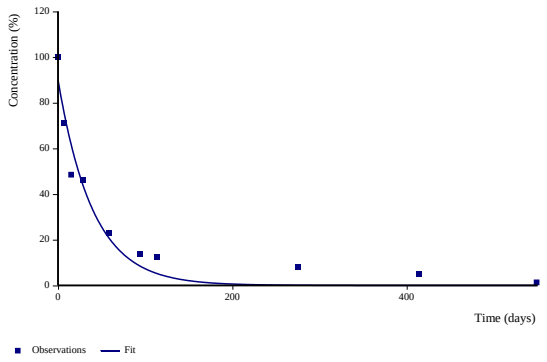
Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.0	k: 0.0251	16.8	k: <0.001	0.0156	k: 0.0350	27.6	91.7
FOMC	Good	99.4	α: 0.9749 β: 17.22	8.7	-[1]	β: 2.1815	β: 32.26	17.8	166
DFOP	Poor	100.3	k1: 0.1543 k2: 0.0148 g: 0.3839	10.3	k1: 0.0817 k2: 0.0039	k1: -0.0835 k2: -0.0056	k1: 0.3920 k2: 0.0240	17.6	123

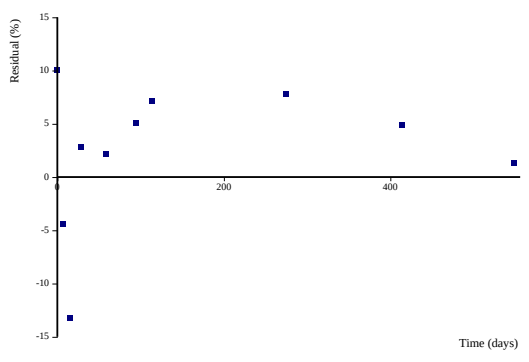
Applicant's conclusion

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The FOMC model provides the best visual and statistical fit.

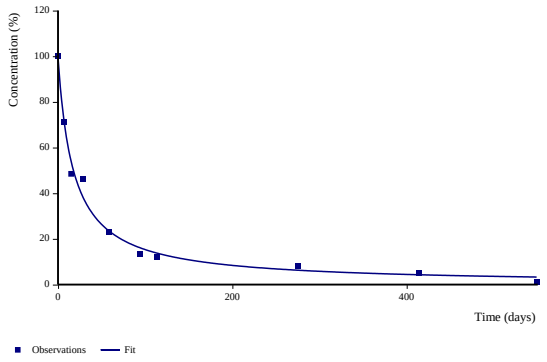
Conclusion: FOMC to be used in pathway fit for trigger endpoints

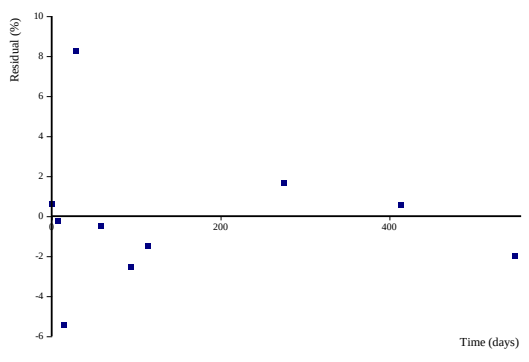
SFO



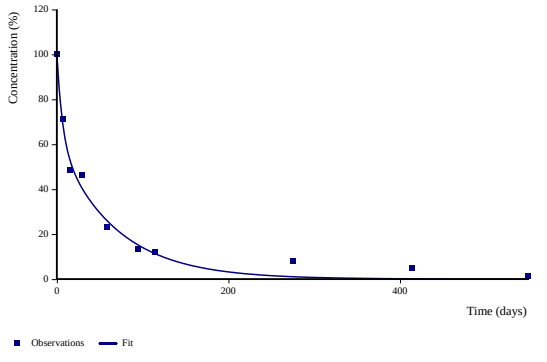


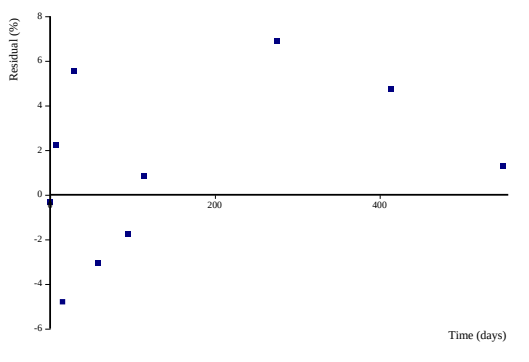
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

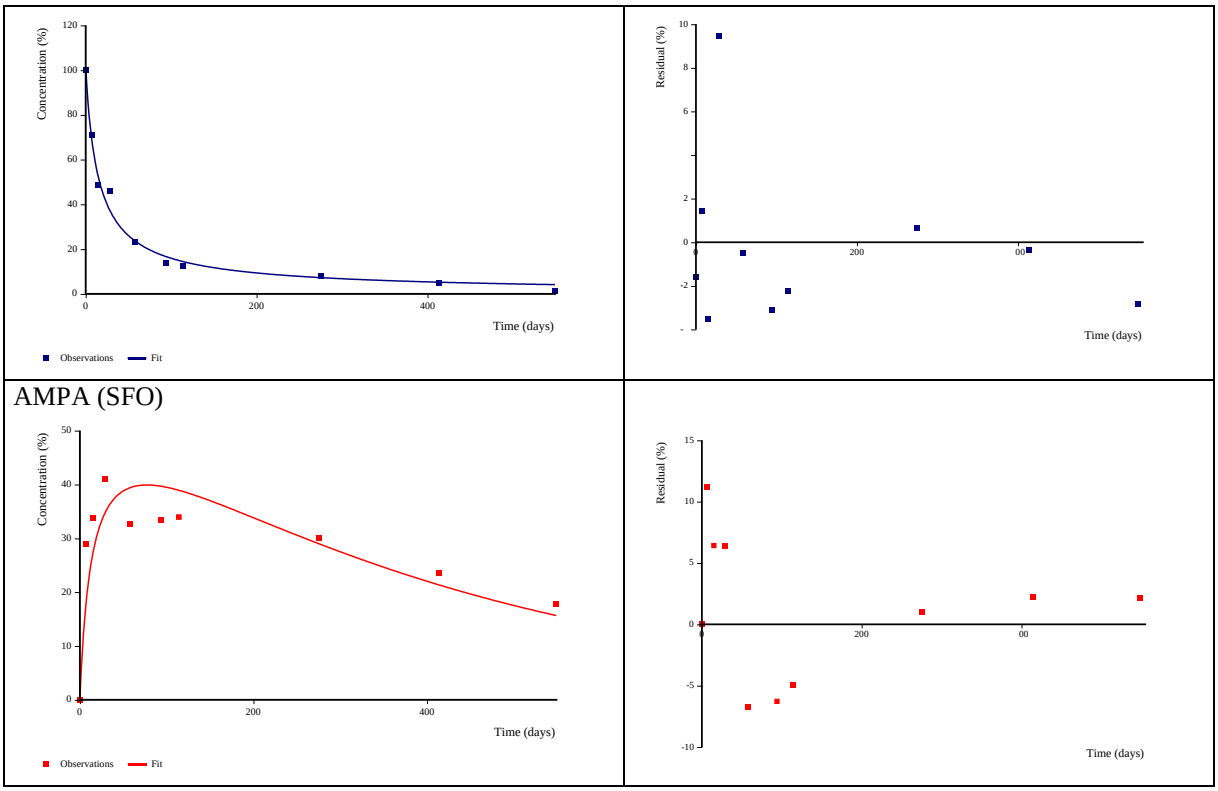
Table 8.6.3.1-22: Kinetic models and goodness-of-fit statistics of pathway fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: FOMC	Good	101.6	α: 0.8344 β: 12.19	9.2	-1	β: 3.0030	β: 21.38	15.8	180
AMPA: SFO	Accep-table	-	k: 0.0025	15.8	k: 0.011	k: 0.0011	k: 0.0040	273	907

Applicant's conclusion:

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The endpoints are statistically reliable.
Conclusion: FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate (FOMC)	



1 t-test not relevant for kinetic parameter β

Determination of modelling endpoints

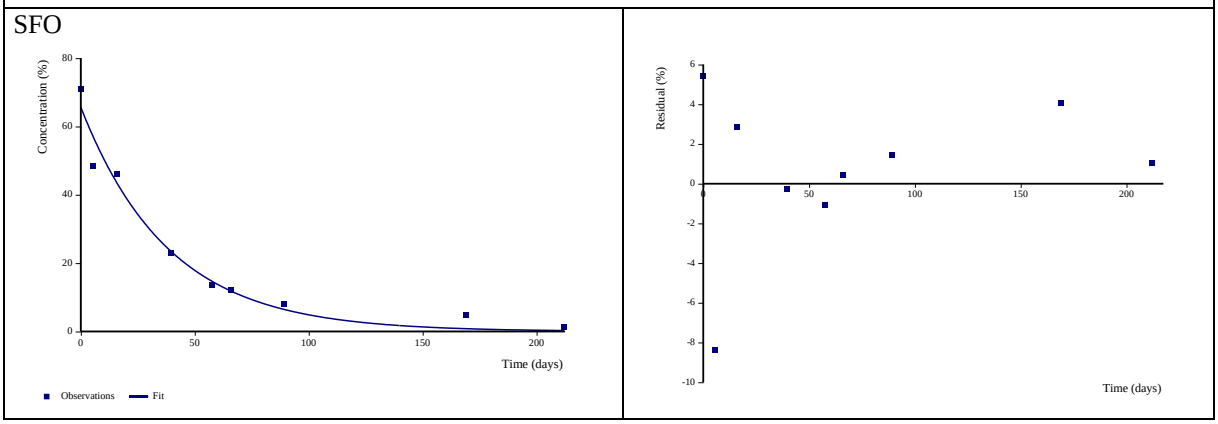
Table 8.6.3.1-23: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Good	65.6	k: 0.0260	11.9	k: <0.001	k: 0.0192	k: 0.0330	26.7	88.5

Applicant's conclusion:

The SFO model well describes the degradation of glyphosate. M0 is accurately represented and residuals are small. Statistically the fit is also acceptable (χ2 error <15 % and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints



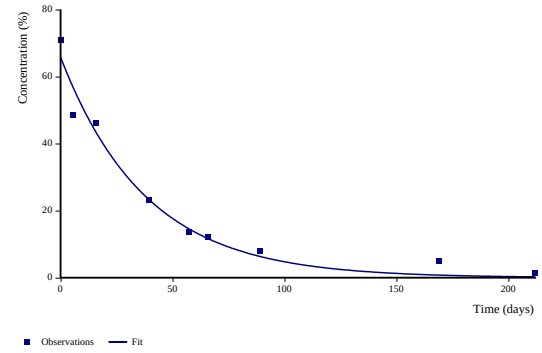
1 Representing DegT_{50} matrix according to EFSA (2014)

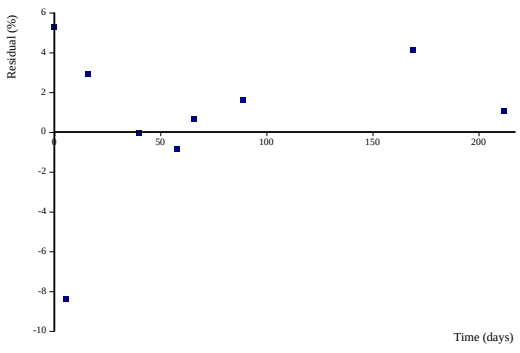
Table 8.6.3.1-24: Kinetic models and goodness-of-fit statistics of pathway fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic Model	Visual assess-ment	M0	Kinetic para-meters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Good	65.8	k: 0.0263	11.9	k: <0.001	k: 0.0198	k 0.0330	26.4	87.6	-
AMPA: SFO	Good	32.2	k: 0.0049	7.2	k: <0.001	k: 0.0024	k: 0.0070	142	473	0.2308 (±0.095)

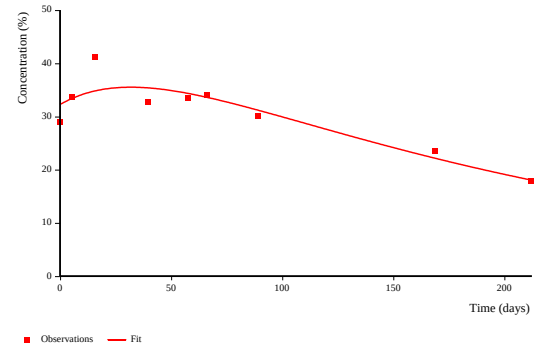
The degradation of glyphosate is described well both visually and statistically by the SFO model in the pathway fit. Similarly, for AMPA, the SFO model describes the data very well visually, and statistically the estimated degradation rates are reliable. Although the metabolite formation phase was not completely included, the estimated parameters are reliable as the metabolite decline occurred after the parent compound has mostly dissipated and, thus, the metabolite degradation rate was estimated independently.
Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

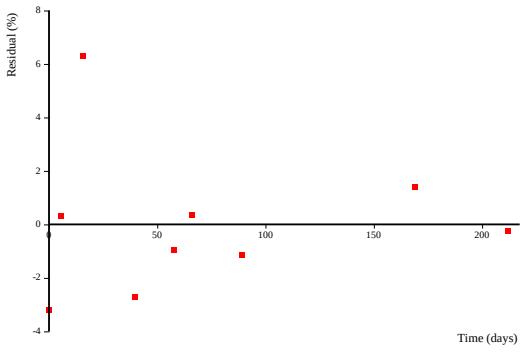
Glyphosate (SFO)





AMPA (SFO)





1 Representing $DegT_{50}$ matrix according to EFSA (2014)

Diegten

DAT (d)	tnorm (d)	tnorm (d) (>10 mm rainfall)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
0	0	-	100.00	0.00
7	4.0	-	45.95	23.51
15	9.0	-	24.41	13.97
30	17.4	0.0	12.37	12.07
62	31.1	13.7	13.61	22.94
194	50.3	32.9	10.05	24.21
282	83.8	66.4	3.57	16.31

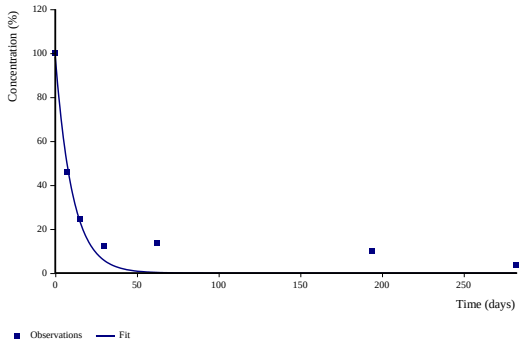
Table 8.6.3.1-25: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992a, CA 7.1.2.2.1/008) – trigger endpoints

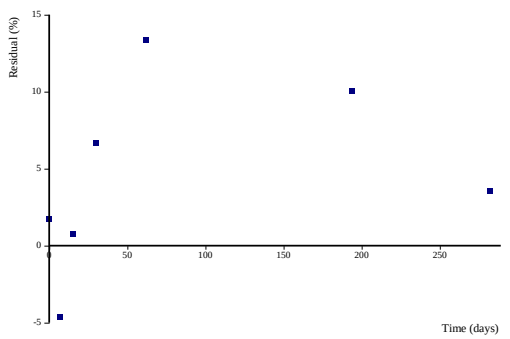
Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	98.2	k: 0.0949	19.0	k: 0.0016	k: 0.0486	k: 0.1410	7.3	24.3
FOMC	Acceptable	100.2	α: 0.7446 β: 3.2231	10.1	-1	β: -2.3487	β: 8.7950	5.0	67.8
DFOP	Good	100.1	k1: 0.1425 k2: 0.0033 g: 0.853	5.0	k1: <0.001 k2: 0.0602	k1: 0.0983 k2: -0.0016	k1: 0.1870 k2: 0.0080	6.1	118
HS	Not calculated								

Applicant's conclusion:

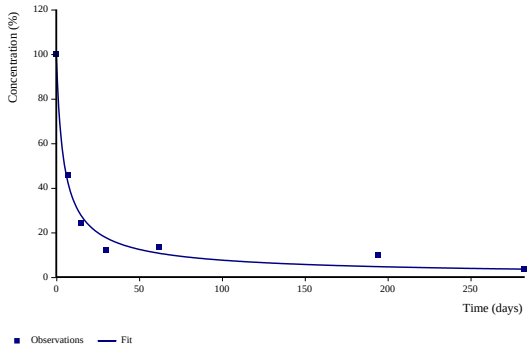
The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best visual fit with smaller residuals, and the t-test for k2 only marginally exceeds the 5 % level and is considered acceptable.
Conclusion: DFOP to be used in pathway fit for trigger endpoints

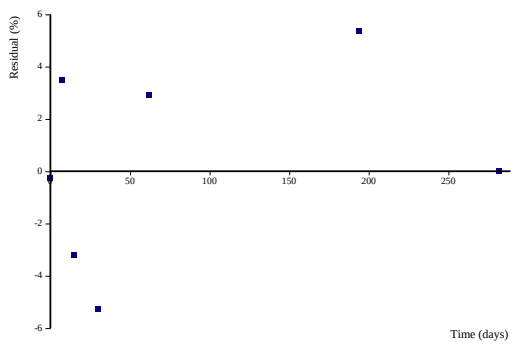
SFO



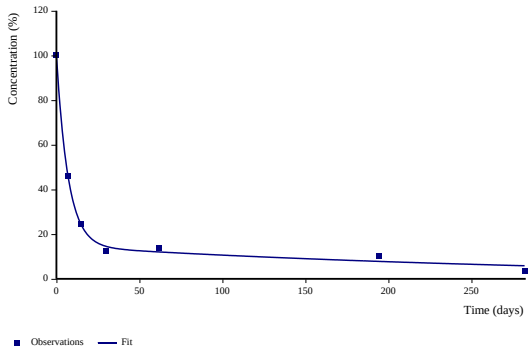


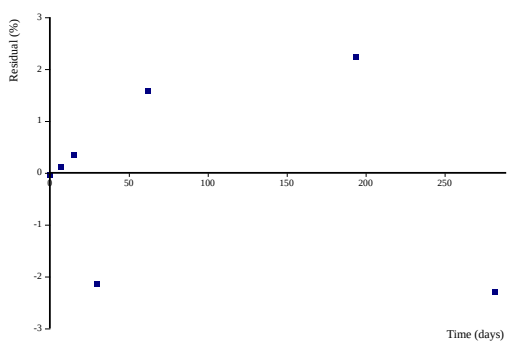
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

Table 8.6.3.1-26: Kinetic models and goodness-of-fit statistics of pathway fits for soil Diegten of study [redacted] (1992a, CA 7.1.2.2.1/008) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	100.1	k1: 0.1434 k2: 0.0033 g: 0.8518	5.0	k1: <0.001 k2: 0.0252	k1: 0.1127 k2: -7.165×10-6	k1: 0.1740 k2: 0.0070	6.1	119
AMPA: SFO	Poor	-	k: 0.0005	26.0	k: 0.3901	k: -0.0038	k: 0.0050	>1000	>1000

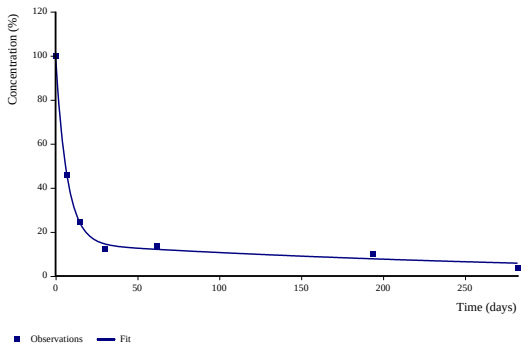
Applicant's conclusion:

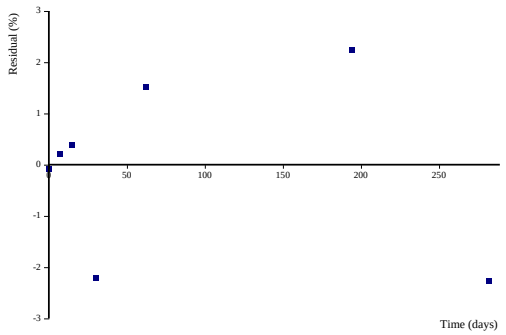
The dissipation of glyphosate is well described by the DFOP model. For AMPA, due to the wide scatter of residue data, the SFO model does not adequately describe the formation of the metabolite. A decline fit for AMPA was not performed, as there is no clear decline phase.

Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate

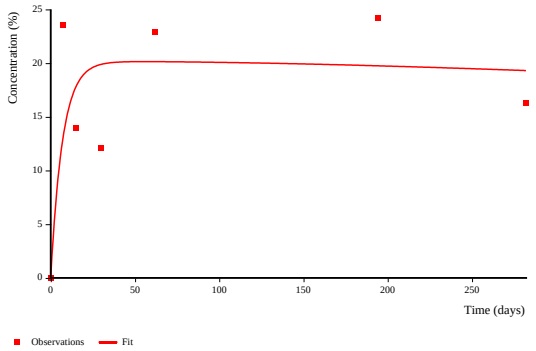
No reliable trigger endpoints for AMPA can be determined

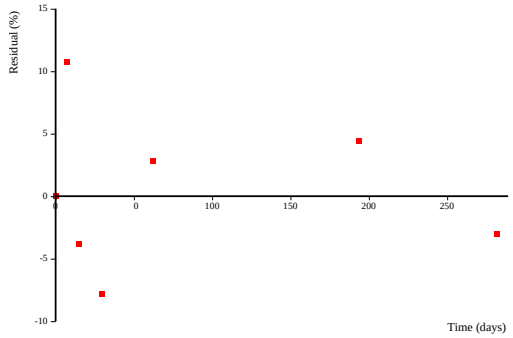
Glyphosate (DFOP)





AMPA (SFO)





Determination of modelling endpoints

Table 8.6.3.1-27: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992a, CA 7.1.2.2.1/008) – modelling endpoints

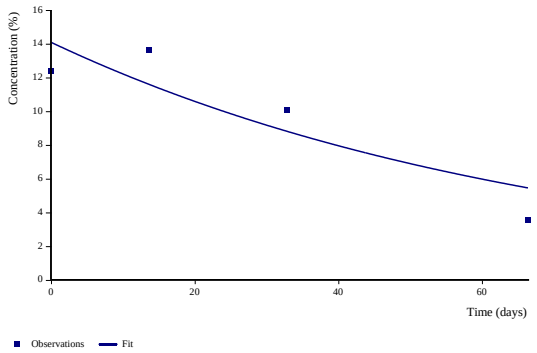
Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	14.1	k: 0.0143	14.4	k: 0.0777	k: -0.0133	k: 0.0420	48.5	161
DFOP (full dataset)	Good	100	k1: 0.2592 k2: 0.0144 g: 0.8205	4.8	k1: 0.0017 k2: 0.0521	k1: 0.1622 k2: -0.0054	k1: 0.3560 k2: 0.0340	48.22	-
HS (full dataset, tb fixed)	Good	99.0	k1: 0.1728 k2: 0.0136 tb: fixed to 10.91	6.8	k1: <0.001 k2: 0.0444	k1: 0.1417 k2: -0.0033	k1: 0.2040 k2: 0.0300	51.02	-

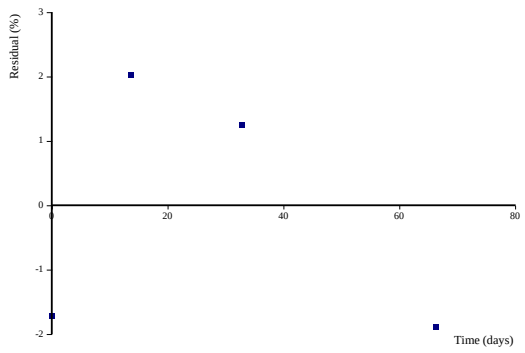
Table 8.6.3.1-27: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992a, CA 7.1.2.2.1/008) – modelling endpoints

Applicant's conclusion:

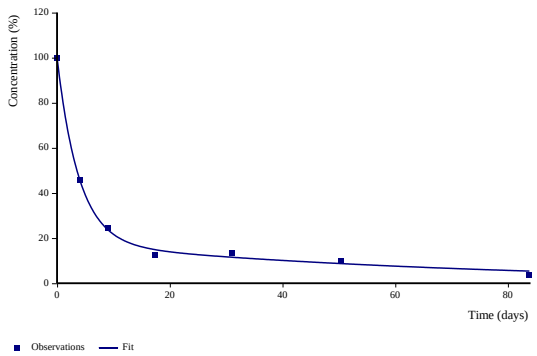
SFO model: visually, given the scatter in the data, the model describes the degradation of glyphosate adequately. But statistically the estimated degradation rate (k) is not significantly different from zero and the confidence interval includes zero. Thus, the DFOP model was alternatively fitted to the whole dataset.
DFOP model: the estimated g value is >0.75. In accordance with the EFSA (2014), the HS model was additionally fitted to the whole dataset.
HS model: in a first model run, the estimated tb was prior to the time >10 mm rain. Therefore, the fitting was repeated with tb fixed to the time when rain was >10 mm (10.91 days, normalised) in accordance with EFSA (2014).
For the repeated fit, the visual fit is good with small randomly scattered residuals, and the parameter k2 is significantly different from zero (at 5 % level).
Conclusion: Slow phase DT_{50} from HS model to be used as modelling endpoint for glyphosate

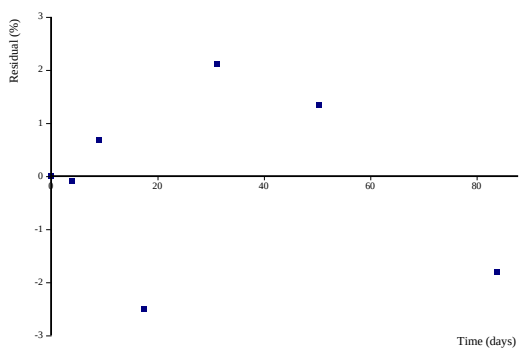
SFO



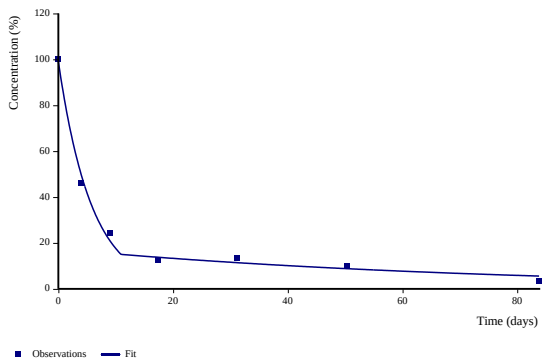


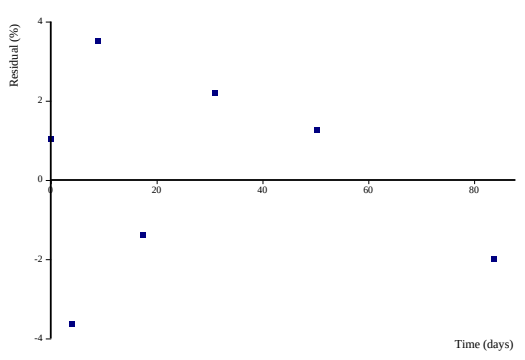
DFOP (all data)





HS (all data, tb fixed)





1 Representing $DegT_{50}$ matrix according to EFSA (2014)
2 Calculated from the slow-phase (k2) according to EFSA (2014)

As the SFO parent-only fit for glyphosate was not acceptable, no pathway fit was tested for soil Diegten. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Ontario ([redacted] 1992) – Only trigger endpoints derived

Table 8.6.3.1-28: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1992, CA 7.1.2.2.1/014)

Time (DAT)	Sum of horizons (0 - 20 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Ontario				
0	2.85[2]	0.00[3]	100.00	0.00
1	2.37	0.18	82.88	9.46
3	0.98	0.40	34.39	21.45
7	0.89	0.36	31.35	19.35
14	0.75	0.52	26.38	27.55
31	0.42	0.40	14.78	21.24
60	0.26	0.40	8.98	21.24
207	0.21	0.35	7.32	18.72
297	0.03	0.10	1.04	5.47
391	-[4]	0.10	-[4]	5.47
577	-[4]	0.03	-[4]	1.58

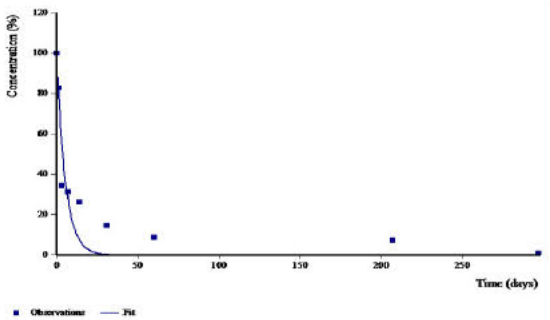
LOD = 0.05 mg/kg
Bold values = maximum residue value; potentially used for decline fit
1 Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
2 Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
3 Residue value of metabolite set to 0 at day 0
4 Data omitted according to FOCUS (2006, 2014)

Table 8.6.3.1-29: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	94.5	k: 0.1819	27.0	k: 0.007	k: 0.0508	k: 0.313	3.8	12.7
FOMC	Good	102.6	α: 0.6375 β: 1.3190	15.8	-1	β: -0.9581	β: 3.596	2.6	47.5
DFOP	Accep-table	103.6	k1: 0.5082 k2: 0.0171 g: 0.7169	14.4	k1: 0.018 k2: 0.122	k1: 0.0526 k2: -0.0162	k1: 0.964 k2: 0.050	2.3	60.7

Applicant's conclusion
The dissipation of glyphosate was best described by bi-phasic models. SFO model does not properly estimate the dissipation. The FOMC model provides the best fit during the whole study period. Thus, FOMC is selected as the best-fit model for parent-only fit.
Conclusion: FOMC to be used in pathway fit for trigger endpoints.

SFO



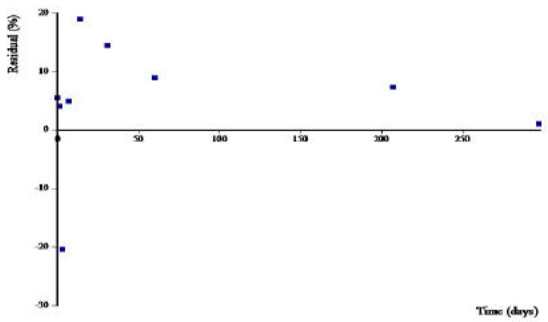
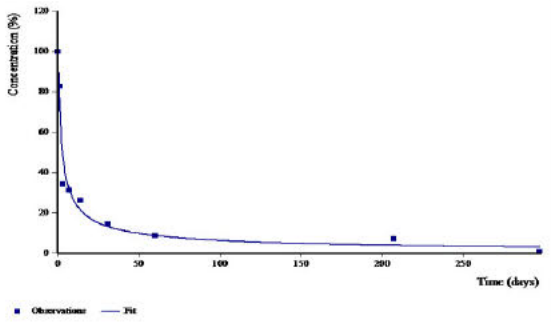
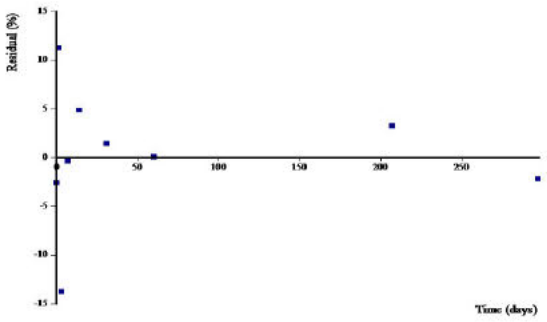


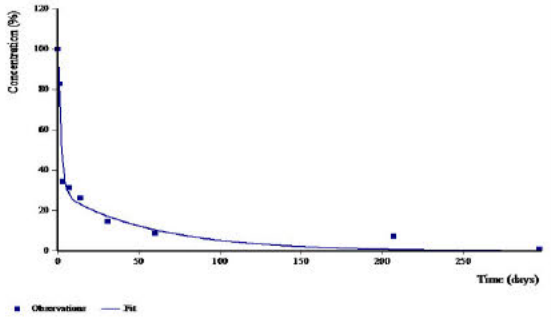
Table 8.6.3.1-29: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

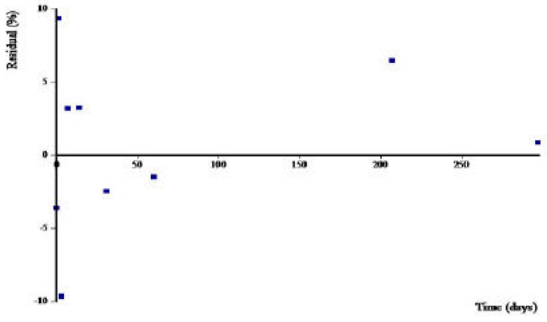
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

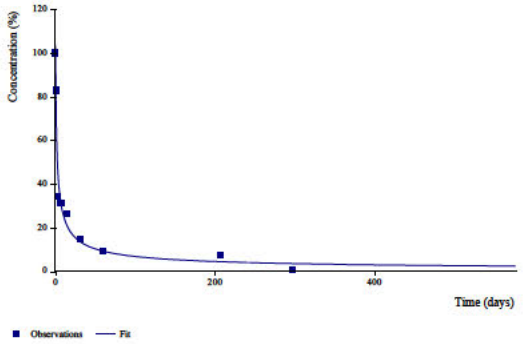
Table 8.6.3.1-30: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	103.8	α: 0.6043 β: 1.1150	15.9	-1	β: -0.2371	β: 2.4670	2.4	49.2	-
AMPA: SFO	Acceptabl e	-	k: 0.0045	16.5	k: <0.001	k: 0.0025	k: 0.0060	155	514	0.309 (±0.040)

Applicant's conclusion

Dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit.
Conclusion: FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate: FOMC



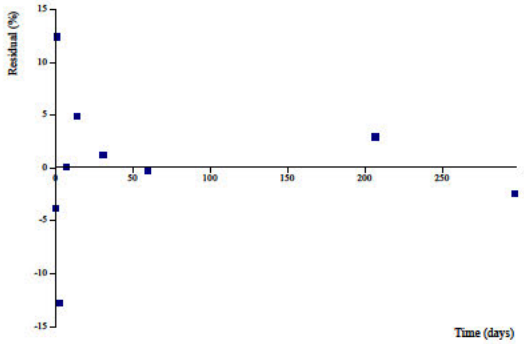
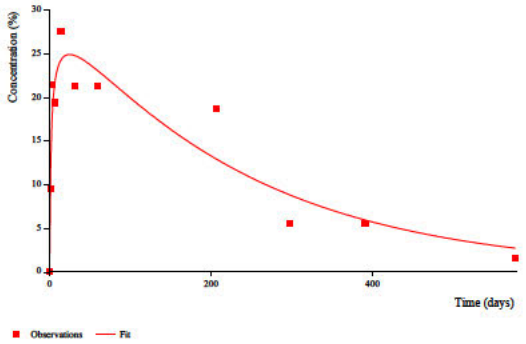
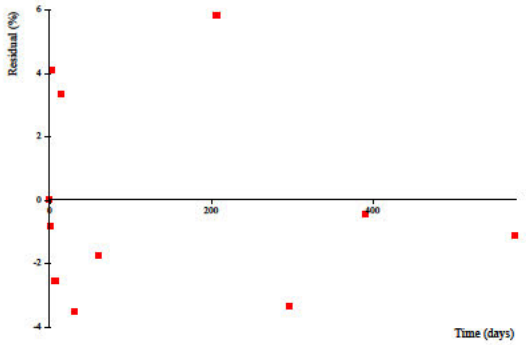


Table 8.6.3.1-30: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
AMPA: SFO										





1 t-test not relevant for kinetic parameter β

California ([redacted] 1989a) – Only trigger endpoints derived

Table 8.6.3.1-31: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1989a, California soil)

Time (DAT)	Sum of horizons (0 - 32 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
California				
0	3.16[2]	0.00[3]	100.00	0.00
1	2.86	0.11	90.70	5.24
3	1.98	0.19	62.82	9.10
7	1.45	0.31	45.80	15.16
14	0.84	0.27	26.61	12.96
31	0.35	0.43	11.04	20.67
59	0.11	0.29	3.44	14.06
205	0.09	0.14	2.72	6.89
366	0.09	0.09	2.72	4.13

LOD = 0.02 mg/kg for glyphosate, 0.04 mg/kg for AMPA

[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite

[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0

[3] Residue value of metabolite set to 0 at day 0

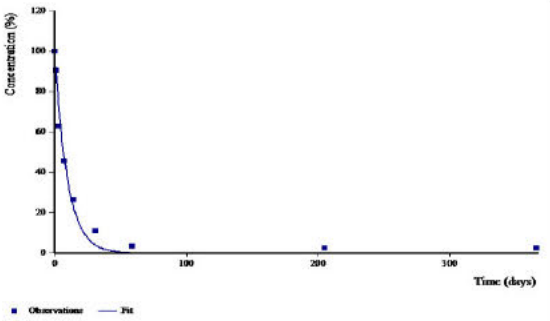
Table 8.6.3.1-32: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1989a, CA 7.1.2.2.1/016) – trigger endpoints

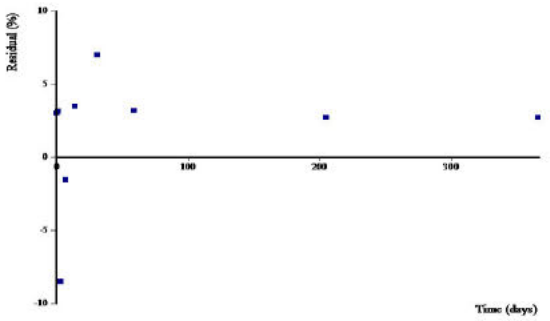
Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	97.0	k: 0.1024	9.3	k: <0.05	-	-	6.8	22.5
FOMC	Good	101.3	α: 1.4810 β: 9.2630	5.0	-[1]	β: 1.3390	β: 17.19	5.5	34.6
DFOP	Good	101.6	k1: 0.2811 k2: 0.0494 g: 0.4847	5.4	k1<0.05 k2<0.05	-	-	5.4	33.2
Applicant's conclusion									

Table 8.6.3.1-32: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1989a, CA 7.1.2.2.1/016) – trigger endpoints

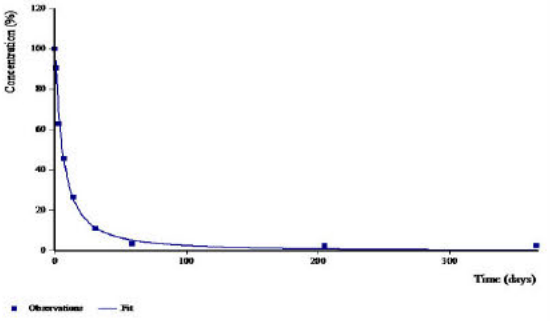
The SFO model provides an acceptable visual and statistically reliable fit. The bi-phasic models further improve the visual fit. The FOMC model provides the best visual fit during the whole study period and the lowest $\chi 2$ error. Thus, FOMC is selected as the best-fit model for parent-only fit. Conclusion: FOMC to be used in pathway fit for trigger endpoints.

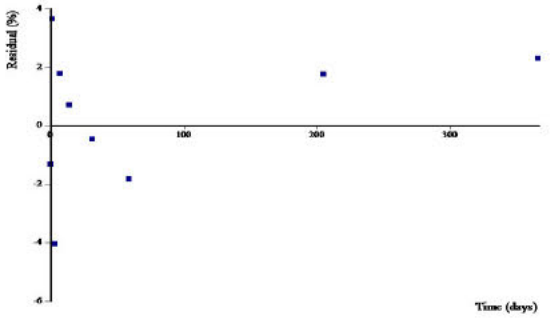
SFO



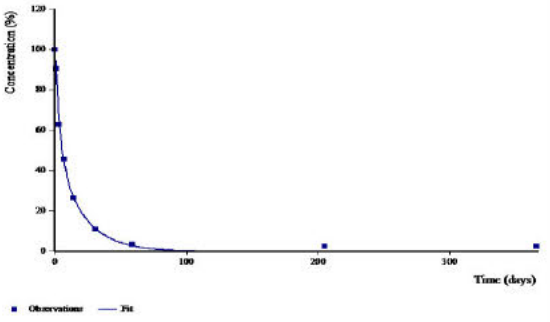


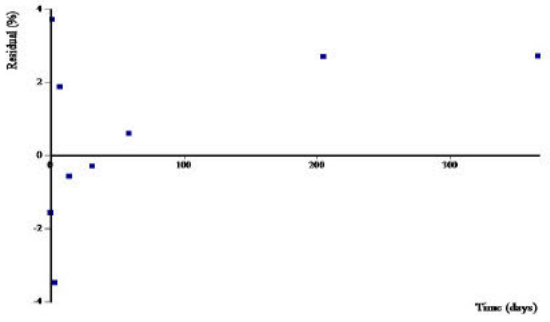
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

Table 8.6.3.1-33: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1989a, CA 7.1.2.2.1/016) – trigger endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	101.8	α: 1.3940 β: 8.3790	5.1	-[1]	β: 2.4360	β: 14.32	5.4	35.3	-

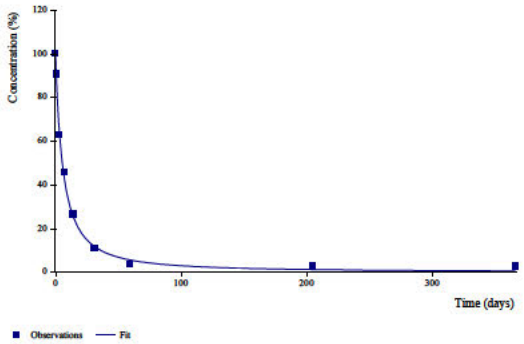
Table 8.6.3.1-33: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1989a, CA 7.1.2.2.1/016) – trigger endpoints

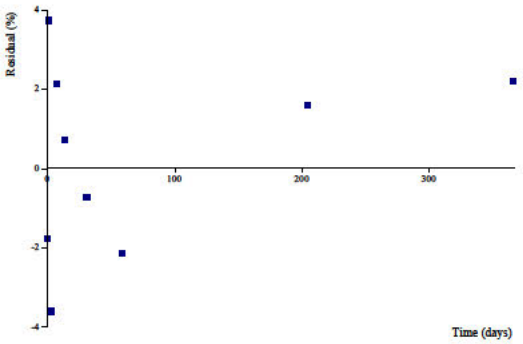
Kinetic model	Visual assessment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
AMPA: SFO	Acceptable	-	k: 0.0062	15.4	k: 0.001	-	-	111	370	0.231 (±0.022)

Applicant's conclusion

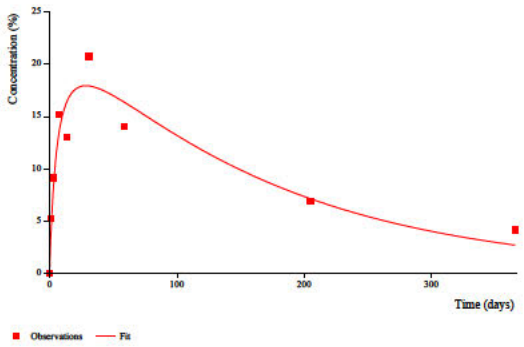
Dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit.
Conclusion: FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

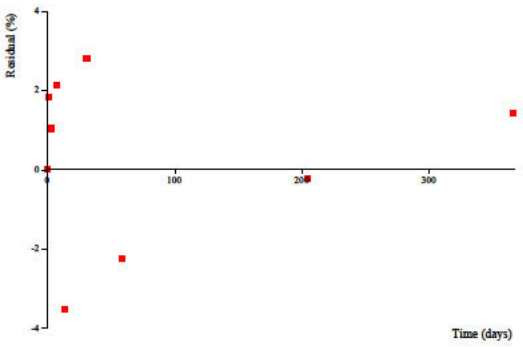
Glyphosate: FOMC





AMPA: SFO





1 t-test not relevant for kinetic parameter β

Normalization not performed.

Arizona

Table 46: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
Arizona				
0	3.612	0.003	100.00	0.00
1	3.60	0.28	99.64	11.73
7	4.03	0.43	111.66	18.32
14	1.04	0.35	28.88	14.66
21	3.42	1.15	94.83	48.33
28	1.36	0.63	37.55	26.38
64	0.61	0.83	16.85	35.18
92	0.26	0.52	7.22	21.99

Table 46: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
122	0.24	0.66	6.74	27.85
184	0.16	0.49	4.32	20.52
364	0.08	0.33	2.17	13.93
462	0.02	0.10	0.48	4.40
553	-4	0.12	-4	5.13

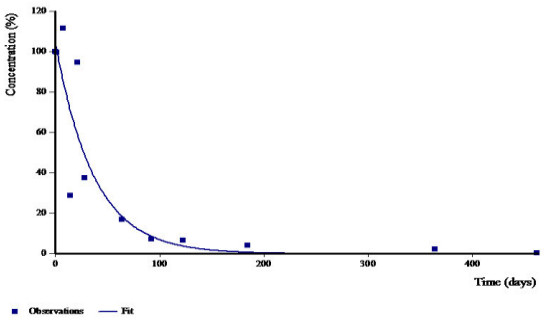
Table 47: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Arizona of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

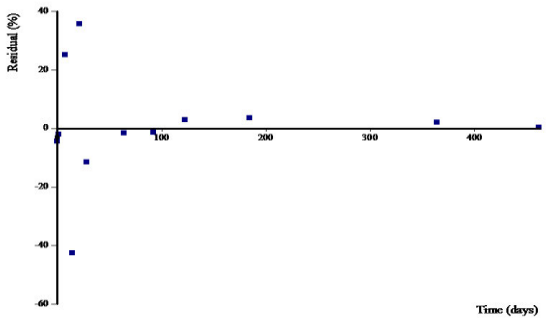
Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	104.4	k: 0.0271	34.4	k: 0.007	k: 0.0068	k: 0.047	25.6	85
FOMC	Poor	104.5	α: 49.25 β: 1800	35.8	-1	β: -1.56 × 105	β: 1.60 × 105	25.5	86
DFOP	Poor	104.8	k1: 0.0285 k2: 0.0009 g: 0.9779	37.3	k1: 0.090 k2: 0.493	k1: -0.0161 k2: -0.1119	k1: 0.073 k2: 0.114	25.1	88

None of the applied kinetic models accurately describe the residue data of glyphosate. The visual fits are poor due to the large residuals of the first five data points and the $\chi 2$ error is high.

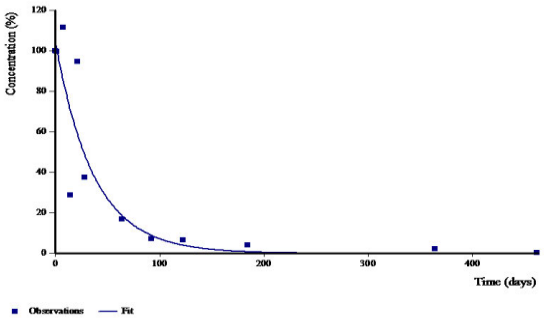
Conclusion: No trigger endpoints can be derived for glyphosate

SFO





FOMC



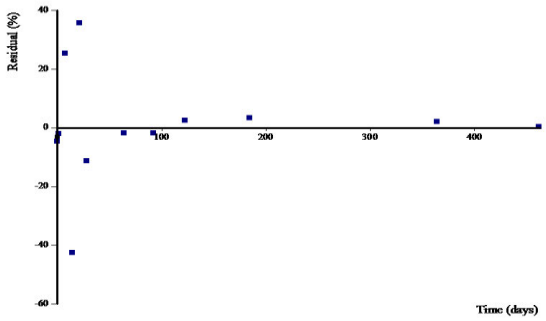
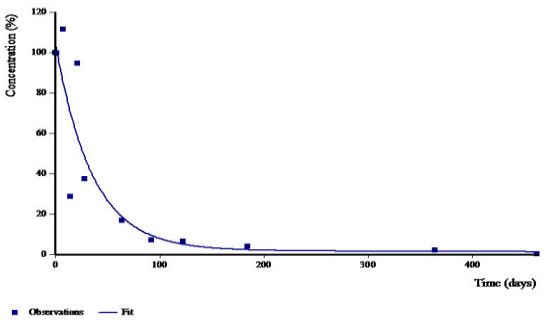
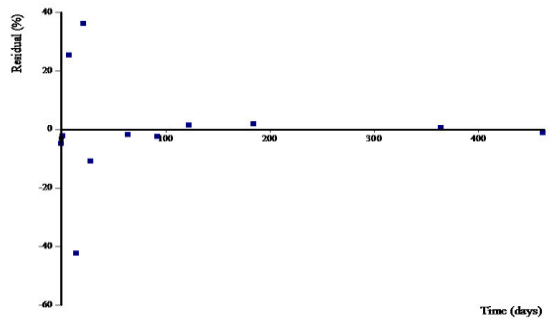


Table 47: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Arizona of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

DFOP





1 t-test not relevant for kinetic parameter β

As for glyphosate, none of the tested models provided an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA for trial Arizona. Thus, a metabolite decline fit was performed.

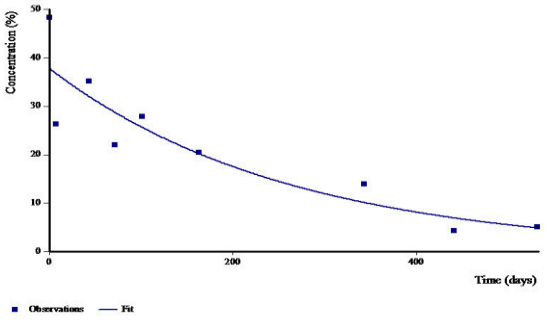
Table 48: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Arizona of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	37.8	k: 0.0038	20.5	k: 0.005	k: 0.0012	k: 0.006	181	601
FOMC	Poor	38.5	α: 4.30 β: 956.2	21.5	-1	β: -1.04 × 104	β: 1.23 × 104	167	677
DFOP	Good	48.3	k1: 1.8490 k2: 0.0030 g: 0.3285	15.3	k1: 0.016 k2: 0.003	k1: 0.2418 k2: 0.0013	k1: 3.457 k2: 0.005	97.6	630

The SFO and FOMC models do not adequately describe the decline of AMPA as M0 is clearly underestimated (measured M0 = 48.3 %). The DFOP model provides a good visual fit with statistically reliable parameters.

Conclusion: DFOP to be used for deriving trigger endpoints for AMPA

SFO



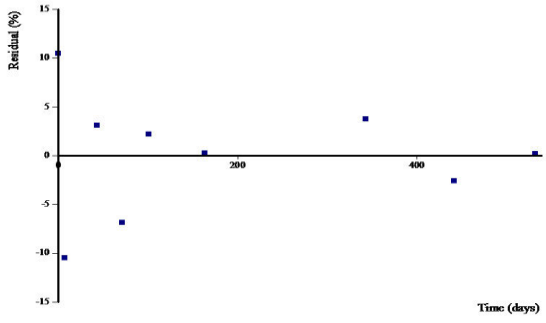
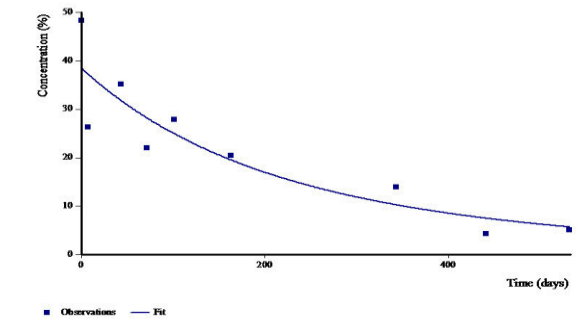
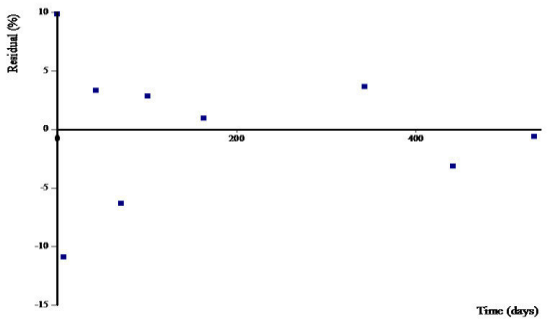


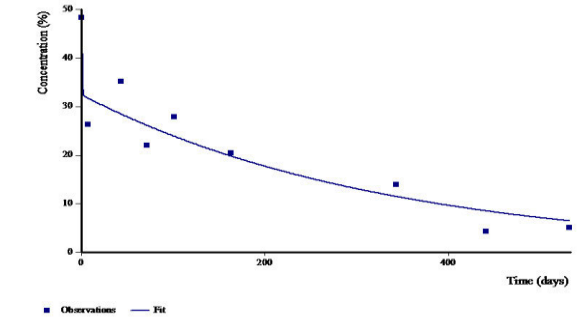
Table 48: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Arizona of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

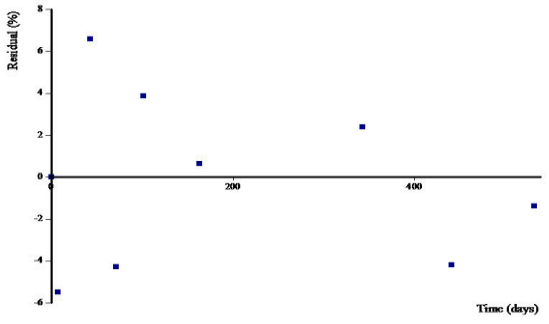
FOMC





DFOP





1 t-test not relevant for kinetic parameter β

Modelling endpoints

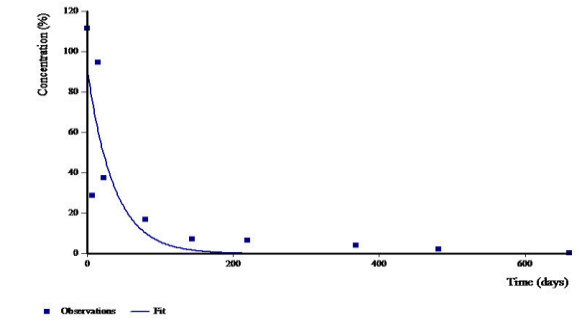
Table 49: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Arizona of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

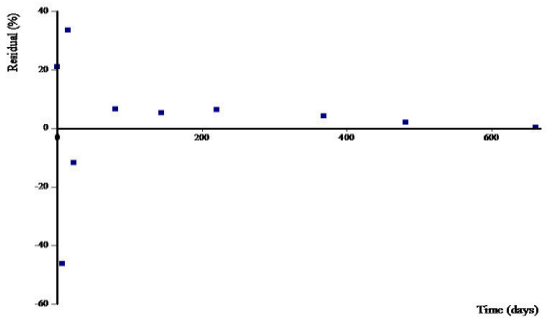
Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5% level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.7	k: 0.0274	51.6	k: 0.070	-1	-1	25.3	84.2

Applicant's conclusion

Due to the large scattering of the residue data and the resulting high χ2 error, the SFO fit is not acceptable.
Conclusion: No modelling endpoints can be derived for glyphosate

SFO





As for glyphosate, the SFO model did not provide an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA for trial Arizona. Thus, a metabolite decline fit was performed.

Table 50: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Arizona of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

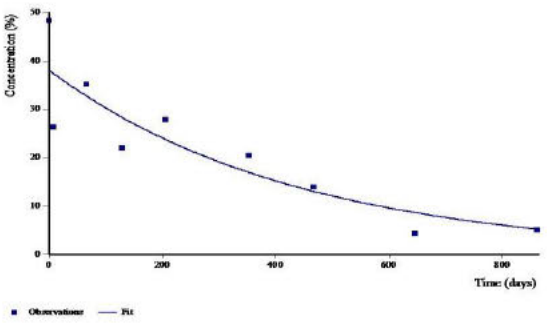
Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5% level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	38.1	k: 0.0023	21.1	k: 0.004	-1	-1	303	>1000

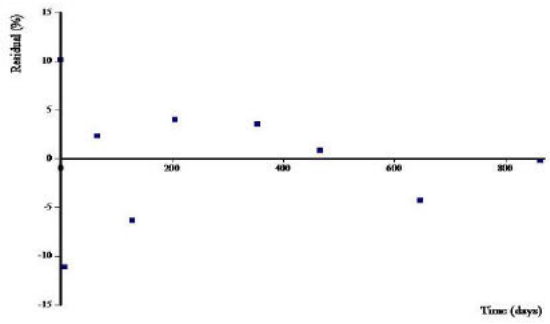
Applicant's conclusion

The SFO model provides a visually acceptable and statistically reliable fit to describe the decline of AMPA.

Conclusion: SFO to be used for deriving modelling endpoints for AMPA

SFO





Iowa

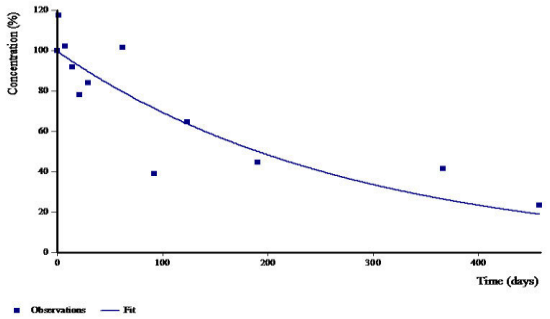
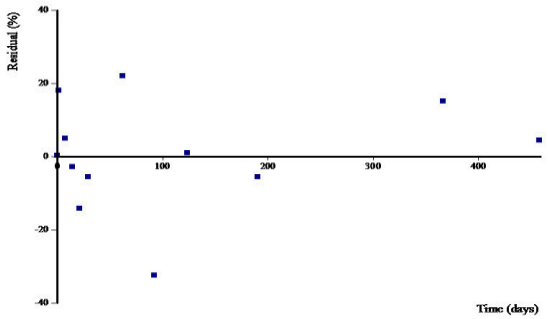
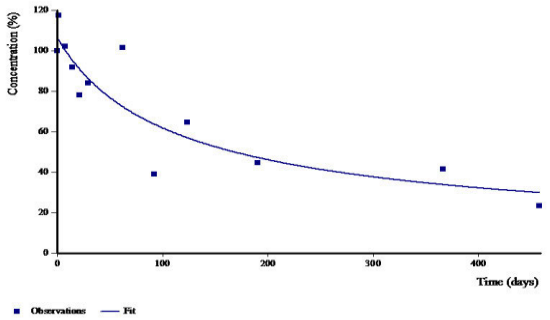
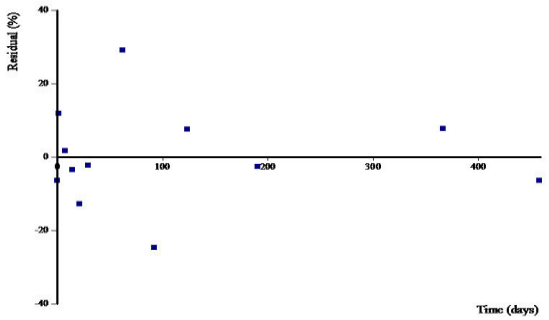
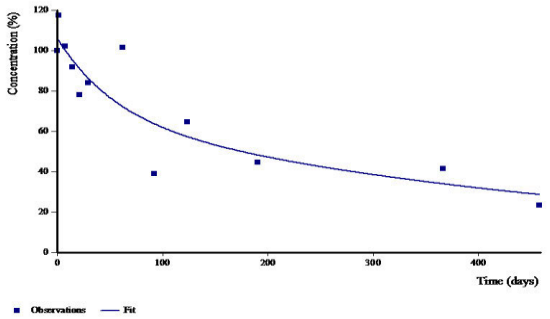
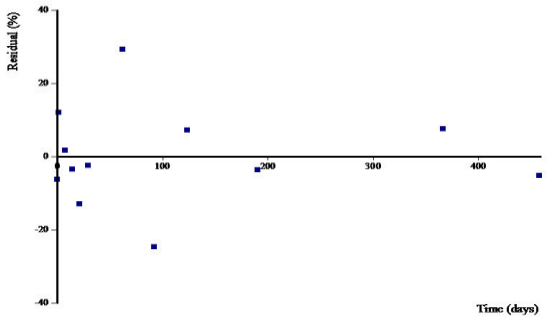
Table 51: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
Iowa				
0	3.202	0.003	100.00	0.00
1	3.76	0.14	117.42	6.81
7	3.27	0.22	102.21	10.55
14	2.95	0.21	92.09	9.80
21	2.50	0.27	78.19	12.79
29	2.69	0.30	84.22	14.28
62	3.25	0.60	101.69	28.48
92	1.25	0.37	39.13	17.58
123	2.08	0.60	64.88	28.61
190	1.43	0.50	44.78	24.00
366	1.34	0.88	41.76	41.93
458	0.76	0.94	23.63	44.92

Table 52: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	99.6	k: 0.0036	15.5	k: 0.001	k: 0.0016	k: 0.006	192	638
FOMC	Accep-table	106.4	α: 0.6571 β: 78.33	14.6	-1	β: -191.1	β: 347.8	147	>1000
DFOP	Accep-table	106.1	k1: 0.0182 k2: 0.0018 g: 0.3689	15.3	k1: 0.313	k1:-0.0644	k1: 0.101 k2: 0.008	152	999

Table 52: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

					k2: 0.241	k2:- 0.0039			
The SFO model provides an acceptable visual and statistically reliable fit. The bi-phasic models further improve the visual fit. The FOMC and DFOP models provide equally good visual fits but the FOMC model has the lowest $\chi 2$. Thus, FOMC is selected as the best-fit model for parent-only fit. Conclusion: FOMC to be used in pathway fit for trigger endpoints.									
SFO									
									
FOMC									
									
DFOP									
									

1 t-test not relevant for kinetic parameter β

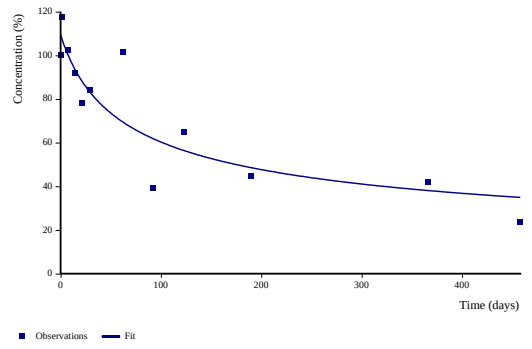
Table 53: Kinetic models and goodness-of-fit statistics of pathway fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

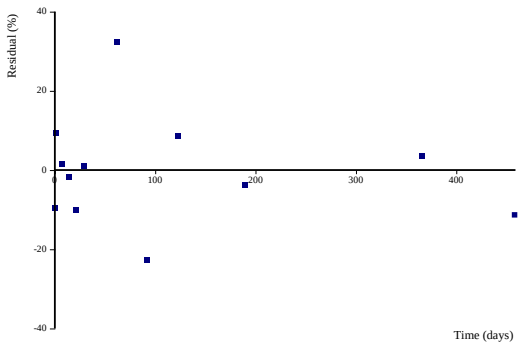
Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Acceptable	109.5	α: 0.4143 β: 31.01	14.9	-1	β: -30.51	β: 92.53	134	>1000	-
AMPA: SFO	Acceptable	-	k: 4.28 × 10-66	19.3	k: 0.5	k: -0.0017	k: 0.002	>1000	>1000	0.542 (±0.163)

Table 53: Kinetic models and goodness-of-fit statistics of pathway fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

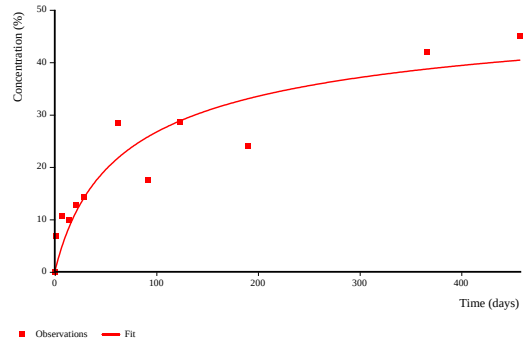
For glyphosate, the FOMC fit is acceptable. The formation of AMPA is well described by the pathway fit but the degradation rate is not significantly different from zero as the metabolite concentration is still increasing towards the end of the study. A decline fit for AMPA was not performed.
Conclusion: Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

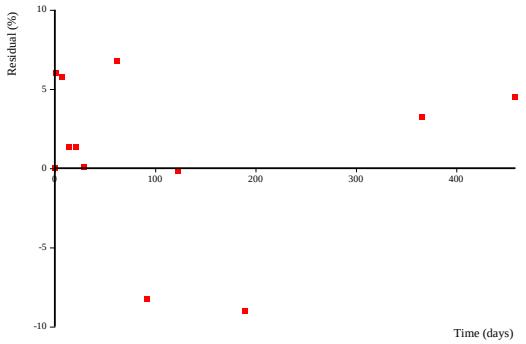
Glyphosate: FOMC





AMPA: SFO





1 t-test not relevant for kinetic parameter β

Modelling endpoints

Table 54: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	χ2 error (%)	Prob > t (5% level)	Lower CI (95%)	Upper CI (95%)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	97.4	k: 0.0038	15.9	k: <0.05	k: 0.0017	k: 0.006	182	605

The SFO model provides a visually acceptable and statistically reliable fit to describe the degradation of glyphosate.
Conclusion: SFO to be used in pathway fit for modelling endpoints

Table 54: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

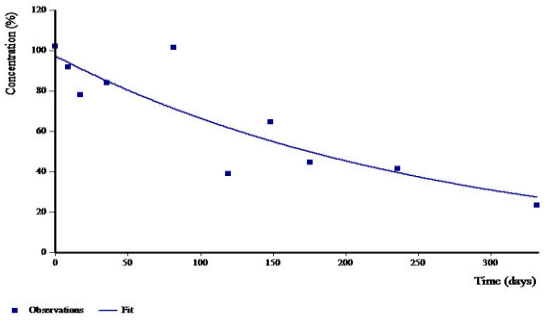
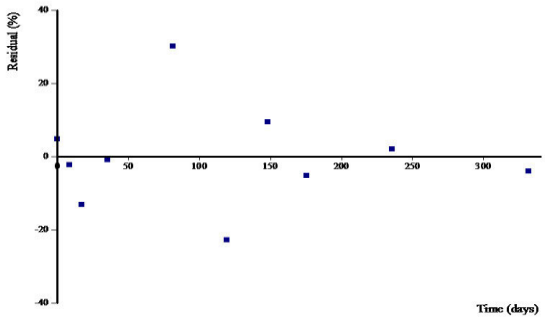
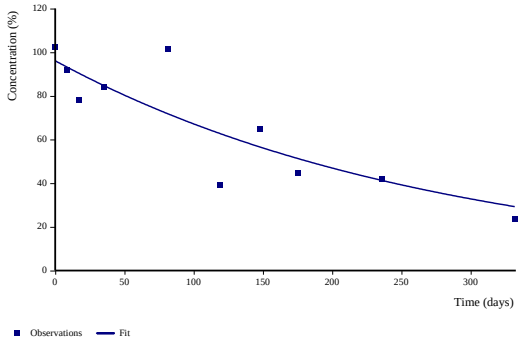
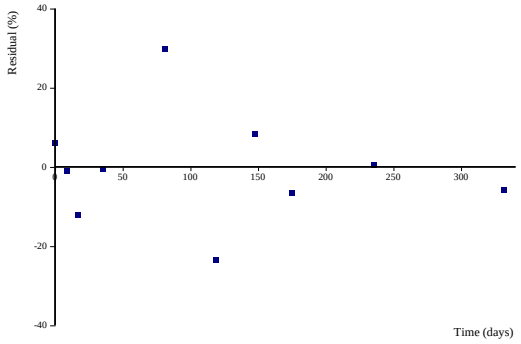
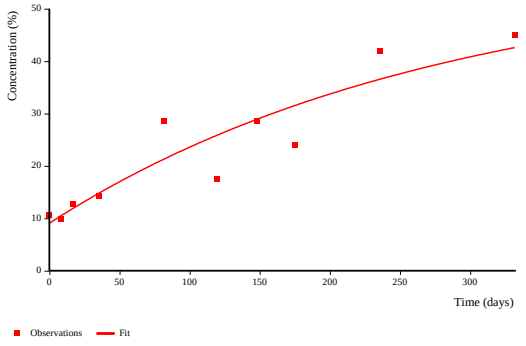
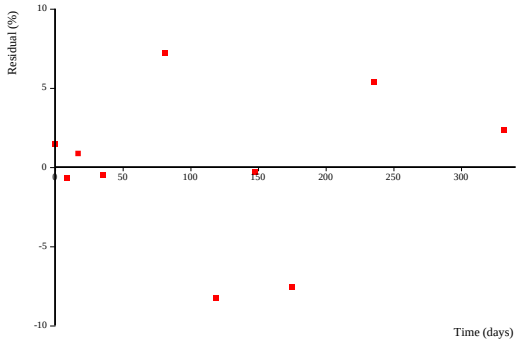
SFO	
	

Table 55: Kinetic models and goodness-of-fit statistics of pathway fits for trial Iowa of study [redacted] (1993a, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assessment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: SFO	Good	96.1	k: 0.0036	15.9	k: <0.001	k: 0.0016	k: 0.006	194	643	-
AMPA: SFO	Good	9.1	k: 0	16.8	k: 0.5	k: -0.0037	k: 0.004	>1000	>1000	0.502 (±0.277)

The degradation of glyphosate is well described by the pathway fit. The formation and decline of AMPA is visually well described by the fit. However, no reliable degradation endpoints can be derived as the metabolite concentration is still increasing towards the end of the study and thus, the estimated k-rate is not significantly different from zero. A decline fit for AMPA was not performed.

Conclusion: The pathway fit for trial Iowa is not considered acceptable for deriving modelling endpoints for parent and metabolite. Thus, modelling endpoints for glyphosate are derived from parent-only fit.

Glyphosate (SFO)	
	
AMPA (SFO)	
	

Minnesota

Table 56: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993a, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)1
Minnesota				
0	2.112	0.003	100.00	0.00
1	2.37	0.36	112.34	26.16
7	2.85	0.46	134.98	33.28
15	3.11	0.40	147.42	28.53
21	2.42	0.48	114.61	34.47
35	1.00	0.63	47.54	45.14
71	0.38	0.69	17.94	49.89
95	0.46	0.74	21.84	53.44
129	0.40	0.71	18.79	51.07
179	0.23	0.58	10.93	41.58
372	0.08	0.28	3.92	20.23
475	0.12	0.41	5.47	29.72

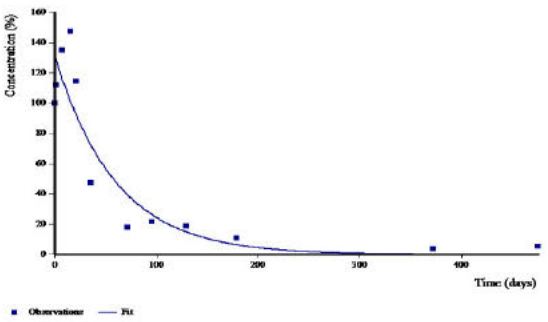
Table 57: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Minnesota of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	130.8	k: 0.0167	27.9	k: 0.003	k: 0.0060	k: 0.027	41.5	138
FOMC	Poor	141.7	α: 359.9 β: 1.35 × 104	29.1	-1	-2	-2	26.0	86.5
DFOP	Poor	130.8	k1: 0.0167 k2: 0.0167 g: 0.1314	30.4	k1: 0.156 k2: <0.001	k1:-0.0190 k2: 0.0097	k1: 0.052 k2: 0.024	41.5	138

None of the applied kinetic models accurately describe the residue data of glyphosate. The visual fits are poor due to an initial increase in glyphosate concentration, and the resulting residuals are large.

Conclusion: No trigger endpoints can be derived for glyphosate.

SFO



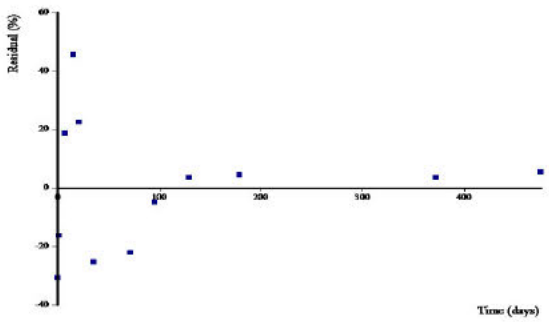
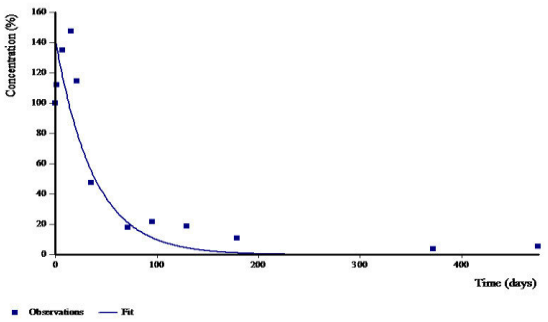
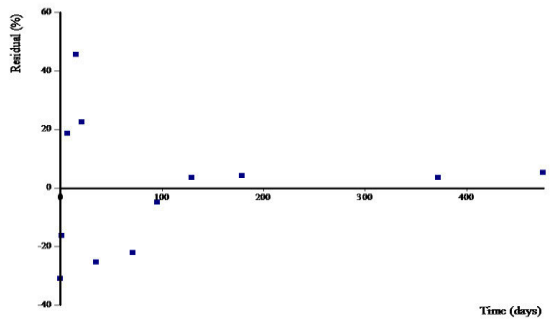


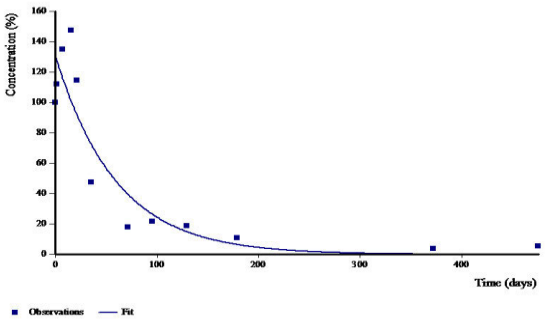
Table 57: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Minnesota of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

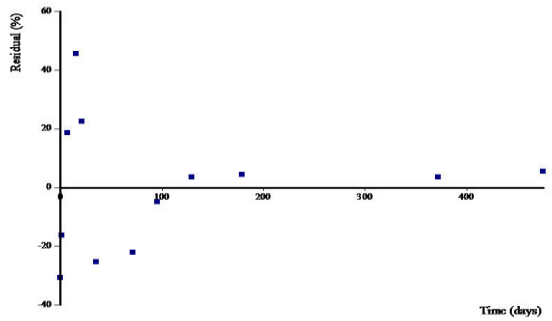
FOMC





DFOP





1 t-test not relevant for kinetic parameter β
2 Errors and t-test values could not be calculated because the covariance matrix could not be created

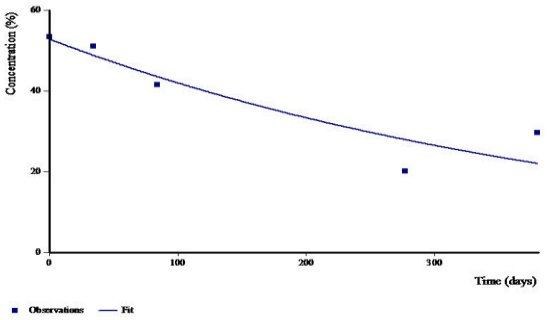
As for glyphosate, none of the tested models provided an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA for trial Minnesota. Thus, a metabolite decline fit was performed.

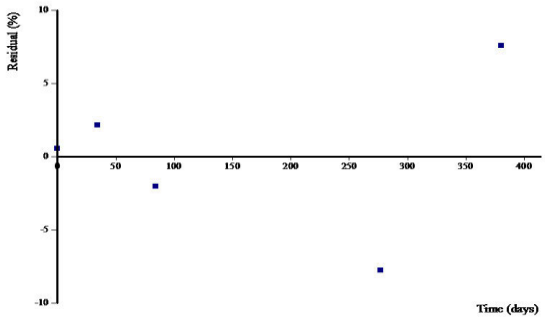
Table 58: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Minnesota of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M0	Kinetic parameters	$\chi 2$ error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Good	52.9	k: 0.0023	10.3	k: 0.020	k: 0.0002	k :0.004	302	>1000
FOMC	Good	55.1	α: 0.6787 β: 156.3	10.4	-1	β: -1768	β: 2080	278	>1000
DFOP	Good	55.6	k1: 0.0074 k2: 0.0000 g: 0.5915	12.0	k1: 0.434 k2: 0.5	k1:-0.4369 k2:-0.2033	k1: 0.452 k2: 0.203	252	>1000
The SFO model adequately describes the degradation behaviour of the measured residue data of AMPA and provides statistically reliable endpoints. The bi-phasic models do not improve the visual or statistical fit of the data. Conclusion: SFO to be used for deriving trigger endpoints for AMPA									

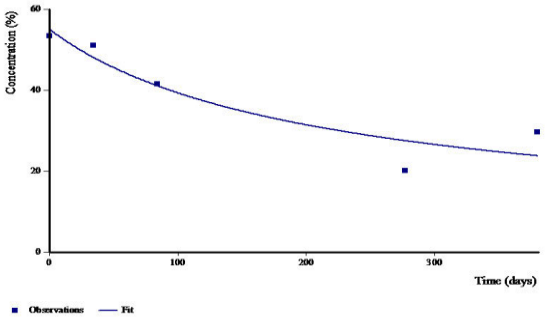
Table 58: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Minnesota of study [redacted] (1993a, CA 7.1.2.2.1/006) – trigger endpoints

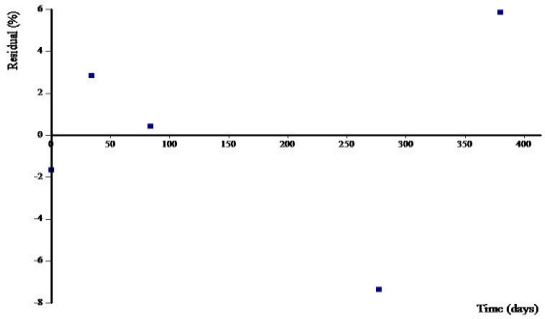
SFO



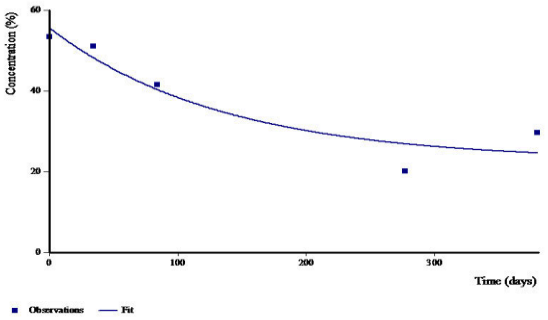


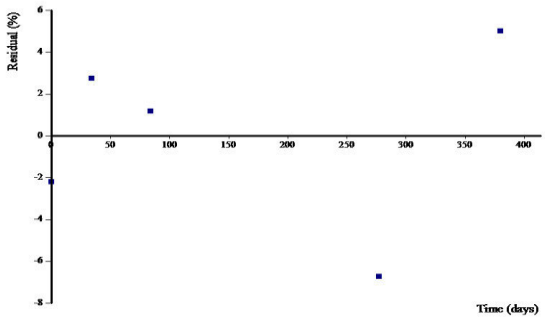
FOMC





DFOP





1 t-test not relevant for kinetic parameter β