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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.9 (PPP) – MON 52276

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.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

Introduction

This section of the RAR presents studies carried out on the representative formulation as well as risk assessments for non-target organisms.

The representative formulation selected by the applicant, MON 52276, is a soluble concentrate (SL) containing 360 g/L glyphosate as isopropylamine salt.

The representative uses covered by the risk assessment are presented in detailed tabular format in Vol 1 of the RAR as well as in the List of endpoints. The content of glyphosate in the list of representative uses is expressed as glyphosate acid, which corresponds to MON 52276 at 360 g/L.

Where applicable, ecotoxicological studies have been conducted with the representative formulation MON 52276 to compare the toxicity of the active substance with that of MON 52276. Ecotoxicological studies conducted with the active substance glyphosate, glyphosate acid, glyphosate salts and its metabolites are evaluated in section B.9 CA of the RAR but irrespective of test item, all endpoints relevant for the risk assessment are presented in this section of the RAR. All endpoints presented for MON 52276 and glyphosate are given in glyphosate acid equivalents (i.e. recalculated to acid equivalents).

Assessment of effects on biodiversity

In addition to the standard risk assessment, the applicant also presented an assessment of effects on biodiversity ([redacted] 2020, Report No. TRR0000305). In the applicant's summary document M-CP Section 10, the biodiversity assessment was summarised separately for each group of organisms (birds and wild mammals, aquatic organisms etc). The RMS has instead chosen to present the assessment of effects on biodiversity for all groups of organisms concentrated in one place of the RAR (see under section B.9.14 of this volume of the RAR), with short summaries of the literature cited by [redacted] (2020) compiled in an Appendix.

The assessment of effects on biodiversity was submitted by the applicant to address the requirement of Article 4(3)(e)(iii) of Regulation (EC) No. 1107/2009. Furthermore, the renewal of the approval of glyphosate in 2017 (Implementing Regulation (EU) 2017/2324), included the condition that Member States shall pay particular attention to "the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions".

However, there is currently no specific guidance or harmonized assessment procedures at the EU level for conducting a comprehensive biodiversity assessment. Therefore, [redacted] (2020) proposed Specific Protection Goals (SPGs) for the assessment that were primarily drawn from existing EU guidance and working documents and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides that used the ecosystems services approach. In addition, the assessment submitted also considered aspects of EFSA Scientific Opinions, since these may form the basis of future guidance documents. Hence, the expectation of the applicant was that the SPGs developed for the glyphosate biodiversity assessment are fit-for-purpose.

The first sections of [redacted] (2020) provide background of the intrinsic properties and environmental profile of glyphosate, a discussion of policy and sustainability solutions that are aimed at protecting and conserving biodiversity, and environmental benefits to biodiversity that can be realized by using glyphosate as a tool for sustainable farming. These issues may be of importance, but policy and agricultural practice are not part of the assessment of applications for (renewal of) approval of active substances under

Regulation (EC) No 1107/2009. Therefore, the RMS has only briefly summarised these sections below (using sub-headings as in [redacted] 2020) for information, without any evaluation of the arguments. The main assessment of glyphosate effects on biodiversity is presented under section B.9.14.

[redacted] (2020) pointed to the different roles of risk assessors and risk managers and stated:
"The purpose of this report is two-fold: (1) provide a biodiversity assessment that principally informs on potential indirect effects through trophic interactions and (2) to inform risk managers on risk mitigation options that are protective of aquatic and terrestrial biodiversity."

Crop production and biodiversity

[redacted] (2020) stated "Reduction of plant biodiversity in cropped areas is inherent to agriculture production irrespective of the cropping system applied, or whether unwanted weeds are controlled mechanically and/or with plant protection products. Thus, the challenge of agriculture is that on the one hand it can provide essential ecosystem services while on the other hand it can also negatively impact aspects of biodiversity." After discussing the need for control of weeds to maintain efficient agricultural production systems, the report pointed to the role of Integrated Weed Management (IWM), e.g. crop rotation and the "threshold approach" which implies that a certain level of weeds can be accepted in the in-field if they cause no adverse impact to the crop. The report claimed that such practices already support biodiversity conservation in many ways, although IWM practices are not yet considered in standard risk assessments.

Next, the report pointed to the changes in land use, which can be related to habitat availability, as a key driver of biodiversity decline. With numerous references to investigations of biodiversity decline, EU policy (e.g. CAP), EU legislation (e.g. Habitats (92/43/EEC) and Birds (79/409/EEC) EU Directives) etc., the report suggested e.g., that "Achieving the right balance between agricultural production and biodiversity conservation will require congruent agriculture and nature conservation policies, which go beyond the existing Plant Protection Products (PPPs) regulatory framework."

The role of glyphosate in effective weed control

According to [redacted] (2020) the unique properties of glyphosate (broad-spectrum and systemic herbicide with no soil activity due to strong binding to minerals and organic matter) makes glyphosate ideally suited to control weeds prior to planting of crops, and the chemical alternative would be a mixture of herbicides that target a relatively broad spectrum of weeds.

Glyphosate's role in enabling the benefits of conservation agriculture

Conservation agriculture is based on three principles: minimal soil disturbance (no-till or reduced tillage), organic soil cover (crop residues), and diversified crop sequences (crop rotations). [redacted] (2020) presented figures on the increasing practice of conservation agriculture, and claimed that cropping with conservation tillage is highly reliant on the use of glyphosate to provide control of weeds in the intercrop period prior to crop establishment. [redacted] (2020) further discussed environmental benefits of glyphosate that can be realized through its use in conservation tillage: improved carbon sequestration; improved soil quality; improved biodiversity due to reduced disturbance and possibly improved dietary resources; improved water quality and reduction of erosion due to increased structural stability of the soil and physical protection from the soil mulch; enabled cover crop management (since glyphosate is the standard herbicide used for terminating cover crops) and thereby protection of the soil surface from erosion, reduced nitrogen leaching, better habitat for soil organisms and wildlife, mitigation of compaction damage of the topsoil and suppression of weeds.

The role of glyphosate on habitat restoration and control of invasive plant species

According to [redacted] (2020) "Glyphosate is highly efficacious against most of the weeds on the EU invasive alien species list of concern and can be leveraged as an important tool to protect EU biodiversity against invasive species." The report mentioned several examples of use of glyphosate in the control of invasive species.

Glyphosate's Environmental Safety Profile

Under this sub-heading, [redacted] (2020) provided a short description of the Environmental Fate profile and the Ecotoxicological profile of glyphosate. These aspects are covered elsewhere in the RAR.

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Relevant and reliable studies for the risk assessment for birds of glyphosate and relevant metabolites are summarised in the tables below. Details of the acute studies are summarised in Volume 3CA, Section B.9.1.1.

Table B.9.1.1-1: Relevant endpoints for risk assessment: Acute oral toxicity of glyphosate and AMPA to birds

Reference in dossier	Substance	Species	Test design	LD_{50} (mg a.e./kg bw)
CA 8.1.1.1	Glyphosate	Bird[1]	Acute oral	Extrapolated LD_{50} = 4334 mg/kg bw/day[2]
[redacted] 1991 CA 8.1.1.1/009	AMPA	*Colinus virginianus*	Acute oral	LD_{50} > 2250 mg/kg bw/day

[1] Tested species: Bobwhite quail (*Colinus virginianus*), Japanese quail (*Coturnix coturnix japonica*), Mallard duck (*Anas platyrhynchos*)

[2] All acute oral bird studies resulted in endpoints > 2000 mg/kg bw (see Vol. 3CA, Section B.9.1.1). Therefore, an extrapolation factor of 2.167 as recommended in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438) was applied.

Details of this reproduction study is summarised in Volume 3CA, Section B.9.1.3.

Table B.9.1.1-2: Relevant endpoints for risk assessment: Reproductive toxicity of glyphosate to birds

Reference in dossier	Substance	Species	Test design	NOAEL (mg a.e./kg feed)	NOAEL (mg a.e./kg bw/d)
[redacted] 1999; 123-187; CA 8.1.1.3/004	Glyphosate technical	*Colinus virginianus*	17 weeks reproduction	1000	116

a.e.: acid equivalents

Risk assessment for metabolites

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1% of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). Available mammalian and avian data on the metabolite AMPA indicate that it is of similar or lower toxicity than glyphosate acid (see Volume 3CA, Section B.6).

Following application to plant tissues, unchanged glyphosate was the only significant residue. In presence of soil as a substrate the active substance is quickly degraded, leaving AMPA at rates comparable or even higher than parent glyphosate. However, the uptake via the roots and the translocation in the plants was very low, not resulting in significant residue levels as confirmed by plant metabolism and confined rotational crop studies Therefore, it can be concluded that the risk to birds will be acceptably low and no further quantitative risk assessment is conducted.

Risk assessment for the representative formulation

An acute oral mammalian study is available with the formulation which is presented in the toxicological section of Volume 3CA. This study shows, that the acute toxicity of the formulation (> 5000 mg/kg bw) is not more elevated than the toxicity of the active substance alone (> 2000 mg/kg bw). Assuming a similar pattern for birds as for mammals, the avian risk assessment for the representative formulation is considered to be covered by the avian risk assessment presented for the active substance glyphosate.

Literature data

Summaries and evaluation by the RMS of the available literature data are presented in Volume 3CA, section 9.2.3. The applicant proposed that no information from published literature have an impact on the selected avian endpoints based on standard data as presented here. However, the RMS notes that some sublethal effects related to feeding behaviour, growth and embryo development were observed in the study by Ruuskanen *et al*. (2020).

Ruuskanen (2020) reported effects on flight feather moult and plumage development in juvenile Japanese quails at a dietary concentration of 164 mg a.s./kg food, indicating a higher sensitivity for this parameter compared to observations in the available standard endpoints.

The RMS proposes that further consideration is needed on possible ecological relevance of these results. However, for the time being, the risk assessment is based on the standard avian data.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Studies considering the toxicity of glyphosate, relevant metabolites and the representative formulation to mammals were assessed for their validity to current and relevant guidelines. A more detailed summary and evaluation by the RMS are provided in Vol 3CA, section 6. The selection of endpoints and the discussion around those used in the risk assessment are presented in Vol 1, section 2.9.4.

Details of the acute oral studies on mammals are summarised in Volume 3CA, section 6.1.

Table B.9.1.2-1: Relevant endpoints for risk assessment: Acute oral toxicity of glyphosate, AMPA and MON 52276 to mammals

Substance	Species	Test design	LD_{50}
Glyphosate acid	Rat/Mice	Acute toxicity	Screening Step: > 2000 mg a.e./kg bw
Glyphosate acid	Rat/Mice	Acute toxicity	Tier 1/Tier 2: 3447 mg a.e./kg bw
AMPA	Mouse	Acute toxicity	> 5000 mg/kg bw
MON 52276	Rat	Acute toxicity	> 5000 mg a.e./kg bw

a.e.: acid equivalents

An acute oral mammalian study is available with the formulation which is presented in the toxicological section (Volume 3CP, section 6). The data shows, that the acute toxicity of the formulation (> 5000 mg/kg bw) is not higher than the toxicity of the active substance alone (> 2000 mg/kg bw). Therefore the

mammalian risk assessment for the representative formulation is considered to be covered by the mammalian risk assessment presented for the active substance glyphosate.

Details of the developmental and reproduction studies on mammals are summarised in Volume 3CA, section 6.6-6.8.

Table B.9.1.2-2: Relevant endpoints for risk assessment: Reproductive toxicity of glyphosate and AMPA to mammals

Substance	Species	Test design	NOAEL
Glyphosate acid	Rabbit	Developmental toxicity (long-term)	Screening: 50 mg a.e./kg bw/d
Glyphosate acid	Rabbit	Developmental toxicity (long-term)	Tier 1 and 2: 100 mg a.e./kg bw/d
AMPA	Rat	13 week oral	150 mg/kg bw/d

a.e.: acid equivalents

Risk assessment for metabolites

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1% of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several mammal toxicity studies which demonstrated that it is of similar or lower toxicity than glyphosate acid (Vol 3CA, section 6).

Following application to plant tissues, unchanged glyphosate was the only significant residue. In presence of soil as a substrate the active substance is quickly degraded, leaving AMPA at rates comparable or even higher than parent glyphosate. However, the uptake via the roots and the translocation in the plants was very low, not resulting in significant residue levels as confirmed by plant metabolism and confined rotational crop studies. Therefore, it can be concluded that the risk to mammals will be acceptably low and no further quantitative risk assessment on the main metabolite is conducted.

Literature data

There are no literature articles and peer-reviewed published data considered to be relevant for the selection of endpoints for glyphosate or its relevant metabolites on wild mammals.

Concerning effects at the ecosystem level – specifically indirect effects on mammals via trophic interactions, and considering impacts on biodiversity at a wider landscape level, a biodiversity assessment is presented at the end of this section. The references cited by the applicant in their evaluation are summarised in Appendix to this document.

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.2.1. Risk assessment for birds

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438); hereafter referred to as EFSA/2009/1438.

The table below summarises how the risk assessment for birds considers all the proposed uses and the application rates presented in the GAP.

Table B.9.2.1-1: Risk assessment strategy for birds

GAP number and summary of use	Application rate considered (28-day interval unless otherwise stated)									
	1 × 540 g/ha	**1 × 720 g/ha**	**1 × 1080 g/ha**	**2 × 720 g/ha**	**1 × 1440 g/ha**	**3 × 720 g/ha**	**1 × 1800 g/ha**	**2 × 1080 g/ha[1]**	**2 × 1440 g/ha**	**2 × 1800 g/ha (90 days apart)**
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre emergence of field crops.		X	X		X					
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of field crops.		X	X	X	X	X		X		
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of field crops.	X									
Use 4 a-c: Applied to weeds (post emergence) below trees in orchards.		X	X	X	X	X		X	X	
Use 5 a-c: Applied to weeds (post emergence) below vines in vineyards		X	X	X	X	X		X	X	
Use 6 a-b: Applied to weeds (post emergence) in field crops BBCH < 20		X	X							
Use 7 a-b: Applied to weeds (post emergence) around railroad tracks							X			X
Use 8 and 9: Applied to invasive species (post emergence) in agricultural and non-agricultural areas							X			
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of field crops		X	X							

X = this use is covered by the application rate indicated.

[1] Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.e./ha plus 1 × 720 g a.e./ha (28 day interval between each application)

B.9.2.1.1. Screening assessment

For the screening assessment: crops that maybe present at time of application to target weeds and the relevant application rates shown in the table above are considered. The acute and long-term screening assessment results are presented below according to the following main uses:

- in **field crops** (covering GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c); pre-sowing, pre-planting pre-emergence, post-harvest. Exposure to birds via grassland, bare soil and field crops is considered and is covered by the general screening scenarios grassland, bare soil and bulb and onion like crops (etc.). It should be noted that the bare soil scenario for birds and mammals is intended for true bare soils. As no foliage is present, no herbivorous birds or mammals are relevant, and also the omnivorous bird/mammal diets lack all foliage components. Glyphosate, as a contact herbicide, will only be applied when weeds are present. Thus, the bare soil scenario is considered to be of low relevance for the bird and mammal risk assessment. Further, grassland is not included in the list of representative field crop uses. However, from the RMS' point of view the grassland may also be relevant for some situations in field crops, especially when glyphosate is used to remove weeds before sowing or after harvest. Although relevance of these scenarios are questionable, the bare soil and grassland scenarios are maintained for the standard screening assessment.

- in **orchards** (covering GAP uses 4 a-c) applied to weeds post emergence exposure below trees; exposure to small insectivorous birds in orchards is considered and is covered by the general screening scenario orchards (etc.)
- in **vineyards** (covering GAP uses 5 a-c) applied to weeds post emergence exposure below vines; exposure to small omnivorous birds in vineyards is considered and is covered by the general screening scenario vineyard.
- in **railroad tracks** (covering GAP uses 7 a-b) and in the **control of invasive species** (covering GAP uses 8 and 9) applied to weeds post emergence; exposure to birds via grassland, bare soil and field crops is considered and is covered by the general screening scenarios grassland, bare soil and bulb and onion like crops (etc.).

Field crops

Table B.9.2.1-2: Screening assessment of the acute risk for birds due to the use of glyphosate in field crops: Uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**4334**					
TER criterion		**10**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.	1 × 1440	Grassland	Large herbivorous birds	30.5	1	43.9	98.7
		Bare soil	Small granivorous birds	24.7	1	35.6	122
		Bulb and onion like crops	Small omnivorous birds	158.8	1	229	19.0
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.	2 × 1080 (28 d)	Grassland	Large herbivorous birds	30.5	1.1	36.2	120
		Bare soil	Small granivorous birds	24.7	1.1	29.3	148
		Bulb and onion like crops	Small omnivorous birds	158.8	1.1	189	23.0
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.	1 × 540	Grassland	Large herbivorous birds	30.5	1	16.5	263
		Bare soil	Small granivorous birds	24.7	1	13.3	325
		Bulb and onion like crops	Small omnivorous birds	158.8	1	85.8	50.5
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato	1 × 720	Grassland	Large herbivorous birds	30.5	1	22.0	197
		Bare soil	Small granivorous birds	24.7	1	17.8	244

Active substance		Glyphosate					
Acute toxicity (mg/kg bw)		4334					
TER criterion		10					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.		Bulb and onion like crops	Small omnivorous birds	158.8	1	114	37.9
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.	2 × 720 (28 d)	Grassland	Large herbivorous birds	30.5	1.1	24.2	179
		Bare soil	Small granivorous birds	24.7	1.1	19.6	222
		Bulb and onion like crops	Small omnivorous birds	158.8	1.1	126	34.5
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.	1 × 1080	Grassland	Large herbivorous birds	30.5	1	32.9	132
		Bare soil	Small granivorous birds	24.7	1	26.7	163
		Bulb and onion like crops	Small omnivorous birds	158.8	1	172	25.3
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet.	3 × 720 (28 d)	Grassland	Large herbivorous birds	30.5	1.1	24.2	179
		Bare soil	Small granivorous birds	24.7	1.1	19.6	222
		Bulb and onion like crops	Small omnivorous birds	158.8	1.1	126	34.5

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table B.9.2.1-3: Screening assessment of the long-term/reproductive risk for birds due to the use of glyphosate in field crops: Uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c

Reprod. Toxicity (mg/kg bw/d)		116					
TER criterion		5					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato	1 × 1440	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	12.4	9.4
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	8.70	13.3

Reprod. Toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds		Bulb and onion like crops	Small omnivorous birds	64.8	1.0 × 0.53	49.5	2.3
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	2 × 1080 (28 d)	Grassland	Large herbivorous birds	16.2	1.1 × 0.53	10.2	11.4
		Bare soil	Small granivorous birds	11.4	1.1 × 0.53	7.18	16.2
		Bulb and onion like crops	Small omnivorous birds	64.8	1.1 × 0.53	40.8	2.8
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 540	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	4.64	25.0
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	3.26	35.6
		Bulb and onion like crops	Small omnivorous birds	64.8	1.0 × 0.53	18.6	6.2
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 720	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	6.18	18.8
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	4.35	26.7
		Bulb and onion like crops	Small omnivorous birds	64.8	1.0 × 0.53	24.7	4.7
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato	2 × 720 (28 d)	Grassland	Large herbivorous birds	16.2	1.1 × 0.53	6.80	17.1
		Bare soil	Small granivorous birds	11.4	1.1 × 0.53	4.79	24.2

Reprod. Toxicity (mg/kg bw/d)		116					
TER criterion		5					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds		Bulb and onion like crops	Small omnivorous birds	64.8	1.1 × 0.53	27.2	4.3
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 1080	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	9.27	12.5
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	6.53	17.8
		Bulb and onion like crops	Small omnivorous birds	64.8	1.0 × 0.53	37.1	3.1
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	3 × 720 (28 d)	Grassland	Large herbivorous birds	16.2	1.2 × 0.53	7.42	15.6
		Bare soil	Small granivorous birds	11.4	1.2 × 0.53	5.22	22.2
		Bulb and onion like crops	Small omnivorous birds	64.8	1.2 × 0.53	29.7	3.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Orchards

Table B.9.2.1-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of glyphosate in orchards: Uses 4 a-c

Active substance		Glyphosate					
Acute toxicity (mg/kg bw)		4334					
TER criterion		10					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Orchards post-emergence of weeds	2 × 1440 (28 d)	Orchards	Small insectivorous birds	46.8	1.1	74.1	58.5
Orchards post-emergence of weeds	1 × 720	Orchards	Small insectivorous birds	46.8	1.0	33.7	129

Orchards post-emergence of weeds	1 × 1080	Orchards	Small insectivorous birds	46.8	1.0	50.5	85.7
Orchards post-emergence of weeds	2 × 720 (28 d)	Orchards	Small insectivorous birds	46.8	1.1	37.1	117
Orchards post-emergence of weeds	1 × 1440	Orchards	Small insectivorous birds	46.8	1.0	67.4	64.3
Orchards post-emergence of weeds	3 × 720 (28 d)	Orchards	Small insectivorous birds	46.8	1.1	37.1	117
Orchards post-emergence of weeds	2 × 1080 (28 d)	Orchards	Small insectivorous birds	46.8	1.1	55.6	78.0
Reprod. Toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Orchards post-emergence of weeds	2 × 1440 (28 d)	Orchards	Small insectivorous birds	18.2	1.1 × 0.53	15.3	7.6
Orchards post-emergence of weeds	1 × 720	Orchards	Small insectivorous birds	18.2	1.0 × 0.53	6.95	16.7
Orchards post-emergence of weeds	1 × 1080	Orchards	Small insectivorous birds	18.2	1.0 × 0.53	10.4	11.2
Orchards post-emergence of weeds	2 × 720 (28 d)	Orchards	Small insectivorous birds	18.2	1.1 × 0.53	7.64	15.2
Orchards post-emergence of weeds	1 × 1440	Orchards	Small insectivorous birds	18.2	1.0 × 0.53	13.9	8.3
Orchards post-emergence of weeds	3 × 720 (28 d)	Orchards	Small insectivorous birds	18.2	1.2 × 0.53	8.33	13.9
Orchards post-emergence of weeds	2 × 1080 (28 d)	Orchards	Small insectivorous birds	18.2	1.1 × 0.53	11.5	10.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Vineyards

Table B.9.2.1-5: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of glyphosate in vineyards: Uses 5 a-c

Active substance	**Glyphosate**

Acute toxicity (mg/kg bw)		**4334**					
TER criterion		**10**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Vineyard post-emergence of weeds	2 × 1440 (28 d)	Vineyard	Small omnivorous birds	95.3	1.1	151	28.7
Vineyard post-emergence of weeds	1 × 720	Vineyard	Small omnivorous birds	95.3	1.0	68.6	63.2
Vineyard post-emergence of weeds	1 × 1080	Vineyard	Small omnivorous birds	95.3	1.0	103	42.1
Vineyard post-emergence of weeds	2 × 720 (28 d)	Vineyard	Small omnivorous birds	95.3	1.1	75.5	57.4
Vineyard post-emergence of weeds	3 × 720 (28 d)	Vineyard	Small omnivorous birds	95.3	1.1	75.5	57.4
Vineyard post-emergence of weeds	1 × 1440	Vineyard	Small omnivorous birds	95.3	1.0	137	31.6
Vineyard post-emergence of weeds	2 × 1080 (28 d)	Vineyard	Small omnivorous birds	95.3	1.1	113	38.3
Reprod. Toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Vineyard post-emergence of weeds	2 × 1440 (28 d)	Vineyard	Small omnivorous birds	38.9	1.1 × 0.53	32.7	3.55
Vineyard post-emergence of weeds	1 × 720	Vineyard	Small omnivorous birds	38.9	1.0 × 0.53	14.8	7.84
Vineyard post-emergence of weeds	1 × 1080	Vineyard	Small omnivorous birds	38.9	1.0 × 0.53	22.3	5.2
Vineyard post-emergence of weeds	2 × 720 (28 d)	Vineyard	Small omnivorous birds	38.9	1.1 × 0.53	16.3	7.12
Vineyard post-emergence of weeds	3 × 720 (28 d)	Vineyard	Small omnivorous birds	38.9	1.2 × 0.53	17.8	6.52

Vineyard post-emergence of weeds	1 × 1440	Vineyard	Small omnivorous birds	38.9	1.0 × 0.53	29.7	3.91
Vineyard post-emergence of weeds	2 × 1080 (28 d)	Vineyard	Small omnivorous birds	38.9	1.1 × 0.53	24.5	4.73

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Railroad tracks and control of invasive species

Table B.9.2.1-6: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of glyphosate on railroad tracks and to control invasive species: Uses 7a-b, 8, 9

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**4334**					
TER criterion		**10**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Grassland	Large herbivorous birds	30.5	1.0	54.9	78.9
		Bare soil	Small granivorous birds	24.7	1.0	44.5	97.5
	1 × 1800	Grassland	Large herbivorous birds	30.5	1.0	54.9	78.9
		Bare soil	Small granivorous birds	24.7	1.0	44.5	97.5
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Grassland	Large herbivorous birds	30.5	1.0	54.9	78.9
		Bare soil	Small granivorous birds	24.7	1.0	44.5	97.5
		Bulb and onion like crops	Small omnivorous birds	158.8	1.0	286	15.2
Reprod. Toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	15.5	7.5
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	10.9	10.6
	1 × 1800	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	15.5	7.5
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	10.9	10.6
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Grassland	Large herbivorous birds	16.2	1.0 × 0.53	15.5	7.5
		Bare soil	Small granivorous birds	11.4	1.0 × 0.53	10.9	10.6
		Bulb and onion like crops	Small omnivorous birds	64.8	1.0 × 0.53	61.8	1.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Conclusions screening assessment

The screening TER_a values for all proposed uses of MON 52276 in field crops, orchards, vineyards, railroad tracks and control of invasive species are greater than the Commission Regulation (EU) No.546/2011 trigger of 10, indicating that acute risk to birds is acceptable following the proposed use patterns for these crops. For the reproductive risk assessment, the screening assessment resulted in a need for further consideration for some scenarios:

Field crops (Uses: 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c)

The screening TER_{lt} values for use of MON 52276 in field crops for the scenarios "bare soil" and "grassland" are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5. For the use rate of 1 × 540 g a.e./ha (*Uses 3 a-b*) acceptable long-term risk for the "bulbs and onion like crops" scenario is concluded in the screening assessment. However, regarding the scenario "bulbs and onion like crops" a Tier 1 risk assessment is necessary for the application rates 1 × 1440 g a.e./ha, 2 × 1080 g a.e./ha, 1 × 720 g a.e./ha, 1 × 1080 g a.e./ha, 2 x 720 g a.e./ha and 3 × 720 g a.e./ha.

Orchards (Uses: 4 a-c)

The screening TER_{lt} values for use of MON 52276 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that the long-term risk to birds is acceptable following the proposed use patterns in orchards.

Vineyards (Uses: 5a-c)

The screening TER_{lt} values for use of MON 52276 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5 for the application rates; 2 × 720 g a.e./ha, 3 × 720 g a.e./ha, indicating that the long-term risk to birds is acceptable following the proposed use patterns in vineyards. For the application rates of 2 × 1440 g a.e./ha and 1 × 1080 g a.e./ha a Tier 1 risk assessment is necessary.

Railroad tracks – application by spray train (Uses: 7a-c)

The screening TER_{lt} values for use of MON 52276 on railroad tracks are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that the long-term risk to birds is acceptable following the proposed use patterns around railroad tracks.

Invasive species in agricultural and non-agricultural areas (Uses: 8 and 9)

The screening TER_{lt} values for use of MON 52276 on invasive species in agricultural and non-agricultural areas for the scenarios "bare soil" and "grassland" are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that the long-term risk to birds is acceptable following the proposed use pattern. Regarding the scenario "bulbs and onion like crops" a Tier 1 risk assessment is necessary for the intended application rate of 1 × 1800 g a.e./ha.

B.9.2.1.2. Tier 1 assessment

The Tier 1 risk assessment is conducted for those proposed uses, for which the calculated TER_{lt} values are below the trigger of 5 in the screening assessment e.g. uses in field crops (except use 3 a-b), uses in vineyards and uses to control invasive species. The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal bird species from Appendix A of EFSA/2009/1438.

Due to the proposed uses of the product MON 52276 in agricultural and non-agricultural areas, justifications were provided considering which scenarios are relevant for the risk assessment. For those proposed uses where a large number of scenarios is relevant (Field crops: Use 2 a-c, 6 a-b, 10 a-c, Control of invasive species: Use 8 - 9) an approach was taken to present only the worst-case risk assessment in this section. Therefore the worst-case scenarios were selected based on the relevant generic focal species with the highest short-cut values as these are considered protective of the other scenarios with lower short-cut values. A full and complete avian Tier I risk assessment that considers all other scenarios and focal species was presented by the applicant in a separate Annex to M-CP 10 in the dossier but is not presented here.

A summary of **all** relevant scenarios and focal species (includes those presented in this section and in the Annex) is provided in table B.9.2.1-7 below. Note that the numbers in brackets refer to the bird scenarios stated in the Appendix A of EFSA/2009/1438.

Field crops (Use 1 a-c, 2 a-c, 6 a-b, 10 a-c)

For the Tier 1 assessment of the crop group "field crops", the intended use of MON 52276 includes several general uses on field crops as described further below. The applications are intended to be made by tractor mounted sprayers (K) or by hand-held equipment (*Uses 10 a-c*).

Use 1 a-c is, the "pre-sowing, pre-planting, pre-emergence" use, where the intention of this use is to prepare a non-agricultural area for agriculture use, meaning that the product is applied when no agricultural crop is present. Therefore, the applicant proposed that the "bare soil", the "grassland" and the "leafy vegetable" scenarios are relevant (regarding the 'bare soil' scenario, this is however not agreed by the RMS, since glyphosate is only applied when weeds are present). Anyway, as an acceptable risk for the "bare soil" and "grassland" scenarios was concluded at the screening assessment, a Tier 1 risk assessment is presented only for "leafy vegetables". The "leafy vegetables" scenario was considered relevant to cover species that feed on broad-leaved weeds; the small granivorous bird "finch" (71, 72), the small omnivorous bird "lark" (79, 81), the medium herbivorous/granivorous bird "pigeon" (82) and the small insectivorous bird "wagtail" (83, 84) are taken into account.

Uses 2 a-c and 10 a-c are the "post-harvest, pre-sowing, pre-planting" use where the product can be applied to existing cropland after harvest for removal of remaining crops. Thus, for this use almost all field crops need to be considered. Only for those crops where safe risk could be concluded in the screening assessment, i.e. "bare soil" and "grassland" and for crops which are generally not considered relevant ("cotton") or for spatial cultures like "bush & cane fruit", "hops", "orchards", "ornamentals/nursery" and "vineyards" a risk assessment is not considered necessary. As the product is applied after post-harvest, late crop stages are taken into account for risk assessment. Frugivorous bird scenarios are not taken into account, as the product is intended to be applied after harvest and will not be applied at typical crop stages when fruits are ripe. For the same reason also the two cereals scenario (late post emergence (May-June), BBCH 71-89 (19); late season, seed heads (35)) and the sunflower scenario (Late (Flowering, seed ripening) BBCH 61-92 (216) are not considered relevant by the applicant. However, the RMS proposes that late cereal and sunflower scenarios (35 and 216) should be included for scenario 2 a-c, due to possible exposure of birds from seed spill remaining in the field at treatment after harvest.

Thus, for the Tier 1 risk assessment for the uses 2 a-c and 10 a-c, the relevant generic focal species with the highest short-cut values at late crop stages across all relevant crop scenarios are taken into account; the medium granivorous bird "gamebird" in maize (101), the medium herbivorous / granivorous bird "pigeon" in maize (117), the small insectivorous bird "dunnock" (120), the small granivorous bird "finch" in oilseed rape (122), the small insectivorous bird "wagtail" in bulbs & onion like crops (18) and the small omnivorous bird "lark" in bulbs & onion like crops (16). These selected scenarios cover the risk for all relevant scenarios.

Uses 6 a-b are the "shielded ground directed inter-row application" uses at crop stages < BBCH 20 and all crops scenarios at early growth stages are taken into account, which are presented in the GAP, i.e.

vegetables (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables and leafy vegetables). To avoid exposure of crops, a shielded sprayer is used to ensure that the product is only applied to grasses and weeds in the inter-row. Therefore, only those vegetables crop scenarios are considered relevant where the generic focal species does not directly feed on the crop. The "grassland" scenario is considered relevant. However, as an acceptable risk was concluded for these scenarios already at the screening assessment the Tier 1 risk assessment is not required.

Thus, for the tier 1 risk assessment for the uses 6a-b, the relevant generic focal species with the highest short-cut values at early crop stages (< BBCH 20) across all relevant crops scenarios are taken into account, i.e. the medium herbivorous/granivorous bird "pigeon" in leafy vegetables (82), the small insectivorous bird "wagtail" in bulbs & onion like crops (17), the small omnivorous bird "lark" in bulbs & onion like crops (14) and the small granivorous bird "finch" in leafy vegetables (71). These selected scenarios cover the risk for all relevant scenarios.

Vineyards (Use 5 a-c)

For the crop grouping "vines" all non-frugivorous bird scenarios are taken into account, i.e. the small insectivorous bird "redstart" (217, 218), the small granivorous bird "finch" (219, 220, 221) and the small omnivorous bird "lark" (231, 232, 233) are taken into account.

Invasive species in agricultural and non-agricultural areas (Use 8-9)

For the use on invasive species in agricultural and non-agricultural areas, almost all crops need to be considered. Only for those crops where safe risk could be proven in the screening assessment, i.e. "bare soil" and "grassland" or which are not considered relevant ("cotton") do not need to be assessed in the Tier 1 risk assessment. In general, those scenarios need to be taken into account, where a downward application of the product is relevant. Frugivorous bird scenarios are not taken into account, as the product is intended to be applied only on the invasive species Giant hogweed (*Heracleum mantegazzianum*) and Japanese knotweed (*Reynoutria japonica*) and due to the specific application method (handheld, spraying shield) fruits will not be exposed to the product. For the same reason also the cereal scenario (late season, seed heads; 35) and the sunflower scenario (Late (Flowering, seed ripening) BBCH 61-92 (216) are not considered relevant.

Thus, for the Tier 1 risk assessment for uses 8 and 9, the relevant generic focal species with the highest short-cut values across all relevant crop scenarios are taken into account, i.e. the large herbivorous bird "goose" in cereals (22), the medium granivorous bird "gamebird" in maize (99), the medium herbivorous granivorous bird "pigeon" in leafy vegetables (82), the small granivorous bird "finch" in leafy vegetables (71), the small insectivorous bird "dunnock" in oilseed rape (120), the small insectivorous bird "finch" in hop (66), the small insectivorous bird "passerine" in cereals (21), the small insectivorous bird "tit" in orchards (141), the small insectivorous bird "wagtail" in bulbs and onion like crops (17), the small insectivorous bird "warbler" in bush and cane fruit (20), the small insectivorous bird "redstart" in vineyards (217), the small insectivorous / worm feeding species "thrush" in maize (102), and the small omnivorous bird "lark" (14). These selected scenarios cover the risk for all relevant scenarios.

Table B.9.2.1-7: Summary of avian scenarios presented for Tier 1. 'Worst case scenarios are indicated in bold and are included in the Tier 1 risk assessment below.

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
Field crops (Pre-sowing, pre-planting, pre-emergence): Use 1 a-c				
No. 71	**Leafy vegetables BBCH 10 - 49**	**Small granivorous bird "finch" Serin (*Serinus serinus*)**	**12.6**	**Vol 3CP, B.9.2.1**

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
No. 72	**Leafy vegetables BBCH ≥ 50**	**Small granivorous bird "finch" Serin (*Serinus serinus*)**	**3.8**	**Vol. 3CP, B.9.2.1**
No. 79	**Leafy vegetables BBCH 10 - 49**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**10.9**	**Vol. 3CP, B.9.2.1**
No. 81	**Leafy vegetables BBCH ≥ 50**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**3.3**	**Vol. 3CP, B.9.2.1**
No. 82	**Leafy vegetables Leaf development BBCH 10 - 19**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7**	**Vol. 3CP, B.9.2.1**
No. 83	**Leafy vegetables BBCH 10 - 19**	**Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)**	**11.3**	**Vol. 3CP, B.9.2.1**
No. 84	**Leafy vegetables BBCH ≥ 20**	**Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)**	**9.7**	**Vol. 3CP, B.9.2.1**
Field crops (Post-harvest, pre-sowing, pre-planting): Use 2 a-c, 10 a-c				
No. 7	Bulb and onion like crops BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	Covered by scenario no. 122
No. 16	**Bulb and onion like crops BBCH ≥ 40**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**6.5**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 18	**Bulb and onion like crops BBCH ≥ 20**	**Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)**	**9.7**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 34	Cereals BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 16
No. 35	**Cereals Late season- Seed heads**	**Small granivorous/insectivorous bird "bunting" Yellowhammer (*Emberiza citronella*)**	**4.7**	**Added by RMS**
No. 49	Fruiting vegetables BBCH ≥ 50	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 7
No. 58	Fruiting vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no 16
No. 61	Fruiting vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 72	Leafy vegetables BBCH ≥ 50	Small granivorous bird "finch" Serin (*Serinus serinus*)	3.8	Covered by scenario no. 7
No. 81	Leafy vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no 16

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
No. 84	Leafy vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 86	Legume forage BBCH ≥ 50	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 7
No. 95	Legume forage BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no 16
No. 98	Legume forage BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 101	**Maize BBCH ≥ 40**	**Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)**	**0.8**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 114	Maize BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (Lullula arborea)	2.7	Covered by scenario no 16
No. 117	**Maize BBCH ≥ 40**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**5.7**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 119	Maize BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	4.8	Covered by scenario no. 18
No. 120	**Oilseed rape Late (with seeds) BBCH 30-99**	**Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)**	**2.7**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 122	**Oilseed rape Late (with seeds) BBCH 80-99**	**Small granivorous bird "finch" Linnet (*Carduelis cannabina*)**	**11.4**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 134	Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	2.7	Covered by scenario no 16
No. 138	Oilseed rape BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	0.9	Covered by scenario no 117
No. 160	Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no 16
No. 162	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 164	Pulses BBCH ≥ 50	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 7
No. 173	Pulses BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no 16

EFSA Appendix A Scenario Number	**Tier 1 scenario**	**Generic focal species**	**SVm**	**Risk assessment presented**
No. 176	Pulses BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 178	Root & stem vegetables BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 7
No. 187	Root & stem vegetables BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no 16
No. 189	Root & stem vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 198	Strawberries BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	4.4	Covered by scenario no 16
No. 201	Strawberries BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 18
No. 216	**Sunflower Late (Flowering, seed ripening) BBCH 61-92**	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	**Added by RMS**
Field crops (Shielded ground directed inter-row application): Use 6a, b				
No. 6	Bulbs and onion like crops BBCH 10 - 39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 14	**Bulbs and onion like crops BBCH 10 - 39**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**10.9**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 17	**Bulbs and onion like crops BBCH 10 - 19**	**Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)**	**11.3**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 48	Fruiting vegetables BBCH 10 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 56	Fruiting vegetables BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 60	Fruiting vegetables BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 71	**Leafy vegetables BBCH 10 - 49**	**Small granivorous bird "finch" Serin (*Serinus serinus*)**	**12.6**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 79	Leafy vegetables BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 82	**Leafy vegetables Leaf development BBCH 10 -19**	**Medium herbivorous/granivorous bird "pigeon"**	**22.7[1]**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
		Wood pigeon (*Columba palumbus*)		
No. 83	Leafy vegetables BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 85	Legume forage BBCH 10 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 93	Legume forage BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 97	Legume forage BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 158	Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 161	Potatoes BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 163	Pulses BBCH 10 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 171	Pulses BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 174	Pulses Leaf development BBCH 10 - 19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	Covered by scenario no. 82
No. 175	Pulses BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 177	Root & stem vegetables BBCH 10 - 39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 185	Root & stem vegetables BBCH 10 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 188	Root & stem vegetables BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 206	Sugar beet Early (spring) BBCH 10 - 19	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 207	Sugar beet BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	5.9	Covered by scenario no. 17
Vineyard: Use 5 a-c				
No. 217	**Vineyard BBCH 10 – 19**	**Small insectivorous bird "redstart"**	**11.5**	**Vol. 3CP, B.9.2.1**

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
		Black redstart (*Phoenicurus ochruros*)		
No. 218	**Vineyard BBCH 20 – 39**	**Small insectivorous bird "redstart" Black redstart (*Phoenicurus ochruros*)**	**9.9**	**Vol. 3CP, B.9.2.1**
No. 219	**Vineyard BBCH 10 – 19**	**Small granivorous bird "finch" Linnet (*Carduelis cannabina*)**	**6.9**	**Vol. 3CP, B.9.2.1**
No. 220	**Vineyard BBCH 20 – 39**	**Small granivorous bird "finch" Linnet (*Carduelis cannabina*)**	**5.7**	**Vol. 3CP, B.9.2.1**
No. 221	**Vineyard BBCH ≥ 40**	**Small granivorous bird "finch" Linnet (*Carduelis cannabina*)**	**3.4**	**Vol. 3CP, B.9.2.1**
No. 231	**Vineyard BBCH 10 – 19**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**6.5**	**Vol. 3CP, B.9.2.1**
No. 232	**Vineyard BBCH 20 – 39**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**5.4**	**Vol. 3CP, B.9.2.1**
No. 233	**Vineyard BBCH ≥ 40**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**3.3**	**Vol. 3CP, B.9.2.1**
Control of invasive species in agricultural and non-agricultural areas: Use 8 – 9				
No. 6	Bulbs and onion like crops BBCH 10 - 39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 7	Bulb and onion like crops BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	Covered by scenario no. 71
No. 14	**Bulbs and onion like crops BBCH 10 - 39**	**Small omnivorous bird "lark" Woodlark (*Lullula arborea*)**	**10.9**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 16	Bulb and onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	Covered by scenario no. 14
No. 17	**Bulbs and onion like crops BBCH 10 - 19**	**Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)**	**11.3**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 18	Bulb and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 20	**Bush & cane fruit Whole season BBCH 00 - 79 Currants**	**Small insectivorous bird "warbler" Willow warbler (*Phylloscopus trochilus*)**	**20.3**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 21	**Cereals Late post-emergence (May-June) BBCH 71 - 89**	**Small insectivorous bird "passerine" Fan tailed warbler**	**22.4**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
No. 22	**Cereals Early (shoots) autumn-winter BBCH 10 - 29**	**Large herbivorous bird "goose" Pink-foot goose (*Anser brachyrhynchus*)**	**16.2**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 31	Cereals BBCH 10 - 29	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 33	Cereals BBCH 30 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	Covered by scenario no. 14
No. 34	Cereals BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 48	Fruiting vegetables BBCH 10 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 49	Fruiting vegetables BBCH ≥ 50	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 71
No. 56	Fruiting vegetables BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 58	Fruiting vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 60	Fruiting vegetables BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 61	Fruiting vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 66	**Hops BBCH 10 - 19**	**Small insectivorous bird "finch" Chaffinch (*Fringilla colebs*)**	**9.1**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 67	Hops BBCH ≥ 20	Small insectivorous bird "finch" Chaffinch (*Fringilla colebs*)	10.6	Covered by scenario no. 66
No. 68	Hops BBCH 10 - 19	Small granivorous bird "finch" Goldfinch (*Carduelis carduelis*)	11.4	Covered by scenario no. 71
No. 69	Hops BBCH 20 - 39	Small granivorous bird "finch" Goldfinch (*Carduelis carduelis*)	5.7	Covered by scenario no. 71
No. 70	Hops BBCH ≥ 40	Small granivorous bird "finch" Goldfinch (*Carduelis carduelis*)	3.4	Covered by scenario no. 71
No. 71	**Leafy vegetables BBCH 10 - 49**	**Small granivorous bird "finch" Serin (*Serinus serinus*)**	**12.6**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 72	Leafy vegetables BBCH ≥ 50	Small granivorous bird "finch" Serin (*Serinus serinus*)	3.8	Covered by scenario no. 71
No. 79	Leafy vegetables BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 81	Leafy vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
No. 82	**Leafy vegetables Leaf development BBCH 10 - 19**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7[1]**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 83	Leafy vegetables BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 84	Leafy vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 85	Legume forage BBCH 10 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 86	Legume forage BBCH ≥ 50	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 71
No. 93	Legume forage BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 94	Legume forage BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 96	Legume forage Leaf development BBCH 21 - 49	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	Covered by scenario no. 14
No. 97	Legume forage BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 98	Legume forage BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 99	**Maize BBCH 10 - 29**	**Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)**	**3.0**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 100	Maize BBCH 30 - 39	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	1.5	Covered by scenario no. 99
No. 101	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	Covered by scenario no 99
No. 102	**Maize Leaf development BBCH 10 - 19**	**Small insectivorous / worm feeding species "thrush" Robin (*Erithacus rubecula*)**	**5.7**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 111	Maize BBCH 10 - 29	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 113	Maize BBCH 30 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	Covered by scenario no. 14

EFSA Appendix A Scenario Number	**Tier 1 scenario**	**Generic focal species**	**SVm**	**Risk assessment presented**
No. 114	Maize BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	2.7	Covered by scenario no. 14
No. 115	Maize BBCH 10 - 29	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	Covered by scenario no. 14
No. 116	Maize BBCH 30 - 39	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	11.4	Covered by scenario no. 14
No. 117	Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	5.7	Covered by scenario no. 14
No. 118	Maize BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 119	Maize BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	4.8	Covered by scenario no. 17
No. 120	**Oilseed rape Late (with seeds) BBCH 30 - 99**	**Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)**	**2.7**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 121	Oilseed rape Early (shoots) BBCH 10 - 19	Large herbivorous bird "goose" Greylag goose (*Anser anser*)	15.9	Covered by scenario no. 22
No. 122	Oilseed rape Late (with seeds) BBCH 80 - 99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 131	Oilseed rape BBCH 10 - 29	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 133	Oilseed rape BBCH 30 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 134	Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	2.7	Covered by scenario no. 14
No. 135	Oilseed rape BBCH 10 - 19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	Covered by scenario no. 14
No. 136	Oilseed rape BBCH 20 - 29	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	3.5	Covered by scenario no. 14
No. 137	Oilseed rape BBCH 30 - 39	Medium herbivorous/granivorous bird "pigeon"	1.1	Covered by scenario no. 14

EFSA Appendix A Scenario Number	**Tier 1 scenario**	**Generic focal species**	**SVm**	**Risk assessment presented**
		Wood pigeon (*Columba palumbus*)		
No. 138	Oilseed rape BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	0.9	Covered by scenario no. 14
No. 139	Oilseed rape BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	5.9	Covered by scenario no. 17
No. 140	Oilseed rape BBCH 20 - 29	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	2.8	Covered by scenario no. 17
No. 141	**Orchard Spring Summer**	**Small insectivorous bird "tit" Bluetit (*Parus caeruleus*)**	**18.2**	**Vol. 3CP, B.9.2.1 (Worst case scenario)**
No. 142	Orchard Not crop directed application all season	Small insectivorous/worm feeding species "thrush" Robin (*Erithacus rubecula*)	2.7	Covered by scenario no. 14
No. 146	Orchard Not crop directed application all season	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	Covered by scenario no. 71
No. 158	Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 160	Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 161	Potatoes BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 162	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 163	Pulses BBCH 10 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 164	Pulses BBCH ≥ 50	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 71
No. 171	Pulses BBCH 10 - 49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 173	Pulses BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 174	Pulses Leaf development BBCH 10 - 19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	Covered by scenario no. 14
No. 175	Pulses BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17

EFSA Appendix A Scenario Number	Tier 1 scenario	Generic focal species	SVm	Risk assessment presented
No. 176	Pulses BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motcailla flava*)	9.7	Covered by scenario no. 17
No. 177	Root & stem vegetables BBCH 10 - 39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 178	Root & stem vegetables BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 71
No. 185	Root & stem vegetables BBCH 10 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 187	Root & stem vegetables BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	Covered by scenario no. 14
No. 188	Root & stem vegetables BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 189	Root & stem vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 196	Strawberries BBCH 10 - 39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 198	Strawberries BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	4.4	Covered by scenario no. 14
No. 200	Strawberries BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 201	Strawberries BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 202	Sugar beet Late (summer / autumn) BBCH 30 - 49	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	Covered by scenario no. 71
No. 206	Sugar beet Early (spring) BBCH 10 - 19	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14
No. 207	Sugar beet BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	5.9	Covered by scenario no. 17
No. 209	Sugar beet BBCH 10 - 19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	5.9	Covered by scenario no. 17
No. 210	Sugar beet BBCH 20 - 49	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	Covered by scenario no. 17
No. 214	Sunflower	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	Covered by scenario no. 14

EFSA Appendix A Scenario Number	**Tier 1 scenario**	**Generic focal species**	**SVm**	**Risk assessment presented**
	Early germination / Leaf development (BBCH 00 - 19)			
No. 215	Sunflower Early germination / Leaf development (BBCH 00 – 19)	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	Covered by scenario no. 17
No. 217	**Vineyard** **BBCH 10 - 19**	**Small insectivorous species "redstart"** **Black redstart "*Phoenicurus ochruros*"**	**11.5**	**Vol. 3CP, B.9.2.1** **(Worst case scenario)**
No. 218	Vineyard BBCH ≥ 20	Small insectivorous species "redstart" Black redstart "*Phoenicurus ochruros*"	9.9	Covered by scenario no. 217
No. 219	Vineyard BBCH 10 - 19	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	Covered by scenario no. 71
No. 220	Vineyard BBCH 20 - 39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	5.7	Covered by scenario no. 71
No. 221	Vineyard BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	Covered by scenario no. 71
No. 231	Vineyard BBCH 10 - 19	Small omnivorous bird "lark" Wood lark (*Lullula arborea*)	6.5	Covered by scenario no. 14
No. 232	Vineyard BBCH 20 - 39	Small omnivorous bird "lark" Wood lark (*Lullula arborea*)	5.4	Covered by scenario no. 14
No. 233	Vineyard BBCH ≥ 40	Small omnivorous bird "lark" Wood lark (*Lullula arborea*)	3.3	Covered by scenario no. 14

Worst case scenarios are indicated in bold and are included in the Tier 1 risk assessment below.

[1] The given short-cut value is corrected and deviates from the short-cut value presented in the Appendix A of the EFSA/2009/1438. In the Appendix A for the wood pigeon (*Columba palumbus*) a short-cut value of 37.0 is stated. This value was calculated by multiplication of the FIR/BW (1.29) with the mean RUD value (28.7). As the correct FIR/BW for the wood pigeon is 0.79, as stated for all other crop scenarios in the Appendix A the risk assessment was done with the corrected short-cut value of 22.7 (28.7 × 0.79).

[2] Same scenario like scenario 207.

The Tier 1 risk assessment is presented in the following tables for the relevant uses in field crops (except use 3 a-b), uses in vineyards and uses to control invasive species, taking into account those generic focal species scenarios which were indicated in bold in the table above.

Field crops

Table B.9.2.1-8: Tier 1 assessment of the long-term/reproductive risk for birds due the use of glyphosate in field crops (Pre-sowing, pre-planting, pre-emergence): Use 1 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Pre-sowing, pre-planting, pre-emergence)	1 × 1440	Leafy vegetables BBCH 10-49	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	1.0 × 0.53	9.62	12.1
		Leafy vegetables BBCH ≥ 50	Small granivorous bird "finch" Serin (*Serinus serinus*)	3.8	1.0 × 0.53	2.90	40.0
		Leafy vegetables BBCH 10-49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1.0 × 0.53	8.32	13.9
		Leafy vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.0 × 0.53	2.52	46.0
		Leafy vegetables Leaf development BBCH 10-19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1.0 × 0.53	17.3	6.7
		Leafy vegetables BBCH 10-19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1.0 × 0.53	8.62	13.5
		Leafy vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.0 × 0.53	7.40	15.7
	1 × 1080	Leafy vegetables BBCH 10-49	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	1.0 × 0.53	7.21	16.1
		Leafy vegetables BBCH ≥ 50	Small granivorous bird "finch" Serin (*Serinus serinus*)	3.8	1.0 × 0.53	2.18	53.2
		Leafy vegetables BBCH 10-49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1.0 × 0.53	6.24	18.6
		Leafy vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.0 × 0.53	1.89	61.4
		Leafy vegetables Leaf development BBCH 10-19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1.0 × 0.53	13.0	8.9

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Leafy vegetables BBCH 10-19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1.0 × 0.53	6.47	17.9
		Leafy vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.0 × 0.53	5.55	20.9
	1 × 720	Leafy vegetables BBCH 10-49	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	1.0 × 0.53	4.81	24.1
		Leafy vegetables BBCH ≥ 50	Small granivorous bird "finch" Serin (*Serinus serinus*)	3.8	1.0 × 0.53	1.45	80.0
		Leafy vegetables BBCH 10-49	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1.0 × 0.53	4.16	27.9
		Leafy vegetables BBCH ≥ 50	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.0 × 0.53	1.26	92.1
		Leafy vegetables Leaf development BBCH 10-19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1.0 × 0.53	8.66	13.4
		Leafy vegetables BBCH 10-19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1.0 × 0.53	4.31	26.9
		Leafy vegetables BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.0 × 0.53	3.70	31.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

Table B.9.2.1-9: Tier 1 assessment of the long-term/reproductive risk for birds due the use of glyphosate in field crops (Post-harvest, pre-sowing, pre-planting): Use 2 a-c, 10 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Post-harvest, pre-sowing, pre-planting)	1 × 1440	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	1.0 × 0.53	0.612	190
		Maize BBCH 10-29 (to cover birds that visit the fields and consume treated grasses and weeds**)**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7**	1.0 × 0.53	17.3	6.7
		Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	5.7	1.0 × 0.53	4.35	26.7
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.0 × 0.53	2.06	56.3
		Oilseed rape Late (with seeds) BBCH 80-99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	1.0 × 0.53	8.70	13.3
		Bulbs and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.0 × 0.53	7.40	15.7
		Bulbs & onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.0 × 0.53	4.96	19.4
		Cereals Late season- Seed heads	**Small granivorous/insectivorous bird "bunting" Yellowhammer (*Emberiza citronella*)**	**4.7**	1.0 × 0.53	3.59	32.3
		Sunflower Late (Flowering, seed ripening) BBCH 61-92	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	1.0 × 0.53	7.63	15.2
	2 × 1080 (28 d)	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	1.1 × 0.53	0.504	230
		Maize BBCH 10-29	**Medium herbivorous/granivorous**	**22.7**	1.1 × 0.53	14.3	8.11

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		(to cover birds that visit the fields, and consume treated grasses and weeds)	**bird "pigeon" Wood pigeon (*Columba palumbus*)**				
		Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	5.7	1.1 × 0.53	3.59	32.3
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.1 × 0.53	1.70	68.2
		Oilseed rape Late (with seeds) BBCH 80-99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	1.1 × 0.53	7.18	16.2
		Bulbs and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.1 × 0.53	6.11	19.0
		Bulbs & onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.1 × 0.53	4.09	28.4
		Cereals Late season- Seed heads	**Small granivorous/insectivorous bird "bunting" Yellowhammer (*Emberiza citronella*)**	**4.7**	1.1 × 0.53	2.96	39.2
		Sunflower Late (Flowering, seed ripening) BBCH 61-92	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	1.1 × 0.53	6.30	83.4
	1 × 720	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	1.0 × 0.53	0.305	380
		Maize BBCH 10-29 (to cover birds that visit the fields, and consume treated grasses and weeds**)**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7**	1.0 × 0.53	8.68	13.4
		Maize BBCH ≥ 40	Medium herbivorous/granivorous	5.7	1.0 × 0.53	2.18	53.2

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
			bird "pigeon" Wood pigeon (*Columba palumbus*)				
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.0 × 0.53	1.13	102.7
		Oilseed rape Late (with seeds) BBCH 80-99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	1.0 × 0.53	4.79	24.2
		Bulbs and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.0 × 0.53	3.70	31.4
		Bulbs & onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.0 × 0.53	2.48	46.8
		Cereals Late season- Seed heads	**Small granivorous/insectivorous bird "bunting" Yellowhammer (*Emberiza citronella*)**	**4.7**	1.0 × 0.53	1.79	64.8
		Sunflower Late (Flowering, seed ripening) BBCH 61-92	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	1.0 × 0.53	3.82	137.9
	2 × 720 (28 d)	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	1.1 × 0.53	0.336	345
		Maize BBCH 10-29 (to cover birds that visit the fields, and consume treated grasses and weeds**)**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7**	1.1 × 0.53	9.52	12.2
		Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	5.7	1.1 × 0.53	2.39	48.5
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.1 × 0.53	1.13	103

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Oilseed rape Late (with seeds) BBCH 80-99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	1.1 × 0.53	4.79	24.2
		Bulbs and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.1 × 0.53	4.07	28.5
		Bulbs & onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.1 × 0.53	2.73	42.5
		Cereals Late season- Seed heads	**Small granivorous/insectivorous bird "bunting" Yellowhammer (*Emberiza citronella*)**	**4.7**	1.2 × 0.53	2.15	54.0
		Sunflower Late (Flowering, seed ripening) BBCH 61-92	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	1.2 × 0.53	4.58	25.3
	1 × 1080	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	1.0 × 0.53	0.458	253
		Maize BBCH 10-29 (to cover birds that visit the fields, and consume treated grasses and weeds**)**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7**	1.0 × 0.53	13.0	8.93
		Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	5.7	1.0 × 0.53	3.26	35.6
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.0 × 0.53	1.55	74.8
		Oilseed rape Late (with seeds) BBCH 80-99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	1.0 × 0.53	6.52	17.8
		Bulbs and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.0 × 0.53	5.55	20.9

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Bulbs & onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.0 × 0.53	3.72	31.2
		Cereals Late season- Seed heads	**Small granivorous/insectivorous bird "bunting" Yellowhammer (*Emberiza citronella*)**	**4.7**	1.0 × 0.53	2.69	43.1
		Sunflower Late (Flowering, seed ripening) BBCH 61-92	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	1.0 × 0.53	5.72	20.3
	3 × 720 (28 d)	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	0.8	1.2 × 0.53	0.366	317
		Maize BBCH 10-29 (to cover birds that visit the fields, and consume treated grasses and weeds**)**	**Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)**	**22.7**	1.2 × 0.53	10.4	11.2
		Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	5.7	1.2 × 0.53	2.61	44.4
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.2 × 0.53	1.24	93.5
		Oilseed rape Late (with seeds) BBCH 80-99	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	11.4	1.2 × 0.53	5.22	22.2
		Bulbs and onion like crops BBCH ≥ 20	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	9.7	1.2 × 0.53	4.44	26.1
		Bulbs & onion like crops BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.2 × 0.53	2.98	38.9
		Cereals Late season- Seed heads	**Small granivorous/insectivorous bird "bunting"**	**4.7**	1.2 × 0.53	2.15	53.4

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		116					
TER criterion		5					
GAP crop	Application rate (g a.e./ha)	Crop scenario Growth stage	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
			Yellowhammer (*Emberiza citronella*)				
		Sunflower Late (Flowering, seed ripening) BBCH 61-92	**Small granivorous/insectivorous bird 'bunting' Yellowhammer (*Emberiza citronella*)**	**10.0**	1.2 × 0.53	4.58	114.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table B.9.2.1-10: Tier 1 assessment of the long-term/reproductive risk for birds due the use of glyphosate in field crops (Shielded ground directed inter-row application): Use 6 a-b

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		116					
TER criterion		5					
GAP crop	Application rate (g a.e./ha)	Crop scenario Growth stage	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Field crops (Shielded ground directed inter-row application)	1 × 1080	Bulbs and onion like crops BBCH 10-19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1.0 × 0.53	6.47	17.9
		Bulbs & onion like crops BBCH 10-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1.0 × 0.53	6.24	18.6
		Leafy vegetables BBCH 10-49	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	1.0 × 0.53	7.21	16.1
		Leafy vegetables Leaf development BBCH 10-19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1.0 × 0.53	13.0	8.9
	1 × 720	Bulbs and onion like crops BBCH 10-19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1.0 × 0.53	4.31	26.9
		Bulbs & onion like crops	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1.0 × 0.53	4.16	27.9

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		116					
TER criterion		5					
GAP crop	Application rate (g a.e./ha)	Crop scenario Growth stage	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
		BBCH 10-39					
		Leafy vegetables BBCH 10-49	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	1.0 × 0.53	4.81	24.1
		Leafy vegetables Leaf development BBCH 10-19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1.0 × 0.53	8.66	13.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_{lt} values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following the proposed use patterns in field crops (Uses 1 a-c, 2 a-c, 10 a-c and 6 a-b).

Vineyard

Table B.9.2.1-11: Tier 1 assessment of the long-term/reproductive risk for birds due the use of glyphosate in vineyards: Use 5 a-c

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		116					
TER criterion		5					
GAP crop	Application rate (g a.e./ha)	Crop scenario Growth stage	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Vineyard post-emergence of weeds	2 × 1440 (28 d)	Vineyard BBCH 10-19	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	11.5	1.1 × 0.53	9.65	12.0
		Vineyard BBCH 20-39	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	9.9	1.1 × 0.53	8.31	14.0
		Vineyard BBCH 10-19	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	1.1 × 0.53	5.79	20.0
		Vineyard BBCH 20-39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	5.7	1.1 × 0.53	4.79	24.2
		Vineyard BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	1.1 × 0.53	2.85	40.7
		Vineyard BBCH 10-19	Small omnivorous bird "lark"	6.5	1.1 × 0.53	5.46	21.2

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
			Woodlark (*Lullula arborea*)				
		Vineyard BBCH 20-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	1.1 × 0.53	4.53	25.6
		Vineyard BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.1 × 0.53	2.77	41.9
	1 × 1080	Vineyard BBCH 10-19	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	11.5	1.0 × 0.53	6.58	17.6
		Vineyard BBCH 20-39	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	9.9	1.0 × 0.53	5.67	20.5
		Vineyard BBCH 10-19	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	1.0 × 0.53	3.95	29.4
		Vineyard BBCH 20-39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	5.7	1.0 × 0.53	3.26	35.6
		Vineyard BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	1.0 × 0.53	1.95	59.5
		Vineyard BBCH 10-19	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.0 × 0.53	3.72	31.2
		Vineyard BBCH 20-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	1.0 × 0.53	3.09	37.5
		Vineyard BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.0 × 0.53	1.89	61.4
	1 × 1440	Vineyard BBCH 10-19	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	11.5	1.0 × 0.53	8.78	13.2
		Vineyard BBCH 20-39	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	9.9	1.0 × 0.53	7.56	15.3
		Vineyard BBCH 10-19	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	1.0 × 0.53	5.27	22.0

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Vineyard BBCH 20-39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	5.7	1.0 × 0.53	4.35	26.7
		Vineyard BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	1.0 × 0.53	2.59	44.8
		Vineyard BBCH 10-19	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.0 × 0.53	4.96	23.4
		Vineyard BBCH 20-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	1.0 × 0.53	4.12	28.2
		Vineyard BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.0 × 0.53	2.52	46.0
	2 × 1080	Vineyard BBCH 10-19	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	11.5	1.1 × 0.53	7.24	16.0
		Vineyard BBCH 20-39	Small insectivorous bird "redstart" Black Redstart (*Phoenicurus ochrurus*)	9.9	1.1 × 0.53	6.23	18.6
		Vineyard BBCH 10-19	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	6.9	1.1 × 0.53	4.34	26.7
		Vineyard BBCH 20-39	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	5.7	1.1 × 0.53	3.59	32.3
		Vineyard BBCH ≥ 40	Small granivorous bird "finch" Linnet (*Carduelis cannabina*)	3.4	1.1 × 0.53	2.14	54.2
		Vineyard BBCH 10-19	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	6.5	1.1 × 0.53	4.09	28.4
		Vineyard BBCH 20-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	1.1 × 0.53	3.40	34.1
		Vineyard BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	3.3	1.1 × 0.53	2.08	55.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_{lt} values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following the proposed use patterns in vineyards (Uses 5 a-c).

Control of invasive species

Table B.9.2.1-12: Tier 1 assessment of the long-term/reproductive risk for birds due the use of glyphosate on invasive species in agricultural and non-agricultural areas: Use 8, 9

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Cereals Early (shoots) autumn-winter BBCH 10 - 29	Large herbivorous bird "goose" Pink-foot goose (*Anser brachyrhynchus*)	16.2	1.0 × 0.53	15.5	7.5
		Maize BBCH 10-29	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	3.0	1.0 × 0.53	2.86	40.6
		Leafy vegetables BBCH 10-19	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1.0 × 0.53	21.7	5.3
		Leafy vegetables BBCH 10-49	Small granivorous bird "finch" Serin (*Serinus serinus*)	12.6	1.0 × 0.53	12.0	9.7
		Oilseed rape Late (with seeds) BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (*Prunella modularis*)	2.7	1.0 × 0.53	2.58	45.0
		Hops BBCH 10-19	Small insectivorous bird "finch" Chaffinch (*Fringilla coelebs*)	9.1	1.0 × 0.53	8.68	13.4
		Cereals Late post-emergence (May-June) BBCH 71 - 89	Small insectivorous bird "passerine" Fan tailed warbler	22.4	1.0 × 0.53	21.4	5.4
		Cereals Early autumn-winter BBCH 10-29	Large herbivorous bird "goose" Pink-foot goose (*Anser brachyrhynchus*)	16.2	1.0 × 0.53	15.5	7.5
		Orchards Spring Summer	Small insectivorous bird "tit" Bluetit (Parus caeruleus)	18.2	1.0 × 0.53	17.4	6.7
		Bulbs and onion like crops BBCH 10-19	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1.0 × 0.53	10.8	10.7

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**116**					
TER criterion		**5**					
GAP crop	**Application rate (g a.e./ha)**	**Crop scenario Growth stage**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Bush and cane fruit Whole season BBCH 00-79 Currants	Small insectivorous bird "warbler" Willow warbler (*Phylloscopus trochilus*)	20.3	1.0 × 0.53	19.4	6.0
		Vineyard BBCH 10-19	Small insectivorous bird "redstart" Black redstart (*Phoenicurus ochruros*)	11.5	1.0 × 0.53	11.0	10.5
		Maize Leaf development BBCH 10-19	Small insectivorous / worm feeding species "thrush" Robin (*Erithacus rubecula*)	5.7	1.0 × 0.53	5.44	21.3
		Bulbs and onion like crops BBCH 10-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1.0	10.4	11.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_{lt} values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following the proposed use patterns for all the crops in the use to control invasive species.

B.9.2.1.3. Higher tier assessment

Since all scenarios in the Tier 1 risk assessment indicated low acute and chronic risk for birds, no higher tier assessment is needed.

B.9.2.1.4. Drinking water exposure

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.

Leaf scenario

The 'Leaf scenario' is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain / irrigation water sufficiently to attract birds, i.e. for the before named crops at BBCH ≥ 41.

Since none of the proposed uses falls into these categories, the leaf scenario does not apply to the use of MON 52276.

Puddle scenario

The 'Puddle scenario' is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is therefore relevant for all uses of MON 52276 and should therefore be assessed.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 (K_{OC} < 500 L/kg) or 3000 (K_{OC} ≥ 500 L/kg), as specified in EFSA/2009/1438.

As pointed out in EFSA/2009/1438, specific calculations of exposure and TER values are only necessary when the ratio of effective application rate (in g a.e./ha) to relevant endpoint (in mg a.e./kg bw/d) exceeds 50 in the case of less sorptive (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive (K_{OC} ≥ 500 L/kg) substances.

For glyphosate, the ratio of highest application rate (1800 g a.e./ha) to lowest relevant endpoint (NOAEL = 116 mg a.e./kg bw/d) is 19. As the geomean $K_{f,OC}$ for glyphosate is 4245 mL/g (See Volume 3CA, section 7) the risk can be considered acceptable without the need for further calculations.

B.9.2.1.5. Effects of secondary poisoning

According to the EFSA/2009/1438, substances with a log P_{OW} ≥ 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

Since the log P_{OW} values of glyphosate is log P_{OW} < -3.2 (pH 2-5, 20 °C), the active substance is deemed to have a low potential to bioaccumulate in animal tissues. No risk assessment from secondary poisoning is therefore required.

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several mammal toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Toxicology section). Furthermore, the log P_{OW} for AMPA – estimated via EpiSuite Program and SMILES code (C(N)P(=O)(O)O) – is -2.47 and does not indicate a potential for bioaccumulation (EFSA Journal 2015;13(11): 4302).

B.9.2.2. Risk assessment for other terrestrial vertebrates

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

The table below summarises how the risk assessment for mammals considers all the proposed uses and the application rates presented in the GAP.

Table B.9.2.2-1: Risk assessment strategy for mammals

GAP number and summary of use	Application rate considered (28 day interval unless otherwise stated)									
	1 × 540 g/ha	**1 × 720 g/ha**	**1 × 1080 g/ha**	**2 × 720 g/ha**	**1 × 1440 g/ha**	**3 × 720 g/ha**	**1 × 1800 g/ha**	**2 × 1080 g/ha[A]**	**2 × 1440 g/ha**	**2 × 1800 g/ha (90 days apart)**
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre-emergence of field crops.		X	X		X					
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of field crops.		X	X	X	X	X		X		
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of field crops.	X									
Use 4 a-c: Applied to weeds (post emergence) below trees in orchards.		X	X	X	X	X		X	X	
Use 5 a-c: Applied to weeds (post emergence) below vines in vineyards		X	X	X	X	X		X	X	
Use 6 a-b: Applied to weeds (post emergence) in field crops BBCH < 20		X	X							
Use 7 a-b: Applied to weeds (post emergence) around railroad tracks							X			X
Use 8 and 9: Applied to invasive species (post emergence) in agricultural and non-agricultural areas							X			
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of field crops		X	X							

X = this use is covered by the application rate indicated.

A Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.s./ha plus 1 × 720 g a.s./ha (28 day interval between each application).

B.9.2.2.1. Screening assessment

For the screening assessment; crops that maybe present at time of application to target weeds and the relevant application rates shown in the table above are considered. The acute and long-term screening assessment results are presented below according to the following main uses:

- in **field crops** (covering GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c); pre-sowing, pre-planting pre emergence, post-harvest. Exposure to mammals via grassland, bare soil and field crops is considered and is covered by the general screening scenarios bare soil, bulb and onion like crops (etc.) and fruiting vegetables (etc.). It should be noted that the bare soil scenario for birds and mammals is intended for true bare soils. As no foliage is present, no herbivorous birds or mammals are relevant, and also the omnivorous bird/mammal diets lack all foliage components. Glyphosate, as a contact herbicide, will only be applied when weeds are present. Thus, the bare soil scenario is considered to be of low relevance for the bird and mammal risk assessment. Further, grassland is not included in the list of representative field crop uses. However, from the RMS' point of view the grassland may also be relevant for some situations in field crops, especially when glyphosate is

used to remove weeds before sowing or after harvest. Although relevance of these scenarios are questionable, the bare soil and grassland scenarios are maintained for the standard screening assessment.

- in **orchards and vineyards** (covering GAP uses 4 a-c, 5a-c) applied to weeds post emergence exposure below trees; exposure to small herbivorous mammals in orchards and vineyards is considered and is covered by the general screening scenario fruiting vegetables (etc.).
- in **railroad tracks** (covering GAP uses 7 a-b) applied to weeds pots emergence; exposure to mammals via grassland, bare soil and field crops (leafy vegetables) is considered and is covered by the general screening scenarios bare soil and fruiting vegetables (etc.).
- in control of **invasive** species (covering GAP uses 8 and 9) applied; exposure to mammals via grassland, bare soil and field crops is considered and is covered by the general screening scenarios bare soil bush and cane fruit, bulb and onion like crops (etc.) and fruiting vegetables (etc.).

Field crops

Table B.9.2.2-2: Screening assessment of the acute risk for mammals due to the use of glyphosate in field crops: Uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**> 2000 (lowest value for the screening step)**					
TER criterion		**10**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Pre-sow, pre-planting, post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 1440	Bare soil	Small granivorous mammal	14.4	1.0	20.7	96.6
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.0	170	11.7
		Fruiting vegetables	Small herbivorous mammal	136.4	1.0	196	10.2
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	2 × 1080 (28 d)	Bare soil	Small granivorous mammal	14.4	1.1	17.1	117
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.1	141	14.2
		Fruiting vegetables	Small herbivorous mammal	136.4	1.1	162	12.3
Pre-sow, pre-planting, post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 540	Bare soil	Small granivorous mammal	14.4	1.0	7.78	257
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.0	63.9	31.3
		Fruiting vegetables	Small herbivorous mammal	136.4	1.0	73.7	27.1

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**> 2000 (lowest value for the screening step)**					
TER criterion		**10**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds Sugar beet. Post-emergence of weeds	1 × 720	Bare soil	Small granivorous mammal	14.4	1.0	10.4	192
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.0	85.2	23.5
		Fruiting vegetables	Small herbivorous mammal	136.4	1.0	98.2	20.4
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds Sugar beet. Post-emergence of weeds	2 × 720 (28 d)	Bare soil	Small granivorous mammal	14.4	1.1	11.4	175
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.1	93.8	21.3
		Fruiting vegetables	Small herbivorous mammal	136.4	1.1	108	18.5
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 1080	Bare soil	Small granivorous mammal	14.4	1.0	15.6	128
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.0	128	15.6
		Fruiting vegetables	Small herbivorous mammal	136.4	1.0	147	13.6
Pre-sow, pre-planting, post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	3 × 720 (28 d)	Bare soil	Small granivorous mammal	14.4	1.1	11.4	175
		Bulb and onion like crops	Small herbivorous mammal	118.4	1.1	93.8	21.3
		Fruiting vegetables	Small herbivorous mammal	136.4	1.1	108	18.5

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table B.9.2.2-3: Screening assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops: Use 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**50**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDD90 (mg/kg bw/d)**	**TERlt**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 1440	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	5.04	9.92
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.0 × 0.53	36.9	**1.36**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	55.2	**0.91**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	2 × 1080 (28 d)	Bare soil	Small granivorous mammal	6.6	1.1 × 0.53	4.16	12.0
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.1 × 0.53	30.4	**1.64**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.1 × 0.53	45.5	**1.10**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 540	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	1.89	26.5
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.0 × 0.53	13.8	**3.62**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	20.7	**2.42**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 720	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	2.52	19.9
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.0 × 0.53	18.4	**2.71**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	27.6	**1.81**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	2 × 720 (28 d)	Bare soil	Small granivorous mammal	6.6	1.1 × 0.53	2.77	18.0
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.1 × 0.53	20.3	**2.47**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.1 × 0.53	30.3	**1.65**

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		50					
TER criterion		5					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Indicator species	SVm	MAFm × TWA	DDD90 (mg/kg bw/d)	TERlt
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	1 × 1080	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	3.78	13.2
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.0 × 0.53	27.7	**1.81**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	41.38	**1.21**
Pre-sow, pre-planting, pre-emergence & post-harvest of: Root and Stem veg, Potato Bulb and onion like crops, fruiting veg, leafy veg, Sugar beet. Post-emergence of weeds	3 × 720 (28 d)	Bare soil	Small granivorous mammal	6.6	1.2 × 0.53	3.02	16.5
		Bulb and onion like crops	Small herbivorous mammal	48.3	1.2 × 0.53	22.1	**2.26**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.2 × 0.53	33.1	**1.51**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Orchards and vineyards

Table B.9.2.2-4: Screening assessment of the acute risk for mammals due to the use of glyphosate in orchards and vineyards: Uses 4 a-c, 5 a-c.

Active substance		Glyphosate					
Acute toxicity (mg/kg bw)		> 2000 (lowest value used for the screening step)					
TER criterion		10					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Indicator species	SV90	MAF90	DDD90 (mg/kg bw/d)	TERa
Orchards / vineyards post-emergence of weeds	2 × 1440 (28 d)	Fruiting vegetables	Small herbivorous mammal	136.4	1.1	216	**9.3**
Orchards / vineyards post-emergence of weeds	1 × 720	Fruiting vegetables	Small herbivorous mammal	136.4	1.0	98.2	20.4
Orchards / vineyards post-emergence of weeds	1 × 1080	Fruiting vegetables	Small herbivorous mammal	136.4	1.0	147	13.6
Orchards / vineyards post-emergence of weeds	2 × 720 (28 d)	Fruiting vegetables	Small herbivorous mammal	136.4	1.1	108	18.5
Orchards / vineyards	3 × 720 (28 d)	Fruiting vegetables	Small herbivorous mammal	136.4	1.1	108	18.5

post-emergence of weeds							
Orchards / vineyards post-emergence of weeds	1 × 1440	Fruiting vegetables	Small herbivorous mammal	136.4	1.0	196	10.2
Orchards / vineyards post-emergence of weeds	2 × 1080 (28 d)	Fruiting vegetables	Small herbivorous mammal	136.4	1.1	162	12.3

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table B.9.2.2-5: Screening assessment of the long-term/reductive risk for mammals due to the use of glyphosate in orchards and vineyards: Uses 4 a-c, 5 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**50**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDD90 (mg/kg bw/d)**	**TERlt**
Orchards / vineyards post-emergence of weeds	2 × 1440 (28 d)	Fruiting vegetables	Small herbivorous mammal	72.3	1.1 × 0.53	60.7	**0.82**
Orchards / vineyards post-emergence of weeds	1 × 720	Fruiting vegetables	Small herbivorous mammal	72.3	1 × 0.53	27.6	**1.81**
Orchards / vineyards post-emergence of weeds	1 × 1080	Fruiting vegetables	Small herbivorous mammal	72.3	1 × 0.53	41.4	**1.21**
Orchards / vineyards post-emergence of weeds	2 × 720 (28 d)	Fruiting vegetables	Small herbivorous mammal	72.3	1.1 × 0.53	30.3	**1.65**
Orchards / vineyards post-emergence of weeds	3 × 720 (28 d)	Fruiting vegetables	Small herbivorous mammal	72.3	1.2 × 0.53	33.1	**1.51**
Orchards / vineyards post-emergence of weeds	1 × 1440	Fruiting vegetables	Small herbivorous mammal	72.3	1 × 0.53	55.2	**0.91**
Orchards / vineyards post-emergence of weeds	2 × 1080 (28 d)	Fruiting vegetables	Small herbivorous mammal	72.3	1.1 × 0.53	45.5	**1.10**

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table B.9.2.2-6: Screening assessment of the acute risk for mammals due to the use of glyphosate on railroad tracks and to control invasive species: Uses 7a-b, 8 and 9

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**> 2000 (lowest value for the screening step)**					
TER criterion		**10**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Bare soil	Small granivorous mammal	14.4	1.0	28.5	77.2
		Fruiting vegetables	Small herbivorous mammal	136.4	1.0	270	8.13
	1 × 1800	Bare soil	Small granivorous mammal	14.4	1.0	25.9	77.2
		Fruiting vegetables	Small herbivorous mammal	136.4	1.0	246	**8.13**
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Bare soil	Small granivorous mammal	14.4	1	25.9	77.2
		Bush and cane fruit	Small herbivorous mammal	81.9	1	147	13.6
		Bulbs and onion like crops	Small herbivorous mammal	118.4	1	213	**9.38**
		Fruiting vegetables	Small herbivorous mammal	136.4	1	246	**8.13**

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table B.9.2.2-7: Screening assessment of the long-term/reproductive risk for mammals due to the use of glyphosate on railroad tracks and to control invasive species: Uses 7a-b, 8 and 9

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**50**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SVm**	**MAFm × TWA**	**DDD90 (mg/kg bw/d)**	**TER_{lt}**
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	6.30	7.94
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	69.0	**0.72**
	1 × 1800	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	6.30	7.94
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	69.0	**0.72**
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Bare soil	Small granivorous mammal	6.6	1.0 × 0.53	6.30	7.94
		Bush and cane fruit	Small herbivorous mammal	43.4	1.0 × 0.53	41.4	**1.21**
		Bulbs and onion like crops	Small herbivorous mammal	48.3	1.0 × 0.53	46.1	**1.09**
		Fruiting vegetables	Small herbivorous mammal	72.3	1.0 × 0.53	69.0	**0.72**

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Conclusion screening assessment

Field crops (Uses: 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c)

The screening TERa values for use of MON 52276 in field crops for all scenarios are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use the proposed use patterns for these crops.
The screening TER_{lt} values for use of MON 52276 in field crops for the scenario "bare soil" are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5. Regarding the scenarios "bulbs and onion like crops" and "fruiting vegetables" a long-term Tier 1 risk assessment is necessary for all intended application rates.

Orchards and vineyards (Uses: 4 a-c and 5 a-c)

The screening TER_a values for use of MON 52276 in orchards and vineyards for the scenario "fruiting vegetables" are above the Commission Regulation (EU) No. 546/2011 trigger of 10 for the application rates 1 × 720 g a.s./ha, 1 × 1080 g a.s./ha, 2 × 720 g a.s./ha, 3 × 720 g a.s./ha, 1 × 1440 g a.s./ha and 2 × 1080 g a.s./ha. For the application rate of 2 × 1440 the TER_a value is slightly below the trigger of 10. Therefore, an acute Tier 1 risk assessment is necessary for this rate.

The screening TER_{lt} values for use of MON 52276 in orchards and vineyards for the scenario "fruiting vegetables" are below the Commission Regulation (EU) No. 546/2011 trigger of 5. Therefore, a long-term Tier 1 risk assessment is necessary for all intended application rates.

Railroad tracks – application by spray train (Uses: 7 a-b)

The screening TER_a and TER_{lt} values for use of MON 52276 on railroad tracks for the scenario "bare soil" are above the Commission Regulation (EU) No. 546/2011 trigger of 10 and 5 respectively. The screening TER_a and TER_{lt} values for the "fruiting vegetables" scenario are below the Commission Regulation (EU) No. 546/2011 trigger of 10 and 5, respectively. Therefore, an acute and long-term Tier 1 risk assessment is necessary for all intended application rates.

Invasive species in agricultural and non-agricultural areas (Uses: 8 and 9)

The screening TER_a values for use of MON 52276 on invasive species in agricultural and non-agricultural areas for the scenarios "bare soil" and "bush and cane fruit" are above the Commission Regulation (EU) No. 546/2011 trigger of 10. The screening TER_a values for the "bulbs and onion like crops" and "fruiting vegetables" scenarios are below the Commission Regulation (EU) No. 546/2011 trigger of 10. Therefore, an acute Tier 1 risk assessment is necessary for the intended application rate of 1 × 1800 g a.s./ha.

The screening TER_{lt} values for use of MON 52276 on invasive species in agricultural and non-agricultural area for the scenario "bare soil" are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5. The screening TER_{lt} values for the "bush and cane fruit", "bulbs and onion like crops" and "fruiting vegetables" scenarios are below the trigger of 5. Therefore a long-term Tier 1 risk assessment is necessary for the intended application rate of 1 × 1800 g a.s./ha.

B.9.2.2.2. Tier 1 assessment

Tier 1 risk assessment is conducted for those intended uses, for which the calculated TER_a or TER_{lt} values were below the trigger of 10 or 5, respectively, e.g. for uses in field crops, uses in orchards and vineyards,

uses on railroad tracks and uses to control invasive species in agricultural and non-agricultural areas. The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal mammalian species from Appendix A of EFSA/2009/1438.

Due to the proposed uses of the product MON 52276 in agricultural and non-agricultural areas, justifications are provided below considering which scenarios are relevant for the risk assessment. For those proposed uses where a large number of scenarios is relevant (Field crops: Use 2 a-c, 6 a, b, 10 a-c, Control of invasive species: Use 8 - 9) an approach has been taken to present only the worst-case risk assessment in this section. Therefore the worst-case scenarios have been selected based on the relevant generic focal species with the highest short-cut values as these are considered protective of the other scenarios with lower short-cut values. For completeness, a full and complete mammalian Tier I risk assessment that considers all other scenarios and focal species was provided by the applicant in a separate Annex to M-CP 10 but is not presented here.

A summary of all relevant scenarios and focal species is provided in the B.9.2.2-8 below. Please note that numbers in brackets refer to the mammals' scenarios stated in the Appendix A of EFSA/2009/1438.

Field crops (Uses: 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c)

For the Tier 1 assessment of the crop group "field crops", the intended use of MON 52276 includes several general uses on field crops as described further below. The applications are intended to be made by tractor mounted sprayers (*Uses 1 a-c, 2 a-c, 3 a-b, 6 a-b*) or by hand-held equipment (*Uses 10 a-c*).

Use 1 a-c is, the "pre-sowing, pre-planting, pre-emergence" use, where the intention of this use is to prepare a non-agricultural area for agriculture use, meaning that the product is applied when no agricultural crop is present. Therefore the applicant proposes that "bare soil", the "grassland" and the "leafy vegetable" scenarios are considered relevant (regarding the 'bare soil' scenario, this is however not agreed by the RMS, since glyphosate is only applied when weeds are present). Anyway, as an acceptable risk for the "bare soil" scenario was concluded at the screening assessment, a Tier 1 risk assessment is presented only for "grassland" and "leafy vegetables". The "grassland" scenario is considered relevant to cover species that feed on grass; the large herbivorous mammal "lagomorph" (72), the small insectivorous mammal "shrew" (73), the small herbivorous mammal "vole" (74) and the small omnivorous mammal "mouse" (75) are taken into account. The "leafy vegetables" scenario is considered relevant to cover species that feed on broad-leaved weeds; the small insectivorous mammal "shrew" (91, 92), the small herbivorous mammal "vole" (93, 94), the large herbivorous mammal "lagomorph" (95) and the small omnivorous mammal "mouse" (102, 103) are taken into account.

Uses 2 a-c, 3 a-b and 10 a-c are the "post-harvest, pre-sowing, pre-planting" use where the product can be applied to existing cropland after harvest for removal of remaining crops. Thus, for this use almost all field crops were considered. Only for the crop where safe risk could be concluded in the screening assessment, i.e. "bare soil" and for crops which are generally not considered relevant ("cotton") or for spatial cultures like "bush & cane fruit", "hops", "orchards", "ornamentals/nursery" and "vineyards" a risk assessment is not considered necessary. As the product is applied after post-harvest, late crop stages are taken into account for risk assessment. Frugivorous mammal scenarios were not taken into account, as the product is intended to be applied after harvest and will not be applied at typical crop stages when fruits are ripe. For the same reason also the pulses scenario (pre harvest seed, BBCH 81-99) is not considered relevant.

Thus, for the Tier 1 risk assessment for the uses 2 a-c, 3 a-b and 10 a-c, the relevant generic focal species with the highest short-cut values at late crop stages across all relevant crop scenarios are taken into account; the small insectivorous mammal "shrew" in bulb and onion like crops (5), the large herbivorous mammal "lagomorph" in grassland (72), the small herbivorous mammal "vole" in grassland (74) and the small omnivorous mammal "mouse" in grassland (75). These selected scenarios cover the risk for all relevant scenarios.

Uses 6 a-b are the "shielded ground directed inter-row application" uses at crop stages < BBCH 20 and all crops scenarios at early growth stages are taken into account, which are presented in the GAP, i.e. vegetables (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables and leafy vegetables). To avoid exposure of crops, a shielded sprayer is used to ensure that the product is only applied to grasses and weeds in the inter-row. Therefore, only those vegetables crop scenarios are considered relevant where the generic focal species does not directly feed on the crop. In addition, the "bare soil" and the "grassland" scenario are considered relevant. However, as an acceptable risk was concluded for the "bare soil" scenario already at the screening assessment the Tier 1 risk assessment is not required for this scenario.

Thus, for the Tier 1 risk assessment for the uses 6 a-b, the relevant generic focal species with the highest short-cut values at early crop stages (< BBCH 20) across all relevant crops scenarios were taken into account, i.e. the small insectivorous mammal "shrew" in bulb and onion like crops (4), the small omnivorous mammal "mouse" (13) in bulbs and onion like crops, the small herbivorous mammal "vole" in fruiting vegetables (62) and the large herbivorous mammal "lagomorph" (95) in leafy vegetables.

Orchards (Uses: 4 a-c)

For the crop grouping "orchards" due to the downward application of the product all generic focal species for not "crop directed" applications were taken into account, i.e. the small insectivorous mammal "shrew" (148), the small herbivorous mammal "vole" (149), the large herbivorous mammal "lagomorph" (154) and the small omnivorous mammal "mouse" (170).

Vineyards (Uses: 5 a-c)

For the crop grouping "vineyards" due to the downward application of the product all generic focal species, for not "crop directed" applications were taken into account, i.e. the large herbivorous mammal "lagomorph" (267, 268, 269, 270), the small insectivorous mammal "shrew" (271, 272), the small herbivorous mammal "vole" (273) and the small omnivorous mammal "mouse" (287).

Railroad tracks – application by spray train (Uses: 7 a-b)

For the use on railroad tracks the same scenarios were selected like for use 1 a-c, i.e. the "bare soil", the "grassland" and the "leafy vegetable" were considered relevant. As an acceptable risk for the "bare soil" scenario was concluded at the screening assessment a Tier 1 risk assessment was presented only for "grassland" and "leafy vegetables". The "grassland" scenario is considered relevant to cover species that feed on grass; the large herbivorous mammal "lagomorph" (72), the small insectivorous mammal "shrew" (73), the small herbivorous mammal "vole" (74) and the small omnivorous mammal "mouse" (75) are taken into account. The "leafy vegetables" scenario is considered relevant to cover species that feed on broad-leaved weeds; the small insectivorous mammal "shrew" (91, 92), the small herbivorous mammal "vole" (93, 94), the large herbivorous mammal "lagomorph" (95) and the small omnivorous mammal "mouse" (102, 103) are taken into account.

Invasive species in agricultural and non-agricultural areas (Uses: 8 - 9)

For the use on invasive species in agricultural and non-agricultural areas, almost all crops need to be considered. Only for the crop where safe risk could be concluded in the screening assessment, i.e. "bare soil" and for crops which are generally not considered relevant ("cotton") do not need to be assessed in the Tier 1 risk assessment. In general, those scenarios need to be taken into account, where a downward application of the product is relevant. Frugivorous mammal scenarios were not taken into account, as the product is intended to be applied only on the invasive species Giant hogweed (*Heracleum mantegazzianum*) and Japanese knotweed (*Reynoutria japonica*) and due to the specific application method (handheld,

spraying shield) fruits will not be exposed to the product. For the same reason also the pulses scenario (pre harvest seed, BBCH 81-99) is not considered relevant.

Thus, for the Tier 1 risk assessment for uses 8 and 9, the relevant generic focal species with the highest short-cut values across all relevant crop scenarios are taken into account, i.e. the small insectivorous mammal "shrew" in bulb and onion like crops (4), the small omnivorous mammal "mouse" in bulb and onion like crops (13), the large herbivorous mammal "lagomorph" in cereals (35) and the small herbivorous mammal "vole" in fruiting vegetables (62). These chosen scenarios cover the risk for all relevant scenarios.

Table B.9.2.2-8: Tier 1 mammalian scenarios. Worst case scenarios are indicated in bold and are included in the Tier 1 risk assessment below.

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
Field crops (Pre-sowing, pre-planting, pre-emergence): Use 1 a-c					
No. 72	**Grassland All season**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**-**	**17.3**	**Vol. 3CP, B.9.2.2**
No. 73	**Grassland Late**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**-**	**1.9**	**Vol. 3CP, B.9.2.2**
No. 74	**Grassland All season**	**Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)**	**-**	**72.3**	**Vol. 3CP, B.9.2.2**
No. 75	**Grassland Late season (seed heads)**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**-**	**6.6**	**Vol. 3CP, B.9.2.2**
No. 91	**Leafy vegetables BBCH 10 - 19**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**-**	**4.2**	**Vol. 3CP, B.9.2.2**
No. 92	**Leafy vegetables BBCH ≥ 20**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**-**	**1.9**	**Vol. 3CP, B.9.2.2**
No. 93	**Leafy vegetables BBCH 40 - 49**	**Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)**	**-**	**72.3**	**Vol. 3CP, B.9.2.2**
No. 94	**Leafy vegetables BBCH ≥ 50**	**Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)**	**-**	**21.7**	**Vol. 3CP, B.9.2.2**
No. 95	**Leafy vegetables All season**	**Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)**	**-**	**14.3**	**Vol. 3CP, B.9.2.2**
No. 102	**Leafy vegetables BBCH 10 – 49**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**-**	**7.8**	**Vol. 3CP, B.9.2.2**

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 103	**Leafy vegetables BBCH ≥ 50**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**-**	**2.3**	**Vol. 3CP, B.9.2.1**
Field crops (Post-harvest, pre-sowing, pre-planting): Use 2 a-c, 3 a-b, 10 a-c					
No. 5	**Bulbs and onion like crops BBCH ≥ 20**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**-**	**1.9**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 6	Bulbs and onion like crops BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	43.4	(Covered by scenario no. 74)
No. 14	Bulbs and onion like crops BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	4.7	(Covered by scenario no. 75)
No. 33	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 34	Cereals BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 46	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 61	Fruiting vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 63	Fruiting vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 71	Fruiting vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 72	**Grassland All season**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**-**	**17.3**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 73	Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 74	**Grassland All season**	**Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)**	**-**	**72.3**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 75	**Grassland Late season (seed heads)**	**Small omnivorous mammal "mouse"**	**-**	**6.6**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
		Wood mouse (*Apodemus sylvaticus*)			
No. 92	Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 94	Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 95	Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	14.3	(Covered by scenario no. 72)
No. 103	Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 105	Legume forage BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 107	Legume forage BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 116	Legume forage BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 118	Maize BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 121	Maize BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	18.1	(Covered by scenario no. 74)
No. 132	Maize BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	1.9	(Covered by scenario no. 75)
No. 134	Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 135	Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	18.1	(Covered by scenario no. 74)
No. 136	Oilseed rape All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	14.3	(Covered by scenario no. 72)
No. 147	Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse"	-	1.9	(Covered by scenario no. 75)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
		Wood mouse (*Apodemus sylvaticus*)			
No. 186	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 187	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 189	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	4.3	(Covered by scenario no. 72)
No. 197	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 199	Pulses BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 201	Pulses BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 203	Pulses BBCH ≥ 50	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	4.3	(Covered by scenario no. 72)
No. 212	Pulses BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 214	Root and stem vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 215	Root and stem vegetables BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	21.7	(Covered by scenario no. 74)
No. 223	Root and stem vegetables BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	2.3	(Covered by scenario no. 75)
No. 225	Strawberries BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 226	Strawberries BBCH ≥ 40	Small herbivorous mammal "vole Common vole (*Microtus arvalis*)	-	28.9	(Covered by scenario no. 74)
No. 228	Strawberries BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	5.7	(Covered by scenario no. 72)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 236	Strawberries BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	3.1	(Covered by scenario no. 75)
No. 238	Sugar beet BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 239	Sugar beet BBCH ≥ 40	Small herbivorous mammal "vole Common vole (*Microtus arvalis*)	-	18.1	(Covered by scenario no. 74)
No. 241	Sugar beet BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	3.6	(Covered by scenario no. 72)
No. 249	Sugar beet BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	1.9	(Covered by scenario no. 75)
No. 251	Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	1.9	(Covered by scenario no. 5)
No. 252	Sunflower BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	-	18.1	(Covered by scenario no. 74)
No. 255	Sunflower BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	3.6	(Covered by scenario no. 72)
No. 266	Sunflower BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	1.9	(Covered by scenario no. 75)
Field crops (Shielded ground directed inter-row application): Use 6 a, b					
No. 4	**Bulbs & onion like crops BBCH 10 – 19**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**-**	**4.2**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 13	**Bulbs & onion like crops BBCH 10 – 39**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**-**	**7.8**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 60	Fruiting vegetables BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 62	**Fruiting vegetables BBCH 10 – 49**	**Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)**	**-**	**72.3**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 70	Fruiting vegetables BBCH 10-49	Small omnivorous mammal "mouse"	-	7.8	(Covered by scenario no. 4)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
		Wood mouse (*Apodemus sylvaticus*)			
No. 91	Leafy vegetables BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 95	**Leafy vegetables All season**	**Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)**	**-**	**14.3**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 102	Leafy vegetables BBCH 10-49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	7.8	(Covered by scenario no. 4)
No. 104	Legume forage BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 115	Legume forage BBCH 10-49	Small omnivorous mammal "mouse" Wood mouse (Apodemus sylvaticus)	-	7.8	(Covered by scenario no. 4)
No. 185	Potatoes BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 188	Potatoes BBCH 10 – 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	14.3	(Covered by scenario no. 95)
No. 196	Potatoes BBCH 10 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	7.8	(Covered by scenario no. 4)
No. 198	Pulses BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 202	Pulses BBCH 10 – 49	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	14.3	(Covered by scenario no. 95)
No. 211	Pulses BBCH 10 – 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	7.8	(Covered by scenario no. 4)
No. 213	Root & stem vegetables BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 222	Root & stem vegetables BBCH 10-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	7.8	(Covered by scenario no. 4)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 237	Sugar beet BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	-	4.2	(Covered by scenario no. 4)
No. 240	Sugar beet BBCH 10-39	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	-	14.3	(Covered by scenario no. 95)
No. 248	Sugar beet BBCH 10-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	-	7.8	(Covered by scenario no. 4)
Orchards: Use 4 a-c					
No. 148	**Orchards Application crop directed BBCH <10 or not crop directed**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**5.4**	**1.9**	**Vol. 3CP, B.9.2.2**
No. 149	**Orchards Application crop directed BBCH <10 or not crop directed**	**Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)**	**136.4**	**72.3**	**Vol. 3CP, B.9.2.2**
No. 154	**Orchards Application crop directed BBCH <10 or not crop directed**	**Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)**	**35.1**	**14.3**	**Vol. 3CP, B.9.2.2**
No. 170	**Orchards Application crop directed BBCH <10 or not crop directed**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**17.2**	**7.8**	**Vol. 3CP, B.9.2.2**
Vineyards: Use 5 a-c					
No. 267	**Vineyard Application ground directed**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**27.2**	**11.1**	**Vol. 3CP, B.9.2.2**
No. 268	**Vineyard BBCH 10-19**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**16.3**	**6.7**	**Vol. 3CP, B.9.2.2**
No. 269	**Vineyard BBCH 20 – 39**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**13.6**	**5.5**	**Vol. 3CP, B.9.2.2**
No. 270	**Vineyard BBCH ≥ 40**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**8.1**	**3.3**	**Vol. 3CP, B.9.2.2**
No. 271	**Vineyard BBCH 10 – 19**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**7.6**	**4.2**	**Vol. 3CP, B.9.2.2**
No. 272	**Vineyard BBCH ≥ 20**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**5.4**	**1.9**	**Vol. 3CP, B.9.2.2**

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 273	**Vineyard Application ground directed**	**Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)**	**136.4**	**72.3**	**Vol. 3CP, B.9.2.2**
No. 287	**Vineyard Application ground directed**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**17.2**	**7.8**	**Vol. 3CP, B.9.2.2**
Railroad tracks – application by spray train: Use 7a-b					
No. 72	**Grassland All season**	**Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)**	**32.6**	**17.3**	**Vol. 3CP, B.9.2.2**
No. 73	**Grassland Late**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**5.4**	**1.9**	**Vol. 3CP, B.9.2.2**
No. 74	**Grassland All season**	**Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)**	**136.4**	**72.3**	**Vol. 3CP, B.9.2.2**
No. 75	**Grassland Late season (seed heads)**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**14.4**	**6.6**	**Vol. 3CP, B.9.2.2**
No. 91	**Leafy vegetables BBCH 10 - 19**	**Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)**	**7.6**	**4.2**	**Vol. 3CP, B.9.2.2**
No. 92	**Leafy vegetables BBCH ≥ 20**	**Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)**	**5.4**	**1.9**	**Vol. 3CP, B.9.2.2**
No. 93	**Leafy vegetables BBCH 40 - 49**	**Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)**	**136.4**	**72.3**	**Vol. 3CP, B.9.2.2**
No. 94	**Leafy vegetables BBCH ≥ 50**	**Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)**	**40.9**	**21.7**	**Vol. 3CP, B.9.2.2**
No. 95	**Leafy vegetables All season**	**Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)**	**35.1**	**14.3**	**Vol. 3CP, B.9.2.2**
No. 102	**Leafy vegetables BBCH 10 – 49**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**17.2**	**7.8**	**Vol. 3CP, B.9.2.2**
No. 103	**Leafy vegetables BBCH ≥ 50**	**Small omnivorous mammal "mouse"**	**5.2**	**2.3**	**Vol. 3CP, B.9.2.1**

EFSA Appendix A Scenario	**Tier 1 scenario given by glyphosate RAR**	**Generic focal species**	**SV90**	**SVm**	**Risk assessment presented under**
		Wood mouse (*Apodemus sylvaticus*)			
Control of invasive species in agricultural and non-agricultural areas: Use 8-9					
No. 4	**Bulbs & onion like crops BBCH 10 – 19**	**Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)**	**7.6**	**4.2**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 5	Bulbs & onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 6	Bulbs & onion like crops BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	81.9	43.4	(Covered by scenario no. 62)
No. 13	**Bulbs & onion like crops BBCH 10 – 39**	**Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)**	**17.2**	**7.8**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 14	Bulbs & onion like crops BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	10.3	4.7	(Covered by scenario no. 13)
No. 15	Bush & cane fruit BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 16	Bush & cane fruit BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 17	Bush & cane fruit BBCH 10-19	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	81.9	43.4	(Covered by scenario no. 62)
No. 18	Bush & cane fruit BBCH 20 – 39	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	68.2	36.1	(Covered by scenario no. 62)
No. 19	Bush & cane fruit BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 29	Bush & cane fruit BBCH 10-19	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	10.3	4.7	(Covered by scenario no. 13)
No. 30	Bush & cane fruit BBCH 20 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	8.6	3.9	(Covered by scenario no. 13)
No. 31	Bush & cane fruit BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 32	Cereals BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 33	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 34	Cereals BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 35	**Cereals Early (shoots)**	**Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)**	**42.1**	**22.3**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 44	Cereals BBCH 10-29	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 45	Cereals BBCH 30 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	8.6	3.9	(Covered by scenario no. 13)
No. 46	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 60	Fruiting vegetables BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 61	Fruiting vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 62	**Fruiting vegetables BBCH 10 – 49**	**Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)**	**136.4**	**72.3**	**Vol. 3CP, B.9.2.2 (Worst case scenario)**
No. 63	Fruiting vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 70	Fruiting vegetables BBCH 10-49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 71	Fruiting vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 72	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	32.6	17.3	(Covered by scenario no. 35)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 73	Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 74	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 75	Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	14.4	6.6	(Covered by scenario no. 13)
No. 77	Hop BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 78	Hop BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 79	Hop BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 88	Hop BBCH 10-19	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 89	Hop BBCH 20 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	8.6	3.9	(Covered by scenario no. 13)
No. 90	Hop BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 91	Leafy vegetables BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 92	Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 93	Leafy vegetables BBCH 40-49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 94	Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 95	Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 102	Leafy vegetables BBCH 10-49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 103	Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 104	Legume forage BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 105	Legume forage BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 106	Legume forage BBCH 40 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 107	Legume forage BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 108	Legume forage Leaf development BBCH 21-49	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 115	Legume forage BBCH 10-49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 116	Legume forage BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 117	Maize BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 13)
No. 118	Maize BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 119	Maize BBCH 10 -29	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 120	Maize BBCH 30 – 39	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	68.2	36.1	(Covered by scenario no. 62)
No. 121	Maize BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	34.1	18.1	(Covered by scenario no. 62)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 130	Maize BBCH 10-29	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 131	Maize BBCH 30 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	8.6	3.9	(Covered by scenario no. 13)
No. 132	Maize BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	4.3	1.9	(Covered by scenario no. 13)
No. 133	Oilseed rape BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 134	Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 135	Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	34.1	18.1	(Covered by scenario no. 62)
No. 136	Oilseed rape All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 145	Oilseed rape BBCH 10-29	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 146	Oilseed rape BBCH 30 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 147	Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	4.3	1.9	(Covered by scenario no. 13)
No. 148	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 149	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 154	Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 170	Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 175	Ornamentals/nursery BBCH 40 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 176	Ornamentals/nursery BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	68.2	36.1	(Covered by scenario no. 62)
No. 185	Potatoes BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 186	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 187	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 188	Potatoes BBCH 10 – 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 189	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	10.5	4.3	(Covered by scenario no. 35)
No. 196	Potatoes BBCH 10 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 197	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 198	Pulses BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 199	Pulses BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 200	Pulses BBCH 40 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 201	Pulses BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 202	Pulses BBCH 10 – 49	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 203	Pulses BBCH ≥ 50	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	10.5	4.3	(Covered by scenario no. 35)
No. 211	Pulses BBCH 10 – 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 212	Pulses BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 213	Root & stem vegetables BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 214	Root & stem vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 215	Root & stem vegetables BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9	21.7	(Covered by scenario no. 62)
No. 222	Root & stem vegetables BBCH 10-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 223	Root & stem vegetables BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	2.3	(Covered by scenario no. 13)
No. 224	Strawberries BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 225	Strawberries BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 226	Strawberries BBCH ≥ 40	Small herbivorous mammal "vole Common vole (*Microtus arvalis*)	54.6	28.9	(Covered by scenario no. 62)
No. 227	Strawberries BBCH 10-39	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 228	Strawberries BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.0	5.7	(Covered by scenario no. 35)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 235	Strawberries BBCH 10-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 236	Strawberries BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.9	3.1	(Covered by scenario no. 13)
No. 237	Sugar beet BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 238	Sugar beet BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 239	Sugar beet BBCH ≥ 40	Small herbivorous mammal "vole Common vole (*Microtus arvalis*)	34.1	18.1	(Covered by scenario no. 62)
No. 240	Sugar beet BBCH 10-39	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 241	Sugar beet BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	8.8	3.6	(Covered by scenario no. 35)
No. 248	Sugar beet BBCH 10-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 249	Sugar beet BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	4.3	1.9	(Covered by scenario no. 13)
No. 250	Sunflower BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 251	Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 252	Sunflower BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	34.1	18.1	(Covered by scenario no. 62)
No. 253	Sunflower BBCH 10-19	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	14.3	(Covered by scenario no. 35)
No. 254	Sunflower BBCH 20 – 39	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	17.6	7.2	(Covered by scenario no. 35)

EFSA Appendix A Scenario	Tier 1 scenario given by glyphosate RAR	Generic focal species	SV90	SVm	Risk assessment presented under
No. 255	Sunflower BBCH ≥ 40	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	8.8	3.6	(Covered by scenario no. 35)
No. 264	Sunflower BBCH 10-19	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)
No. 265	Sunflower BBCH 20 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	8.6	3.9	(Covered by scenario no. 13)
No. 266	Sunflower BBCH ≥ 40	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	4.3	1.9	(Covered by scenario no. 13)
No. 267	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	27.2	11.1	(Covered by scenario no. 35)
No. 268	Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	16.3	6.7	(Covered by scenario no. 35)
No. 269	Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	13.6	5.5	(Covered by scenario no. 35)
No. 270	Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	8.1	3.3	(Covered by scenario no. 35)
No. 271	Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	4.2	(Covered by scenario no. 4)
No. 272	Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.9	(Covered by scenario no. 4)
No. 273	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	72.3	(Covered by scenario no. 62)
No. 287	Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	7.8	(Covered by scenario no. 13)

Worst case scenarios are indicated in **bold**.

The Tier 1 risk assessment is presented in the following tables for the relevant uses in field crops, orchards, vineyards, for the uses on railroad tracks and for the uses to control invasive species in agricultural and non-agricultural areas, taking into account those generic focal species scenarios which were indicated in bold in the table above.

Field crops

Table B.9.2.2-9: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops (Pre-sowing, pre-planting, pre-emergence); Uses 1 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Pre-sowing, pre-planting, pre-emergence)	1 × 1440	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	13.2	7.58
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.45	69.0
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	5.04	19.8
		Leafy vegetables BBCH 10 - 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	3.21	31.2
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.45	69.0
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	21.7	1.0 × 0.53	16.6	6.02
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	10.9	9.17
		Leafy vegetables BBCH 10 - 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	5.95	16.8
		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	2.3	1.0 × 0.53	1.76	56.8
	1 × 1080	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	9.90	10.1
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.09	91.7
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	3.78	26.5
		Leafy vegetables BBCH 10 - 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	2.40	41.7
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.09	91.7
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	21.7	1.0 × 0.53	12.4	8.06
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	8.19	12.2
		Leafy vegetables BBCH 10 - 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	4.47	22.4
		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	2.3	1.0 × 0.53	1.32	75.8
	1 × 720	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	6.60	15.1
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (Sorex araneus)	1.9	1.0 × 0.53	0.73	137.0
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	2.52	39.7
		Leafy vegetables BBCH 10 - 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	1.60	62.5
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	0.73	137.0
		Leafy vegetables	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		100					
TER criterion		5					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
		BBCH 40 - 49					
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	21.7	1.0 × 0.53	8.28	12.1
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	5.46	18.3
		Leafy vegetables BBCH 10 - 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	2.98	33.6
		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	2.3	1.0 × 0.53	0.88	113.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in field crops (Pre-sowing, pre-planting, pre-emergence, Uses 1 a-c) except for the scenarios marked in bold in the table above, where a refined risk assessment is required.

Table B.9.2.2-10: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops (Post-harvest, pre-sowing, pre-planting): Use 2 a-c, 3 a-b, 10 a-c

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		100					
TER criterion		5					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Field crops (Post-harvest, pre-sowing, pre-planting)	1 × 1440	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.45	69.0
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	13.2	7.58
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	5.04	19.8

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
	2 × 1080 (28 d)	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	1.20	83.3
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.1 × 0.53	10.9	9.17
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	45.5	**2.20**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.1 × 0.53	4.16	24.0
	1 × 540	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	0.544	183.8
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	4.95	20.2
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	20.7	**4.83**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	1.89	52.9
	1 × 720	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	0.725	137.9
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	6.60	15.1
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	2.52	39.7

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
	2 × 720 (28 d)	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	0.798	125.3
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.1 × 0.53	7.26	13.8
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	30.4	**3.29**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.1 × 0.53	2.77	36.1
	1 × 1080	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.09	91.7
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	9.90	10.1
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	3.78	26.5
	3 × 720 (28 d)	Bulbs and onion like crops BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.2 × 0.53	0.870	114.9
		Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.2 × 0.53	7.92	12.6
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.2 × 0.53	33.1	**3.02**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.2 × 0.53	3.02	33.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in field crops (Post-harvest, pre-sowing, pre-planting, Use 2 a-c, 3 a-b, 10 a-c) except for some scenarios, marked in bold in the table above, where a refined risk assessment is required.

Table B.9.2.2-11: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops (Shielded ground directed inter-row application): Use 6 a-b

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Shielded ground inter-row application)	1 × 1080	Bulbs & onion like crops BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	2.40	41.7
		Bulbs & onion like crops BBCH 10 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	4.46	22.4
		Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.4**
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	8.19	12.2
	1 × 720	Bulbs & onion like crops BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	1.60	62.5
		Bulbs & onion like crops BBCH 10 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	2.98	33.6
		Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.6**
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	5.46	18.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in field crops (Shielded ground directed inter-row application, uses 6 a-b) except for some scenarios, marked in bold in the table above, where a refined risk assessment is required.

Orchards

Table B.9.2.2-12: Tier 1 assessment of the acute risk for mammals due to the use of glyphosate in orchards: Uses 4 a-c

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**3447 (geomean)**					
TER criterion		**10**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Orchard Post-emergence of weeds	2 × 1440 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.1	8.55	403
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	1.1	216	16.0
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	1.1	55.6	62.0
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	1.1	27.2	126.7

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_a values are above the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following the proposed use patterns in orchards (Uses 4 a –c).

Table B.9.2.2-13: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in orchards: Use 4 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Vineyard	2 × 1440 (28 d)	Orchards Application crop directed BBCH	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	1.60	62.5

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Post-emergence of weeds		<10 or not crop directed					
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	60.7	**1.65**
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.1 × 0.53	12.0	8.33
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.1 × 0.53	6.55	15.3
	1 × 720	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	0.725	137.9
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	5.46	18.3
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	2.98	33.6
	1 × 1080	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.09	91.7
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
		Orchards Application crop directed BBCH	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	8.19	12.2

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		<10 or not crop directed					
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal “mouse” Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	4.47	22.4
	2 × 720 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal “shrew” Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	1.90	52.6
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal “vole” Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	72.3	**1.38**
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal “lagomorph” Rabbit (*Oryctolagus cuniculus*)	14.3	1.1 × 0.53	14.3	6.99
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal “mouse” Wood mouse (*Apodemus sylvaticus*)	7.8	1.1 × 0.53	7.80	12.8
	3 × 720 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal “shrew” Common shrew (*Sorex araneus*)	1.9	1.2 × 0.53	0.87	114.9
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal “vole” Common vole (*Microtus arvalis*)	72.3	1.2 × 0.53	33.1	**3.02**
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal “lagomorph” Rabbit (*Oryctolagus cuniculus*)	14.3	1.2 × 0.53	6.55	15.3
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal “mouse” Wood mouse (*Apodemus sylvaticus*)	7.8	1.2 × 0.53	3.57	28.0
	1 × 1440	Orchards Application crop directed BBCH	Small insectivorous mammal “shrew” Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.45	69.0

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		100					
TER criterion		5					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
		<10 or not crop directed					
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	10.9	9.17
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	5.95	16.8
	2 × 1080 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	1.20	83.3
		Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	45.5	**2.20**
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.1 × 0.53	9.00	11.1
		Orchards Application crop directed BBCH <10 or not crop directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.1 × 0.53	4.91	20.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in orchards (Uses 4 a-c) except for the scenarios marked in bold in the table above, where a refined risk assessment is required.

Vineyards

Table B.9.2.2-14: Tier 1 assessment of the acute risk for mammals due to the use of glyphosate in vineyards: Use 5 a-c

Active substance	Glyphosate
Acute toxicity (mg/kg bw)	3447 (geomean)

TER criterion		**10**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Vineyard Post-emergence of weeds	2 × 1440 (28 d)	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	27.2	1.1	43.1	80.0
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	16.3	1.1	25.8	133.6
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	13.6	1.1	21.5	160.3
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	8.1	1.1	12.8	269.3
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	1.1	12.0	287.3
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.1	8.55	403.2
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	1.1	216	16.0
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	1.1	27.2	126.7

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_a values are above the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following the proposed use patterns in vineyards (Uses 5 a-c).

Table B.9.2.2-15: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in vineyards: Use 5 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Vineyard Post-emergence of weeds	2 × 1440 (28 d)	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.1 × 0.53	9.32	10.7
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.1 × 0.53	5.62	17.8

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.1 × 0.53	4.62	21.6
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.1 × 0.53	2.77	36.1
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.1 × 0.53	3.53	28.3
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	1.60	62.5
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	60.7	**1.6**
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.1 × 0.53	6.55	15.3
	1 × 720	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.0 × 0.53	4.24	23.6
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.0 × 0.53	2.56	39.1
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.0 × 0.53	2.10	47.6
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.0 × 0.53	1.26	79.4
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	1.60	62.5
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	0.725	137.9
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.6**

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	2.98	33.6
	1 × 1080	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.0 × 0.53	6.35	15.7
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.0 × 0.53	3.84	26.0
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.0 × 0.53	3.15	31.7
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.0 × 0.53	1.89	52.9
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (Sorex araneus)	4.2	1.0 × 0.53	2.40	41.7
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.09	91.7
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.4**
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	4.47	22.4
	2 × 720 (28 d)	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.1 × 0.53	4.66	21.5
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.1 × 0.53	2.81	35.6
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.1 × 0.53	2.31	43.3

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.1 × 0.53	1.39	71.9
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.1 × 0.53	1.76	56.8
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	0.798	125.3
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	30.4	**3.3**
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus)*	7.8	1.1 × 0.53	3.27	30.6
	3 × 720 (28 d)	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.2 × 0.53	5.08	19.7
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.2 × 0.53	3.07	32.6
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.2 × 0.53	2.52	39.7
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.2 × 0.53	1.51	66.2
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.2 × 0.53	1.92	52.1
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.2 × 0.53	0.87	114.9
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.2 × 0.53	33.1	**3.0**
		Vineyard Application	Small omnivorous mammal "mouse"	7.8	1.2 × 0.53	3.57	28.0

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		100					
TER criterion		5					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
		ground directed	Wood mouse (*Apodemus sylvaticus*)				
	1 × 1440	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.0 × 0.53	8.47	11.8
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.0 × 0.53	5.11	19.6
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.0 × 0.53	4.20	23.8
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.0 × 0.53	2.52	39.7
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	3.21	31.2
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.45	69.0
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.8**
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	5.95	16.8
	2 × 1080 (28 d)	Vineyard Application ground directed	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	11.1	1.1 × 0.53	6.99	14.3
		Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	6.7	1.1 × 0.53	4.22	23.7
		Vineyard BBCH 20 – 39	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	5.5	1.1 × 0.53	3.46	28.9
		Vineyard BBCH ≥ 40	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	3.3	1.1 × 0.53	2.08	48.1

Active substance		Glyphosate					
Reprod. toxicity (mg/kg bw/d)		100					
TER criterion		5					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
		Vineyard BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.1 × 0.53	2.64	37.9
		Vineyard BBCH ≥ 20	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.1 × 0.53	1.20	83.3
		Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	45.5	**2.2**
		Vineyard Application ground directed	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.1 × 0.53	4.91	20.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in vineyards (Uses 5 a-c) except for the scenarios marked in bold in the table above, where a refined risk assessment is required.

Railroad tracks – application by spray train

Table B.9.2.2-16: Tier 1 assessment of the acute risk for mammals due to the use of glyphosate on railroad tracks: Use 7 a-b

Active substance		Glyphosate					
Acute toxicity (mg/kg bw)		3447 (geomean)					
TER criterion		10					
GAP crop	Application rate (g a.s./ha)	Crop scenario	Generic focal species	SV90	MAF90	DDD90 (mg/kg bw/d)	TERa
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	32.6	1.0	58.7	58.7
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.0	9.72	354.6
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	136.4	1.0	246	14.0
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	14.4	1.0	25.9	133.1
		Leafy vegetables	Small insectivorous mammal "shrew"	7.6	1.0	13.7	251.6

		BBCH 10 - 19	Commnon shrew (*Sorex araneus*)				
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	5.4	1.0	9.72	354.6
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	136.4	1.0	246	14.0
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	40.9	1.0	73.6	46.8
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	1.0	63.2	54.5
		Leafy vegetables BBCH 10 – 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	1.0	31.0	111.2
		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	1.0	9.36	368.3
	1 × 1800	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	32.6	1.0	58.7	58.7
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	5.4	1.0	9.72	354.6
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	136.4	1.0	246	14.0
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	14.4	1.0	25.9	133.1
		Leafy vegetables BBCH 10 - 19	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	7.6	1.0	13.7	251.6
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	5.4	1.0	9.72	354.6
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	136.4	1.0	246	14.0
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	40.9	1.0	73.6	46.8
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	35.1	1.0	63.2	54.5
		Leafy vegetables BBCH 10 – 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	1.0	31.0	111.2
		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.2	1.0	9.36	368.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_a values are above the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following the proposed use patterns on railroad tracks (Uses 7a-b).

Table B.9.2.2-17: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate on railroad tracks: Use 7 a-b

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	16.5	6.1
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.81	55.2
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	6.30	15.9
		Leafy vegetables BBCH 10 - 19	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	4.2	1.0 × 0.53	4.01	24.9
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.81	55.2
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	21.7	1.0 × 0.53	20.7	**4.8**
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	13.6	7.4
		Leafy vegetables BBCH 10 – 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	7.44	13.4

		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	2.3	1.0 × 0.53	2.19	45.7
	1 × 1800	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.53	16.5	6.1
		Grassland Late	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.81	55.2
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
		Grassland Late season (seed heads)	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	6.6	1.0 × 0.53	6.30	15.9
		Leafy vegetables BBCH 10 - 19	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	4.2	1.0 × 0.53	4.01	24.9
		Leafy vegetables BBCH ≥ 20	Small insectivorous mammal "shrew" Commnon shrew (*Sorex araneus*)	1.9	1.0 × 0.53	1.81	55.2
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	21.7	1.0 × 0.53	20.7	**4.8**
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.53	13.6	7.4
		Leafy vegetables BBCH 10 – 49	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	7.8	1.0 × 0.53	7.44	13.4
		Leafy vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	2.3	1.0 × 0.53	2.19	45.7

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns on railroad tracks (Uses 7 a-b)
except for the scenarios marked in bold in the table above, where a refined risk assessment is required.

Control of invasive species

Table B.9.2.2-18: Tier 1 assessment of the acute risk for mammals due to the use of glyphosate on invasive species in agricultural and non-agricultural areas: Uses 8, 9

Active substance		**Glyphosate**					
Acute toxicity (mg/kg bw)		**3447 (geomean)**					
TER criterion		**10**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SV90**	**MAF90**	**DDD90 (mg/kg bw/d)**	**TERa**
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Bulbs & onion like crops BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	1.0	13.7	251.6
		Bulbs & onion like crops BBCH 10 – 39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	1.0	31.0	111.2
		Cereals Early (shoots)	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	42.1	1.0	75.8	45.5
		Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	1.0	246	14.0

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 1 TER_a values are above the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following the proposed use patterns on invasive species (Uses 8 and 9).

Table B.9.2.2-19: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate on invasive species in agricultural and non-agricultural areas: Uses 8, 9

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Invasive species in agricultural and non-agricultural areas. Post emergence	1 × 1800	Bulbs & onion like crops BBCH 10 – 19	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1.0 × 0.53	4.01	24.9
		Bulbs & onion like	Small omnivorous mammal "mouse"	7.8	1.0 × 0.53	7.44	13.4

of invasive species.		crops BBCH 10 – 39	Wood mouse (*Apodemus sylvaticus*)				
		Cereals Early (shoots)	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	22.3	1.0 × 0.53	21.3	**4.7**
		Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 1 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns on invasive species (Uses 8 and 9) except for the scenarios marked in bold in the table above, where a refined risk assessment is required. Since no specific EU-agreed guideline exists for non-crop uses, the above calculations rely on the standard assumptions for field use. It is noted however, that the assessment for non-crop uses (e.g. railroad tracks) and invasive species can be considered as unduly conservative. For example, instead of a full application rate, the drift rate for field crops (e.g. 2.7%) depositing in the proximity of railroad tracks would be a more realistic exposure rate along the marginal habitat.

Overall conclusion of Tier 1

To sum up, the following scenarios did not meet the acceptability trigger of 5 for the long term risk assessment.

Table B.9.2.2-20: Scenarios that did not meet the trigger for acceptability in long-term risk assessment

Application rate (g a.s./ha)	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Uses 1 a-c; Field crops (Pre-sowing, pre-planting, pre-emergence)						
1 × 1440	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
1 × 1440	Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
1 × 1080	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
1 × 1080	Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
1 × 720	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**
1 × 720	Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**

Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Use 2 a-c, 3 a-b, 10 a-c; Field crops (Post-harvest, pre-sowing, pre-planting)						
1 × 1440	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
2 × 1080 (28 d)	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	20.7	**4.83**
1 × 720	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**
2 × 720	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	30.4	**3.29**
1 × 1080	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
3 × 720 (28 d)	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.2 × 0.53	33.1	**3.02**
Use 6 a-b; Field crops (Shielded ground inter-row application)						
1 × 1080	Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.4**
1 × 720	Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.6**
Use 4 a-c; Vineyard, Post-emergence of weeds						
2 × 1440 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	60.7	**1.65**
1 × 720	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.62**
1 × 1080	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.42**
2 × 720 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	72.3	**1.38**
3 × 720 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.2 × 0.53	33.1	**3.02**

Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
1 × 1440	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.81**
2 × 1080 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	45.5	**2.20**
Use 5 a-c; Vineyard Post-emergence of weeds						
2 × 1440 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	60.7	**1.6**
1 × 720	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	27.6	**3.6**
1 × 1080	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	41.4	**2.4**
2 × 720 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	30.4	**3.3**
3 × 720 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.2 × 0.53	33.1	**3.0**
1 × 1440	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	55.2	**1.8**
2 × 1080 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.1 × 0.53	45.5	**2.2**
Use 7 a-b; Railroad tracks – application by spray train. Post emergence of weeds (90d apart).						
2 × 1800 (90 d)	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
2 × 1800 (90 d)	Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
2 × 1800 (90 d)	Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	21.7	1.0 × 0.53	20.7	**4.8**
1 × 1800	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**

Application rate (g a.s./ha)	Crop scenario	Generic focal species	SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
1 × 1800	Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**
1 × 1800	Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	21.7	1.0 × 0.53	20.7	**4.8**
Uses 8, 9; Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.						
1 × 1800	Cereals Early (shoots)	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	22.3	1.0 × 0.53	21.3	**4.7**
1 × 1800	Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.53	69.0	**1.4**

B.9.2.2.3. Higher tier assessment (Tier 2)

Long-term Tier 2 exposure was calculated for those intended uses, for which the Tier 1 risk assessment indicates the need for a refined long-term risk assessment. As indicated in the tables above further refinements are needed for herbivorous mammals, i.e. the small herbivorous mammal "vole" and (in one case only) the large herbivorous mammal "lagomorph" (rabbit).

In Tier 2, TWA and MAF values for glyphosate can be refined based on measured residues on grass foliage. The methodology used to calculate the TWA for glyphosate on grass foliage for the long-term risk assessment follows the procedure described in the Guidance Document on Terrestrial Ecotoxicology (2002). According to the approach outlined in the Guidance Document on Terrestrial Ecotoxicology, the dissipation of glyphosate in grass was estimated using the standard first-order dissipation model:

$Ct = Ci \times e^{-kt}$

k = first order rate constant
Ci = initial residue concentration
Ct = residue concentration at time t

The decline of glyphosate residue on grass was characterized using data from 22 residue trials each of which had a day 0 value. Based on this data, the k value for grass foliage was calculated to be 0.2476 $days^{-1}$ (Renewal Assessment Report for glyphosate, 29 January 2015, Volume 3, Annex B.9, B.9.13).

Residue half-life times (DT_{50}) in days were calculated with following equation:

$$DT_{50} = \frac{-\ln 0.5}{k}$$

The average DT_{50} for grass foliage was **2.8 days**.

The 21-day time weighted average (TWA) for glyphosate on grass foliage has been calculated according to the following formula:

$$TWA = \frac{\left(1 - e^{-kt} \right)}{kt}$$

The 21-day TWA is calculated to be **0.19** for the active substance glyphosate acid and grass. For the refined risk assessment this value is applied for the small herbivorous mammal "vole" Common vole (*Microtus arvalis*), the large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*) and the large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*). Although the calculated 21-day TWA of 0.19 is based on residue decline on "grass" the applicant proposed that this may be considered to be valid for "non-grass herbs" as well. This assumption was supported by Ebeling & Wang (2018)[1], who evaluated the residue dissipation of 30 active substances (including glyphosate) on grasses / cereals (177 trials) and non-grass herbs (101 trials). No significant difference between residue dissipation on grasses / cereals and non-grass herbs was found.

Foliar dissipation half lives were calculated and potential factors determining dissipation were analysed in the study, such as crop group, residue zone or rainfall. From the results, the strongest source of variability was reported to be found between individual trials, while other factors, including the residue zone or crop groups were concluded to not have a significant impact on dissipation. Only heavy rainfall (>6.5 mm/day, i.e. the 95th percentile rainfall) had a statistically significant influence (explained about five percent of the overall variability). The differences in DT_{50} between crops and residue zones were neither marked nor statistically significant.

The statistical analysis by Ebeling and Wang (2018) was based on results from 278 residue trials (after selection criteria had been applied to the initial number of 396 trials) for 30 different compounds (active substances). The selection criteria were as follows:

1. Trials must be conducted on leafy substrate that could be assigned to the plant category grasses and cereals or non-grass herbs
2. Data should preferentially include an initial measurement on the day of application
3. Replicate trials should be available to facilitate a comparison of plant categories and different locations
4. Trials with measurements for fewer than 3 time points are disregarded
5. Only trials are accepted for the final evaluation which provided a DT_{50} value based on a SFO fit that passed a visual assessment, had a $\chi 2$ error below 25 and passed a t-test.

With regard to criteria 4 and 5 above, it should be noted that at least five data points are required to obtain a reliable kinetic fit according to FOCUS Kinetic (2014), which refers to the EC Guidance Document on Persistence in Soil (DG VI - 9188/VI/97 - Rev 8 of 12.07.2000). In addition, the selection of trials/DT_{50} values for the statistical analysis does not mention the evaluation of the residuals. One could have a good visual fit with acceptable values for χ^2 test and t-test and have systematic errors in the residual plot at the same time. As a consequence, it is not clear that all DT_{50} values included in the analysis are sufficiently robust and some trials could have been omitted from the final selection. Also note that there is no information on timing of the sampling points, which for most residue tests do normally not focus on the first few days after application.

Data were evaluated using 3 different approaches:

- In approach A, data from all individual trials (and compounds) were pooled (resulting in 177 trials in grasses and cereals with 25 compounds and 101 trials in non-grass herbs with 12 compounds).
- In approach B, for each compound and crop category a geometric mean DT_{50} was calculated to eliminate bias attributable to different numbers of trials for different compounds and crops

[1] Ebeling, M., Wang, M. Dissipation of Plant Protection Products from Foliage. Environmental Toxicology and Chemistry (2018). Wiley Online Library.

(resulting in 177 trials in grasses and cereals with 25 compounds and 101 trials in non-grass herbs with 12 compounds).

- Approach C was the same as approach B, but only crop–compound combinations were used for which a geometric DT50 was available for the EU-N and EU-S residue zones (resulting in 134 trials in grasses and cereals with 14 compounds and 86 trials in non-grass herbs with 7 compounds).

The DT_{50} values of crop groups or residue zones were log (natural)–transformed and compared with a 2-sided t-test (approaches A and B) and a paired t-test (approach C, comparison between residue zones) with a significance level of α=0.05. Mean, median, geometric mean, and 90th percentile DT_{50} values were calculated.

It is confirmed that the grouping of trials for the three different approached in general fitted the purpose of the study (to investigate the possibility of extrapolation of the residue decline DT_{50} between different crop types and between different zones). However, it would be more beneficial to compare the residue decline DT_{50} for grasses and cereals to DT_{50} for non-herbal crops for a number of individual compounds which had DT_{50} values in both groups. This type of analysis would provide more useful insights for the regulatory purposes. By comparing a group of substances with only DT_{50} values in the 'grass group' to a different group of substances with only a DT_{50} on the 'vegetable' group the data points might not be comparable. The disaggregated results, using only substances with values in both groups, could act as a stronger evidence to the extrapolation possibilities.

It is not explicitly mentioned in Birds and Mammals (2009) that the extrapolation of the residue decline DT_{50} between of different crop types is not possible. Moreover, like the default DT_{50}, the refinement of the residue decline DT_{50} does not aim at plant-specific kinetics, but at a value that can be used also for plant food items not tested in the analysis, according to Birds and Mammals (2009).

As long as the analysis itself has been done correctly (using the same plant item, e.g. whole plant vs green parts), the possibility of extrapolation between different crops is not entirely excluded by Birds and Mammals (2009).

On the contrary, the EFSA Recurring issues document (June, 2019) states specifically that it is generally not considered appropriate to extrapolate the data from monocotyl to dicotyl crops and vice-versa. It is implied that considering the fact that plant morphology, type of formulation and application technique have an influence on the measured residue levels, it is considered acceptable to extrapolate between similar crops (i.e. morphologically similar, pertaining to the same group), provided that the same formulation and application technique as per GAP is used.

At the same time, for plant residues the default datasets for DT_{50} values can be considered to cover a broad spectrum of external conditions. They suggest a relatively low variability of DT_{50} values between sites/plants for one single substance as well as for all substances in the dataset.

This low variability of DT_{50} values have been confirmed by Ebeling and Wang (2018), provided that the statistical method used (a 2-sided t-test) is acceptable for such study.

The resulting geomean DT_{50} values for grass and cereals were not significantly different from the geomean DT_{50} values for non-grass herbs for all 3 approaches.

The study of Ebeling and Wang (2018) has certain shortcomings, but the outcomes of the statistical analysis support the possibility of extrapolation of DT_{50} values between grass and cereals and non-grass herbs. However, it would be more beneficial to compare the residue decline DT_{50} for grasses and cereals to DT_{50} for non-herbal crops for a number of individual compounds. This type of analysis would provide more useful insights for the regulatory purposes. The disaggregated results could act as a stronger evidence to the extrapolation possibilities.

Hence, further consideration related to the analysis of the data may be needed, and also an overall discussion on the acceptability of the proposed approach in relation to the current EFSA guidance document (2009).

For the calculation of the overall DT_{50} (based on the individual DT_{50} values from all acceptable trials), the geometric mean should be used. However, this geometric mean can only be used in the risk assessment if it is shown that there have been enough replicates (separate DT_{50} values) to cover the expected variation in plant degradation studies. Possible ways to address this have been proposed, for example in the Northern Zone GD, or the EFSA proposal, as in Appendix B of the report of the outcome of the recurring issues meeting (EFSA, 2019).

MAF_{90} and MAF_m values for the application intervals of 28 and 90 days and based on the measured foliar half-life were calculated using the formula in Appendix H of EFSA/2009/1438. Resulting MAF values for two and three applications are presented in the following table.

Table B.9.2.2-21: MAF_{90}, MAF_m and MAF_m × TWA values based on a measured foliar DT_{50} of 2.8 days

Number of applications	Application interval (d)	Measured foliar DT_{50} (d)	MAF_{90}	MAF_m	MAFm × TWA
2	28	2.8	1.00	1.00	0.19
3	28	2.8	1.00	1.00	0.19
2	90	2.8	1.00	1.00	0.19

The available residue trials included in the estimated residue decline estimation are summarised as presented by the applicant in the tables below.

Table B.9.2.2-22: Glyphosate residues in grass following a single treatment of Roundup® (MON 2139, SL/360). Source: Monsanto Field Residue Studies

Country, Year Trial, ID	**App. Rate (kg a.s./ha)[1]**	**NRG 100% of DM[2]**	**% of Day 0 a.s. residue**	**DAT[3]**	**R^2**	**k ($days^{-1}$)**	**DT_{50} days**	**Glyphosate Monograph reference; Monsanto Report No.**
Great Britain, 1981								
SU 8125	1.08	101	100	1h	0.99	0.4106	1.7	RIP95-01242MLL 30.080
		27	26.7	3				
		12	11.9	7				
SU 8125	2.88	67	100	1h	0.997	0.3251	2.1	
		27	40.3	3				
		5	7.5	7				
SU 30117	1.08	247	100	1h	0.997	0.9587	0.72	
		14	5.7	3				
		8	3.2	7				
		7	2.8	9				
		6	2.4	10				
		3	1.2	14				
SU 30117	2.88	130	100	1h	0.976	0.7063	0.98	
		14	10.8	3				
		11	8.5	7				
		9	6.9	9				
		10	7.7	10				
		3	2.3	14				
SU 30119	1.08	193	100	1h	0.809	0.1456	4.8	
		175	90.7	4				
		38	19.3	9				
		9	4.7	11				

SU 30119	2.88	161	100	1h	0.901	0.155	4.5	
		123	76.4	4				
		30	18.6	9				
		13	8.1	11				
France, 1981								
811	0.72	168	100	0	0.976	0.4576	1.5	RIP95-01245MLL 30.082
		9	5.4	5				
		23	13.7	8				
		5	3	12				
811	1.08	134	100	0	0.95	0.3768	1.8	
		9	6.7	5				
		27	20.1	8				
		5	3.7	12				
Netherlands, 1982								
NL 8207	1.44	682	100	0	0.998	0.423	1.6	RIP95-01264MLL 30.101
		77	11.3	5				
		31.7	4.6	10				
Denmark, 1981								
Villbach (GE)-1981-0181 Vi	1.8	162.9	100	0	0.844	0.1415	4.9	RIP95-01273MLL 30.132
		36	22.3	7				
		52.6	32.3	14				
Villbach (GE)-1981-0281 Vi	1.8	496.3	100	0	0.994	0.1537	4.5	
		184.4	37.2	7				
		37	7.5	14				
Lettgunbrunn (GE)-1981-0981LE	1.8	437.9	100	0	0.961	0.2616	2.6	
		51.2	11.7	7				
		69.4	15.8	14				
Villbach (GE)-1981-0481 Vi	1.8	190.7	100	0	0.937	0.1098	6.3	
		69	36.2	7				
		59	30.9	14				
Denmark, 1983								
Vogach (GE)-19B	1.44	158.9	100	0	0.995	0.9083	0.76	RIP95-01273MLL 30.132
		9.9	6.2	3				
		8.3	5.2	7				
		3.3	2.1	10				
		4.4	2.8	14				
Untermehlhausen (GE)-1983	1.44	169.6	100	0	0.99	0.2852	2.4	
		16.4	9.7	7				
		16.2	9.6	10				
		13	7.7	14				
Schoneberg	1.44	257.2	100	0	*	*	104	
		155.8	60.6	3				
		144.6	56.2	7				
		123.9	48.2	10				
		151	58.7	14				
Utphe (GE)-1983	1.44	354.9	100	0	0.961	0.1718	4	
		78.7	22.2	7				
		62.7	17.7	14				
		39	11	21				
Meiling (GE)-1983	1.44	253.9	100	0	0.997	0.9014	0.77	
		16.6	6.5	3				
		6	2.4	7				
		6.3	2.5	10				
		8.3	3.3	14				

1 a.s. = glyphosate acid.
2 NRG 100% of DM = residual glyphosate mg/kg normalized to 1 kg a.s./ha and corrected to 100% dry matter content.
Values taken directly from Monsanto reports.

3 DAT = Days After Treament.
4 Estimated DT_{50} value based on time when approximately 50% dissipation was reached.
* Did not fit standard 1st order dissipation model.

B.9.2.2-23: Glyphosate residues in grass following a single treatment of CHE 3607 (SL/360). Source: Cheminova Field Residue Studies (cited in Glyphosate Monograph)

App. Rate (kg a.s./ha)[1]	Residue (mg a.s./kg wet weight)	% of Day 0 a.s. Residue	DAT[2]	R^2	k ($days^{-1}$)	DT_{50} (days)	Glyphosate Monograph reference; Cheminova Report No.
Great Britain, 1992							
2.16	237.6	100	4h	0.987	1.9629	0.35	RIP95-01308 IF-93/04572-01
	45	18.9	1				
	19.6	8.2	3				
	9.6	4	5				
1.08	87.6	100	4h	0.937	2.0879	0.33	
	14.6	16.7	1				
	14.3	16.3	3				
	8.3	9.5	5				
2.16	252.3	100	4h	0.951	0.4885	1.4	RIP95-01312 IF-93/13842-01
	131	51.9	1				
	72.1	28.6	3				
	36.6	14.6	5				
1.08	90.4	100	4h	*	*	33	
	142.8	158	1				
	39.8	44	3				
	17.3	19.1	5				

[1] a.s. = glyphosate acid.
[2] DAT = Days After Treament.
[3] Estimated DT_{50} value based on time when approximately 50% dissipation was reached.
* Did not fit standard 1st order dissipation model.

The available residue trials were evaluated by Germany and was accepted for the previous evaluation and have not been re-evaluated in detail for this assessment. The dosing regime is similar to the representative use of MON 52276, the representative EU-formulation for the renewal. The trial sites appear to be representative for the Northern, Central and Southern zone of the EU. Given the findings by Ebeling and Wang (2018, see above), information on possible heavy rainfall events in the studies would be useful since this factor seemed to influence the declination rate.

The available residue data indicate that the default declination DT_{50} of 10 days is probably overly conservative. However, the applicant did not present study information sufficient to re-evaluate the quality of the data according to current standard.

No detailed evaluation of the kinetic data was presented by the applicant, and the RMS was not able to confirm the proposed average DT_{50} for residue decline. According to the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019), goodness of fit from residue trials used to refine the fTWA should be assessed using four indicators, all of which should be clearly reported. These indicators should be evaluated together and not in a hierarchical manner:

- Visual fit → plot of time vs concentration should be provided. Ideally, the fitted line should pass through (or in the vicinity of) the measurement points.
- Residual plot → Plot of time vs residuals against the y = 0 line should be provided. Points should ideally be scattered around the zero line. Regular patterns are generally indicative that the kinetic model used is not appropriate. Underestimation of the last time points is indicative of an under-conservative kinetic.

- Chi-square (χ^2) % should be reported and should ideally be < 15 %. Chi-square should be calculated using the mean of true replicates.
- t-test and/or confidence intervals of individual model parameters should be reported. t-test for rate constant resulting in p-values > 0.05 (or confidence intervals including zero) indicate large uncertainty in the estimation of the model parameters and such results should not be accepted.

It is proposed that this information is included to confirm the appropriateness of the selected DT_{50} for the refinement of residue decline in the risk assessment for wild mammals. The applicant is further referred to FOCUS Kinetics (2014) as to the presentation of visual and statistical fits. The general recommendations on data quality and data handling issues described in chapter 6 of FOCUS Kinetics are also considered relevant for residue dynamics, with the exception of the section on experimental artefacts which concern degradation studies.

Further quality criteria for the residue decline studies are provided in Risk Assessment for Birds and Mammals (2009) and in the Central Zone recommendations for conducting studies on residue levels and dissipation on food items for birds and mammals. According to the birds and mammal guidance (chapter 6 .1.4.1), residue measurements should focus on the first few days after application, especially if a low DT_{50} is expected. The guidance recommends 0, 1, 2, 5 days. None of the available residue tests seems to fulfil this criterium.

In addition, the following information regarding the refinement of residue decline of glyphosate on grass should be provided and analyzed before a decision on the acceptability of the refinement can be made.

In general, the study report should contain the following information: plot dimensions, plant density, trial site history, treatments, application equipment details, weather data at application (including average temperature and rainfall in the region), method of sampling, sampling collection, sampling storage stability, analytical method and validation, crop health, growing conditions, cultivation, maintenance chemicals during trial period, irrigation, weather conditions for the duration of the trial, and a comparison to long-term monthly weather conditions (Central zone recommendations, based on the NZ GD, 2014). The most important requirements are specified below.

1. The treatment used in the study must be in line with the GAP. Not only the crop and application rate must be provided, but also the BBCH stage. If the study is not performed according to the GAP, adequate support should be provided that these derivations had no impact on the DT_{50} estimation.

2. The material on which dissipation is determined must refer to the same plant item. It should be clearly stated per each trial which source material has been used at each individual sampling point (here: green foliage vs whole plant).

3. The trial sites and climatological conditions should be representative for the proposed use in the respective country where authorization is being sought. The applicant should provide argumentation to support this and the applicability to the requested area of use will be determined by the assessor.

4. The sampling scheme followed should always be justified based on the available information on the substance. Sampling points should primarily cover the first few days after application e.g. at least the following data points: 0, 1, 2, 5, 10, 14 and 20 DAA. A sample taken at 0 DBA is also recommended as a control for residues from previous applications. Ideally, in FOCUS Kinetics (2014) the number of data points remaining after the elimination of a lag phase, non-detects or outliers should not be smaller than five in accordance with the EC Guidance Document on Persistence in Soil (DG VI - 9188/VI/97 - Rev 8 of 12.07.2000).

5. The sampling method should be acceptable. A clear description should be provided regarding how the representative samples were taken from the crop, which parts were sampled and how these were stored

before analysis. If weeds or other vegetation is sampled, the amount of monocot and dicot plant material should be reported. It is recommended that damaged plant parts not be sampled. Taking samples at the beginning or extreme end of the plot should be avoided. The above ground portion of the plant should be sampled.

6. It should be stated whether the reported residue values are true replicates, or pseudo replicates. If the reported values are of true replicates, these individual values should be reported. If values of pseudo replicates are given, the average value can be reported. It should be noted that different trial sites are not replicates. Within one site replicate subplots need to be sampled for a replicated sampling strategy. In the OECD Guideline for TFD studies (terrestrial field dissipation) at least three subplots are required.

At Tier 2, the endpoint of 100 mg/kg bw/d is used for the chronic risk assessment. Detailed discussions on the selected chronic endpoints are presented in Volume 1, section 2.9.4.

Although the refinement of residue decline requires further confirmation as proposed above, the calculated TWA below is tentatively included in the Tier 2 calculations below.

Field crops

Table B.9.2.2-24: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops (Pre-sowing, pre-planting, pre-emergence): Use 1 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Pre-sowing, pre-planting, pre-emergence)	1 × 1440	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.19	4.73	21.1
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	21.7	1.0 × 0.19	5.94	16.8
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.19	3.91	25.6
	1 × 1080	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	21.7	1.0 × 0.19	4.45	22.5

	1 × 720	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED BY FURTHER INFORMATION**); DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 2 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in field crops (Pre-sowing, pre-planting, pre-emergence, Uses 1 a-c).

Table B.9.2.2-25: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops (Post-harvest, pre-sowing, pre-planting): Use 2 a-c, 3 a-b, 10 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Post-harvest, pre-sowing, pre-planting)	1 × 1440	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.19	4.73	21.1
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
	2 × 1080 (28 d)	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.19	3.55	28.2
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74
	1 × 540	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	7.42	13.5
	1 × 720	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	2 × 720 (28 d)	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	1 × 1080	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74
	3 × 720 (28 d)	Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED**); DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table B.9.2.2-26: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in field crops (Shielded ground directed inter-row application): Use 6 a-b

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Field crops (Shielded ground inter-row application)	1 × 1080	Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74
	1 × 720	Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED**); DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 2 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in field crops (Uses 6 a-b); shielded ground directed inter-row application.

Orchards

Table B.9.2.2-27: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in orchards: Uses: 4 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Orchard Post-emergence of weeds	2 × 1440 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.19	3.91	25.6
	1 × 720	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	1 × 1080	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74

	2 × 720 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	3 × 720 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	1 × 1440	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
		Orchards Application crop directed BBCH <10 or not crop directed	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.19	3.91	25.6
	2 × 1080 (28 d)	Orchards Application crop directed BBCH <10 or not crop directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED**); DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 2 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in orchards (Uses 4 a-c).

Vineyards

Table B.9.2.2-28: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate in vineyards: Use 5 a-c

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Vineyard Post-emergence of weeds	2 × 1440 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
	1 × 720	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	1 × 1080	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74

	2 × 720 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	10.1
	3 × 720 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	9.89	5.06
	1 × 1440	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	19.8	5.06
	2 × 1080 (28 d)	Vineyard Application ground directed	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	14.8	6.74

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED**); DDD: daily dietary dose; TER: toxicity to exposure ratio.

The Tier 2 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns in vineyards (Uses 5 a-c).

Railroad tracks – application by spray train

Table B.9.2.2-29: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate on railroad tracks: Use 7 a-b

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Railroad tracks – application by spray train. Post emergence of weeds (90d apart).	2 × 1800 (90 d)	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.19	5.92	16.9
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.19	24.7	**4.04**
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.19	24.7	**4.04**
		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	21.7	1.0 × 0.19	7.42	13.5
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.19	4.89	20.5
	1 × 1800	Grassland All season	Large herbivorous mammal "lagomorph" Brown hare (*Lepus europaeus*)	17.3	1.0 × 0.19	5.92	16.9
		Grassland All season	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.19	24.7	**4.04**
		Leafy vegetables BBCH 40 - 49	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	72.3	1.0 × 0.19	24.7	**4.04**

		Leafy vegetables BBCH ≥ 50	Small herbivorous mammal "vole" Common vole (*Microtulus arvalis*)	21.7	1.0 × 0.19	7.42	13.5
		Leafy vegetables All season	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	14.3	1.0 × 0.19	4.89	20.5

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED**); DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 2 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns on railroad tracks (uses 7a-b) except for the following scenarios where a refined risk assessment is required for all intended application rates:

Grassland; the small herbivorous mammal "vole" common vole (2 × 1800 g a.s./ha, 1 × 1800 g a.s./ha).

Leafy vegetables; the small herbivorous mammal "vole" common vole (2 × 1800 g a.s./ha, 1 × 1800 g a.s./ha).

Control of invasive species

Table B.9.2.2-30: Tier 2 assessment of the long-term/reproductive risk for mammals due to the use of glyphosate on invasive species in agricultural and non-agricultural areas: Uses 8 and 9

Active substance		**Glyphosate**					
Reprod. toxicity (mg/kg bw/d)		**100**					
TER criterion		**5**					
GAP crop	**Application rate (g a.s./ha)**	**Crop scenario**	**Generic focal species**	**SVm**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Invasive species in agricultural and non-agricultural areas. Post emergence of invasive species.	1 × 1800	Cereals Early (shoots)	Large herbivorous mammal "lagomorph" Rabbit (*Oryctolagus cuniculus*)	22.3	1.0 × 0.19	7.63	13.1
		Fruiting vegetables BBCH 10 – 49	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1.0 × 0.19	24.7	**4.04**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor (**note by RMS: TO BE CONFIRMED**); DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The Tier 2 TER_{lt} values are above the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following the proposed use patterns on invasive species (Uses 8 and 9) except for the following scenarios where a refined risk assessment is required for the intended application rate:

Fruiting vegetables; the small herbivorous mammal "vole" common vole (1 × 1800 g a.s./ha).

B.9.2.2.4. Higher tier – Long-term mammalian refined (Tier 3) assessment

As indicated in the tables above, based on the applicant's evaluation, further refinements of the long-term mammal risk assessment were required for the small herbivorous mammal "vole" considering two exposure

scenarios, namely the 'Grassland - all season' scenario and the leafy vegetable (BBCH 40-49) scenario for applications to control invasive and noxious weeds and for application to railroad tracks at 1800 g/ha.

In addition to the refined TWA and MAF values applied for the Tier 2 assessment (tentatively included above), use specific considerations and a further refined chronic mammalian endpoint was considered by the applicant.

Discussion on refinement of the chronic mammalian endpoint is presented in Volume 1, section 2.9.4.

The applicant proposed that the observed maternal effects in rabbit are not resulting from systemic exposure to glyphosate, but are due to GI-tract irritation resulting from the dosing route. An additional endpoint was therefore presented based on the results of seven rat developmental toxicity studies, where an endpoint of 300 mg/kg bw/day was concluded. The results of multi-generational studies in rats, with resulting NOAEL of 700 mg/kg bw/day, were also discussed by the applicant. In this type of study, animals are exposed via the diet, which would be the route of exposure in the field.

The RMS concluded, however, that ecological relevance of the observed effects in rabbits cannot be ruled out. Further, according to the standard approach the most sensitive tested species should be selected for the risk assessment in order to represent the range of possibly sensitive species from all mammals present in the field. Differentiated endpoints for lagomorphs and rodents is therefore not considered appropriate.

Further considerations were proposed by the GRG to support an acceptable chronic exposure risk to mammals for all proposed GAP table uses of MON 52276 are presented below

Railroad tracks

The application of the product on railroad tracks is done by spray trains. These trains are equipped with high resolution cameras and are able to identify weeds on the tracks. The product is applied very targeted to the weeds and only on those sections where weeds are present. Thus this application method is not comparable to a standard broadcast application where application takes place on the whole area. In general railroad tracks are placed on aggregate, i.e. small rocks, providing an environment for plants which are adapted to dryer conditions. Due to management and rather dry and hostile conditions that a railroad track provides, it is not expected that dense and long grass vegetation would be present, thus creating an uninviting habitat for small mammals to exist, feed and burrow.

According to Le Louarn & Quere (2003)[2] the common vole is a grassland species and inhabit meadows, set-aside land, flower strips as primary habitats. It lives in shallow burrows rarely more than about 30 cm deep (Stein, 1958)[3] . These primary habitats provide food and shelter from predators so that monthly survival of voles in primary habitats like set-aside grasslands is about 0.5 – 0.6, while being close to zero in arable fields (Jacob & Halle 2001)[4]. According to Stein (1958)[3] secondary habitats for voles are cropped areas such as grain cereals, oilseed rape, peas, beans, carrots and occasionally sugar beet and potato fields. Jacob et al. (2014)[5] conclude that those secondary habitats may be invaded by voles when the

[2] Le Louarn, H., Quéré, J. P. Les Rongeurs de France. Faunistique et biologie. INRA Editions, Paris, France, pp. 1-256 (2003).

[3] Stein, G.H.W. Die Feldmaus. Franckh'sche Verlagshandlung, Stuttgart, Germany (1958).

[4] Jacob, J., Halle, S. The importance of land management for population parameters and spatial behaviour in common voles (Microtus arvalis). Advances in Vertebrate Pest Management II. Filander Verlag, Fürth, Germany, pp. 319-330 (2001).

[5] Jacob, J., Manson, P., Barfknecht, R., Fredricks, T. Common vole (Microtus arvalis) ecology and management: implications for risk assessment of plant protection products. Published online in Wiley Online Library (15th January 2014).

carrying capacity (critical population density) of primary habitats is exceeded. According to Frank (1957)[6] and Briner et al (2005)[7] common voles of both sexes tend to be highly territorial, when population densities are low.

Railroad tracks might be occasionally visited by voles when population densities are high in [9]primary habitats but it can be assumed that they don't spend much time in such hostile environments. Due to disturbance, rather dry conditions and the risk from predators, typical primary or secondary habitats provide better environmental conditions for voles than railroad tracks. Therefore the small herbivorous mammal "vole" should not be regarded as a relevant focal species on railroad tracks. Therefore, to provide a conservative approach for the application on railroad tracks 50% of the application rate could be taken into account for an alternative refined chronic risk assessment.

By virtue of the very high residues per unit dose (RUD) value for common voles feeding on 100% grasses as stated in the EFSA /2009/1438 guidance document, the vole is considered the worst-case exposure model / focal species. An acceptable risk assessment for the common vole is considered protective of all focal mammal species in the EFSA guidance. It is highly probable that other mammal species may frequent the habitats associated with railroad tracks. However, the Tier I level of the risk assessment – for both the small omnivorous (e.g., woodmouse) and large herbivorous mammals (e.g. rabbits and hares) was considered acceptable across all proposed GAP table uses.

An additional point is that across the EU, different vole species exist and for some EU member states, different small mammal species are considered more relevant to the risk assessment, based on the local situation or due to the level of protection for this particular being considered differently in different member states. (Jacobs et al., 2014)[8].

A full risk assessment covering all focal mammal species is presented in the Annex M-CP 10-03 to this dossier section that covers all mammal focal species feeding guilds. Worst case representative focal species from each of the feeding guilds across all mammal species in the EFSA guidance are considered in the presented assessment above.

<u>Control of invasive species</u>

For the use on invasive species on agricultural and non-agricultural areas (Uses 8-9) the product MON 52276 is intended to be applied on the two invasive species; Giant hogweed (Heracleum montegazzianum) and Japanese knotweed (Reynoutrica japonica). Both species are easily recognisable, are usually well known by operators and can reach impressive sizes (more than 2 m height).

Control of invasive plant species that pose a risk to man and society, may be achieved by direct targeted overspray of the plant or by first cutting back the plants and applying directly to fresh regrowth. In both cases, the aim is to achieve exposure of the plant systemically, targeting all growing areas of the plant. The type of plant to be controlled and the density of plants in the target area, will dictate the management approach that is ultimately used. In all cases, the spray applications made, will be directed and targeted to a specific plant or stand of plants. This approach contrasts with a boom spray application where the entire area under the boom is exposed, whether there is a target plant present or not. It is therefore appropriate when considering applications made to control invasive species, that the total applied area considered in the risk calculation, is reduced compared to a boom spray application, given the very directed and targeted

[6] Frank, F. The causality of microtine cycles in Germany. The Journal of Wildlife Management 21(2): 113-121 (1957).

[7] Briner, T., Nentwig, W, Airolid, J.P. Habitat quality of wildflower strips for common voles (Microtus arvalis) and its relevance for agriculture. Agriculture, Ecosystems & Environment 105:173-179 (2005).

[8] Jacob, J., Manson, P., Barfknecht, R., Fredricks, T. (2014) Common vole (*Microtus arvalis*) ecology and management: implications for risk assessment of plant protection products. Pest Management Science 70:869-878.

application method used, which includes use of shielded sprayers that further reduces the risk to non-target plants.

When spraying invasive species, different plant density scenarios are applicable. A small reduction in the application rate (10-30% reduction) would reflect a scenario where a high density of invasive species can be expected. Such a scenario is considered relevant in non-agricultural fields where higher densities of the invasive species Giant hogweed or Japanese knotweed may occur. Therefore, as a conservative worst case approach a reduction of the application rate to 90% can be taken into account for an alternative chronic risk assessment in non-agricultural areas.

In agricultural areas farmers won't tolerate higher amounts of invasive species in their fields. Thus, the density in comparison to non-agricultural fields is much lower and plants are more dispersed as they are not allowed to spread over several years. In case the product is applied by hand-held equipment to invasive species at BBCH stages when the intended crop is present it can be expected that only few invasive species are present and that the operator avoids exposure of cultured crops. In conclusion, to address the lower plant density of invasive species in agricultural fields, a 40% reduction in the application rate based on the reduced total area can be applied in an alternative risk assessment. This is also considered appropriate to cover the chronic risk to mammals.

Assessment and conclusion by RMS:
The RMS finds the justification presented by the applicant reasonable for these uses, especially since the product applications in most situations are directed to specific areas of unwanted vegetation in contrast to large scale field applications. It is reasonable to assume that, instead of a full application rate, the drift rate for field crops (e.g. 2.7%) depositing in the proximity of railroad tracks would be a more realistic exposure rate along the marginal habitat. It is also noted that for treatments at 80% of the maximum dose, stated to be sufficiently effective in some situations, the risk was considered as low without these further justifications. This conclusion is however also pending further information needed to support the refined residue decline DT_{50} in plants (see above).

B.9.2.2.1. Drinking water exposure

Only the puddle scenario is relevant for risk assessment for mammals through drinking water.

Puddle scenario

The 'Puddle scenario' is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is therefore relevant for all uses of MON 52776 and should therefore be assessed.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 (K_{OC} < 500 L/kg) or 3000 ($K_{OC} \geq$ 500 L/kg), as specified in EFSA/2009/1438.

As pointed out in EFSA/2009/1438, specific calculations of exposure and TER values are only necessary when the ratio of effective application rate (in g a.s./ha) to relevant endpoint (in mg a.s./kg bw/d) exceeds 50 in the case of less sorptive (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive ($K_{OC} \geq$ 500 L/kg) substances.

For glyphosate, the ratio of highest application rate (1800 g a.s./ha) to lowest relevant endpoint (NOAEL = 100 mg a.s./kg bw/d) is 18. As the geomean $K_{f,OC}$ for glyphosate is 4245 mL/g (See Environmental fate) the risk can be considered acceptable without the need for further calculations.

B.9.2.2.2. Effects of secondary poisoning

According to the EFSA/2009/1438, substances with a log $P_{OW} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

Since the log P_{OW} values of glyphosate is log $P_{OW} < -3.2$ (pH 2–5, 20 °C), the active substance is deemed to have a negligible potential to bioaccumulate in animal tissues. No formal risk assessment from secondary poisoning is therefore required.

The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is eliminated unchanged and only a small amount (less than 1 % of the applied dose) is transformed to aminomethylphosphonic acid (AMPA). The metabolite AMPA has been tested in several mammal toxicity studies which demonstrated that it is of lower toxicity than glyphosate acid (see Toxicology section). Furthermore, the log P_{OW} for AMPA – estimated via EpiSuite Program and SMILES code (C(N)P(=O)(O)O) – is -2.47 and does not indicate a potential for bioaccumulation (EFSA Journal 2015;13(11): 4302).

B.9.2.3. Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No studies were specifically conducted to address the risk assessment for amphibians. The study by [redacted] (2012), included in the standard data submission is an Amphibian Metamorphosis assay for the detection of thyroid active substances, but also provides information on general toxicity to *Xenopus laevis*. The study was conducted at water concentrations up to 90 mg a.e./L, and although a slight increase was observed in the wet weight of *Xenopus laevis* tadpoles at 90 mg a.e./L, there were no other effects observed in the study, with no effects on growth and development, no mortality and no effects on the thyroid, following a 21-day exposure period.

In the current literature review to support the 2020 submission for renewal of approval in the EU, the applicant proposed that only one study from the public domain literature is relevant and reliable for inclusion in the ecotoxicological risk assessment. However, from the initial search, the RMS has identified further studies that may provide additional information relevant for the evaluation. The available acute and chronic endpoints for the aquatic stages on amphibians are summarised in the tables below. In addition, a study on reptiles was also identified as useful and included in the discussion. A detailed assessment of the relevance and reliability of these studies is found together with the respective study summaries, in the Appendix to Vol 3 CP.

Table B.9.2.3-1: Ecotoxicological endpoints on amphibians, based on studies from the open literature considered relevant for the risk assessment by the RMS

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
Amphibians						
CA 8.2.8/001; Daam, M.A. *et al*. 2019	*Physalaemus cuvieri* (tadpoles Gs 25)	glyphosate	96 h	LC_{50}	115 mg a.s./L	Reliable with restrictions
	Hypsiboas pardalis (tadpoles Gs 25)		96 h	LC_{50}	106 mg a.s./L	
CA 8.1.4 Turhan D. Ö *et al*. 2020	*Xenopus laevis* (embryos stage 8 and tadpoles Gs 46)	glyphosate	96 h	LC_{50}	>403 mg glyphosate/L	Reliable with restrictions

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
CA 8.1.4 Bach N. C. *et al*. 2016	*Leptodactylus latrans* (tadpoles Gs 25 and 36)	glyphosate	96 h	LC_{50}	>300 mg glyphosate/L	Reliable
			96 h	LOEC	15 mg glyphosate/L (development and growth, Gs 25) 30 mg glyphosate/L (morphological abnormalities, Gs 25 and 36)	
CA 9 Babalola O. O. *et al*. 2019	*Xenopus laevis* (embryos)	Enviro	96 h	LC_{50}	446 mg a.e./L	Reliable
CA 9 Lajmanovich R. C. *et al*. 2011	*Rhinella arenarum* tadpoles Gs 36-38)	Roundup Ultra-Max	48 h	LC_{50}	2.42 mg a.e./L	Reliable with restrictions
CA 9 Lajmanovich R. C. *et al*. 2013	*Rhinella arenarum* (tadpoles Gs 29-30)	Ultra-Max®	48 h	LC_{50}	13.20 mg a.i./L	Reliable with restrictions
CA 9 Wagner N. *et al*. 2017	*Xenopus laevis* (larvae NF stage 47)	Roundup® UltraMax	96 h	LC_{50}	7.04 mg a.i./L	Reliable
	Xenopus laevis (embryos NF stage 8-11)		96 h	LC_{50}	25.82 mg a.i./L	
	Discoglossus pictus (larvae Gs 25)		96 h	LC_{50}	18.29 mg a.i./L	
	Discoglossus pictus (embryos Gs 8-9)		96 h	LC_{50}	128.2 mg a.i./L	
CA 9 Rissoli Zanelli R. *et al*. 2016	*Lithobates catesbeianus* (tadpoles Gs 25)	glyphosate	96 h	LOEC (NOEC n.d.[a])	1 mg a.e/L (epidermis morphology and O_2 uptake)	Reliable with restrictions
CA 9 Slaby S. *et al*. 2019	*Xenopus laevis* (stage VI oocytes)	glyphosate	overnight	NOEC	1480 µM a.e. (cell abnormalities)	Reliable with restrictions*

NOEC could not be determined (n.d.) because:
[a] only one glyphosate concentration was tested and resulted in a significant effect;
*Not relevant for the standard risk assessment, but for ED-assessment.

Table B.9.2.3-2: Ecotoxicological endpoints on amphibians and reptiles, based on studies from the open literature considered less relevant but supplementary by the RMS. These studies will be used in a WoE

Reference	Species/life stage		Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
Amphibians							
CA 8.1.4. Lenkowski J. R. *et al*. 2010	*Xenopus laevis* (embryo NF stage 41)		Roundup	48 h	NOEC	1 mg a.i./L (intestinal malformations)	Reliable with restrictions
CA 8.1.4. Williams B. K. *et al*. 2010	Tadpoles Gs 25	*Pseudacris triseriata*	Roundup WeatherMax	Chronic (not specified)	NOEC	0.0006 mg a.i. (survival)	Reliable with restrictions
		Bufo americanus			NOEC	0.0006 mg a.i. (time to metamorphosis) 0.7 mg a.i (survival)	
		Hyla versicolor			NOEC	0.7 mg a.i. (survival)	
	Tadpoles	*Pseudacris triseriata*	Roundup Original Max	Chronic (not specified)	NOEC	0.7 mg a.i. (survival and time to metamorphosis)	

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
	Bufo americanus			NOEC	0.0006 mg a.i. (time to metamorphosis) 0.7 mg a.i (survival)	
	Hyla versicolor			NOEC	0.700 mg a.i. (survival)	
CP 10.1.1 & 10.1.2 Edge C. *et al* 2011	*Lithobates clamitans* (juveniles)	VisionMax®	14 days	Correlation	2.03 - 10.21 kg a.e./ha (application rate negatively correlated to liver somatic index and fungal infection rates)	Reliable (see Annex biodiversity)
CP 10.1.1 & 10.1.2 Edge C. 2013	*Lithobates clamitans* (juveniles)	Roundup WeatherMax™	16 days	NOED	8.64 kg a.e./ha (survival, body condition, liver somatic index)	Reliable with restrictions (see Annex biodiversity)
	Lithobates pipiens (juveniles)					
CA 9 Agostini M. G. *et al*. 2020	*Boana pulchellus* (tadpoles Gs 37-42)	Glyphosate commercial formulation (unspecified)	24 h	NOEC	179.3 µg glyphosate/L (survival)	Reliable
				LOEC (NOEC n.d.[b])	54.5 µg glyphosate/L (mobility)	
	Rhinella arenarum (tadpoles Gs 37-40)		24 h	NOEC	315.5 µg glyphosate/L (survival)	
				LOEC (NOEC n.d.[b])	214.5 µg glyphosate/L (mobility)	
CA 9 Babalola O. O. *et al*. 2019	*Xenopus laevis* (embryos)	Kilo Max	96 h	LC_{50}	207 mg a.e./L	Reliable
CA 9 Edge C. *et al*. 2014	*Lithobates sylvaticus* (tadpoles Gs 25)	Roundup WeatherMax	96 h	LC_{50}	6.01 mg a.e./L (geomean of 4 populations)	Reliable
		Roundup Weed and Grass Control	96 h	LC_{50}	0.65 mg a.e./L (geomean of 4 populations)	
CA 9 Fuentes L. *et al*. 2011	Tadpoles Gs 25: *Rana sphenocephala*	Roundup® WeatherMAX	96 h	LC_{50}	1.33 mg a.e./L	Reliable
	Tadpoles Gs 25: *Bufo fowleri*		96 h	LC_{50}	1.96 mg a.e./L	
	Tadpoles Gs 25: *Rana catesbeiana*		96 h	LC_{50}	1.97 mg a.e./L	
	Tadpoles Gs 25: *Rana pipiens*		96 h	LC_{50}	2.27 mg a.e./L	
	Tadpoles Gs 25: *Rana clamitans*		96 h	LC_{50}	2.77 mg a.e./L	
	Tadpoles Gs 25: *Hyla chrysoscelis*		96 h	LC_{50}	3.26 mg a.e./L	
CA 9 Jones D. K. *et al*. 2010	*Rana sylvatica* (tadpoles Gs 26)	Roundup Original MAX®	18 days exposure, with product application on day 0, 7 or 14	LC_{50}	2.10 mg a.e./L (application day 0) 2.44 mg a.e./L (application day 7) 4.27 mg a.e./L (application day 14)	Reliable

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
				NOEC	1 mg a.e./L (body mass, application day 14)	
	Bufo americanus (tadpoles Gs 25)		Product application on day 0, 7 or 14, observations on day 18	LC_{50}	2.31 mg a.e./L (application day 0) 2.30 mg a.e./L (application day 7) 3.93 mg a.e./L (application day 14)	
				NOEC	1 mg a.e./L (body mass, days 7 and 14)	
CA 9 Jones D. K. *et al*. 2011	*Rana catesbeiana*	Roundup Original MAX®	Product application on day 7, mortality assessed day 16	LC_{50} (at 'low' competition)	2.18 mg a.e./L	Reliable
	Hyla versicolor				2.04 mg a.e./L	
	Rana clamitans				2.58 mg a.e./L	
CA 9 Krynak K. L. *et al*.2017	*Acris blanchardi* (tadpoles Gs 42, juveniles 11-18 days old)	Rodeo™ Original MAX®	12 days exposure, observations depending on life stage	NOEC	1.5 mg a.e./L (mortality and skin bacterial community, tadpoles) 2.5 mg a.e./L (mortality, juveniles)	Reliable with restrictions
CA 9 Lajmanovich R. C. *et al*. 2011	*Rhinella arenarum* tadpoles Gs 36-38)	Infosato	48 h	LC_{50}	38.76 mg a.e./L	Reliable with restrictions
		Glifoglex	48 h	LC_{50}	73.77 mg a.e./L	
		C-K YUYOS FAV	48 h	LC_{50}	77.52 mg a.e./L	
CA 9 Lanctot C. *et al*. 2014	*Lithobates sylvaticus* (tadpoles Gs 25 -1st pulse and Gs 30-2nd pulse)	Roundup WeatherMax®	96 h exposure (1st pulse), observation after 16 days	NOEC	0.21 mg a.e./L (mortality)	Reliable
			96 h exposure (1st pulse), observation after 18 days	LOEC (NOEC n.d.[c])	≤0.21 mg a.e./L (weight increase)	
			2 x 96 h exposure (2nd pulse), observation after > 18 days	LOEC (NOEC n.d.[c])	≤0.21 mg a.e./L (snout-vent length)	
CA 9 Munoz L. M. H. *et al*. 2015	Tadpoles Gs 25 *Hypsiboas crepitans*	Roundup® Active	96 h	LC_{50}	1.41 mg a.e./L	Reliable with restrictions
	Tadpoles Gs 25 *Rhinella marina*		96 h	LC_{50}	1.42 mg a.e./L	
	Tadpoles Gs 25 *Rhinella humboldti*		96 h	LC_{50}	2.44 mg a.e./L	
	Tadpoles Gs 25 *Engystomops pustulosus*		96 h	LC_{50}	2.79 mg a.e./L	
CA 9	Embr *Rhinella marina*		96 h	LC_{50}	1.42 mg a.e./L	

Reference	Species/life stage		Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
Triana Velasquez T. M. *et al*. 2013		*Hypsiboas crepitans*	Roundup® Active	96 h	LC_{50}	2.15 mg a.e./L	Reliable with restrictions
		Rhinella humboldti		96 h	LC_{50}	2.9 mg a.e./L (lab) 40.8 mg a.e./L (microcosm)	
		Engystomops pustulosus		96 h	LC_{50}	3.03 mg a.e./L (lab) 74.7 mg a.e./L (microcosm)	
CA 9 Brodeur J. C. *et al*. 2014	*Rhinella arenarum* (tadpoles Gs 25)		Glifosato Atanor	96 h	LC_{50}	19.4 mg a.e./L	Reliable
			Glifoglex	96 h	LC_{50}	72.8 mg a.e./L	
Ca 9 Navarro-Martín L. *et al*. 2014	*Lithobates sylvaticus* (tadpoles Gs 25)		VisionMax®	~41 days (average time to reach Gs 46)	NOEC	1.1 mg a.e./L (mortality, development rate and metamorphic success)	Reliable
CA 9 Relyea R. A. 2018	Tadpoles	*Hyla versicolor*	Roundup Original Max	17 days	LC_{50}	2.3 mg a.e./L	Reliable
		Rana catesbeiana		17 days	LC_{50}	3 mg a.e./L	
		Rana clamitans		17 days	LC_{50}	>3 mg a.e./L	
Reptiles							
CA 9 Poletta G. L. *et al*.2011	*Caiman latirostris* (embryos)		Roundup Full II (sprayed)	5 days exposure; observations after 3 months	LOEC (NOEC n.d.[a])	17.25 g glyphosate/L (total length and SVL)	Reliable

NOEC could not be determined (n.d.) because:
[a] only one glyphosate concentration was tested and resulted in a significant effect;
[b] significant effects were observed in all treated ponds. Thus, LOEC is based on the lowest measured concentration of glyphosate;
[c] significant effects were observed in the lowest tested concentration.

For amphibians, acute exposure (up to 96 hours) to glyphosate technical or glyphosate-based products resulted in LC_{50} values ranging from 0.75 mg a.e./L to >403 mg glyphosate/L for 19 species tested. Sublethal effects observed in these short-term studies included reduced mobility and malformations, with an overall lowest acute NOEC of <0.54 mg glyphosate/L. In chronic exposures, the most sensitive parameters were time to metamorphosis and survival, with a NOEC of 0.0006 mg glyphosate/L.

In a study on the **reptile species** *Caiman latirostris* (Poletta *et al*. 2011), the test material was applied as a spray solution on the eggshell surface. It seems less likely that eggs of reptile species would be present on the ground surface of treated fields, and therefore this exposure pathway is considered extreme. However, the study showed effects on total length and snout vent length (SVL) at a treatment level of 17.25 g glyphosate/L, which is only slightly higher than the highest recommended concentration of glyphosate in the spray liquid according to the representative GAP (1.35 – 14.4 g a.e./L). Hence, based on the available data, potential risk to reptiles cannot be excluded following exposure via overspray in the treated field.

In the previous RAR for renewal of approval (2015), a review was presented that considered acute and chronic amphibian toxicity studies in the public domain literature, conducted with glyphosate and/ or commercial glyphosate-based formulations. The previous RMS considered acute effects based on studies with 96 hours or less duration. Chronic studies were evaluated that focused mostly on lethality effects, with some studies considering effects of glyphosate formulations on body weights and/or performance at metamorphosis. There were very few studies from the previous literature search considering effects on terrestrial stages of amphibians. There were several acute toxicity endpoints presented in the RAR (2015) for amphibians exposed to glyphosate and its salts, ranging from >17.9 to >466 mg a.s./L. The studies from the previous literature search have, however, not been re-visited for the current evaluation by the RMS.

In the conclusions drawn by the previous RMS, it is indicated that the findings from the reviewed public literature data on amphibians pointed towards toxicity of surfactants in the glyphosate-based formulations. In some cases, the experimental difficulties or set-ups were considered contributing factors, but overall, the results indicate effects of ethoxylated surfactants (e.g., polyoxyethoxylated alkylamines, POEA) on amphibians and that there were implications for registering glyphosate-based products containing these types of surface-active chemicals. The representative formulation does not contain POEA or ethoxylated surfactants known to be of toxic concern to amphibians.

The RMS considers that, when the tested formulations differ from MON 52276, the results may be useful in a Weight of Evidence approach, as long as these formulations do not include substances that are not allowed within the EU (Regulation (EU) 2016/1313 and DRAFT Regulation amending Annex III of Regulation (EC) 1107/2009). In cases where the active substance and a formulated product were tested in the same study, the results from the active ingredient test are considered to be the most relevant.

Based on the publicly available data retrieved from the current literature search, the toxicity of glyphosate to amphibians does not seem to be covered by the risk assessment for aquatic organisms. Hence, further consideration is needed on possible risk to amphibians following the representative uses of glyphosate. Further consideration is also needed on possible risk to reptiles following exposure via direct overspray in the field. It is acknowledged that there is no agreed EU guidance on how to carry out the risk assessment for these groups, however, some useful advice and recommendations are available in the EFSA opinion from 2018: Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles - - 2018 - EFSA Journal - Wiley Online Library.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Data point	CP 10.2.1/001
Report author	[redacted]
Report year	1992
Report title	MON 52276: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Test Conditions
Report No	J9108002b
Document No	[redacted]-91-296
Guidelines followed in study	US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *Major:* *- Fish were acclimatised 48 hours prior to the test (7 days are required)* *Minor:* *- Observations occurred after 24h, 48h and 96h instead of twice/day* *- pH of the highest concentration (5.9) was slightly below the specified range of 6.0-8.5.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid and reliable

Summary
The effects of MON 52276 (30.95% glyphosate acid) on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour flow-through toxicity test. Two groups of ten fish each were exposed for 96 hours to nominal concentrations of MON 52276 at 0 (control), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. At 0, 48 and 96 hours, samples of test medium were taken for the analysis of glyphosate content.

Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation.

Mortality to one fish was observed at the lowest test concentration (119 mg/L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration.

Based on mean measured concentrations, the 96-hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to MON 52276 in a flow-through test system was > 989 mg/L (> 306 mg glyphosate/L, arithmetic mean measured). The corresponding no observed effect concentration (NOEC) was = 989 mg/L (= 306 mg glyphosate/L, arithmetic mean measured), based on the absence of mortality and abnormal sublethal effects at this concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Active substance:	Glyphosate
Description:	Amber liquid
Lot/Batch #:	LLN-9105-3135F
Purity:	30.95%

2. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juveniles
Size:	Length: 3.1 – 4.1 cm
Loading:	10 test individual for 15 L test solution
Source:	[redacted]
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	0.35 – 0.95 g (mean = 0.60 ± 0.16 g)
Food	live brine shrimp, nauplii and flake until 48h prior to test initiation

3. Environmental conditions:

Temperature:	11.5 – 13.8°C
Photoperiod:	16 hours, 392 – 500 lux
pH:	8.1-8.3 (control); 5.8 – 7.6 (test item concentrations)
Dissolved oxygen:	≥7.1 mg/L (≥ 67% of saturation)
Conductivity:	382 – 705 µmhos/cm
Hardness:	38 - 116 mg CaCO3/L
Alkalinity:	57 - 77 mg CaCO3/L
Dissolved oxygen saturation	10.8 mg/L at 12°C

4. Dates of experimental work: October 7th to October 11th 1991

B. STUDY DESIGN

Experimental treatments: Two groups of ten fish each were exposed under flow-through conditions in a proportional diluter system 4.8 cycles/h (approx. 5.4 volume addition every 24h) for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. For flow-through system, the recommended maximum loading is 0.5 g wet weight fish/L per 24 hours. Taking into account a 15 L tank with a flow rate of 5.4 tank volumes per 24 hours, a total of 81 L passed through the tank in 24 hours. With 0.6 g fish and ten fish per tank (= 6 g), this was corresponding to 6 g in 81 L in 24 hours equivalent to 0.07 g/L in 24 hours.

The test water was a blend of treated municipal water and treated well water. During the 14-day holding period prior to test initiation, fish were fed daily and were in good health. There were two vessels per treatment, each containing ten fish (appr.24 L glass vessels containing 15 L test medium).

Observations: Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation. Water temperature in a control vessel was measured hourly throughout the test, and water pH and dissolved oxygen were measured daily in all test vessels. Hardness, total alkalinity and specific conductivity were measured at test initiation and test termination. At 0, 48 and 96 hours, samples of test medium were taken for quantification of glyphosate by HPLC.

Statistical calculations: LC_{50} values were calculated along with the 95% confidence limits using non-linear interpolation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The arithmetic mean measured concentrations during the 96-hour exposure ranged from 119 to 989 mg MON 52276/L and from 92 to 100% of nominal. The results are provided based on mean measured concentrations.

Table B.9.3-1: Analytical results

Nominal concentration [mg MON 52276/L]	Measured concentration [mg MON52276/L]			Mean (±SD) [mg MON 52276/L]	% of nominal
	0hr	48hr	96hr		
Control	ND	ND	ND	-	-
130	124 119	114 112	123 123	119 (5.1)	92
216	202 244	190 172	195 246	208 (30.2)	96
360	368 357	339 348	373 385	362 (16.9)	100
600	584 599	520 545	598 639	581 (42.4)	97
1000	1030 994	921 937	1010 1040	989 (49.1)	99

ND = not detection, limit of detection 2.6 mg/L.

The LC_{50} and NOEC values are given below based on mean measured concentrations.

Table B.9.3-2: Endpoints

Endpoints (96 h)	MON 52276 [mg/L]	Glyphosate [mg/L]*
LC_{50} (95% C.I.)	>989	>306.1
NOEC	989	306.1

* MON 52276 is 30.95% glyphosate as active ingredient.

B. OBSERVATIONS

Mortality and signs of toxicity in control and treated groups are reported in the table below. Mortality to one fish was observed at the lowest test concentration (119 mg/L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration.

Table B.9.3-3: Acute toxicity of MON 52276 to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions

MON 52276 [mg /L] [1]	Time point [h]	Abnormalities/ Sublethal Effects	Mortality[2]	Cumulative % mortality
0	24 48 72 96	None observed	0	0
119	24 48 72 96	None observed	1	5
208	24 48 72 96	None observed	0	0
362	24 48 72 96	None observed	0	0
581	24 48 72 96	None observed	0	0
989	24 48 72 96	None observed	0	0

[1] Mean measured values.
[2] Number of dead fish of 20 total.

All validity criteria according to OECD 203 were fulfilled, as no mortality was observed in control group, dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

According to the applicant, the following points deviated from current guideline:

- Fish were acclimatised 48 hours prior to the test instead of the 7 required
- Observations occurred after 24h, 48h, 72h and 96h. The requirements are the following a minimum of 2 observations within the first 24 hours of the study and on days 2-4 of the test, all vessels with living fish inspected twice per day (preferably early morning and late afternoon to best cover the 24-hour periods).
- The pH in the highest concentration outside of accepted range of 6.0-8.5 so the stock solution should have been adjusted to lie within this specified range.

These deviations are not considered to have a negative impact on the study.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Based on mean measured concentrations, the 96-hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to MON 52276 in a flow-through test system was > 989 mg/L (> 306 mg glyphosate/L, arithmetic mean measured). The corresponding no observed effect concentration (NOEC) was ≥ 989 mg/L (≥ 306 mg glyphosate/L, arithmetic mean measured), based on the absence of mortality and abnormal sublethal effects at this concentration.

The study is considered to be valid and suitable for use in the risk assessment.

Assessment and conclusion by RMS:

Test item: MON 52276 (EU representative formulation of current RAR)
The study was performed under Flow-Through Test Conditions

The pH values at the highest tested concentration (989 mg/L) was slightly below the recommended range (recommended pH 6-8.5). The actual pH values were of 5.8. As noted by the applicant, the guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, a test without pH adjustment is considered relevant by RMS. Indeed, the pH of 5.8 at the highest tested dose is slightly below the recommended value and no mortality was observed. This deviation is acceptable.

The applicant also noted that fish were acclimatised only 48 hours prior to the test instead of the 7 required and that observations should have been conducted twice during the first day. RMS considers these 2 deviations as minor and not likely to affect the outcome of the study.

The water temperature differed by more than 2°C (2.3°C) during the first 24 h between test vessels. RMS considers the deviation acceptable. The temperature (11.5-13.8°C) was as recommended range for this species (10-14°C) in OECD 203 (2019).

RMS notes that 20 fishes were used (instead of 7) for each treatment. The loading of the tanks correspond to the recommendations for flow-through test design.

This study is valid according to validity criteria.

Acute LC50 value for rainbow trout exposed to MON 52276 > 989 mg MON 52276/L (> 306 mg a.s./L) (mean measured).
NOEC after 96 h = 989 mg MON 52276/L (306 mg a.s./L) (mean measured).

Data point:	CP 10.2.1/002
Report author	[redacted]
Report year	1992
Report title	MON 52276: Acute Toxicity To The Common Carp, *Cyprinus carpio,* Under Flow-Through Test Conditions
Report No	J9108002c
Document No	[redacted]-91-298
Guidelines followed in study	OECD guideline 203
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *Major:* 1. *Dissolved oxygen concentration dropped under 60% of saturation (from 8.7 mg/L to 2.5 mg/L = 28.7%)* *Minor:* 2. *Temperature range should not vary more than ±1°C and should be within the range 20-24°C (current values: 21.7-23.8°C).* 3. *Observations occurred after 24h, 48h and 96h instead of twice/day* 4. *Fish length ranged from 2.7 – 5 cm, outside the recommended length of 2.0 – 4.0 cm.* 5. *pH of the highest concentration (5.7) was not in the specified range of 6.0-8.5.* 6. *The test concentrations were not maintained within 80% of nominal concentrations at 96 h (current values from 52 to 84%).*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid and reliable

Summary
The effects of MON 52276 (30.95% glyphosate acid) on common carp (*Cyprinus carpio*) were evaluated in a 96-hour flow-through toxicity test. Two groups of ten fish each were exposed for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. At 0, 48 and 96 hours, samples of test medium were taken for the analysis of glyphosate content.

Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation.

No treatment related mortality or sublethal effects were observed in common carp at any test concentration.

Based on arithmetic mean measured concentrations, the 96-hour LC_{50} for common carp (*Cyprinus carpio*) exposed to MON 52276 in a flow-through test system was > 895 mg/L (> 277 mg glyphosate/L). The corresponding no observed effect concentration (NOEC) was = 895 mg/L (= 277 mg glyphosate/L, arithmetic mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Active substance	Glyphosate
Description:	Amber liquid
Lot/Batch #:	LLN-9105-3135F
Purity:	30.95%

2. Test organism:

Species:	Common carp (*Cyprinus carpio*)
Age:	Juveniles
Size:	2.7 – 5.0 cm
Loading:	10 test individuals for 15 L test solution (0.93 g fish/L)
Source:	[redacted]
Acclimation period:	14 days prior to the test initiation
Body weight of the animals:	0.57 – 2.97 g (mean of 1.39 g)
Food	brine shrimp, nauplii and flake until 48h prior test initiation

3. Environmental conditions:

Temperature:	21.7 – 23.8°C
Photoperiod:	16 hours light, 350 - 425 lux
pH:	7.2-8.1 (control); 7.1 to 5.2 (test item concentrations)
Dissolved oxygen:	6.7 – 8.7 mg/L (8.7 mg/L is 100% saturation) in control
	1.5 – 8.2 mg/L in test item concentrations
Conductivity:	1614 - 1688 µmhos/cm
Hardness:	184 - 192 mg $CaCO_3$/L
Alkalinity:	34 - 45 mg $CaCO_3$/L

4. Dates of experimental work: November 19th to November 23rd 1991

B. STUDY DESIGN

Experimental treatments: Two groups of ten fish each were exposed under flow-through conditions using a proportional diluter system (3.8 daily volume turnover) for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. During the 14-day holding period prior to test initiation, fish were fed daily and were in good health. There were two vessels per treatment, each containing ten fish (appr. 24 L glass vessels containing 15 L test medium).

Observations: Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation. Water temperature in a control chamber was measured hourly throughout the test, and water pH and dissolved oxygen were measured daily in all test chambers. Hardness, total alkalinity and specific conductivity were measured at test initiation and test termination. At 0, 48 and 96 hours, samples of test medium were taken for quantification of glyphosate by HPLC.

Statistical calculations: LC_{50} values were calculated along with the 95% confidence limits using non-linear interpolation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The arithmetic mean measured concentrations during the 96 hour exposure ranged from 98 to 895 mg test item/L and from 75 to 90% of nominal on the overall period. The results were determined based on mean measured concentrations.

Table B.9.3-4: Analytical results

Nominal concentration [mg MON 52276/L]	Measured concentration [mg MON52276/L]			Mean (±SD) [mg MON 52276/L]	% of nominal
	0hr	48hr	96hr		
Control	ND	ND	ND	-	-
130	111 112	117 107	74 67	98 (21.7)	75
216	171 235	188 219	125 116	176 (48.4)	81
360	395 371	366 390	215 302	340 (69.6)	94
600	570 619	592 649	481 403	552 (92.8)	92
1000	1020 1047	1002 1010	677 615	895 (194.6)	90

ND = not detection, limit of detection 1.9 mg/L.

The LC_{50} and NOEC values are given below based on mean measured concentrations.

Table B.9.3-5: Endpoints

Endpoints (96 h)	MON 52276 [mg/L]	Glyphosate [mg/L]*
LC_{50} (95% C.I.)	> 895	> 277
NOEC	895	277

*MON 52276 is 30.95% glyphosate as active ingredient.

B. OBSERVATIONS

Mortality and signs of toxicity in control and treated groups are reported below. No mortality and no sublethal effects were observed at any test concentrations.

Table B.9.3-6: Acute toxicity of MON 52276 to Common carp (*Cyprinus carpio*) under flow-through conditions

MON 52276 (mg/L) [1]	Time point (h)	Abnormalities/ Sublethal Effects	Mortality[2]	Cumulative % mortality
0	24 48 72 96	None observed	0	0
98	24 48 72 96	None observed	0	0
176	24 48 72 96	None observed	0	0
340	24 48 72 96	None observed	0	0
552	24 48 72 96	None observed	0	0
895	24 48 72 96	None observed	0	0

[1] Mean measured values.
[2] Number of dead fish of 20 total.

For an estimated period of 4-6 hours, beginning at 8 hours prior to test termination, only dilution water was delivered to test chambers due to a malfunction in the diluter system. Since there were no indications of stress or any other effects, it is unlikely that the reduction in exposure concentration for this short period had any effect on the outcome of the test.

During the test period, the dissolved oxygen during the test fell below 60% of the air saturation value in at least one replicate at every dose level and in both replicates at the two highest dose levels; the fish did not appear stressed as a result.

The following validity criteria according to the OECD 203 (2019) were fulfilled:

- The control mortality was lower than 10 % at the end of the study.
- Analytical measurement of the test concentrations was reported.

The following validity criterion according to the OECD 203 (2019) was not fulfilled:

- The dissolved oxygen concentration was below the trigger value of ≥ 60 % of the air saturation value (ranging from 8.7 mg/L to 1.5 mg/L).

The applicant also noted the following points:

- Observations occurred after 24h, 48h, 72h and 96h. The requirements are the following a minimum of 2 observations within the first 24 hours of the study and on days 2-4 of the test, all

vessels with living fish inspected twice per day (preferably early morning and late afternoon to best cover the 24-hour periods).

- The pH in the highest concentration outside of accepted range of 6.0-8.5 so the stock solution should have been adjusted to lie within this specified range (see RMS opinion in commenting box below).
- Dissolved oxygen concentration dropped under 60% of saturation (from 8.7 mg/L to 1.5 mg/L)
- Temperature range should not vary more than ±1°C and should be within the range 20-24°C (current values: 21.7-23.8°C).
- Fish length ranged from 2.7 – 5 cm, outside the recommended length of 2.0 – 4.0 cm.
- The test concentrations were not maintained within 80% of nominal concentrations at 96h (current values from 52 to 84%). The endpoints have been based on the overall mean measured concentrations.

The applicant considers that these deviations do not have a negative impact on the study. RMS agrees (see commenting box below).

III. CONCLUSIONS

Assessment and conclusion by applicant:
Based on arithmetic mean measured concentrations, the 96-hour LC_{50} for common carp (*Cyprinus carpio*) exposed to MON 52276 in a flow-through test system was > 895 mg/L (> 277 mg glyphosate/L). The corresponding no observed effect concentration (NOEC) was ≥ 895 mg/L (≥ 277 mg glyphosate/L, arithmetic mean measured).

The study is considered to be valid and suitable for use in the risk assessment.

Assessment and conclusion by RMS:
Test item: MON 52276 (EU representative formulation of the current RAR)
The study is performed under flow-through conditions

The applicant noted that observations should have been conducted twice during the first day. RMS considers this deviation as minor.

The pH values at the highest tested concentration (895 mg/L) was slightly below the recommended range (recommended pH 6-8.5). The actual pH values were of 5.7. As noted by the applicant, the guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, a test without pH adjustment is considered relevant by RMS. Indeed, the pH at the highest tested concentrtations pH is slightly below the recommended value and no mortality was observed. This deviation is acceptable.

The dissolved oxygen concentration was below the trigger value of ≥ 60 % of the air saturation value (ranging from 8.7 mg/L to 1.5 mg/L, so a minimum value of 17.2% was measured). A minimum of 5.2 mg/L (i.e. 60% saturation) was necessary for this criteria to be fulfilled. This was not the case as for all concentrations, at least in one replicate at every dose level and in both replicates at the two highest dose levels. However carp did not show any symptoms of stress associated with low dissolved oxygen. RMS considers the deviation acceptable.

The water temperature varied by more than 2°C (2.1°C) during the first 48 h between test vessels. RMS considers the deviation acceptable. The temperature (21.7-23.8°C) was as recommended range for this species (20-24°C) in OECD 203 (2019).

Fish length ranged from 2.7 – 5 cm, outside the recommended length of 2.0 – 4.0 cm. This deviation is acceptable.

For an estimated period of 4-6 hours, beginning at 8 hours prior to test termination, only dilution water was delivered to test chambers due to a malfunction in the diluter system. Since there were no indications of stress or any other effects, it is unlikely that the reduction in exposure concentration for this short period had any effect on the outcome of the test.

RMS notes that 20 fishes were used (instead of 7) for each treatment.

The loading rate of fish in test containers was 0.93 g of fish per liter of test solution. The flow rate provided 3.8 daily volume turnovers. Then the loading of the tanks correspond to the recommendations for flow-through test design.

This study is considered valid and acceptable for risk assessment.

Acute LC50 value for common carp (*Cyprinus carpio*) exposed to MON 52276 > 895 mg MON 52276/L (> 277 mg a.s./L) (mean measured).

NOEC after 96 h = 895 mg MON 52276/L (277 mg a.s./L) (mean measured).

Data point:	CP 10.2.1/003
Report author	[redacted]
Report year	1992
Report title	MON 52276: Acute toxicity to the water flea, *Daphnia magna*, under flow-through test conditions
Report No	J9108002a
Document No	TO-91-295
Guidelines followed in study	US EPA FIFRA 72-2 (1982), OECD 202 (1984), and EEC Method C.2 (1992).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current OECD 202 guideline (2004):* *Major:* *- none* *Minor:* *- The pH of the test system was correlated with MON 52276 concentration and varied by more than 1 unit across the 5 dose levels.* *- The temperature was slightly higher and ranged from 20.0 – 23.8 °C instead of 18.0 – 22.0°C. This did not have a negative effect on the study and validity criteria are met.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid and reliable

Summary

The effects of MON 52276 (30.95% w/w glyphosate acid) on *Daphnia magna* were evaluated in a 48-hour flow-through toxicity test. Neonates of *Daphnia magna* were exposed to nominal concentrations of MON 52276 at 130, 216, 360, 600, and 1000 mg/L and a negative control consisting of dilution water. The test consisted of two replicates per treatment group and control. 10 Daphnids were exposed per replicate and were not fed during the test. Total number of *Daphnia magna* exhibiting immobility and other clinical signs of toxicity was recorded at 24 and 48 hours after test initiation.

Temperature, pH-values and dissolved oxygen concentrations were measured at the beginning, at approximately 24 hours during the test and at the end of the test. At 0 and 48 hours, samples of test medium were taken for quantification of glyphosate by HPLC. The analysed test concentrations ranged between 95 and 105% of the nominal values.

No mortality to *Daphnia magna* from exposure to MON 52276 was observed at test concentrations $\leq$ 356 mg/L. At 580 mg/L, 20% mortality was observed at 48 hours, with 100% mortality observed at 948 mg/L. Sublethal effects were observed only at the 580 mg/L concentration.

Based on mean measured concentrations, the 48-hour EC_{50} for *Daphnia magna* exposed to MON 52276 in a flow-through test system was 676 mg/L (95% confidence limits of 580 and 948 mg/L), (equivalent to 209 mg glyphosateL). The corresponding no observed effect concentration (NOEC) was 356 mg/L (107 mg glyphosate/L), based on the lack of mortality and sublethal effects at this concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	MON 52276
Active substance:	Glyphosate
Purity:	30.95%
Lot/Batch #:	LLN-9105-3135F
Appearance:	Amber liquid

2. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	1 daphnid per 30 mL test medium
Source:	In-house culture (originally from: U.S. Environmental Protection Agency, Duluth, Minnesota)
Diet/Food:	none
Acclimation period:	Not stated

3. Environmental conditions:

Temperature:	20.0 – 23.8 °C
Photoperiod:	16 hours light, 384 - 517 lux
pH:	5.9 – 8.3
Dissolved oxygen:	7.4 – 8.7 mg O_2/L

Conductivity: 436 - 644 µS/cm

Hardness: 60 – 96 mg $CaCO_3$/L

5.Dates of experimental work: Oct 16th to Oct 18th 1991

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of MON 52276 (30.95% w/w glyphosate acid) on neonates of *Daphnia magna* were evaluated in a 48-hour flow-through toxicity test using a proportional diluter system (1.6 cycles/h). Twenty Daphnids (2 replicates of 10 animals per test beaker) were exposed to nominal concentrations of MON 52276 at 130, 216, 360, 600, and 1000 mg/L dissolved in a blend of treated municipal water and treated well water (corresponding to 133, 227, 356, 580 and 948 mg/L of the measured concentrations). In addition, a control group was exposed to test water without test substance (blank control).

2. Observations: Total number of immobile *Daphnia magna* was recorded 24 h and 48 h after test initiation. In addition, specimens were observed for clinical signs of toxicity.

Water temperature was measured at 0 and 48 hours in each test chamber, as well as hourly in one negative control replicate. Water pH and dissolved oxygen were recorded at test start then every 24 hours. Hardness, alkalinity and specific conductance were measured once in the dilution water at test initiation.

At 0 and 48 hours, samples of test medium were taken for quantification of glyphosate by HPLC.

The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 percent of the daphnids should have been immobilised or show other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

3. Statistical calculations: EC_{50} values including 95% confidence limit were determined by non-linear interpolation.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 95 and 105% of the nominal values. The results were determined based on mean measured concentrations.

Table B.9.3-7: Analytical results

Nominal concentration [mg MON 52276/L]	Measured concentration [mg MON52276/L]			Mean (±SD) [mg MON 52276/L]	% of nominal
	0hr	24hr	48hr		
Control	ND	ND	ND	-	-
130	122 139	125 136	123 153	133 (12.1)	102
216	217 228	221 217	236 240	227 (9.9)	105
360	373 370	346 328	362 359	356 (16.8)	99
600	593 612	512 550	593 621	580 (41.4)	97
1000	969 961	911 870	985 994	948 (48.1)	95

ND = not detected, limit of detection 1.9 mg/L.

B. OBSERVATIONS

No mortality to *Daphnia magna* from exposure to MON 52276 was observed at test concentrations ≤ 356 mg/L. At 580 mg/L, 20% mortality was observed at 48 hours, with 100% mortality observed at 948 mg/L. Sublethal effects were observed only at the 580 mg/L concentration.

Table B.9.3-8: Acute toxicity of MON 52276 to *Daphnia magna* under flow-through conditions

Measured concentration MON 52276 (mg/L) [1]	Time point (h)	Abnormalities/ Sublethal Effects	No. of *Daphnia* immobilised or dead[2]	Cumulative % mortality
0	24 48	None observed	0 0	0 0
133	24 48	None observed	0 0	0 0
227	24 48	None observed	0 0	0 0
356	24 48	None observed	0 0	0 0
580	24 48	None observed 3 lethargic	0 4	0 20
948	24 48	-- --	11 20	55 100

[1] Mean measured values.
[2] Of 20 total *Daphnia* in group.

All validity criteria according to the OECD 202 were fulfilled, as no immobility of Daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

The applicant noted that the following points deviated from current guideline:
- the pH of the test system was correlated with MON 52276 concentration and varied by more than 1 unit across the 5 dose levels. Within each test concentration, the pH variation was less than one unit.
- The temperature range during the test was 3.8 ºC, rather than the maximum range of 2 ºC specified in the guideline.

The applicant considers that these deviations do not have a negative impact on the study. RMS agrees (see commenting box below).

III. CONCLUSIONS

Assessment and conclusion by applicant:
Based on mean measured concentrations, the 48-hour EC_{50} for *Daphnia magna* exposed to MON 52276 in a flow-through test system was 676 mg/L (95% confidence limits of 580 and 948 mg/L), (equivalent to 209 mg glyphosate/L). The corresponding no observed effect concentration (NOEC) was 356 mg/L (107 mg glyphosate/L), based on the lack of mortality and sublethal effects at this concentration.

The study is considered to be valid and suitable for use in the risk assessment.

Assessment and conclusion by RMS:
Test item: MON 52276 (EU representative formulation of current RAR).
The study has been conducted under flow-through conditions.

The pH of the test system was correlated with MON 52276 concentration and varied by more than 1 unit across the 5 dose levels. The pH values at the highest tested concentration (948 mg/L) was slightly below the recommended range (recommended pH 6-8.5). The actual pH values was of 5.8. This deviation is considered acceptable by RMS.

The water temperature varied by more ±1°C during the first 48 h between test vessels but remained within the recommended range of 18-22°C. RMS considers the deviation acceptable.

This study is considered acceptable.

Acute LC50 value for *Daphnia magna* exposed to MON 52276 = 676 mg MON 52276/L (209 mg a.e./L) (mean measured).

NOEC after 48 h = 356 mg MON 52276/L (110 mg a.e./L) (mean measured).

Data point	CP 10.2.1/004
Report author	[redacted]
Report year	1992
Report title	Alga, growth inhibition test. Effect of MON 52276 on the growth of *Selenastrum capricornutum*
Report No	WE-06-057
Document No	TO-91-298
Guidelines followed in study	OECD Guideline 201 (1981) EU Directive 87/302/EEC, Part C (1987) NEN 6506, Delft (1984)
Deviations from current test guideline	Deviation from current OECD 201 guideline (2011): Major: - The test concentrations were not verified. Minor: - none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid but not reliable

Summary

The effects of MON 52276 on *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. Algal cells were exposed to five nominal MON 52276 concentrations of 50, 90, 160, 290 and 500 mg test item/L. In addition, a control group was prepared with algae added to test medium without test substance.

Six replicate vessels were prepared for the control and three replicates for each test concentration. Each vessel was inoculated with an initial algal cell density 1 x 10^4 cells/mL.

After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements and cell counting. The concentration resulting in 50% inhibition of cell growth (biomass) and reduction of cell growth rate (E_bC_{50} & E_rC_{50} values respectively) were then calculated. as well as the associated NOEC values.

The authors concluded that the 72 hour E_bC_{50} for MON 52276 was calculated to be 150 mg/L and the 72 hour E_rC_{50} was calculated to be 393 mg/L, with a corresponding NOEC determined to be 90 mg/L.

The RMS concluded that no reliable endpoint could be set as no analytical measurements were made to check the nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	MON 52276
Lot No.:	LLN 260491 B
Chemical purity:	31 % glyphosate acid equivalent, as 41 % isopropylamine salt of glyphosate
Physical state:	Light amber-brown liquid
Density:	1.16 mg/cm^3

Test organism:

Species:	*Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*)
Initial cell concentration:	1 x 10^4 cells/mL
Source:	Inoculum obtained from a 4 day incubated laboratory pre-culture, prepared at the performing laboratory (Original parent culture source is the Culture Centre for Amoeba and Protozoa in the UK. Strain No. CCAP 278/4)

Environmental conditions:

Temperature:	20.9 – 23.1°C (Required: 21 to 25ºC ± 2ºC)
Photoperiod:	24 h light
Light intensity:	8875 ± 125 lux
pH:	8.31 – 8.97 (control), 6.38 – 8.89 at 50, 160 and 290 mg/L 7.32 – 8.99 at 90 mg/L- deviated by more than 1 pH unit (1984 guideline requirement, but within 1.5 pH units (current OECD 201 guideline requirement). 5.88 – 5.98 at 500 mg/L
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates: 15 October – 18 October 1991

Experimental treatments

Based on a range finding test, the definitive algal growth inhibition test was performed with five concentrations (50, 90, 160, 290 and 500 mg test item/L) prepared by appropriate dilution of a 10 g/L stock solution. In addition, a control was also prepared where algae were exposed to algal medium only without test substance (blank control). OECD 201 recommended algal medium was used as the diluent. For each MON 52276 concentration, three replicate vessels were prepared, and six replicate vessels were prepared for the control group. (150 mL Erlenmeyer glass flasks with cotton wool bungs.) To each test or control vessel, 100 mL of the test medium was added, and all replicates vessels were then inoculated with algal cells, at an initial algal cell density of 1 x 10^4 cells/mL.

Observations
After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements (absorbance measurement). In addition, the algal cell concentrations were also determined by microscopic counting at 48 hours and 72 hours. Inhibition of cell growth and reduction of cell growth rate were derived graphically, by plotting the average algal cell concentrations for each test concentration against time. Concentrations resulting in 50 % reduction of growth rate (E_rC_{50}) and 50 % inhibition of cell growth (E_bC_{50}) were determined, as well as the associated NOEC values. The endpoints were calculated for the absorbance and cell counting method. Temperature and the light intensity were recorded daily during the test, while the pH was measured in one replicate of each test concentration at the start and end of the test.

Statistical calculations
The median effect concentration is determined using the logit model of Chou and Chou (1985).

II. RESULTS AND DISCUSSION

A. FINDINGS

The E_rC_{50}, E_bC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.3-9: Toxicity of MON 52276 to *Selenastrum capricornutum*

Endpoint	MON 52276 [mg test item/L]	
	absorbance	cell counting
0 - 72 h E_rC_{50}	393	284
0 - 72 h E_bC_{50}	150	178
NOEC	90	

B. OBSERVATIONS
Based on cell counting, reduction of algal growth rate increased with increasing concentration of MON 52276 from a nominal concentration of 160 mg test item/L upwards. For the two lowest test concentrations of 50 mg test item/L and 90 mg test item/L, increases of algal growth rate of 13.6 % and 8.4 %, respectively, were observed, with nearly 100% inhibition in cell growth at the highest nominal concentration, compared to the control. Reduction of growth rate and cell growth results are below.

Table B.9.3-10: Percentage reduction of growth rate and inhibition of cell growth of *Selenastrum capricornutum* exposed for 72 hours to MON 52276

	Control	MON 52276 [mg test item/L]				
Test parameters	**-**	**50**	**90**	**160**	**290**	**500**
Mean absorbance (0-72 h)	0.260	0.419	0.391	0.128	0.027	0.015
Cell growth rate reduction (0-72 h) [%] based on absorbance	-	-13.6	-8.4	10.9	42.8	58.2
Cell growth inhibition (0-72 h) [%] based on absorbance	-	-36.9	-27.7	50.3	81.5	89.6
Mean cell densities (0-72 h) (× 1000 cells/mL)	644	741	663	315	45	33
Cell growth rate reduction (0-72 h) [%] based on cell counting	-	-3.4	-0.7	17.5	64.8	72.5
Cell growth inhibition (0-72 h) [%] based on cell counting	-	-1.7	8.3	54.1	84.7	93.2

III. CONCLUSIONS

Based on absorbance, the 72 h E_rC_{50} and the 72 h E_bC_{50} for *Selenastrum capricornutum* exposed to MON 52276 were calculated to be 393 mg test item/L and 150 mg test item/L. The NOEC was determined to be 90 mg test item/L. For cell counting method, 72 h E_rC_{50} and 72 h E_bC_{50} for *Selenastrum capricornutum* exposed to MON 52276 were calculated to be 284 mg test item/L and 178 mg test item/L, respectively. The NOEC was determined to be 90 mg test item/L.

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	59
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤35 %	20.4 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7 %.	≤7 %	4.1 %

The biomass in the control cultures increased by a factor of ≥16 (actual: 59), the coefficient of variance for section specific growth rates was ≤ 35 % (actual: 20.4 %) and the coefficient of variance for the whole test period it was ≤ 7 % (actual: 4.1 %).

This study was performed according to the valid test guideline at the time of conduct. In the last Annex I renewal, this study was evaluated and considered acceptable for use in risk assessment. In the current submission dossier, a re-evaluation of the study against the current test guideline validity criteria was conducted (at least a 16 fold increase in biomass, a mean coefficient of variation for section-by-section growth rates in the control being <35% and a coefficient of variation of the average specific growth rate over the test period in the controls being <7%) and against these criteria, the study was considered valid. Chemical analysis was not conducted during the study. However, glyphosate is very water soluble (>10 g/L) and stable under conditions of exposure in laboratory algal studies is supported by more recent studies performed with alga. The principal route of degradation of glyphosate is via microbial action. Degradation of glyphosate over a short exposure period is not expected. Glyphosate is stable under conditions of continuous illumination (see results of the photolysis studies presented in the Environmental Fate section (see M-CA Section 7). Therefore, the losses of glyphosate from the test system following 72 or 96 hr exposure would not be expected. The study should therefore be considered strongly supportive of the risk assessment. The endpoints achieved in the MON 52276 algal study were 72 hr ErC50 = 284 mg test item/L; 72 hr EbC50 = 178 mg test item/L and NOEC = 90 mg test item/L.

Assessment and conclusion by RMS:

RMS checked the validity criteria and found that all validity criteria were met according to the OECD 201 (2011) guideline. The biomass in the control cultures increased by a factor of 58.6, the mean coefficient of variation for section-by-section specific growth rate is 25.5% and the coefficient of variation of average specific growth rates is 4.9%.

However, no analytical measurements were made to check the nominal concentrations. In the other aquatic studies available with the product, concentrations have been satisfactorily maintained except in the acute toxicity study with *Cyprinus carpio*. Therefore, as concentrations could not be checked, RMS considers this study may only be suitable for weight of evidence in risk assessment. However, the endpoints expressed based on absorbance are not considered relevant to RMS. Indeed, the absorbance values were directly used for determining effects on algae growth and no calibration curve has been used in order to relate absorbance to cell density.
No reliable endpoint can be set.

Data gap : Toxicity study on alga with the representative formulation.

Data point:	CP 10.2.1/005
Report author	[redacted]
Report year	2002
Report title	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed *Lemna gibba*.
Report No	GA-2002-051
Document No	20021186/01-AALg
Guidelines followed in study	OECD 221 (draft of October 2000)
Deviations from current test guideline	Deviation from current OECD 221 guideline (2006): Major: - Bacterial contamination occurred in test concentrations 2.4 and 6.8 mg/L. Minor: - none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid and acceptable for risk assessment purpose

Summary
The effects on the growth of the aquatic plant *Lemna gibba* G3 exposed to MON 52276 (30.9% w/w glyphosate acid) were determined in a seven-day semi-static study. For the main test, three replicates of 12 fronds in AAP Medium for *Lemna gibba* were exposed in glass beakers under continuous illumination to nominal MON 52276 concentrations of 0 (control), 0.9, 2.4, 6.8, 19.1, 53.6 and 150 mg/L, equivalent to 0.278; 0.742; 2.10; 5.90; 16.6; 46.4 mg glyphosate acid/L. Renewal of the test media was performed on day 3 and 5 after test initiation. Direct counts of number of fronds were conducted on day 3, 5 and 7. Observations of changes in plant development, frond size, appearance, necrosis or other abnormalities were also performed at those times. The effect on biomass production was evaluated by determining the final dry weights of the plants. The growth rate inhibition was determined by counting the number of fronds produced for each test concentration and control group. The effect on biomass production was evaluated by determining the final dry weights of the plants. Samples from all the test concentrations were collected for analysis of glyphosate by HPLC on Days 0, 3, 5 and 7.

Significant inhibitory effects of MON 52276 were observed at 53.6 and 150 mg/L (43%) for frond numbers, growth rate and biomass increase. These were equivalent to 16.6 and 46.4 mg glyphosate acid/L respectively.

The authors concluded : The EC_{50} for frond number, biomass and growth rates based on frond number and biomass for MON 52276 were determined to be 66.58, 118.16 and >150 mg MON 52276/L, respectively. The overall NOEC was determined to be 19.1 mg MON 52276/L. Hence, The EC_{50} for frond number, biomass and growth rates based on frond number and biomass were determined to be 20.57, 36.51 and > 46.35 mg glyphosate acid/L, respectively. The overall NOEC was determined to be 5.9 mg glyphosate acid/L.

RMS concluded that EC50 for growth rate and yield based on frond number are > 150 and 66.58 mg MON 52276/L, respectively. The EC50 based on dry weight for yield is 118.16 mg MON 52276/L (36.51 mg a.e./L). The NOEC for growth rate is 19.1 mg MON 52276/L (5.90 mg a.e./L). However, for biomass, given the consistence of percentage reduction given for mean frond number, mean dry weight and mean biomass increase, RMS considered that a NOEC of 19.1 mg/L even if significant statistically is not biologically relevant. As only a 7d EC50 based on yield was calculated for dry weight in the study report, 7d ECx (EC10, EC20 and EC50) based on growth rates should also be calculated for this parameter (data gap).
The validity criteria according to guideline OECD 221 are fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	MON 52276
Description:	Light amber-brown liquid formulation
Lot/Batch #:	A1C1204104
Purity:	30.9% glyphosate acid equivalent, as 41.5% isopropylamine salt of glyphosate

2. Test organism:

Species:	Young *Lemna gibba* G3, 2 – 5 fronds
Source:	Institut für Pflanzenökologie und Ökotoxikologie, University of Hohenheim, Stuttgart, Germany

3. Environmental conditions:

Temperature:	22 - 25 °C
Light intensity:	Continuous illumination, 7000 lux
pH:	7.49 – 9.42 (adjusted to 7.5)
Conductivity:	not stated
Hardness:	Not stated

4. Dates of experimental work: May 24th to June 15th 2002

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed with six concentration levels, 0.9, 2.4, 6.8, 19.1, 53.6 and 150 mg MON 52276/L, equivalent to 0.278; 0.742; 2.10; 5.90; 16.6; 46.4 mg glyphosate acid/L, with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Colonies consisting of 2-5 fronds totalling 12 fronds per replicate were added to each replicate test chamber. The plants were placed in 100 mL test vessels containing 50 mL 20X-AAP test media. The pH of the test medium was adjusted at

each test media renewal to 7.5, to avoid extreme pH values. The test was conducted under a 7-day static-renewal test conditions. The renewal of the test media was performed on day 3 and 5 after test initiation.

2. Observations:

Biological data: Observations were made on the number and the condition of the fronds on Days 3, 5 and 7. The growth rate inhibition was determined by counting the number of fronds produced for each test concentration and control group. The effect on biomass production was evaluated by determining the final dry weights of the plants.

Physical data: pH and temperature of the test vessels were measured on days 0, 3, 5 and 7. Samples from all the test concentrations were collected for analysis of glyphosate by HPLC on Days 0, 3, 5 and 7.

3. Statistical calculations: The 7-day EC_{50} value for frond counts and growth rates based on frond counts and biomass were determined by probit analysis and the calculation of statistical significance was determined by using one-way analysis of variance (ANOVA) and Dunnett's test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The mean measured glyphosate concentrations were 82.9% to 112% of nominal over the test period. The test substance remained stable, therefore the results are based on the nominal concentrations.

Table B.9.3-11: Analytical results

Nominal concentration [mg MON 52276/L]	Nominal concentration [mg glyphosate acid/L]	Mean measured [mg glyphosate acid/L]	% of nominal
Control		-	-
0.9	0.278	0.231	82.9
2.4	0.742	0.701	94.5
6.8	2.10	2.11	101
19.1	5.90	6.62	112
53.6	16.6	17.4	105
150	46.4	48.5	104

Results were based on nominal MON 52276 concentrations.

Table B.9.3-12: Endpoints

Endpoint	Frond number [mg/L]	Growth rate based on frond number [mg/L]	Biomass [mg/L]
	Nominal concentration of MON 52276 [mg/L]		
EC_{50} (7 days)	66.58 (56.30 – 79.66)	>150	118.16 (91.37 – 171.37)
NOEC (7 days)	19.1	19.1	19.1
	Nominal concentration of glyphosate a.e. [mg/L]		
EC_{50} (7 days)	20.57 (17.39-24.61)	>46.35	36.51 (28.23-52.95)
NOEC (7 days)	5.9	5.9	5.9

B. OBSERVATIONS

Observations: Significant inhibitory effects were observed at 2.4 and 6.8 mg/L for frond numbers and growth rates, and at 6.8 mg/L for biomass. However, these effects were not dose-related and were considered to be due to a reduced uptake of nutrients following a root decay caused by a bacterial infection. Additional dose-related significant inhibitory effects were observed at 53.6 and 150.0 mg/L for frond numbers, growth rates and biomass increase.

Table B.9.3-13: Toxicity of MON 52276 to *Lemna gibba* under semi-static conditions

MON 52276 concentration (mg/L) [1]	Mean frond number [2]			Mean dry weight (mg) [3]	Average specific growth rate (μ)	Mean biomass increase (based on dry weight)
	Day 3	Day 5	Day 7	Day 7	0 - 7 days	0 - 7 days
0 (control)	44	120	270	32.4	0.444	31.0
0.9	45	116	234	28.5	0.4233	27.2
2.4	43	100	204*	27.8	0.4010*	26.5
6.8	40	98	193*	26.3	0.3961*	25.0*
19.1	49	119	242	28.3	0.4284	27.0
53.6	39	84	157*	24.6	0.3668*	23.3*
150.0	27	48	71*	14.1	0.2533*	12.8*

[1] Nominal values.
[2] Initial mean frond number: 12
[3] Initial mean dry weight: 1.3 mg
* Statistically significant compared to control

Based on nominal concentrations, the EC_{50} for frond count of *Lemna gibba* exposed to MON 52276 under semi-static test conditions for 7 days was 66.58 mg MON 52276/L (95% confidence limits of 56.30 and 79.66 mg MON 52276/L), equivalent to 20.57 mg a.e./L. Since the percentage inhibition compared to control was only 43% at the highest MON 52276 concentrations tested, the E_rC_{50} was estimated to be > 150 mg MON 52276/L, equivalent to 46.35 mg a.e./L. Based on nominal concentrations, the E_bC_{50} was 118.16 mg MON 52276/L (95% confidence limits of 91.37 and 171.37mg MON 52276/L), equivalent to 36.51 mg a.e./L. The no-observed-effect-concentration (NOEC) was 19.1 mg MON 52276/L, equivalent to 5.90 mg a.e./L.

The doubling time of frond numbers in the control was less than 2.5 days (37.4 hours). The validity criteria according to the current guideline OECD 221 are therefore fulfilled.

III. CONCLUSION

Assessment and conclusion by applicant:

Based on nominal concentrations, the EC_{50} for frond count of *Lemna gibba* exposed to MON 52276 under semi-static test conditions for 7 days was calculated to be 66.58 mg/L (95% confidence limits of 56.30 and 79.66 mg MON 52276/L), equivalent to 20.57 mg a.e./L. Since the percentage inhibition compared to control was only 43% at the highest MON 52276 concentrations tested, the E_rC_{50} was estimated to be > 150 mg MON 52276/L, equivalent to 46.35 mg a.e./L. Based on nominal concentrations, the E_bC_{50} was 118.16 mg MON 52276/L (95% confidence limits of 91.37 and 171.37mg MON 52276/L), equivalent to 36.51 mg a.e./L. The no-observed-effect-concentration (NOEC) was 19.1 mg MON 52276/L, equivalent to 5.90 mg a.e./L.

This study was conducted according to the draft OECD 221 test guideline from October 2000. The currently adopted test guideline is largely unchanged from the draft guideline. In the last Annex I renewal, this study was evaluated and considered acceptable for use in risk assessment. For this submission, the study has been re-evaluated. The study was conducted at nominal rates of 0.9, 2.4, 6.8, 19.1, 53.6 and 150 mg MON 52276/L. Chemical analysis was conducted during the study with mean measured concentrations of product between 82.9 and 104% of nominal achieved. The study was considered valid with a doubling time of < 48 hours compared to the required < 2.5 days in the test guideline. The report identifies bacterial infection in some test cultures, most notably in the two lowest exposure concentrations. Relative to the control group, there was no significant difference in the frond number inhibition (%) at the end of the study across the four lowest exposure concentrations. However, there was a significant inhibition in frond number at the highest exposure concentration (150 mg MON 52276/L), where there was 43% inhibition. Despite the apparent bacterial infection which was not confirmed in the study report – only based on observation, the study should be considered supportive for use in risk assessment.

Assessment and conclusion by RMS:

All validity criteria were met according to OECD Guideline 221. According to the study report, the significant effects observed at 2.4 and 6.8 mg prod/L tested rates were due to root decays caused by a bacterial infection. However RMS considered that the effect reported at the two highest doses cannot be disregarded.

Given the results of percentage of reduction of average specific growth rate and of mean biomass increase, the EC50 values for growth rate and biomass are agreed. RMS agreed to set a NOEC for growth rate at 19.1 mg/L given the effects observed on percentage of reduction of average specific growth rate of 3.5% at 19.1 mg/L and 4.7% ate 0.9 mg/L. However for biomass , given the consistence of percentage reduction given for mean frond number, mean dry weight and mean biomass increase, RMS considered that a NOEC of 19.1 mg/L even if significant statistically is not biologically relevant.

MON 52276 concentration (mg/L)	Mean frond number	% reduction of mean frond number	Mean dry weight (mg)	% reduction of mean dry weight	Average specific growth rate (μ)	% reduction of average specific growth rate	Mean biomass increase (based on dry weight)	% reduction of mean biomass increase
	Day 7	Day 7	Day 7	Day 7	0 - 7 days	0 - 7 days	0 - 7 days	0- 7 days
0 (control)	270	-	32.4	-	0.444	-	31	-
0.9	234	13.3	28.5	12.0	0.4233	4.7	27.2	12.3
2.4	204*	24.4	27.8	14.2	0.401*	9.7	26.5	14.5
6.8	193*	28.5	26.3	18.8	0.3961*	10.8	25*	19.4
19.1	242	10.4	28.3	12.7	0.4284	3.5	27	12.9
53.6	157*	41.9	24.6	24.1	0.3668*	17.4	23.3*	24.8
150	71*	73.7	14.1	56.5	0.2533*	43.0	12.8*	58.7

Frond number :

7d-E_rC_{50} > 150 mg MON 52276/L (>46.35 mg a.e./L) (nom)
7d-NOErC = 19.1 mg MON 52276/L (5.90 mg a.e./L).

7d-E_yC_{50} = 66.58 mg MON 52276/L (20.57 mg a.e./L) (nom)
7d-NOEyC = 19.1 mg MON 52276/L (5.90 mg a.e./L).

The EbC50 presented in the study summary is in fact an EyC50 as it is based on the biomass gain based on dry weight measurements.

Dry weight :

7d-E_yC_{50} = 118.16 mg MON 52276/L (36.51 mg a.e./L)
7d-NOEyC = 19.1 mg MON 52276/L (5.90 mg a.e./L).

As only a 7d EC50 based on yield was calculated for dry weight in the study report, 7d ECx (EC10, EC20 and EC50) based on growth rates should also be calculated for this parameter (data gap).

Data point:	CP 10.2.1/006
Report author	[redacted]
Report year	2012
Report title	Effect of MON52276 (Glyphosate formulation) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment, with a subsequent Recovery Period.
Report No	CHE-016/4-80/A
Document No	-
Guidelines followed in study	Maltby, L., et al. (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
Deviations from current test guideline	None according to Maltby *et al*. (2008)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid and reliable

Summary

The toxicity of MON52276 on growth of *Myriophyllum aquaticum* was evaluated in a 14 day static toxicity test, with subsequent 7 day recovery test, performed at concentrations of 0.78, 3.91, 19.6, 97.8, 489 and 2445 mg MON52276/L, equivalent to 0.24, 1.2, 6.0, 30, 150 and 750 mg glyphosate acid equivalent/L. A negative control (Smart & Bako medium) was prepared in parallel.

Two sets of vessels (exposure and recovery set) were prepared, with each set comprising three replicates for each test concentration and six replicates for the controls. Test vessels were 2-L beakers, each containing five individual plants potted in individual pots containing artificial sediment. Shoot length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days and after 21 days (recovery vessels). At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants. At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root.

Test media were analysed for Glyphosate content at test start and end of exposure and recovery periods. The measured concentrations ranged from 83.9-145% of nominal. Glyphosate was not detected in the control group.

Result showed a significant inhibition of fresh weight of 20.7% at the lowest test concentration of 0.3 mg glyphosate acid equivalent/L. Shoot length increase and growth rate were unaffected at this concentration. Relative to the control group, at the highest treatment rate (723 mg test item/L) there was 93.8% growth inhibition based on fresh weights, shoot length increase was inhibited by 94.1 growth rate by 90.2%. The recovery period demonstrated that *Myriophyllum aquaticum* pre-exposed to up to 26.80 mg MON52276/L were able to recover to control levels of growth, in untreated culture medium within 7 days of transfer.

The study fulfilled the validity criteria of achieving at least 50% increase in control plant growth in terms of length within 7 days of test initiation. The test was therefore considered to be valid.

MON52276 significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a mean measured concentration of <0.3 mg glyphosate acid equivalent/L. Shoot length was inhibited at or above

mean measured concentrations of 5.16 mg glyphosate acid equivalent/L. The 14-d EyC_{50} value for fresh weight inhibition was 4.4 mg glyphosate acid equivalent/L and for shoot length it was 13.44 mg glyphosate acid equivalent/L. The 14-d ErC_{50} value for fresh weight inhibition was 10.33 mg glyphosate acid equivalent/L and for shoot length it was 42.79 mg glyphosate acid equivalent/L.*Myriophyllum aquaticum* pre-exposed for 14 days to up to 26.80 mg glyphosate acid equivalent/L were able to recover in untreated culture medium after a 7 day recovery period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate SL formulation (MON52276)
Description:	Clear, yellow, viscous liquid
Lot/Batch #:	A9K0106104
Purity:	358.8 ± 4.0 g glyphosate acid equivalent/L (30.68% w/w)

2. Test organism:

Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany

3. Environmental conditions:

Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat
	20% kaolin clay
	75-76% quartz sand
	$CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5)
	Based on artificial soil used in OECD guideline 219
	Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	20.0 °C
Photoperiod:	16 h light
Light intensity	7295-7518 lux

	pH: Values recorded at test start and end (in brackets) of 14 day exposure period:
	Controls = 7.97 (8.78-8.82)
	0.3 mg/L = 8.25 (8.82)
	1.1 mg/L = 8.01 (8.82)
	5.16 mg/L = 8.15 (8.82)
	26.8 mg/L = 7.79 (8.81-8.82)
	145 mg/L = 7.26 (6.11-8.82)
	723 mg/L = 5.86 (6.09-6.82)
	Values at start and end of 7 day recovery period:
	Recovery period start = 6.0 – 9.2
	Recovery period end = 8.3 – 9.8
	Oxygen saturation Values recorded at test start and end (in brackets) of 14 day exposure period:
	Controls = 96% (102-108%)
	0.3 mg/L = 90% (107-108%)
	1.1 mg/L = 96% (107-111%)
	5.16 mg/L = 91% (114-132%)
	26.8 mg/L = 95% (100-104%)
	145 mg/L = 90% (116-122%)
	723 mg/L = 96% (4-9%)
	Values at start and end of 7 day recovery period:
	Controls = 103-110% (99-109%)
	0.3 mg/L not included in the recovery period
	1.1 mg/L = 108-114% (103-110%)
	5.16 mg/L = 111-113% (115-121%)
	26.8 mg/L = 123-130% (123-126%)
	145 mg/L = 127-137% (104-143%)
	723 mg/L = 6-33% (107-111%)
4. Dates of experimental work:	Oct 28th to Nov 18th 2010

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 0.24, 1.2, 6.0, 30, 150 and 750 mg glyphosate acid equivalent/L, equivalent to 0.78, 3.91, 19.6, 97.8, 489 and 2445 mg MON52276/L, with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups. Two sets of vessels (exposure and recovery) were prepared at the start of the test.

The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate.

After 14 days exposure another set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, were transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event.

2. Observations: Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 5, 8, 11 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was recorded at test start and after 14 days.

Analytical control measurements of the actual concentration of the glyphosate were performed by means of LC/MS-MS analysis at test start, after 14 (after exposure phase) and 21 days (after recovery phase).

3. Statistical calculations: The EC_{10}, EC_{20} and EC_{50} and its 95% confidence interval were calculated by probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Williams' t-test, Dunnett's t-test or Welch's t-test (p = 0.05).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements of the actual concentration of the glyphosate were performed at test start, after 14 and 21 days (after recovery phase). The measured concentrations ranged from 83.9-145% of nominal at test start and 88.1 to 110% of nominal at test end. Except for the lowest treatment level the test item was stable during the test period. The results were evaluated using the geometric mean measured concentrations.

Table B.9.3-14: Analytical results

Nominal concentration [mg glyphosate a.e./L]	Test start [mg glyphosate/L]		Test end [mg glyphosate/L]		Geometric mean [mg glyphosate/L]	
	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal
Control	<LOQ	-	<LOQ	-	-	-
0.24	0.35	145.0	0.26	110.0	0.30	125.0
1.2	1.15	95.6	1.05	87.8	1.10	91.7
6.0	5.03	83.9	5.29	88.1	5.16	86.0
30	26.3	87.5	27.4	91.5	26.8	89.3
150	145.0	96.5	145.0	96.4	145.0	96.7
750	722.0	96.3	723.0	96.4	723.0	100.4

LOQ = 0.25 mg/L.

The EC_{50} and NOEC values after 14 day growth inhibition test are given below based on geometric mean measured concentrations.

Table B.9.3-15: 14-day endpoints

Endpoint	**Concentration in glyphosate a.e. [mg/L]**			
	14 Day EC_{10} *	**14 Day EC_{20} ***	**14 Day EC_{50} ***	**14 Day NOEC**
Shoot length/yield	0.43 (0.1-1.06)	1.41 (0.48-2.8)	13.44 (7.72 – 23.74)	5.16
Shoot length/growth rate	1.07 (0.23-2.67)	3.81 (1.29-7.61)	42.79 (24.74 – 76.48)	5.16
Fresh weight/yield	0.11 (0.01-0.33)	0.39 (0.09-0.9)	4.44 **(**2.28 – 8.51)	< 0.30
Fresh weight/ growth rate	0.16 (0.03-0.46)	0.66 (0.19-1.48)	10.33 (5.59 – 19.21)	< 0.30
Dry weight/yield	n.d.	n.d.	>145	145
Dry weight/ growth rate	0.44 (n.d.-7.50)	3.23 (n.d.-30.52)	143.3 (10.06 – n.d.)	145
Root length/yield	1.05 (0.59-1.53)	1.89 (1.24-2.53)	5.84 (4.65 – 7.37)	1.10
Root length/growth rate	2.23 (1.10-3.75)	6.33 (3.77-9.39)	46.50 (34.75 – 62.52)	1.10
	Equivalence in concentration in MON52276 [mg/L]			
	14 Day EC_{10} *	**14 Day EC_{20} ***	**14 Day EC_{50} ***	**14 Day NOEC**
Shoot length/yield	1.39 (0.32-3.43)	4.60 (1.56-9.13)	43.81 (25.2-77.4)	16.82
Shoot length/growth rate	3.46 (0.74-8.64)	12.42 (4.20-24.8)	139.5 (80.6-249.3)	16.82
Fresh weight/yield	0.36 (0.03-1.07)	1.27 (0.29-2.93)	14.47 (7.43-27.7)	<0.98
Fresh weight/ growth rate	0.518 (0.10-1.49)	2.15 (0.62-4.82)	33.67 (18.2-62.6)	<0.98
Dry weight/yield	n.d.	n.d.	n.d.	473
Dry weight/ growth rate	1.42 (n.d.-24.27)	10.52 (n.d.-99.5)	467.1 (32.8-n.d.)	473
Root length/yield	3.40 (1.91-4.95)	6.16 (4.04-8.25)	19.04 (15.2-24.0)	3.59
Root length/growth rate	7.22 (3.56-12.14)	20.63 (12.3-30.6)	151.6 (123.0-203.8)	3.59

* (CI) = 95% confidence interval
n.d.: not determined due to mathematical reasons or inappropriate data; highlighted value indicates most sensitive measured parameter

The EC_{50} and NOEC values after 7 day recovery period are given below based on geometric mean measured concentrations.

Table B.9.3-16: 7-day recovery endpoints

Endpoint	Concentrations in glyphosate a.e. [mg/L]	
	7 Day Recovery EC_{50}	**7 Day Recovery NOEC**
Shoot length/relative increase	n.d.	26.80
Shoot length/growth rate	n.d.	26.80
Fresh weight/relative increase	n.d.	≥723
Fresh weight/ growth rate	n.d.	≥723
Dry weight/relative increase	n.d.	≥723
Dry weight/ growth rate	n.d.	≥723
Root length/relative increase	n.d.	≥723
Root length/growth rate	n.d.	≥723
	Equivalence in concentration in MON52276 [mg/L]	
Shoot length/relative increase	n.d.	87.35
Shoot length/growth rate	n.d.	87.35
Fresh weight/relative increase	n.d.	≥2357
Fresh weight/ growth rate	n.d.	≥2357
Dry weight/relative increase	n.d.	≥2357
Dry weight/ growth rate	n.d.	≥2357
Root length/relative increase	n.d.	≥2357
Root length/growth rate	n.d.	≥2357

n.d.: not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS

There was a concentration dependent effect on growth, root length, fresh and dry weight of *Myriophyllum aquaticum.* Growth was significantly reduced at 5.16 mg glyphosate acid equivalent /L, fresh weight at <0.3 mg glyphosate acid equivalent/L, dry weight at 145 mg glyphosate acid equivalent/L and root length at 1.10 mg glyphosate acid equivalent L during the 14 day exposure test. In the subsequent recovery test it was shown that *Myriophyllum aquaticum*, pre-exposed to up to 26.80 mg glyphosate acid equivalent/L were able to recover to control levels of growth in untreated culture medium within 7 days of the exposure period.

Table B.9.3-17: Percentage of inhibition of *Myriophyllum aquaticum* exposed for 14 days to MON52276

Test parameters	Glyphosate a.e.[mg/L] (mean measured)					
	0.3	**1.1**	**5.12**	**26.8**	**145**	**723**
Inhibition of shoot length increase (%)	-3.5	5.1	30.5	74.1	70.3	94.1
Inhibition of shoot length growth rate (%)	-2.6	2.0	17.5	58.1	53.6	88.3
Inhibition of fresh weight increase (%)	20.7	19.2	61.2	80.1	77.6	93.8
Inhibition of fresh weight growth rate (%)	14.6	13.3	49.4	70.9	67.8	90.2
Inhibition of dry weight increase (%)	14.7	18.2	34.3	15.8	-6.9	106.6
Inhibition of dry weight growth rate (%)	11.1	14.4	29.6	19.6	-4.7	112.3
Inhibition of root length increase (%)	-6.8	-3.9	52.0	82.9	94.5	98.3
Inhibition of root length growth rate (%)	-1.7	-0.9	18.3	43.9	66.7	86.8

For *Myriophyllum aquaticum,* plant fresh weight measurements are relevant for risk assessment as lower variability is associated with individual plant measurement compared to procedure used for dry weights which attracts a greater variability - with all plants pooled according to treatment and then compared to dry weights established at test start using a separate set of plants. Furthermore, root length measurements are considered semi-quantitatively, as only the length of the longest roots have been measured. The number of side roots and total number have not been determined given the practical constraints associated with the sediment *Myriophyllum* test design. Effects on roots are considered to be reflected in fresh weight measurements.

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group; with an increase of biomass (shoot length) in controls was > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

III. CONCLUSIONS

Assessment and conclusion by applicant:

MON52276 significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days. Based on geometric mean measured concentrations, the 14-d E_rC_{50} value for fresh weight inhibition was 10.33 mg glyphosate acid equivalent/L and for shoot length it was 42.79 mg glyphosate acid equivalent/L. *Myriophyllum aquaticum* pre-exposed for 14 days to up to 26.80 mg glyphosate acid equivalent/L were able to recover in untreated culture medium after a 7 day recovery period.

The study is considered to be valid and suitable for risk assessment purposes.

Assessment and conclusion by RMS:

RMS checked validity criteria according to OECD Guideline 239. The mean total shoot length and mean total shoot fresh weight in control plants doubled during the exposure phase of the test. The coefficient of variation for yield based on measurements of shoot fresh weight in the control cultures did not exceed 35% between replicates (16.5%). The test design differed from the guideline in the number of plants per replicate, which was of 5 instead of 3 for the same vessel size (2L). Nevertheless, this is not considered to have influenced the results of the study as the control was shown to behave as expected (validity criteria met). Thus, the study is considered valid.

Shoot length

14d NOErC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L)
14d ErC10 = 1.07 mg glyphosate acid/L (mm) (equivalent to 3.46 mg MON52276/L)
14d ErC20 = 3.81 mg glyphosate acid/L (mm) (equivalent to 12.42 mg MON52276/L)
14d ErC50 = 42.79 mg glyphosate acid/L (mm) (equivalent to 139.5 mg MON52276/L)

14d NOEyC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L)
14d EyC10 = 0.43 mg glyphosate acid/L (mm) (equivalent to 1.39 mg MON52276/L)
14d EyC20 = 1.41 mg glyphosate acid/L (mm) (equivalent to 4.60 mg MON52276/L)
14d EyC50 = 13.44 mg glyphosate acid/L (mm) (equivalent to 43.81 mg MON52276/L)

Shoot fresh weight

14d NOErC < 0.3 mg glyphosate acid/L (mm) (equivalent to <0.98 mg MON52276/L)
14d ErC10 = 0.16 mg glyphosate acid/L (mm) (equivalent to 0.518 mg MON52276/L)
14d ErC20 = 0.66 mg glyphosate acid/L (mm) (equivalent to 2.15 mg MON52276/L)
14d ErC50 = 10.33 mg glyphosate acid/L (mm) (equivalent to 33.67 mg MON52276/L)

14d NOEyC < 0.3 mg glyphosate acid/L (mm) (equivalent to <0.98 mg MON52276/L)
14d EyC10 = 0.11 mg glyphosate acid/L (mm) (equivalent to 0.36 mg MON52276/L)
14d EyC20 = 0.39 mg glyphosate acid/L (mm) (equivalent to 1.27 mg MON52276/L)
14d EyC50 = 4.44 mg glyphosate acid/L (mm) (equivalent to 14.47 mg MON52276/L)

Shoot dry weight

14d NOErC = in view of the results, the ErC10 is deemed more appropriate (even if lower limit of 95% CI is not determined)
14d ErC10 = 0.44 mg glyphosate acid/L (mm) (equivalent to 1.42 mg MON52276/L)
14d ErC20 = 3.23 mg glyphosate acid/L (mm) (equivalent to 10.52 mg MON52276/L)
14d ErC50 = 143.3 mg glyphosate acid/L (mm) (equivalent to 467.1 mg MON52276/L)

14d NOEyC = not reliable (no inhibition reported at 145 mg glyphosate acid/L (mm) but inhibition of yied at lower concentrations ranged from 14.7 to 34.3% and is more than 100% at 723 mg glyphosate acid/L).
14d EyC50 > 145 mg glyphosate acid/L (mm) (equivalent to >473 mg MON52276/L)
EyC10 expected to be lower than 0.3 mg a.e./L (equivalent to <0.98 mg MON52276/L)
EyC20 not determined.

Root length

14d NOErC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L)
14d ErC10 = 2.23 mg glyphosate acid/L (mm) (equivalent to 7.22 mg MON52276/L)
14d ErC20 = 6.33 mg glyphosate acid/L (mm) (equivalent to 20.63 mg MON52276/L)
14d ErC50 = 46.5 mg glyphosate acid/L (mm) (equivalent to 151.6 mg MON52276/L)

14d NOEyC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L)
14d EyC10 = 1.05 mg glyphosate acid/L (mm) (equivalent to 3.40 mg MON52276/L)
14d EyC20 = 1.89 mg glyphosate acid/L (mm) (equivalent to 6.16 mg MON52276/L)

14d EyC50 = 5.84 mg glyphosate acid/L (mm) (equivalent to 19.04 mg MON52276/L)

A recovery could be expected after 7 days without exposure to active substance for plants exposed up to and including 26.8 mg a.e./L (mm).

Data point:	CP 10.2.1/007
Report author	Gabriel, U.U. *et al.*
Report year	2010
Report title	Toxicity of roundup (a glyphosate product) to fingerlings of *Clarias gariepinus*
Document No	ISSN: 159 – 3115
Guidelines followed in study	None
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Not applicable*
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (literature publication)
Acceptability/Reliability (RMS):	Less relevant but supplementary and reliable with restrictions (supportive data)

The summary can be found in Appendix to Volume 3 CA B.9 related to literature data on ecotoxicology, under B.9.2.1.2.

Assessment and conclusion by applicant:

The effects of Roundup containing 360 g/l glyphosate (equivalent to 480g/L isopropylamine salt) were tested in an acute test with *C. gariepinus* fingerlings. The 96 hour-LC_{90} was determined to be 19.91 mg product/L.

There is no analytical verification of test concentrations reported and thus the reliability of the endpoint is questionable. The appearance of mucus accumulation on the skin and gills and skin pigmentation recorded in fish in the holding / stock vessels is a clear indicator of stress. Therefore, the condition of the fish used in the test is questionable. The study was not conducted in accordance with a recognised test guideline and was not performed under conditions of GLP. Furthermore, the purity of the formulation roundup is not clearly given as the specification in the full text contains some typing errors. The study is considered reliable with restrictions.

Assessment and conclusion by RMS:

The conclusion reported above by the applicant is partial. Not only lethal effect but also sublethal, i.e. opercular beat frequency, tail beat frequency, are measured in this study.
The relevance of these sublethal effects for the risk assessment cannot be established by RMS as no quantitative link can be made between these parameters and the potential adverse effect at population level (this latter being the specific protection goal). So only results on mortality were considered in deep by RMS. Nevertheless, a link between these abnormal behaviors may exist and may be indicative of mortality and/or potential adverse effect at population level in natural conditions. So, the results for sublethal effects were

also reported in the summary and may be considered in future, together with other data available for the active substance.

The present study assessed the acute toxicity (lethal and sublethal) of the glyphosate based formulation Roundup. It is then not known if the high toxicity measured in this study is due to the formulation (and its co-formulants) or a species-specific sensitivity.
The study was conducted with the African catfish *Clarias gariepinus*. RMS considers that the sensitivity of this species can be considered representative of European catfish species.

The applicant notes that there is no analytical verification of test concentrations reported and thus the reliability of the endpoint is questionable. RMS agrees that the absence of analytical verification is a severe drawback of the study. Dose relationship was observed indicating that dosing was somehow adequate, nevertheless uncertainty remains on the actual concentrations.
The applicant notes that mucus accumulation on the skin and gills and skin pigmentation were recorded in fish in the holding / stock vessels. To RMS understanding, "recorded" only means that it was part of the study design (not that it was observed in control). The study author noted that mucus accumulation was concentration-dependant and minimal in the control.

The authors derived what they called "Safe concentration" by multiplying the lethal concentration by a factor 0.1. RMS does not consider these values relevant for risk assessment.

The dissolved oxygen value reported in the study is of 0.01 ± 0.05 mg/l. RMS considers this as a typing error (control fish would have not survived).

The 96 hour LC50 of Roundup on the fish was 15.88 mg/l (equivalent to approximately 5.7 mg glyphosate acid equivalent/L). However, the similarity of the formulations (Roundup vs. MON 52276) is not established.

RMS considers this study being less relevant but supplementary (formulation issue). The data are considered not reliable. RMS cannot discard higher sensitivity of this species (which can be considered representative of European catfish species). However, the similarity of the formulations (Roundup vs. MON 52276) is not established.

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Available acute toxicity data on glyphosate acid and the representative product MON 52276 to fish, aquatic invertebrates, algae and aquatic macrophytes did not indicate significantly enhanced toxicity of the formulated product MON 52276 in comparison to the active substance glyphosate. Therefore, based on the results of these studies the performance of any further study is not deemed necessary.

B.9.3.3. Further testing on aquatic organisms

Given the outcome of the risk assessment under B.9.4 below, further testing are not deemed required.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

Relevant and reliable studies for the risk assessment of aquatic organisms from the active substance glyphosate and the relevant metabolites (AMPA and HMPA) are summarised in the tables below, presenting all available endpoints for each organism group. Details of these studies are summarised in the document Vol. 3 CA B.9.2and relevant endpoints for the risk assessment are provided in the tables below.

All endpoints for glyphosate and its representative formulation have been expressed in mg glyphosate acid equivalent per litre in order to allow comparison.

Table B.9.4-1: Studies on acute toxicity to fish of glyphosate and metabolites

Annex point	Study	Substance(s)	Test species	Study type	LC_{50} (mg a.e./L)	NOEC (mg a.e./L)	Status	Remark
CA 8.2.1/001	[redacted] 2003	Glyphosate K-salt	*Oncorhynchus mykiss*	Acute / static	> 1193 (nom)	149	valid	-
CA 8.2.1/002	[redacted] 1995	Glyphosate acid	*Oncorhynchus mykiss*	Acute / static	>100 (nom)	32	Valid with restrictions	pH induced effects at 180 mg/L
CA 8.2.1/003	[redacted] 1995	Glyphosate technical	*Oncorhynchus mykiss*	Acute / static	>100	100	Not assessed	No study report available. Data from RAR (2015)
CA 8.2.1/004	[redacted] 1993	Glyphosate IPA-salt	*Oncorhynchus mykiss*	Acute/ static	1001 (nom)	236	Valid	-
CA 8.2.1/005	[redacted] 1990	Glyphosate technical	*Oncorhynchus mykiss*	Acute / static	>87.7 (gm)	87.7	Valid with restrictions	pH issue (pH of 5.6 at 87.7 mg/L with no mortality)
CA 8.2.1/006	[redacted] 1981	Glyphosate IPA-salt	*Salmo gairdneri (Oncorhynchus mykiss)*	Acute / static	>463 (nom)	463	supportive	No analytical test verifications, exposure cannot be confirmed. Other small deviations (pH, fish lengths)
CA 8.2.1/007	[redacted] 1978	Glyphosate technical	*Salmo gairdneri (Oncorhynchus mykiss)*	Acute / static	71.4 (nom)	34.9 (nom)	supportive	No analytics. pH issue
CA 8.2.1/008	[redacted] 1972	Glyphosate acid (CP 65573)	*Oncorhynchus mykiss* *Lepomis macrochirus*	Acute / static	-	-	Not reliable	No analytics Dissolved oxygen <60%
CA 8.2.1/009	[redacted] 1995	Glyphosate acid	*Lepomis macrochirus*	Acute / static	**>32 (nom)**	32	Valid with restrictions	pH issue (pH outside the recommended range at all tested concentration. Endpoints set at the highest

								dose without mortality)
CA 8.2.1/010	[redacted] 1991	Glyphosate technical	*Lepomis macrochirus*	Acute / static	>119 (gm)	119	Supportive	Results can not be considered for acute risk assessment as fish are bigger than recommended. pH issue (endpoint set at highest concentration without effets)
CA 8.2.1/011	[redacted] 1981	Glyphosate IPA-salt	*Lepomis macrochirus*	Acute / static	-	-	Invalid	No analytics. Dissolved oxygen <60%
CA 8.2.1/012	[redacted] 1978	Glyphosate technical	*Lepomis macrochirus*	Acute / static	>100, < 140 (nom)	100	supportive	No analytical test verifications, exposure cannot be confirmed
CA 8.2.1/013	[redacted] 2006	Glyphosate acid	*Cyprinus carpio*	Acute / semi-static	> 100 (nom)	100	Valid	-
CA 8.2.1/014	[redacted] 1973	Glyphosate acid	*Cyprinus carpio*	Acute / static	115	-	Not assessed	No study report available. Data from RAR (2015)
CA 8.2.1/015	[redacted] 2000	Glyphosate technical	*Brachydanio rerio (Danio rerio)*	Acute / semi-static	123 (nom)	56	supportive	Insufficient analytical test verifications, exposure cannot be confirmed
CA 8.2.1/016	[redacted] 1993	Glyphosate IPA-salt	*Leuciscus idus*	Acute / static	> 2282 (nom)*	2282*	supportive	Not listed in the recommended species of OECD 203. Sensitivity of individuals of that size size (5.90 cm) is not known.
CA 8.2.1/017	[redacted] 1998	AMPA	*Oncorhynchus mykiss*	Acute / static	**> 100 (nom)**	100	Valid	-
CA 8.2.1/018	Anonymous, 1994	AMPA	*Oncorhynchus mykiss*	Acute / static	>180	8	Not assessed	No study report available.

								Study of DAR 2001. Not mentioned in RAR (2015)
CA 8.2.1/019	[redacted] 1991	AMPA	*Oncorhynchus mykiss*	Acute / static	520	-	invalid	analytical results not found in separate report [redacted]-90-403, no validation data for analytical method was available (see Volume 3 (AS) B.5)
CA 8.2.1/020	[redacted] 1993	AMPA	*Oncorhynchus mykiss*	Acute / static	> 180 (nom)	18	Valid	-
CA 8.2.1/021 Literature data	Antunes *et al*., 2017.	Glyphosate	*Poecilia reticulata*	Acute / static	68.78 mg/L (male) 70.87 mg/L (female)		Relevant and reliable with restrictions	No analytical verification. Mature individual used.
		AMPA	*Poecilia reticulata*	Acute / static	180 mg/L (male) 164.3 mg/L (female)		Relevant and reliable with restrictions	No analytical verification. Mature individual used.
				Sublethal concentrations of glyphosate and metabolite AMPA induced severe damage to the liver and gills of the guppies.				
CA 8.2.1/022 CA 8.2.1/023 Literature data	Gholami *et al*., 2013.	glyphosate	*Cyprinus carpio*	Acute / static	6.75 mg/L		Relevant and reliable with restrictions	No analytical verification. Control mortality not reported (validity of results questionable).
				Cholinesterase activity was inhibited in the fingerlings treated with sublethal concentrations of glyphosate.				

a.e.: acid equivalents
nom: nominal, gm : geometric mean measured
Endpoint in **bold** is used for risk assessment
*to consider as additionnal endpoint as sensitivity of individuals of 5.90 cm is unknown

From the literature data available on acute toxicity to fish (see Appendix to Volume 3 CA B.9 on general literature data on ecotoxicology), the lowest LC_{50} value is obtained with the study of Gholami et al., 2013 (CA 8.2.1/022 and CA 8.2.1/023, see summary in Appendix to Volume 3 CA B.9 on general literature on ecotoxicology). In this study the 96h-LC_{50} for common carp fingerlings was found to be 6.75 mg glyphosate/L. However, the study is considered of low reliability given that the control mortality was not reported so that it is difficult to know the sensitivity of the individuals. Given that the lowest regulatory

acute RAC is 0.32 mg/ (based on 96h-LC_{50} of 32 mg/L on *Lepomis macrochirus*), the potential most sensitive species can be considered to be covered by this acute RAC.

Table B.9.4-2: Studies on chronic toxicity to fish of glyphosate and metabolites

Annex point	Study	Substance(s)	Test species	Study type	NOEC (mg a.e./L)	Status	Remark
CA 8.2.2.1/001	[redacted] 2010	Glyphosate acid	*Oncorhynchus mykiss*	Chronic, flow-through	-	Valid but endpoint not reliable	Not to be used as critical endpoint as only 2 replicates were used See RMS comment in study summary
CA 8.2.2.1/002 CA 8.2.2.1/003	[redacted] 2000 [redacted] 2020	Glyphosate acid	*Brachydanio rerio*	Chronic, semi-static	**1 (nom)**	Valid	-
CA 8.2.2.1/004	[redacted] 2011	AMPA	*Pimephales promelas*	Chronic, flow-through	12 (mm)	Valid	- (data gap : A statistical power analysis as presented in appendix 5 of the OECD 210 guideline)
CA 8.2.2.1/005 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Rodrigues *et al.*, 2019*).*	*Glyphosate*	*Danio rerio* embryo	acute toxicity to zebrafish embryos	96h-LC50 > 100 mg/L	Relevant and reliable with restrictions	No analytical verification
		AMPA	*Danio rerio* embryo	acute toxicity to zebrafish embryos	96h-LC50 > 100 mg/L	reliable with restrictions	No analytical verification
		Morphological abnormalities (from 10 mg/L to 100 mg/L), including pericardial and yolk sac edemas, spinal curvature, head and tail deformities in different exposure times; not statistically significant.					
CA 8.2.2.1/006 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Schweizer *et al.*, 2019.	glyphosate	*Danio rerio* embryo	Based on OECD 236 Acute toxicity to zebrafish embryos.	LC_{50} (96 hpf): 98.4 mg a.s./L (unbuffered medium)	reliable with restrictions	Fertilisation rate of the batch of eggs not reported. No analytical verification

				Heart rates: EC10 = 7.27 mg a.s./L. Hatching rate: 96 hpf -EC10 and EC50 = 26.2 mg a.s./L and 37.9, respectively. Developmental delays: at 24 hpf the EC10 = 21.3 mg a.s./L. Malformations found in embryos of all glyphosate treatments but with rates below 20%. EC10 = 30.2 mg a.s./L.			
CA 8.2.1 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Gaur H. et al. 2019	glyphosate	*Danio rerio* Embryo (5h post fertilisation)		48h-LD50 = 66.04 mg/L	Relevant and reliable with restrictions	No analytical verification
				50 and 100 mg/L glyphosate showed abnormalities like pericardial edema, yolk sac edema and tail bending in the treated embryos. Hatching was significantly delayed at concentrations of 50 mg/mL and above.			
CA 8.2.2, CA 8.2.3,CP 10.2.2, CP 10.2.3 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Uren Webster T. M. et al., 2014	glyphosate	*Danio rerio*	No NOEC 10 mg/L glyphosate reduced egg production but not fertilization rate in breeding colonies. increased early stage embryo mortalities and premature hatching. Effect assumed to be primarily by exposure during gametogenesis.			
CA 8.2.1 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Zhang S . et al., 2017	glyphosate	*Danio rerio*	NOEC for morphological alterations =10 mg/L (epiboly process and body length, eye and head area) NOEC Surface tension of chorion < 1mg/L (not concentration dependant), the study author claims that it is not significant at concentrations below 1mg/L but the data are not shown in this study NOEC hatching rate = 200mg/L (increase with concentration) NOEC larvae abnormality = 10 mg/L			
CA 8.2.2.2/001	Anonym., 1975	Glyphosate acid	*Pimephales promelas*	Chronic, 255 d FFLC, flow-through	25.7	supportive	Analytical method validation not available.

							Indirect quantification of glyphosate. Some parameters show high variability. Statistics not reliable.
CA 8.2.2.3/001 CA 8.2.2.3/002	[redacted] 1989 (part 1) [redacted] 1989 (part 2)	Radiolabelled glyphosate acid	*Lepomis macrochirus*	BCF (part 1): 56 d /flow-through BCF (part 2): 56 d /flow-through	No BCF can be set. Indicative of low potential for bioaccumulation.	Supportive	Cf RMS comment in study summary

Literature data on aquatic vertebrates

Regarding information on literature data for aquatic organisms, please note that RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

In RAR 2015, 24 studies were recognised as supporting information for aquatic vertebrates. The synthesis of these supportive information is reported here:
Various studies deal with sub-lethal endpoints such as histological alterations of gill, liver and further organ tissues, such as neurotoxic endpoints and genetic biomarkers (Guilherme et al., 2010, Salbego et al., 2010; Soso et al., 2007; De Menezes et al., 2011; Kreutz et al., 2011; Cavalcante et al., 2008; Ferreira et al. 2010; Cattaneo et al., 2011; Modesto et al., 2010).
In a few studies (Evrard et al., 2010; Langiano et al., 2008) histological alterations in the gills and liver or in liver gene expressions or in methionine metabolism, lipid transport and metabolisms related to oxidative stress were observed. Most of these endpoints measured can be taken as early warning indicators of genotoxic and oxidative stress at the individual level but could not be used in traditional environmental risk assessment, which takes into account the population levels. Moreover, a few alterations like the enhancement of stress related genes and enzymes are of general character since linked to the metaboolic response towards abiotic and biotic factors of the experimental environment. In most cases they are not considered to be life-threatening or have evident effects on population level. In cases where strong histological changes were observed, which might lead to impaired organ functioning (e.g Zhidenko et al., 2007; Ortiz-Ordoñez et al., 2011), the commercial formulation tested was likely to contain POEA as surfactant. The toxicological studies testing the the commercial formulation Roundup® are of limited validity regarding effects of glyphosate-based formulations that do not contain POEA. Although Roundup as the most important herbicide formulation world-wide has been tested frequently, most of the authors have not stated exactly the contents of acid equivalents, POEA or other surfactants in the formulation used. Concerns on side-effects of glyphosate formulations containing POEA as surfactants raised in particular early studies (Folmar et al., 1979, Smith et al., 2004, Haller et al., 2003), wheras testing on technical grade glyphosate have seldom been conducted. One example for a test with glyphosate technical is the study by Tierney et al. (2006), who evaluated the effect of relatively low doses of glyphosate on the olfactorial sense of salmons.
Several studies investigated changes in the metabolic and enzymatic state in aquatic organsims (Fan, et al. 2013, Sandrini, et al 2013, Syedkolaei, et al. 2013, Gholami-Seyedkolaei, et al. 2013). It seems that theses changes in biochemical parameters could be used as biomarkers, because a dose-response association

between commercial formulation treatment and enzymtic activity was found in the different tissues. For an adequate appreciation of the ecological relevance of biochemical, metabolic and histopathological effects, their impact on population structure and function remains to be elucidated further. All studies have been supporting character for traditional environmental risk assessment, because the concentrations tested are exceeding the environmental concentrations of the active ingredient and endpoints are covered by the risk assessment.
Many tests using fishes were conducted in order to investigate the genotoxic and cytotoxic potential of glyphosate towards different aquatic organisms (Nwani et al. 2013, Moreno,et al. 2014, De Souza Filho, et al. 2013, de Castilhos Ghisi, et al. 2012, Vera-Candioti, et al. 2013, Guilherme, et al. 2012 and 2014). Most of the studies were performed with ecologically realistic concentration of the herbicide. Nevertheless, in most cases, again commercial formulations have been used which do not allow to discriminate which compound of the commercial formulation could be responsible for the observed effects. It has also been reported that glyphosate itself caused oxidative DNA damage in cells of A. anguilla exposed under laboratory conditions (Guilherme et al., 2012). At present, it seems evident that more information is required to understand and clarify the risk of genotoxicity of glyphosate containing herbicides.
RAR 2015 further stated that these results revealed that both glyphosate itself as well as the formulated products should be carefully monitored considering their potential impact on aquatic biota. It was suggested that a transition from traditional ecotoxicological methods determining acute toxicity with endpoints on mortality and reproduction can be complemented by far subtler methods taking into account biochemical parameters, but the studies available had limited value to conclude on the relevance on the population level. None of the studies that were evaluated in detail reported the statistical power of the respective test design. There were no acute mortality endpoints on fish reported in the peer-reviewed open literature that raise particular new concerns compared to the standard studies submitted with the notification of the active substance glyphosate. Most studies were conducted with commercially available formulations that did not allow for keeping apart the effects of the parent active substance glyphosate, its metabolites and the surfactants.

Here below is an overview of the studies on fish retrieved in the literature review 2020 that were considered relevant and reliable/reliable with restrictions after detailed assessment by RMS. When endpoints relevant for the risk assessment or information useful for weight of evidence are available from these articles, the results are reported in the tables B.9.4-1 to B.9.4.-2. Please note that RMS identified some studies in Volume 3 CA B.9 under the table “List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant” and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

Antunes, A. M. et al., 2017, assessed acute mortality on mature guppies *Poecilia reticulata*. The sensitivity of juveniles might not be covered by this study. Sublethal concentrations of glyphosate and metabolite AMPA induced severe damage to the liver and gills of the guppies. Morphological changes on gills seem to be defense responses in the gills (proliferation of the interlamellar epithelium, partial/total fusion of the secondary lamellae, edema). The study suggests they may affect the breathing process leading to hypoxia. Histopathological changes in gills were similar for the males and females. The liver showed mainly regressive changes, such as steatosis, pyknotic nuclei and high distribution of collagen fibers. The liver response was different between the genders. The hepatic inflammatory changes were more common in males. The study is considered as relevant and reliable with restriction as no analytical verifications of test concentrations were reported.

Gholami, S.J. et al., 2013, investigated effects of lethal concentrations and sublethal concentrations (determined by acetylcholinesterase assay) of glyphosate on fingerlings of the common carp (Cyprinus carpio, Linnaeus, 1758). Cholinesterase activity was inhibited in the fingerlings treated with sublethal concentrations of glyphosate. Respiratory disorders were observed (on fingerlings exposed to glyphosate). This study states that durations of exposure to the pesticides had greater effects on the treated fingerlings than their concentrations.

Rodrigues, L.B. et al.,, 2019, assessed the acute toxicity and genotoxicity of the glyphosate based formulation Atanor 48 (ATN) and its major constituents glyphosate, surfactant polyethoxylated tallow amine (POEA), as well as the metabolite of glyphosate AMPA, on fish embryo. Acute toxicity test conducted with zebrafish (Danio rerio), while genotoxic effects were investigated in the comet assays with cells from zebrafish larvae and rainbow trout gonad-2 (RTG-2). Glyphosate and AMPA caused no acute toxic effect (LC50-96 h > 100 mg/L) in zebrafish. Glyphosate induced some morphological abnormalities (from 10 mg/L to 100 mg/L), including pericardial and yolk sac edemas, spinal curvature, head and tail deformities in different exposure times; however, these malformations were not statistically significant when compared to their respective negative control. Potential effects on hatching were not investigated. No analytical verification of test concentrations were reported, RMS considers this study as reliable with restrictions.

Schweizer, M. et al, 2019, aims to differentiate the effects of glyphosate-induced acidification of the medium and those exerted by the compound itself (independent of low pH) on embryonic and early larval development of Danio rerio. Acute endpoints based on developmental delay and heart rate are not directly in the scope of EU risk assessment for Annex I renewal purposes. However a potential adverse effect on these parameters may indirectly represent an adverse effect on fish populations in natural conditions. The results from this study are considered reliable with restrictions (no analytical verification) and are reported in the table of endpoints above. Globally the study demonstrates that the severe effects detected seemed to be mainly caused by a low (glyphosate induced) pH, the compound glyphosate itself affects embryonic development in Danio rerio at a sublethal level.

Gaur H. et al., 2019, investigated effect on the hatching rate and mortality of zebrafish embryo. Zebrafish embryos treated with 50 and 100 mg/L glyphosate showed abnormalities like pericardial edema, yolk sac edema and tail bending in the treated embryos. Hatching was significantly delayed in zebrafish embryos exposed to glyphosate at concentrations of 50 mg/mL and above. Glyphosate significantly reduced the heartbeat in a time and concentration-dependent manner indicating cardiotoxicity. The results from this study are considered reliable with restrictions (no analytical verification) and are reported in the table of endpoints above.

In Uren Webster T. M. et al., 2014, 10 mg/L glyphosate reduced egg production but not fertilization rate in breeding colonies. increased early stage embryo mortalities and premature hatching. However, exposure during embryogenesis alone did not increase embryo mortality, suggesting that this effect was caused primarily by exposure during gametogenesis. No NOEC could be determined, then this study provides no endpoint usable for the risk assessment. The study authors claim that early stage mortality was not the result of direct toxicity of the chemical exposure on embryos. Their assumption is based on the fact that exposed embryos originating from a control population of untreated adults exposed at concentrations of up to 10 mg/L of Roundup and 10 mg/L glyphosate had no effect on embryo survival at <3.5 or 3.5−24 hpf. However RMS notes that the chosen glyphosate concentration of 10 mg/L is clearly above the NOEC based on mortality on zebrafish of 1 mg/L ([redacted] 2000 where mortality was of 26.7% at the tested concentration (nominal) of 10 mg/L).

Zhang S. et al., 2017, investigated the effects of glyphosate on early development of larval zebrafish via morphological, biomechanics, behavioral and physiological analyses. The following was stated:

NOEC for morphological alterations =10 mg/L (epiboly process and body length, eye and head area)
NOEC Surface tension of chorion < 1mg/L (not concentration dependant), the study author claims that it is not significant at concentrations below 1mg/L but the data are not shown in this study
NOEC hatching rate = 200mg/L (increase with concentration)
NOEC larvae abnormality = 10 mg/L

A 48-h locomotion test revealed that embryonic exposure to glyphosate significantly elevated locomotor activities especially at 0.01-1 mg/L. The study authors hypothetised that the decreased surface tension of

chorion and the increased locomotive activities may contribute to the hatching rates after glyphosate treatment. The study is relevant and reliable with restrictions.

Here below are listed the studies retrieved in the literature review 2020 that were considered less relevant and considered in a weight of evidence assessment.

Lopes F. M. et al., 2014, investigated the effect of glyphosate on sperm quality of the fish Danio rerio after 24 and 96 h of exposure at concentrations of 5 mg/L and 10 mg/L. No significant differences in sperm concentration were observed. Sperm motility and the motility period were reduced after exposure to both glyphosate concentrations during both exposure periods. The mitochondrial functionality and membrane and DNA integrity were also reduced at the highest concentration during both exposure periods. The test item is not clearly defined (formulation or active substance). No analytical verification was available.The study is considered less relevant but supplementary (due to the uncertainty on the test item) and reliable with restrictions.

Sulukan E. et al., 2017 assessed the effects of a glyphosate containing formulation (not identified) on enzyme activity of carbonic anhydrase, production of reactive oxygen species, cell apoptosis and body morphology during the embryonic development of zebrafish. Embryos were exposed. The survival rates, hatching rates, body malformations under the stereo microscope were evaluated. The main objective was to explain the underlying mechanism of the abnormalities. ROS, enzyme activity of carbonic anhydrase and cellular death were detected end of the 96th hour. The data obtained show that glyphosate treatment inhibited CA activity, caused production of ROS especially branchial regions, triggered cellular apoptosis and caused several types of malformations including pericardial edema, yolk sac edema, spinal curvature and body malformation in a dose-dependent manner. The study authors associate the observed body malformations with cellular apoptosis caused by ROS and inhibition of CA, as a result of glyphosate treatment. These effects were observed even at lowest concentration tested of 1mg/L.
The use of the results to assess the toxicity of glyphosate as formulated in MON52276 is questionable. No analytical verification was conducted. RMS also notes that effects on embryos survival was not concentration dependant and were at comparable (high) levels among all tested concentrations (except control). Hatching success seems also high, 100% success at 100 mg/L. RMS doubts the reliability of the results on these parameters. The study nevertheless showed significant effects on malformations (concentration dependant including lowest concentration of 1 mg/L), indicating that zebrafish embryos are sensitive to glyphosate exposure. RMS considers that this study is less relevant but supplementary (due to formulation issue) and reliable with restrictions for use in risk assessment purpose.

Table B.9.4-3: Studies on acute toxicity to aquatic invertebrates of glyphosate and metabolites

Annex point	Study	Substance(s)	Test species	Study type	LC_{50} (mg a.e./L)	NOEC (mg a.e./L)	Status	Remark
CA 8.2.4.1/001	[redacted] 2003	Glyphosate K - salt	*Daphnia magna*	48 hour acute static	278 am	148.8	Valid	-
CA 8.2.4.1/002	[redacted] 2000	IPA salt	*Daphnia magna*	48 hour acute static	> 471 im	471	Valid	-
CA 8.2.4.1/003	[redacted] 2000	Glyphosate technical	*Daphnia magna*	48 hour acute static	>334 im	179.56	Valid with restriction	pH issues (endpoints set at doses without mortality/effects)
CA 8.2.4.1/004	[redacted] 1996	Glyphosate acid	*Daphnia magna*	48 hour acute static	>100 nom	100	Valid with restriction	pH issues (endpoints set at doses without mortality/effects)

CA 8.2.4.1/005	[redacted] 1995	Glyphosate acid	*Daphnia magna*	48 hour acute	**40**	18	Not assessed but used as critical for Daphnids	Report not available Cf RMS comment in study summary The endpoint measured in this study is the lowest acute toxicity endpoint for Daphnia magna.
CA 8.2.4.1/006	[redacted] 1995	Glyphosate	*Daphnia magna*	48 hour acute static	> 100 nom	100	Valid	-
CA 8.2.4.1/007	[redacted] 1994	IPA salt	*Daphnia magna*	48 hour acute static	> 45.64 nom	45.64	Valid	-
CA 8.2.4.1/008	[redacted] 1993	IPA salt	*Daphnia magna*	48 hour acute	>1000	-	Not assessed	Report not available Data from RAR (2015)
CA 8.2.4.1/009	[redacted] 1990	Glyphosate technical	*Daphnia magna*	48 hour acute static	>62.5 nom	62.5 nom	Valid with retsriction	pH issue Endpoints set at doses with no effects due to impact of pH
CA 8.2.4.1/010	[redacted] 1981	IPA salt	*Daphnia magna*	48 hour acute	581	200	Supportive	No analytical verification of test concentrations
CA 8.2.4.1/011	[redacted] 1978	Glyphosate	*Daphnia magna*	48 hour acute	-	-	Not reliable	No analytical verification of test concentrations. No pH values available.
CA 8.2.4.1/012	[redacted] 1998	AMPA	*Daphnia magna*	48 hour acute static	> 100 nom	100	Valid	-
CA 8.2.4.1/013	[redacted] 1994	AMPA	*Daphnia magna*	48 hour acute static	**>180 nom**	180	Valid	-
CA 8.2.4.1/014	[redacted] 1991	AMPA	*Daphnia magna*	48 hour acute static	690 nom	320	Supportive	Analytical separate report (ML-90-403/EHL-90187-Daphnia) with no results reported on analytics. No validation data for analytical method was available (see Volume 3 (AS) B.5).
CA 8.2.4.1/015	[redacted] 2011	HMPA	*Daphnia magna*	48 hour acute static	**>100 nom**	100	Valid	-

CA 8.2.4.2/001	[redacted] 1996	Glyphosate acid	*Mysidopsis bahia*	96 hour acute static	80 nom	32	Valid with restriction	pH issue (endpoints based with pH of 6 at 100mg/L and 4.5 at 180 mg/L)
CA 8.2.4.2/002	[redacted] 1978	Glyphosate	*Mysidopsis bahia*	96 hour acute	-	-	Not reliable	No analytical verification of test concentrations. Only one replicate per treatment. Age of shrimps (6-8 days old). Temperature at 20°C. heterogenous salinity. Low dissolved oxygen.
CA 8.2.4.2/003	[redacted] 1996	Glyphosate acid	*Crassostrea gigas*	48 hour acute	**40 nom**	32	Valid	-
CA 8.2.4.2/004	[redacted] 1985	Glyphosate technical	*Crassostrea gigas*	48 hour acute	-	-	Not reliable	No analytical verification of test concentrations. No information about dissolve oxygen. pH values not available.
CA 8.2.4 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Demetrio P. M. et al., 2012	glyphosate	*Hydra attenuate*	Acute, 96 h	18.2 (LC50)	-	Reliable with restrictions	Results insufficiently detailed.
CA 8.2.8 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Mottier A. et al., 2013	glyphosate	*Crassostrea gigas*	Acute, 48h	>100 (LC50)		reliable	-
				EC50 = 27.1 (Abnormality rates in D-shaped larvae). EC10 = 13.457,				
		AMPA	*Crassostrea gigas*	Acute, 48h	>100 (LC50)		reliable	-
				EC50 = 46.1 (Abnormality rates in D-shaped larvae,). The EC10 = 10.299 mg/L				
CA 9 Literature data	Xu Yanggui et al., 2017	glyphosate	*Pomacea canaliculata*	Acute, 96 h	174.7 (LC50)	-	Reliable with restrictions	No analytical verification

(see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)								

From the literature data available on acute toxicity to aquatic invertebrates, the lowest LC_{50} value is obtained with the study of Demetrio P. M. et al., 2012 (see summary in Appendix to Volume 3 CA B.9 on general literature on ecotoxicology). In this study the 96h-LC_{50} for *Hydra attenuate* was found to be 18.2 mg glyphosate/L. However, the study is considered of low reliability. Given that the lowest regulatory acute RAC is 0.40 mg/ (based on 48h-LC_{50} of 40 mg/L on *Crassostrea gigas*), the potential most sensitive species can be considered to be covered by this RAC value.

Table B.9.4-4: Studies on chronic toxicity to aquatic invertebrates of glyphosate and metabolites

Annex point	Study	Substance(s)	Test species	Study type	EC_{50} (mg a.e./L)	NOEC (mg a.e./L)	Status	Remark
CA 8.2.5.1/001	[redacted], 1999	Glyphosate acid	*Daphnia magna*	21 d Reproduction semi-static	100 nom	**12.5**	Valid	-
CA 8.2.5.1/002	[redacted] 1995	Glyphosate	*Daphnia magna*	21 d Reproduction semi-static	> 100 nom	56	Valid with restriction	pH issue (pH of 5-6 at 100 mg/L, impact on endpoint considered low)
CA 8.2.5.1/003	[redacted] 1993	IPA salt	*Daphnia magna*	21 d Reproduction semi-static	267.93 nom	42.90	Valid	-
CA 8.2.5.1/004	[redacted] 1990	Glyphosate	*Daphnia magna*	21 d Reproduction semi-static	-	EC10 = 22.65 nom	Valid	-
CA 8.2.5.1/005	[redacted] 1989	Glyphosate	*Daphnia magna*	21 d Reproduction semi-static	> 100 nom	100	Valid	-
CA 8.2.5.1/006	[redacted] 1982	Glyphosate	*Daphnia magna*	21-day flow-through	-	41 am	Valid	-
CA 8.2.5.1/007	[redacted] 2011	AMPA	*Daphnia magna*	21 d Reproduction semi-static	-	**Reproduction: 15 nom**	Valid	-

CA 8.2.5.3/001	[redacted] 2020	Glyphosate acid	*Chironomus riparius*	Water spiked	-	1000	Supportive	No analytical verification in sediment. No report for analytical method was available (see Volume 3 (AS) B.5)
CA 8.2.4 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Avigliano L. et al., 2014	glyphosate	*Cherax quadricarinatus* juveniles	Chronic, 60 days, semi-static	-	33 % mortality at 40 mg/L of glyphosate; 35% decrease in weight gain at 40 mg/L.	Reliable with restrictions	Results insufficiently detailed.
CA 8.2.4 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Avigliano L. et al., 2018	glyphosate	*Neohelice granulate* adult females	Chronic, 3-month pre-reproductive period	-	NOEC < 0.02 mg/L for body weight gain	Reliable with restrictions	Results insufficiently detailed.
CA 9 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Canosa I. S. et al., 2019	glyphosate	*Neohelice granulate* adult males	Chronic, 30 d	-	NOEC < 1.27 mg/L for body weight gain	Reliable with restrictions	Results insufficiently detailed.

a.e.: acid equivalents
nom: nominal
Endpoint in **bold** is used for risk assessment.

Based on its fate characteristics, glyphosate and AMPA are considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint.

However according to the current EFSA guidance on aquatic organisms (2013) and the EFSA opinion on sediment organisms (2015), sediment toxicity studies are triggered when the water–sediment study indicates that > 10 % of the applied radioactivity is present in the sediment at or after day 14 and the outcome of a chronic Daphnia test (or another comparable study with insects) results in an EC10 (or NOEC) < 0.1 mg/L. Since the lowest chronic Daphnia endpoint is greater than 0.1 mg/L, this study is not considered necessary for risk assessment purpose.
However for compliance with the EU Reg 283/2013, further information to assess the effects of glyphosate and AMPA on sediment dwelling organisms is required (data gap).
Moreover, in relation with e-fate data gap, further information to assess the risk assessment for metabolite 1-oxo-AMPA for sediment dwelling organisms is necessary. For details, please refer to Volume 3 CA B.8 point B.8.2.2.5.

Literature data on aquatic invertebrates

In RAR 2015, 18 studies were recognised as supporting information for **aquatic invertebrates**. Most of the cited studies were performed with formulated products and not with the active ingredient alone. Those studies, which investigated the effect of glyphosate itsef or the glyphosate IPA-salt obtained LC50 values ranging from 49.3 mg acid equivalents /L for the marine copepod Acartia tonsa to 415 mg acid equivalents /L for the cladoceran Ceriodaphnia dubia (Tsui, 2003; Le, 2010; Tsui et al., 2004; Dominguez-Cortinas et al., 2008; Bringolf et al., 2007; Mottiera et al., 2013; Frontera, 2011). However, more sensitive species like the coelenterate Hydra attenuata showed lower sensibility and LC50 values were determined to be 18.2 mg/L for the active ingredient glyphosate. These organisms are generally not considered in Tier 1 risk assessment, but is was shown that they are exposed to toxicants to a higher extent due their anatomical and physiological structure (Demetrio, 2012). Moreover, sublethal effects were observed at much lower concentrations of glyphosate in comparison to lethal effects (Mottiera, 2013).
In general, the formulations are of higher ecotoxicological relevance than the active ingredient glyphosate itself. One of the main commercial formulations is Roundup ®, which in addition to the active ingredient glyphosate contains polyoxyethoxylated alkylamines (POEA) as a surfactant. A few studies investigate the effects of the formulation versus the surfactant POEA. These studies have shown that formulations containing POEA are several times more toxic (3 to 5 fold more toxic than Roundup®) to aquatic invertebrates than the active ingredient glyphosate acid or formulations without POEA.
There were no critical data in the literature review of RAR 2015 that could directly be included in the environmental risk assessment for the active substance glyphosate.

Here below is an overview of the studies retrieved in the literature review 2020 that were considered relevant and reliable/reliable with restrictions after detailed assessment by RMS. When endpoints relevant for the risk assessment are available from these articles these are reported in the tables B.9.4-3 and -4. Please note that RMS identified some studies in Volume 3 CA B.9 under the table “List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant” and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

Avigliano L. et al., 2014, assessed the effects of sublethal concentrations of glyphosate on early juvenile of the crayfish Cherax quadricarinatus, in terms of growth rate, metabolic rate and energy reserves levels, to determine how glyphosate affects the activity level of key metabolic enzymes, such as pyruvate kinase and to determine the levels of both alanine and aspartate aminotransferase activities (ALAT and ASAT respectively) as indicative of tissue damage. The highest mortality value (33 %) was seen in animals exposed to 40 mg/L of glyphosate; A significant decrease in weight gain (35 % lower than control) was seen after the first month of exposure to 40 mg/L of glyphosate. Significant decrease in total protein content in both muscle, at 40 mg/L, and hepatopancreas, at both assayed concentrations. Besides, a significant decrease in total lipid content was observed in muscle. At the 10 mg/L exposure, muscle pyruvate kinase

activities were significantly lower (while no differences were seen in the hepatopancreas. Both lipids and proteins are closely involved with the energy available for crustacean growth. This study states that glyphosate is able to reduce growth rates and protein and lipid reserves in chronically exposed (60 days, semi-static, concentrations were maintained) early juvenile crayfish at concentrations of 40 mg/L. Some effects (decrease in protein reserves in hepatopancreas and an apparent metabolic depression in muscle) were observed at 10 mg/L. Overall, RMS considers this study as relevant and reliable with restrictions.

Avigliano L. et al., 2018, exposed adult females of the estuarine crab Neohelice granulata during the 3-month pre-reproductive period (winter) to the herbicide glyphosate, at three different concentrations (0.02, 0.2, and 1 mg/L, as active ingredient). A decrease in the body weight gain on adult female crab was observed by effect of pure glyphosate, at all concentrations assayed (NOEC < 0.02 mg/L). It is likely due to treatment but does not appear concentration related. Concentrations were analytically verified but only graphs were presented. Concerning the potential impact of using wild-caught organisms, RMS then cannot discard the presence of other toxicants in the estuary from which these were caught. The results are reliable with restrictions.

Canosa I. S. et al., 2019, exposed males of the estuarine crab (Neohelice granulate) to pure glyphosate. The in vivo assays comprised the exposure for 30 d to 1 mg/L of the herbicide, until finally assessing weight gain, levels of energy reserves, sperm number per spermatophore, proportion of abnormal spermatophores, and sperm viability. Overall, decrease in weight gain and muscle protein levels and higher incidence of abnormal spermatophores may be attributed to glyphosate at the concentration of 1.27 mg/L. Concentrations were analytically verified. Concerning the potential impact of using wild-caught organisms, RMS then cannot discard the presence of other toxicants in the estuary from which these were caught. The results are reliable with restrictions. The study is considered reliable with restrictions (for effects on bodyweight gain, not reliable for endocrine properties). RMS however notes that only bodyweight gain is reported not bodyweight itself. So the magnitude of the effect is uncertain and potentially low.

Demetrio P. M. et al., 2012, assessed the lethal effects of glyphosate and glyphosate formulation Roundup® Max on the Hydra attenuate (96 hours). This study indicates relative sensitivity of this species. (96h-LC50 glyphosate a.i =18.2 mg a.i/L, 96h-LC50 RoundupMax® =21.8 mg a.i/L (considered less relevant by RMS due to the different formulation tested)). The study seems well conducted (despite the absence of specific guideline) however there are no details of biological observations reported in the paper. Thus, the observed mortality and the LC50 calculation cannot be confirmed by RMS. This study is reliable with restrictions.

Mottier A. et al., 2013, assessed the toxicity of glyphosate, AMPA and two commercial formulations, Roundup Express® (REX) and Roundup Allées et Terrasses® (RAT), containing glyphosate as the active ingredient, on the early life stages of the Pacific oyster, Crassostrea gigas (marine species). This is an embryotoxicity bioassay. The EC50 values were 27.1 and 46.1 mg/Lfor glyphosate and AMPA, respectively for the parameter development (Abnormality rates in D-shaped larvae, measured concentrations). The EC10 values were 13.457 and 10.299 mg/L for glyphosate and AMPA.

Xu Yanggui et al., 2017, investigated the effect of glyphosate an alien invasive species, the golden apple snail Pomacea canaliculata in China. Snails were kept in the water. An endpoint for mortality was set : 96h LC50 = 174.7 mg/L (95% CI: 174.7-175.6). Long-term exposures to glyphosate at 20 and 120 mg/L caused inhibition of food intake, limitation of growth performance and alterations in metabolic profiles of the snail. Glyphosate at 2 mg/L benefited growth performance in P. canaliculata. The study is considered reliable with restrictions.

Here below are listed the studies retrieved in the literature review 2020 that were considered less relevant but supplementary (studies performed with a formulation, relation to the EU representative formulation not defined). None of them was considered sufficiently relevant/reliable for a use in a quantitative risk assessment. These may only be considered in a weight of evidence assessment:

- Mugni H. et al., 2014, assessed the acute toxicity of Roundup Full II® (66.2% glyphosate), to *Hyalella curvispina* in laboratory and field assessments. The mean estimated 48-h LC50 of Roundup Full II® was 9.9 ± 1.7 mg/L. This LC50 value seems low in comparison with the overall dataset available for aquatic invertebrates from regulatory studies. RMS also notes that the lowest LC1 was of 3.8 mg/L (lowest of 6 independent experiments) which may be assimilated to a NOEC, LC10 was 5.5 mg/L. 6 independent assays showed high reproducibility. The study design seems adequate and the results seems robust. However, no biological data are presented in the study report (only LCx values).
  In a field experiment Roundup Full II® was applied to soybean plots. Simulated rain was generated the following day by means of irrigation sprinkler equipment. H. curvispina was exposed to runoff water and soy leaves. No mortality was observed.
  The study states that further studies are needed for juveniles, likely to be more sensitive. This study is considered less relevant but supplementary (due to the different formulation tested) and reliable with restrictions.

- Reno U. et al., 2014, analyzed the acute effects of a glyphosate based herbicide (Eskoba®) on the cladoceran *Simocephalus vetulus*, and the copepod Notodiaptomus conifer, and evaluated the recovery ability of the surviving microcrustaceans. Survival, age of first reproduction, and fecundity were used as endpoints for S. vetulus, while survival and time to reach the adult stage were used as endpoints for N. conifer. The study is considered as less relevant but supplementary (formulation issue).
  S. vetulus: 48-hour EC50 = 21 mg/L
  N. conifer: 48-hour EC50 = 95 mg/L
  In post-exposure experiments, microcrustaceans reduced their life expectancy, S. vetulus decreased its fertility, and N. conifer inhibited its sexual maturity. These results are considered reliable with restrictions.

- Omran N. E. et al., 2016 investigates the response of the snail *Biomphalaria alexandrina* (Mollusca: Gastropoda) as a bioindicator for endocrine disrupters in terms of steroid levels (testosterone (T) and 17b-estradiol (E)), alteration of microsomal CYP4501B1-like immunoreactivity, total protein (TP) level, and gonadal structure after exposure to sublethal concentrations of glyphosate for 3 weeks. According to the study authors, observations on cellular and tissue-level endpoints are relevant for the ED assessment. RMS considers this study as not relevant in the sense of the EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 since results are not based on the active ingredient but to a formulation different than the representative formulation. The dose tested being equivalent to LC10, lower concentrations would have been necessary for investigation of endocrine disruption properties. No analytical confirmation of test concentrations was performed (LC10 was targeted). (for more details refer to appendix to Volume 3 CA B.9 related to literature data on ecotoxicology)
  This study generated a 24 h LC50 value of 41.6 ppm in the snail *B. alexandrina* which is relevant for the aquatic risk assessment. In this study only the formulation "Herfosate" was used and no pure active substance glyphosate. ''Herfosate'' contains 48% w/v of glyphosate IPA, and inert ingredients equal 52% w/v. No additional information is provided on the nature of these co-formulants. No analytical confirmation of test concentrations was performed. Results on mortality are only graphically presented and raw data are not presented.
  In fig 1, mortality is ranged between probit 4-6.5, corresponding to approximately 15-95% mortality. However given that mortality are only graphically presented and raw data are not presented given the LC50 value of 41.6 ppm should be considered with caution together with other available values in a weight of evidence.

Table B.9.4-5: Studies on effects of glyphosate and metabolites to algae

Annex point	Study	Study type	Test species	Substance(s)	Status	Endpoints
CA 8.2.6.1/001	[redacted] 2002	96 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	IPA salt	valid	72h NOErC = 2.21 mg a.e./L (mm) 72h ErC10 = 4.23 mg a.e./L (mm) 72h ErC20 = 7.6 mg a.e./L (mm) 96h NOErC = 4.87 mg a.e./L (mm) 96h ErC10 = 7.11 mg a.e./L (mm) 96h ErC20 = 10.8 mg a.e./L (mm) 96h ErC50 = 23.7 mg a.e./L (mm) 72h NOEyC = 2.21 mg a.e./L (mm) 72h EyC10 = 2.17 mg a.e./L (mm) 72h EyC20 = 3.22 mg a.e./L (mm) 72h EyC50 = 6.85 mg a.e./L (mm) 96h NOEyC = 2.21 mg a.e./L (mm) 96h EyC10 = 3.05 mg a.e./L (mm) 96h EyC20 = 4.19 mg a.e./L (mm) 96h EyC50 = 7.63 mg a.e./L (mm)
CA 8.2.6.1/002	[redacted] 2002	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	Glyphosate K-salt	invalid	-

CA 8.2.6.1/003 CA 8.2.6.1/004	[redacted] 2000 [redacted] 2020	96 h algae inhibition	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	Glyphosate technical	Supportive (No analytical verification of test concentrations throughout the test)	72h NOErC= 5.6 mg a.e./L (nom) 72h ErC10 = 62.6 mg a.e./L 72h ErC20 = 132 mg a.e./L 72h ErC50 = 469 mg a.e./L 72h NOEyC= 5.6 mg a.e./L (nom) 72h EyC10 = 5.54 mg a.e./L 72h EyC20 = 14.6 mg a.e./L 72h EyC50 = 75.9 mg a.e./L
CA 8.2.6.1/005 CA 8.2.6.1/006	[redacted] 1995 [redacted] 2020	120 h algae inhibition	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	Glyphosate acid	valid	72h NOErC = 10 mg a.e./L (nom) 72h ErC10 = 5.74 mg a.e./L (nom) 72h ErC20 = 8.91 mg a.e./L (nom) 72h ErC50 = 17.3 mg a.e./L (nom) 72h NOEyC = 10 mg a.e./L (nom) 72h EyC10 = 4.84 mg a.e./L (nom) 72h EyC20 = 7.59 mg a.e./L (nom) 72h EyC50 = 16.4 mg a.e./L (nom)
CA 8.2.6.1/007	[redacted], 1995	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	Glyphosate	valid	72h NOErC = 32 mg a.e./L (nom) 72h ErC10 = 33 mg a.e./L (nom) 72h ErC50 = 54 mg a.e./L (nom) 72h NOEbC = 10 mg a.e./L (nom) 72h EbC10 = 18 mg a.e./L (nom) 72h EbC50 = 48 mg a.e./L (nom)
CA 8.2.6.1/008	[redacted] 1995	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	Glyphosate	Not reliable (report not available)	-

CA 8.2.6.1/009 CA 8.2.6.1/010	[redacted] 1987 [redacted] 2020	168 h algae inhibition	*Selenasstrum capricornutum* *(Raphidocelis subcapitata)*	Glyphosate technical	valid	72h ErC10 < 10 mg a.e./L (nom) 72h ErC20 = 10.8 mg a.e./L (nom) 72h ErC50 = 20.1 mg a.e./L (nom) 72h EyC10 < 10 mg a.e./L (nom) 72h EyC20 = 10.25 mg a.e./L (nom) 72h EyC50 = 12.11 mg a.e./L (nom)
CA 8.2.6.1/011	[redacted] 1995	72 h algae inhibition	*Desmodesmus subspicatus*	Glyphosate acid	Not assessed. Report not available. Data from DAR (2001) considered relied upon in RAR (2015)	-
CA 8.2.6.1/012	[redacted] 1994	72 h algae inhibition	*Desmodesmus subspicatus*	IPA salt	Not reliable Data from DAR (2001) considered relied upon in RAR (2015)	-
CA 8.2.6.1/013	[redacted] 1993	72 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	IPA salt	invalid	-
CA 8.2.6.1/014	[redacted] 1990	96 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	Glyphosate	invalid	-
CA 8.2.6.1/015	[redacted] 1990	96 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	Glyphosate	Invalid Coefficient of variation for section specific growth rate: > 35%	-
CA 8.2.6.2/001	[redacted] 1996	120 h algae inhibition	*Anabaena flos-aquae*	Glyphosate acid	Not reliable (Correlation between biomass and optical density cannot be demonstrated. Validity criteria can	-

					not be checked)	
CA 8.2.6.2/002 CA 8.2.6.2/003	[redacted] 1987 [redacted] 2020	168 h algae inhibition	*Anabaena flos-aquae*	Glyphosate technical	valid	72h ErC10 = 7.63 mg a.e./L (nom) 72h ErC20 = 12.7 mg a.e./L (nom) 72h ErC50 = 33.4 mg a.e./L (nom) 72h EyC10 = 9.97 mg a.e./L (nom) 72h EyC20 = 11.8 mg a.e./L (nom) 72h EyC50 = 16.4 mg a.e./L (nom) Data gap for 96h endpoints
CA 8.2.6.2/004	[redacted] 1996	120 h algae inhibition	*Navicula pelliculosa*	Glyphosate acid	Invalid Coefficient of variation for section specific growth rate: > 35%	-
CA 8.2.6.2/005	[redacted] 1987	168 h algae inhibition	*Navicula pelliculosa*	Glyphosate technical	Valid	Data gap (EC10, EC20 and EC50 values should be calculated for 72h based on yield and growth rate)

CA 8.2.6.2/006 CA 8.2.6.2/007	[redacted] 1996 [redacted] 2020	96 h algae inhibition	*Skeletonema costatum*	Glyphosate acid	valid	72h NOErC = 5.6 mg a.e./L (nom) 72h ErC10 = 1.87 mg a.e./L (nom) 72h ErC20 = 2.98 mg a.e./L (nom) **72h ErC50 = 13.5 mg a.e./L (nom)** 72h NOEyC = 5.6 mg a.e./L (nom) 72h EyC10 = 5.22 mg a.e./L (nom) 72h EyC20 = 6.38 mg a.e./L (nom) 72h EyC50 = 8.99 mg a.e./L (nom)
CA 8.2.6.2/008	[redacted] 1987	168 h algae inhibition	*Skeletonema costatum*	Glyphosate technical	Invalid Biomass increase in control cultures: <16 and coefficient of variation for section specific growth rate: > 35%	-
CA 8.2.6.2/009	[redacted] 1978	96 h algae inhibition	*Skeletonema costatum*	Glyphosate intermediate	Invalid No information on validity criteria. No analytical measurments.	-
CA 8.2.6.2/010	[redacted] 1996	96 h algae inhibition	*Nitzschia palea*	Glyphosate technical	Invalid validity criteria not met	-
CA 8.2.6.1/016 CA 8.2.6.1/017	[redacted] 1998 [redacted] 2020	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	AMPA	valid	72h NOErC = 100 mg AMPA/L (nom) 72h ErC10 = 92.8 mg AMPA/L (nom) 72h ErC20 = 119 mg AMPA/L (nom)

						72h ErC50 = 191 mg AMPA/L (nom) 72h NOEyC = 46 mg AMPA/L (nom) 72h EyC10 = 58.2 mg AMPA/L (nom) 72h EyC20 = 72.5 mg AMPA/L (nom) 72h EyC50 = 110 mg AMPA/L (nom)
CA 8.2.6.1/018	[redacted] 1994	72 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	AMPA	invalid	-
CA 8.2.6.1/019 CA 8.2.6.1/020	[redacted] 2011 [redacted] 2020	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	HMPA	valid	72h NOErC = 60 mg HMPA/L (nom) 72h ErC10 >120 mg HMPA/L (nom) 72h ErC20 >120 mg HMPA/L (nom) **72h ErC50 >120 mg HMPA/L (nom)** 72h NOEyC = 60 mg HMPA/L (nom) 72h EyC10 = 57.8 mg HMPA/L (nom) 72h EyC20 = 80.4 mg HMPA/L (nom) 72h EyC50 > 120 mg HMPA/L (nom)

Values in **bold** are the lowest endpoints for the active substance/metabolites based on growth rates.

BTable B.9.4-6: Studies on toxicity of glyphosate to aquatic macrophytes

Annex point	Study	Study type	Test species	Substance(s)	Status	Endpoints
CA 8.2.7/001 CA 8.2.7/002	[redacted] [redacted] 2002 [redacted] 2020	7-day, static	*Lemna minor*	IPA salt	Valid	Frond number 7d NOErC = 8.65 mg a.e./L (nom) 7d ErC10 = 8.16 mg a.e./L (nom)

						7d ErC20 = 12.8 mg a.e./L (nom) **7d ErC50 = 30.3 mg a.e./L (nom)** 7d NOEyC = 8.65 mg a.e./L (nom) 7d EyC10 = 7.8 mg a.e./L (nom) 7d EyC20 = 10.3 mg a.e./L (nom) 7d EyC50 = 16.5 mg a.e./L (nom) Dry weight 7d NOEyC = 8.65 mg a.e./L (nom) 7d EyC10 = 5.72 mg a.e./L (nom) 7d EyC20 = 10.3 mg a.e./L (nom) 7d EyC50 = 32.1 mg a.e./L (nom) Phytotoxicity NOEC = 8.65 mg a.e./L (nom) Data gap: ErCx values based on dry weight
CA 8.2.7/003 CA 8.2.7/004	[redacted] 1999 [redacted] 2020	14-d, semi static	*Lemna gibba*	IPA salt	Not reliable. Actual exposure questionable	-
CA 8.2.7/005 CA 8.2.7/006	[redacted] 1996 [redacted] 2020	14-d, semi static	*Lemna gibba*	Glyphosate acid	Valid	Frond number 7d NOErC = 12 mg a.e./L (nom) 7d ErC10 = 13.3 mg a.e./L (nom) 7d ErC20 = 18.7 mg a.e./L (nom) 7d ErC50 = 36.0 mg a.e./L (nom) 7d NOEyC = 6 mg a.e./L (nom) 7d EyC10 = 10.5 mg a.e./L (nom) 7d EyC20 = 14.2 mg a.e./L (nom) 7d EyC50 = 24.0 mg a.e./L (nom) Phytotoxicity NOEC = 1.5 mg a.e./L (nom)

CA 8.2.7/007 CA 8.2.7/008	[redacted] 1987 [redacted] 2020	14-d, static	*Lemna gibba*	Glyphosate Technical	Valid	Frond number 7d NOErC = 16.6 mg a.e./L (mm) 7d ErC10 = 20.8 mg a.e./L (mm) 7d ErC20 = 31.9 mg a.e./L (mm) 7d ErC50 > 49.4 mg a.e./L (mm) 7d NOEyC = 16.6 mg a.e./L (mm) 7d EyC10 = 18.2 mg a.e./L (mm) 7d EyC20 = 20.3 mg a.e./L (mm) 7d EyC50 = 25 mg a.e./L (mm) Phytotoxicity Not recorded
CA 8.2.7/009	[redacted] 1987	Toxicity to Lemna gibba	*Lemna gibba*	Glyphosate Technical	Invalid (Report not available)	-
CA 8.2.7/010	[redacted] 2012	14-d, static	*Myriophyllum aquaticum*	Glyphosate acid	Invalid coefficient of variation for yield based on measurements of shoot fresh weight > 35%	-
CA 8.2.7/011	[redacted] 2012	14-d static	*Myriophyllum aquaticum*	AMPA	Valid	Shoot length 14d NOErC = 14.3 mg AMPA/L (mm) 14d ErC10 = 6.1 mg AMPA/L (mm) 14d ErC20 = 22.5 mg AMPA/L (mm) 14d ErC50 > 94.6 mg AMPA/L (mm) 14d NOEyC = 5.43 mg AMPA/L (mm) 14d EyC10 = 1.3 mg AMPA/L (mm) 14d EyC20 = 5.8 mg AMPA/L (mm) 14d EyC50 > 94.6 mg AMPA/L (mm) Shoot fresh weight 14d NOErC = 14.3 mg AMPA/L (mm) 14d ErC10 = 24.2 mg AMPA/L (mm)

						14d ErC20 = 39 mg AMPA/L (mm) 14d ErC50 > 94.6 mg AMPA/L (mm) 14d NOEyC = 14.3 mg AMPA/L (mm) 14d EyC10 = 19.7 mg AMPA/L (mm) 14d EyC20 = 30.6 mg AMPA/L (mm) 14d EyC50 = 70.8 mg AMPA/L (mm) Shoot dry weight 14d NOErC = 37.1 mg AMPA/L (mm) 14d ErC10 = 38.4 mg AMPA/L (mm) 14d ErC20 = 47.6 mg AMPA/L (mm) **14d ErC50 = 72 mg AMPA/L (mm)** 14d NOEyC = 37.1 mg AMPA/L (mm) 14d EyC10 = 33.9 mg AMPA/L (mm) 14d EyC20 = 42 mg AMPA/L (mm) 14d EyC50 = 63.2 mg AMPA/L (mm) Root length 14d NOErC = 14.3 mg AMPA/L (mm) 14d ErC10 = 17 mg AMPA/L (mm) 14d ErC20 = 35.9 mg AMPA/L (mm) 14d ErC50 > 94.6 mg AMPA/L (mm) 14d NOEyC = 2.23 mg AMPA/L (mm) 14d EyC10 = 5.1 mg AMPA/L (mm) 14d EyC20 = 9.5 mg AMPA/L (mm) 14d EyC50 = 31.1 mg AMPA/L (mm)
CA 8.2.7/012	[redacted] 2011	7-d, semi-static	*Lemna gibba*	HMPA	Valid	Frond number/biomass dry weight 7d NOECr = 123 mg HMPA/L (nom)

						7d ErC10 > 123 mg HMPA/L (nom) 7d ErC20 > 123 mg HMPA/L (nom) **7d ErC50 > 123 mg HMPA/L (nom)** 7d NOECy = 123 mg HMPA/L (nom) 7d EyC10 > 123 mg HMPA/L (nom) 7d EyC20 > 123 mg HMPA/L (nom) 7d EyC50 > 123 mg HMPA/L (nom)
CA 8.2.7/013 Literature data (see Appendix to Vol 3 CA B.9 on literature data on ecotoxicology)	Yanhui *et al.*, 2015	OECD 221 7-d semi-static	*Spirodela polyrhiza*	Glyphosate	Relevant but reliability not assignable (data gap : provide an English certified translation) Report in chinese. No translation available. no analytical test verifications	7d-EC_{50} = 12.817 mg/L.

Values in **bold** are the lowest endpoints for the active substance/metabolites based on growth rates.

Based on its fate characteristics, glyphosate is considered as persistant in sediment. Thus exposure of rooted aquatic plants is expected. RMS therefore considered that further information to assess the effects of glyphosate on rooted aquatic macrophytes is required (data gap).

RMS noted that glyphosate is only slightly toxic for macrophytes in the available toxicity test. A potential explanation might be that glyphosate was dissolved in the test media while in the case of a contact herbicide the substance should be sprayed to the surface of the test system (see OECD guideline).
Given that glyphosate is a contact herbicide, it could be questioned whether the results of test with glyphosate dissolved in water cover the one resulted from exposure following spraying of glyphosate products. Indeed, dissolving the active substance in the medium could underestimate its toxicity to aquatic plants since it is less efficient in this mode of exposure. This is supported by literature studies as Sesin et al. 2020 “Glyphosate Toxicity to Native Nontarget Macrophytes Following Three Different Routes of Incidental Exposure” (published in septembre 2020, after submission of the active substance and therefore not in the literature review). Therefore RMS considered that there is a need to have results for emergent macrophytes available with a different exposure design (overspray) (data gap).

Literature data on algae and aquatic macrophytes

In RAR 2015, 15 studies were recognised as supporting information for algae and aquatic macrophytes.
For algae treated with glyphosate (technical grade), a wide range of EC50 and IC50 values was found. The EC50 values ranged from 2.3 mg/l for Skeletonema costatum (Tsui, 2003) to 70 mg/L for Scenedesmus

quadricauda (Ma, 2006) and the marine diatom Skeletonema costatum seems to be the most sensitive species towards glyphosate. Regarding macrophytes, similar EC50 values compared to algae were reported in the peer reviewed open literature of RAR 2015. IC50 and EC50 values ranged from 0.22 mg a.s./L for Myriophyllum aquaticum (based on chlorophyll a content in Turgut & Fomin, 2002, 36% a.s. product) to 46.9 mg/L for Lemna minor (Cedergreen & Streibig, 2005).
The literature review in RAR 2015 also provided a few studies that were performed on the natural aquatic community in order to assess indirect effects towards algae. Mesocosm studies showed differences at 6 mg glyphosate containing product/L in the structure of phytoplankton and periphyton assemblages in treated mesocosms compared to controls. Total micro- and nanophytoplankton decreased in abundance, whereas the abundance of picocyanobacteria increased (Perez, 2007). Similar effects were observed by Vera et al. (2010), who could also show that despite the mortality of algae, mainly diatoms, cyanobacteria were favored in treated mesocosms. However, it must be considered that in both studies commercial products containing surfactants were used, and therefore the toxicity is determined by the joint effect of both glyphosate and the surfactants of the commercial formulations. Commercial products containing specific formulation ingredients additionally to the active ingredient were shown to be more toxic towards algae than glyphosate acid (Cedergreen & Streibig, 2005; Tsui, 2003).
There was no critical data in the open literature of RAR 2015 that could be directly included in an environmental risk assessment for the active substance glyphosate. Endpoints reported have been detected in the same magnitude or it was not possible to distinguish between the effects of the technical glyphosate and the surface-active substances added to the commercial formulations in the experimental designs used.

Here below are listed the studies retrieved in the literature review 2020 that were considered less relevant but supplementary by RMS. These may only be considered in a weight of evidence assessment. Please note that RMS identified some studies in Volume 3 CA B.9 under the table “List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant” and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

Reno U. et al., 2014, analyzed the acute effects of a glyphosate based herbicide (Eskoba®) on the microalgae Chlorella vulgaris: 72-hour EC50 = 58.59 mg/ L. Despite the growth of C. vulgaris stimulated after 24 hours of exposure to the commercial formulation of glyphosate Eskoba®, it was inhibited after 48 hours by all the concentrations tested. These results are considered reliable with restrictions.

Lam C. H. et al., 2020, investigated the effect of glyphosate on natural isolates of phytoplankton and cyanobacteria. Three species of microalgae found in the San Francisco Estuary (SFE)/Sacramento-San Joaquin River Delta (Delta) (Microcystis aeruginosa, Chlamydomonas debaryana, and Thalassiosira pseudonana) were exposed at a range of concentrations (0, 0.7, 7 and 70 mg glyphosate/L) for 5–8 days. Roundup Custom (meant for aquatic uses) was used. The study is considered less relevant but supplementary due to formulation issue. Glyphosate inhibited algal growth only at the highest concentrations tested, which was 4.9×10^4 µg /L for M. aeruginosa and 7.0×10^4 µg /L for T. pseudonana (NOEC = 7 mg/L for both species). At 700 µg /L, glyphosate significantly enhanced T. pseudonana growth by almost 50% over the control (hormetic effect is hypothetised by authors). Analytical verifications have been made. Only graphics are available (no biological data were reported).
The study is considered reliable with restrictions.

Overall there is no studies that may impact the outcome the risk assessment of direct effects. However, some studies from the previous and current literature studies (for example, Turgut and Formin 2002 and Smedbol E. et al. 2018), studied the effects of formulations to freshwater phytoplancton community. They reported effects on chlorophyll and carotenoid contents. These observations could not be directly related to a measured parameters of current guidelines. However, regulatory studies available for glyphosate did not show a significant toxicity to algae and aquatic plants which is not expected for a herbicide. Moreover, effects on carotenoids being key in light energy absorbtion for use in photosynthesis, and in photoprotection via non-photochemical quenching, RMS considered that the studies from the public literature should be

part of the risk assessment, although done with different formulations. The applicant is asked to explain the differences in toxicity between the studies for the dossier and the public literature and to further investigate herbicide effects of glyphosate to phytoplankton, algae and macrophytes (data gap).

Studies on effects of the representative formulation MON 52276 on aquatic organisms to fulfil the data requirements according to EU Regulation No 284/2013 are presented in the following. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Studies considering the effects of MON 52276 on aquatic organisms were assessed for their validity to current and relevant guidelines and are presented in the following tables. In order to make a direct comparison of toxicity between studies conducted with MON 52276 and those conducted with IPA salt, glyphosate technical and glyphosate acid, the endpoints from all these studies have been converted to acid equivalents (a.e.). This conversion has been made by the acid equivalent purity of the test item stated in the reports.

Table B.9.4-7: Studies on toxicity of formulation MON52276 to aquatic organisms

Annex point	Study	Substance(s)	Test species	Study type	LC/EC_{50}	Status
CP 10.2.1/001	[redacted] 1992	MON-52276	*Oncorhynchus mykiss*	Acute, 96 h, static	> 989 mg MON 52276/L >306 mg a.e./L (am)	Valid
CP 10.2.1/002	[redacted], 1992	MON-52276	*Cyprinus carpio*	Acute, 96 h, static	> 895 mg MON 52276/L **> 277 mg a.e./L** (am)	Valid
CP 10.2.1/003	[redacted] 1992	MON-52276	*Daphnia magna*	Acute, 48 h flow-through	676 mg MON 52276/L **209 mg a.e./L** (am)	Valid
CP 10.2.1/004	[redacted] 1992	MON-52276	*Selenastrum capricornutum (Raphidocelis subcapitata)*	Acute, static	- Data gap: Toxicity study on alga with the representative formulation	Valid but not reliable*
CP 10.2.1/005	[redacted] 2002	MON 52276	*Lemna gibba*	Acute, semi-static	**Frond number** **7d-ErC50 > 150 mg MON 52276/L (>46.35 mg a.e./L) (nom)** 7d-NOErC = 19.1 mg MON 52276/L (5.90 mg a.e./L). 7d-EyC50 = 66.58 mg MON 52276/L (20.57 mg a.e./L) (nom) 7d-NOEyC = 19.1 mg MON 52276/L (5.90 mg a.e./L). **Dry weight** 7d-EyC50 = 118.16 mg MON 52276/L (36.51 mg a.e./L) 7d-NOEyC = 19.1 mg MON 52276/L (5.90 mg a.e./L). Data gap (EC10, EC20 and EC50 values should be calculated based on growth rate for dry weight)	Valid
CP 10.2.1/006	[redacted], 2012	MON 52276	*Myriophyllum aquaticum*	Acute, static	**Shoot length**	Valid

					14d NOErC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L) 14d ErC10 = 1.07 mg glyphosate acid/L (mm) (equivalent to 3.46 mg MON52276/L) 14d ErC20 = 3.81 mg glyphosate acid/L (mm) (equivalent to 12.42 mg MON52276/L) 14d ErC50 = 42.79 mg glyphosate acid/L (mm) (equivalent to 139.5 mg MON52276/L) 14d NOEyC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L) 14d EyC10 = 0.43 mg glyphosate acid/L (mm) (equivalent to 1.39 mg MON52276/L) 14d EyC20 = 1.41 mg glyphosate acid/L (mm) (equivalent to 4.60 mg MON52276/L) 14d EyC50 = 13.44 mg glyphosate acid/L (mm) (equivalent to 43.81 mg MON52276/L) **Shoot fresh weight** 14d NOErC < 0.3 mg glyphosate acid/L (mm) (equivalent to <0.98 mg MON52276/L) 14d ErC10 = 0.16 mg glyphosate acid/L (mm) (equivalent to 0.518 mg MON52276/L) 14d ErC20 = 0.66 mg glyphosate acid/L (mm) (equivalent to 2.15 mg MON52276/L) **14d ErC50 = 10.33 mg glyphosate acid/L (mm) (equivalent to 33.67 mg MON52276/L)** 14d NOEyC < 0.3 mg glyphosate acid/L (mm)	

					(equivalent to <0.98 mg MON52276/L) 14d EyC10 = 0.11 mg glyphosate acid/L (mm) (equivalent to 0.36 mg MON52276/L) 14d EyC20 = 0.39 mg glyphosate acid/L (mm) (equivalent to 1.27 mg MON52276/L) 14d EyC50 = 4.44 mg glyphosate acid/L (mm) (equivalent to 14.47 mg MON52276/L) **Shoot dry weight** 14d ErC10 = 0.44 mg glyphosate acid/L (mm) (equivalent to 1.42 mg MON52276/L) 14d ErC20 = 3.23 mg glyphosate acid/L (mm) (equivalent to 10.52 mg MON52276/L) 14d ErC50 = 143.3 mg glyphosate acid/L (mm) (equivalent to 467.1 mg MON52276/L) 14d EyC50 > 145 mg glyphosate acid/L (mm) (equivalent to >473 mg MON52276/L) EyC10 < 0.3 mg a.e./L (equivalent to <0.98 mg MON52276/L) **Root length** 14d NOErC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L) 14d ErC10 = 2.23 mg glyphosate acid/L (mm) (equivalent to 7.22 mg MON52276/L) 14d ErC20 = 6.33 mg glyphosate acid/L (mm) (equivalent to 20.63 mg MON52276/L) 14d ErC50 = 46.5 mg glyphosate acid/L (mm)	

					(equivalent to 151.6 mg MON52276/L) 14d NOEyC = 1.1 mg glyphosate acid/L (mm) (equivalent to 3.59 mg MON52276/L) 14d EyC10 = 1.05 mg glyphosate acid/L (mm) (equivalent to 3.40 mg MON52276/L) 14d EyC20 = 1.89 mg glyphosate acid/L (mm) (equivalent to 6.16 mg MON52276/L) 14d EyC50 = 5.84 mg glyphosate acid/L (mm) (equivalent to 19.04 mg MON52276/L)	

* The product study on algae [redacted] 1992) was performed according to the valid test guideline at the time of conduct. In the last Annex I renewal, this study was evaluated and considered acceptable for use in risk assessment. See study summary for more details (CP 10.2.1/004).

Concerning the product study performed on *Lemna gibba* ([redacted] 2002), the study was conducted according to the draft OECD 221 test guideline from October 2000. The currently adopted test guideline is largely unchanged from the draft guideline. In the last Annex I renewal, this study was evaluated and considered as supportive for use in risk assessment. See study summary for more details (CP 10.2.1/005).

Comparison of the toxicity values between MON 52276 and the active substance shows that the formulation is less toxic than the active substance for fish, aquatic invertebrates and the aquatic macrophyte *Lemna gibba*. For algae and other aquatic macrophytes, the comparison is not possible as no valid study with algae is available for the product MON 52276 and with *Myriophyllum aquaticum* for the active substance.

The endpoint to aquatic plants from MON 52276 (E_rC_{50} = 10.33 mg a.e./L, *Myriophyllum aquaticum* fresh weight) is lower compared to the lower toxicity endpoint shown by the active substance (E_rC_{50} = 30.3 mg a.e./L, *Lemna minor*, frond number). Therefore, the lower endpoint from the study with MON 52276 is used in the risk assessment as a worst case. Moreover as glyphosate is persistant in sediment, RMS considered that a test with a rooted macrophytes is necessary to finalise the risk assessment of aquatic plants.

Thus the risk assessment presented below is considered as not finalized for both algae and aquatic plants. Indeed for algae it can not be confirmed that the risk assessment based on active substance data is protective as the toxicity of the product is not known. For aquatic plants, the test design of the Lemna studies (mix in media) is considered not appropriate for a contact herbicide (see above). There is a need to have results for emergent macrophytes available with a different exposure design (overspray) Moreover a test with Myriophyllum is required with the active substance. Therefore, a data gap is set for aquatic plants.

Risk assessment for aquatic organisms

The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009 (EFSA Journal 2013; 11(7):3290); hereafter referred to as EFSA/2013/3290.

As commented by RMS in Vol.3 B.8.5, PECsw/sed calculations provided by the applicant are not considered acceptable. In order to provide a 1st informative estimation of PECsw for the peer review, STEP 1-2 PECsw were recalculated by RMS for the worst-case application pattern.

In addition, endpoints used for risk assessment below are temporary since several data gaps were identified by RMS in studies for aquatic organisms. Therefore, these endpoints and PEC/RAC ratios may change after further information is submitted.

The relevant PEC_{SW} for risk assessments covering the proposed use pattern are taken from Vol.3 CP B.8.4.

The derivation of RAC values for the risk assessment is presented in the following tables. The most sensitive endpoint between the active substance (glyphosate, glyphosate acid or glyphosate salt) and the representative formulation MON 52276 is used to provide the representative RAC for each organism group and exposure (acute and chronic).

Table B.9.3.34-8: Derivation of RAC values used in the risk assessment – glyphosate and relevant metabolites

Species	Substance	Exposure	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Glyphosate					
Lepomis macrochirus	Glyphosate acid	96 h	LC_{50} = 32000	100	320
Brachydanio rerio	Glyphosate acid	85 d	NOEC = 1000	10	100
Crassostrea gigas	Glyphosate acid	48h static	EC_{50} = 40000	100	400
Daphnia magna	Glyphosate acid	168 h	NOEC = 12500	10	1250
Skeletonema costatum	Glyphosate acid	72h static	E_rC_{50} = 13500	10	1350
Myriophyllum aquaticum	MON 52276	14 d static	E_rC_{50} = 10330	10	1033
AMPA					
Oncorhynchus mykiss	AMPA	96 h static	LC_{50} = 100000	100	1000
Pimephales promelas	AMPA	33 d flow through	NOEC = 12000	10	1200
Daphnia magna	AMPA	48 h static	EC_{50} > 180000	100	1800
Daphnia magna	AMPA	21 d semi static	EC_{50} = 15000	10	1500
Pseudokirchneriella subcapitata	AMPA	72 h	ErC50 = 191000	10	19100
Myriophyllum aquaticum	AMPA	14 d	E_rC_{50} = 72000	10	7200
HMPA					
Daphnia magna	HMPA	48 h	EC_{50} > 100000	100	1000
Pseudokirchneriella subcapitata	HMPA	72 h	E_rC_{50} > 120000	10	12000
Lemna gibba	HMPA	14 d	EC_{50} > 123000	10	12300

As commented by RMS in Vol.3 B.8.5, PECsw/sed calculations provided by the applicant are not considered acceptable. In order to provide a 1st informative estimation of PECsw for the peer review, STEP 1-2 PECsw were recalculated by RMS for the worst-case application pattern.In addition, endpoints used for risk assessment below are temporary since several data gaps were identified by RMS in studies for aquatic organisms. Therefore, these endpoints and PEC/RAC ratios may change after further information is submitted.

In the following tables, the ratios between predicted environmental concentrations of glyphosate in surface water (PEC_{SW}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use (as described in below) for each FOCUS scenario and for each organism group.

Table B.9.3.3-9: $FOCUS_{sw}$ step 1-2 – PEC/RACs for glyphosate – field uses at 2 x 1440 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Lepomis macrochirus	*Brachydanio rerio*	*Crassostrea gigas*	*Daphnia magna*	*Skeletonema costatum*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		32000 µg/L	1000 µg/L	40000 µg/L	12500 µg/L	13500 µg/L	10330 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		320	100	400	1250	1350	1033
Scenario	**PEC global max (µg L)**						
FOCUS Step 1							
	167.72	0.52	**1.68**	0.42	0.13	0.12	0.16
FOCUS Step 2							
North Europe	69.95	0.22	0.70	0.17	0.06	0.05	0.07
South Europe	56.86	0.18	0.57	0.14	0.05	0.04	0.06

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table B.9.3.3-10: FOCUS$_{sw}$ step 1-2 - TERs for AMPA – field uses at 2 x 1440 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		100000 µg/L	12000 µg/L	100000 µg/L	15000 µg/L	191000 µg/L	72000 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		1000	1200	1000	1500	19100	7200
Scenario	**PEC global max (µg L)**						
FOCUS Step 1							
	111.02	0.11	0.09	0.11	0.07	0.01	0.02
FOCUS Step 2							
North Europe	52.47	0.05	0.04	0.05	0.03	0.003	0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table B.9.3.3-11: FOCUS$_{sw}$ step 1-2 – PEC/RACs for HMPA – field uses at 2 x 1440 g a.s./ha

		Aquatic invertebrates	**Algae**	**Higher plant**
		Daphnia magna	*Pseudokirchneriella subcapitata*	*Lemna gibba*
		EC_{50}	ErC_{50}	EC_{50}
		> 100000 µg/L	> 120000 µg/L	> 123000 µg/L
		100	10	10
		> 1000	> 12000	> 12300
Scenario	**PEC global max (µg L)**			
FOCUS Step 1				
	58.06	0.06	0.005	0.005
FOCUS Step 2				
North Europe	52.47	0.05	0.004	0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table B.9.3.3-12: –PEC/RACs for glyphosate – railways at 1 x 3600 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Lepomis macrochirus	*Brachydanio rerio*	*Crassostrea gigas*	*Daphnia magna*	*Skeletonema costatum*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		32000 µg/L	1000 µg/L	40000 µg/L	12500 µg/L	13500 µg/L	10330 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		320	100	400	1250	1350	1033
Scenario	**PEC global max (µg L)**						
Railway ditch	9.458	0.03	0.09	0.02	0.01	0.01	0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table B.9.3.3-13: – PEC/RACs for AMPA – railways at 1 x 3600 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		100000 µg/L	12000 µg/L	100000 µg/L	15000 µg/L	191000 µg/L	72000 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		1000	1200	1000	1500	19100	7200
Scenario	**PEC global max (µg L)**						
Railway ditch	6.210	0.01	0.01	0.006	0.004	0.0003	0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table B.9.3.3-14: –PEC/RACs for HMPA – railways at 1 x 3600 g a.s./ha

		Aquatic invertebrates	**Algae**	**Higher plant**
		Daphnia magna	*Pseudokirchneriella subcapitata*	*Lemna gibba*
		EC_{50}	ErC_{50}	EC_{50}
		> 100000 µg/L	> 120000 µg/L	> 123000 µg/L
AF		100	10	10
RAC (µg/L)		> 1000	> 12000	> 12300
Scenario	**PEC global max (µg L)**			
Railway ditch	0.627	> 0.001	> 0.0001	> 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

A summary of the risk assessment regarding aquatic biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

B.9.5.1.1. Acute toxicity to bees

B.9.5.1.1.1. Acute oral toxicity to bees

Data point:	CP 10.3.1.1.1/001
Report author	[redacted]
Report year	2001
Report title	Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the honeybee, *Apis mellifera*
Report No	MON-00-2 version 2
Document No	-
Guidelines followed in study	EPPO Guideline on test methods for evaluating the side-effects of plant protection products on honeybees. No. 170 (1992).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current guideline OECD 213 (1998):* *Major:* - *none* *Minor:* *3 to 4 hours starvation instead of 1 to 2 hours recommended* *Humidity was slightly outside the expected range: 46 - 83% instead of 50 - 70%* *4 hours assessment was not carried out* *These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The acute oral toxicity of the formulated product MON 52276 to worker bees (*Apis mellifera* L.) was determined in a limit test at the nominal dose of 103 µg glyphosate isopropylamine/bee (a.s.), equivalent to 77 µg glyphosate acid equivalent/bee (a.e.) for oral exposure. Bees were also exposed to dimethoate at concentrations from 0.075 to 0.3 µg dimethoate/bee (reference toxicant group) or to an aqueous sucrose solution (negative control). The test comprised 5 replicate groups of 10 bees for the test treatments and the control group. Further 3 replicate cages containing each 10 bees were prepared for the reference group. Bee condition was assessed after 1, 3, 24 and 48 hours.

After 48 hours, there were no sub-lethal effects observed. Mortality did not reach or exceed 50 %. The control and treatment group mortality were both 4 %. In the oral test, the 48 h LD_{50} for honey bees exposed to MON 52276 was >103 µg a.s./bee, equivalent to >77 µg a.e./bee, the maximum amount consumed over a 5 h period.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276
Formulation type	Soluble concentrate (SL)
Description:	Dark yellow-coloured fluid
Active substance	glyphosate isopropylamine salt
Lot/Batch #:	100399
Purity:	41.5 % w/w glyphosate isopropylamine
	30.3 % w/w glyphosate acid equivalent (measured)
Density:	1.168 g/cm^3 (nominal)

Positive control: BASF Dimethoate 40 (400 g dimethoate/L)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Roselea Apiaries, East Wellow, Hampshire, UK

Environmental conditions:

Temperature:	24 - 26°C
Humidity:	46 – 83 %
Photoperiod:	24 h dark

Experimental dates: Not stated in the report

B. STUDY DESIGN

Experimental treatments

For the oral test, the test treatments and negative control group comprised five groups of 10 bees, maintained in stainless steel coated 2 – 2.5mm wire mesh cylinders measuring 140 mm deep × 40 mm in diameter, closed by polyurethane foam bungs at both ends. For the reference toxicant, 3 groups of 10 bees were held in mesh cages of the same design, for each of the treatment groups.

Worker honey bees were collected from a queen right hive on the morning of the tests. All bees were lightly anaesthetised using humidified carbon dioxide and added to cages in groups of ten and allowed to recover. Honeybees for the oral test remained unfed during recovery.

In the oral test, honeybees were exposed to MON 52276 dispersed in a 50 % sucrose solution delivered to the cages using a glass feeding tube inserted through one of the polyurethane bungs. A 200 µL volume of solution was provided and assumed that each bee would consume at least 20 µL of solution over a 5 h exposure period. After 5 h, the feeding tube was replaced with a tube containing 50 % sucrose solution only, which was replenished *ab libitum* for the 48 h duration of the test.

The reference item group was prepared in the same way as for the treatment groups. The reference item group was evaluated in two stages, the highest application rate was tested alongside the treatment and control groups, with the lower two treatment rate evaluated five days later with an additional control group included for comparison.

All cages were maintained in the dark in an incubator for the duration of the test.

Observations

In the oral test, the feeding vials were weighed prior to treatment and again after 5 h to establish the actual dose per bee consumed. An assessment of the condition of the bees was made 1, 3, 24 and 48 hours after treatment. The bees were classified as being live, affected, moribund/dead.

Statistical calculations

The data from the definitive bio assays were not suitable for Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

The oral LD_{50} and NOEL values for honeybees exposed to MON 52276 are given below.

Table B.9.5-1: Toxicity of MON 52276 to honey bees (*Apis mellifera* L.) in an oral toxicity test

Endpoints (48 h)	MON 52276 glyphosate acid equivalent [µg a.e./bee]	MON 52276 glyphosate isopropylamine [µg a.s./bee]
LD_{50} oral	>77	>103
NOEL oral	77	103

B. OBSERVATIONS

The mortality in control and in the treatment groups was 4% in the 48-hour exposure. There were no observations of treated bees being sick or behaving abnormally (only one bee affected out of 50 in both control and treated group).

Table B.9.5-2 : Oral toxicity of MON 52276 to honey bees (*Apis mellifera* L.)

Exposure	Mortality [%]		Corrected mortality[b] [%]
	Control	MON 52276 103 µg a.s./bee [a] 77 µg a.e/bee [a]	
1 h	0	0	-
3 h	0	0	-
24 h	0	0	-
48 h	4	4	0

[a] Based on mean weight of test solution of 5 µg/µL consumed per cage of 10 bees, corrected for the density of the 50 % w/w sugar solution

[b] Corrected mortality according to Abbott (1925)

a.e = glyphosate acid equivalent, a.s.= glyphosate isopropylamine

For the reference group (BASF Dimethoate 40), 100 % and 33 % mortality were observed in 0.3 and 0.15 µg dimethoate/bee concentrations after 24 hours exposure, respectively. The LD_{50}-24h was in the range 0.10 - 0.35 µg a.s./bee requested in the guideline and was in line with published values (Gough et al., 1994), indicating that the test insects were suitably sensitive.

The mortality in the control treatments did not exceed 10 %.
All the validity criteria according to guideline OECD 213 were therefore fulfilled.

The applicant noted the following points are deviated from the current guideline:

- 3 to 4 hours starvation instead of 1 to 2 hours recommended.
- Humidity was slightly outside the expected range: 46-83 % instead of 50 -70 %.
- 1 and 3 hours assessments were carried out instead of the 4 hours requested.

The applicant considers that these deviations are not expected to have any negative on the study validity. RMS agrees with the reported deviations (see commenting box below).

III. CONCLUSION

Assessment and conclusion by applicant:
The LD_{50} (48 h) for honey bees exposed to MON 52276 was determined to be >103 µg a.s./bee, equivalent to >77 µg a.e./bee for oral exposure.

This study is considered valid and suitable for risk assessment purposes.

Assessment and conclusion by RMS:
This study has already been submitted and assessed in the 2015 RAR.

The test item is MON 52276 (EU representative formulation)

As also reported by the applicant, the following points are deviated from the current guideline:
- *3 to 4 hours starvation instead of 1 to 2 hours recommended.*

Such delay (1-2 hours) is recommended by OECD 213 so that all bees are equal in terms of their gut contents at the start of the test. Therefore, RMS considers this deviation acceptable.

- *Humidity was slightly outside the expected range: 46-83 % instead of 50 -70 %.*

The recommended range is only slightly exceeded, RMS considers this acceptable.

- *1 and 3 hours assessments were carried out instead of the 4 hours requested.*

No effects of the test item were observed during the test (1, 3, 24 and 48 h) so any effect are not expected to have occurred at 4 h.

RMS considered that these minor deviations will not impact the outcome of the test. No other deviation were noted.

The test is considered valid according to OECD 213 validity criteria as mortality in the negative control did not exceed 10 % and the LD50 of the toxic standard met the range specified.

48 h oral LD50 >103 µg glyphosate IPA/bee, equivalent to >77 µg glyphosate acid/bee

B.9.5.1.1.2. Acute contact toxicity to bees

Data point	CP 10.3.1.1.2/001
Report author	[redacted]
Report year	2001
Report title	Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the honeybee, *Apis mellifera*
Report No	MON-00-2 version 2
Document No	-
Guidelines followed in study	EPPO Guideline on test methods for evaluating the side-effects of plant protection products on honeybees. No. 170. (1992).

Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current guideline OECD 214 (1998):* *Major:* - *none* *Minor:* - *Humidity was slightly outside the expected range: 46 - 83% instead of 50 - 70%* - *4 hours assessment was not carried out* *These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Yes

Summary

The acute contact toxicity of the formulated product MON 52276, to young adult worker bees (*Apis mellifera* L.) was determined in a limit test at the equivalent of a single nominal dose of 134 µg glyphosate isopropylamine salt/bee, equivalent to 100 µg glyphosate acid equivalent (a.e.)/bee. Bees were also exposed to dimethoate at concentrations of 0.075 and 0.3 µg dimethoate/bee (reference toxicant group) or to an aqueous sucrose solution (negative control). The test comprised 5 replicate groups of 10 bees for the test treatments and the control group. Further 3 replicate cages containing each 10 bees were prepared for the reference group. Bee condition was assessed after 1, 3, 24 and 48 hours.

After 48 hours, there were no sub-lethal effects observed. Mortality did not reach or exceed 50 %. After 48 hours control and treatment group mortality were 2% and 12% respectively.

The 48 h LD_{50} for honeybees exposed to MON 52276 was >134 µg a.s./bee, equivalent to >100 µg a.e./bee for contact exposure.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276
Formulation type	Soluble concentrate (SL)
Description:	Dark yellow-coloured fluid
Active substance	glyphosate isopropylamine salt
Lot/Batch #:	100399
Purity:	41.5 % w/w glyphosate isopropylamine 30.3 % w/w glyphosate acid equivalent (measured)
Density:	1.168 g/cm^3 (nominal)
positive control:	BASF Dimethoate 40 (400 g dimethoate/L)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Young adult worker bees
Source:	Roselea Apiaries, East Wellow, Hampshire, UK

Environmental conditions:

Temperature:	24 - 26°C

Humidity: 46 – 83 %
Photoperiod: 24 h dark

B. STUDY DESIGN

Experimental dates: No dates reported

Experimental treatments
For the contact tests, the test treatments and negative control group comprised five groups of 10 bees, maintained in stainless steel coated 2 – 2.5mm wire mesh cylinders measuring 140 mm deep × 40 mm in diameter, closed by polyurethane foam bungs at both ends. For the reference toxicant, 3 groups of 10 bees were held in mesh cages of the same design, for each of the treatment groups.
Worker honey bees were collected from a queen right hive on the morning of the tests. All bees were lightly anaesthetised using humidified carbon dioxide and added to cages in groups of ten and allowed to recover. Bees for the contact test were provided with sucrose solution during the recovery period.
For the contact test, the bees were again lightly anaesthetised with humidified carbon dioxide and then in groups of 10 were turned onto their back using lightweight forceps, and a 1 µL volume of test solution (MON 52276 dispersed in 0.01% v/v Farmon blue – used to facilitate application to the hydrophobic hairs on the thorax) was applied to the ventral thorax using a micro-applicator and the bees were returned to the cages. The bees were fed 50 % sucrose solution *ad libitum* via a glass feeding tube inserted through one bung for the 48 h duration of the test
The reference item group was prepared in the same way as for the treatment groups. The reference item group was evaluated in two stages, the highest application rate was tested alongside the treatment and control groups, with the lower treatment rate evaluated five days later with an additional control group included for comparison.
All cages were maintained in the dark in an incubator for the duration of the test.

Observations

An assessment of the condition of the bees was made 1, 3, 24 and 48 hours after treatment. The bees were classified as being live, affected, moribund/dead.

Statistical calculations
The data from the definitive bio assays were not suitable for Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

The contact LD_{50} and NOEL values for honeybees exposed to MON 52276 are given below based on nominal concentrations.

Table B.9.5-3: Endpoints

Endpoints (48 h)	MON 52276 glyphosate acid equivalent [µg a.e./bee]	MON 52276 glyphosate isopropylamine [µg a.s./bee]
LD_{50} contact	>100	>134
NOEL contact	100	134

B. OBSERVATIONS

After 48-hour exposure, the mortality was 2% and 6% in the control and treatment groups, respectively. The corrected mortality was 4 % after 48 hours of exposure. There were no observations of treated bees being sick or behaving abnormally so the study author considered that the 4% mortality were not treatment related.

Table B.9.5-4: Contact toxicity of MON 52276 to honey bees (*Apis mellifera* L.)

Exposure	Mortality [%]		Corrected mortality[a] [%]
	Control	MON 52276 134 µg a.s/bee 100 µg a.e/bee	
1 h	0	0	-
3 h	0	0	-
24 h	0	0	-
48 h	2	6	4

[a:] Corrected mortality according to Abbott (1925)
a.e = glyphosate acid equivalent, a.s.= glyphosate isopropylamine

For the reference group (BASF Dimethoate 40), 100 % and 22 % mortality were observed in 0.3 and 0.075 µg dimethoate/bee concentrations after 24 hours exposure, respectively. The LD_{50}-24h was in the range 0.10 - 0.35 µg a.s./bee requested in the guideline and was in line with published values (Gough et al., 1994), indicating that the test insects were suitably sensitive.

The mortality in the control treatments did not exceed 10%. The validity criteria according to guideline OECD 214 were therefore fulfilled.

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The contact LD_{50} (48 h) for honey bees exposed to MON 52276 was determined to be > 134 µg a.s./bee, equivalent to > 100 µg a.e./bee.

This study is considered valid and suitable for risk assessment purposes.

Assessment and conclusion by RMS:
This study has already been submitted and assessed in the 2015 RAR.

The test item is the EU representative formulation MON 52276.

As also reported by the applicant, RMS noted that the following points are deviated from the current guideline:
- *Humidity was slightly outside the expected range: 46-83 % instead of 50 -70 %.*

The recommended range is only slightly exceeded, RMS considers this acceptable.

- *1 and 3 hours assessments were carried out instead of the 4 hours requested.*

No effects of the test item were observed during the test (1, 3, 24 and 48 h) so any effect are not expected to have occurred at 4 h.

RMS considered that these minor deviations will not impact the outcome of the test. No other deviation were noted by RMS.

The test is considered valid according to OECD 214 as mortality in the negative control did not exceed 10 % and the LD50 of the toxic standard met the range specified.

48 h contact LD50 >134 µg glyphosate IPA/bee, equivalent to >100 µg glyphosate acid/bee

B.9.5.1.1.3. Chronic toxicity to bees

According to Regulation (EU) No 284/2013, when exposure to bees can not be excluded, testing shall be required if the toxicity of a plant protection product cannot be reliably predicted to be either the same or lower than the active substance tested, in accordance with the requirements set out in points 8.3.1 and 8.3.2 of Part A of the Annex to Regulation (EU) No 283/2013. In view of the acute toxicity data for bees available for the active substance and the formulation MON 52276, chronic toxicity to bees can be reliably predicted from active substance data in accordance with Regulation (EU) No 284/2013.

B.9.5.1.1.4. Effects on honey bee development and other honey bee life stages

According to Regulation (EU) No 284/2013, when exposure to bees can not be excluded, testing shall be required if the toxicity of a plant protection product cannot be reliably predicted to be either the same or lower than the active substance tested, in accordance with the requirements set out in points 8.3.1 and 8.3.2 of Part A of the Annex to Regulation (EU) No 283/2013. In view of the acute toxicity data for bees available for the active substance and the formulation MON 52276, toxicity to bee brood can be reliably predicted from active substance data in accordance with Regulation (EU) No 284/2013.

B.9.5.1.1.5. Sub-lethal effects

In view of the available information and the outcome of the risk assessment, further studies assessing sub-lethal effects on honeybees for the representative EU formulation MON 52276 are not considered required.

B.9.5.1.1.6. Cage and tunnel tests

In view of the available information and the outcome of the risk assessment, further studies such as cage or tunnel tests with honeybees for the representative EU formulation MON 52276 are not considered required.

Data point	CP 10.3.1.5/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions
Report No	V7YH1002
Document No	-
Guidelines followed in study	None; tailor made study
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Not applicable field study*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary (as proposed by the applicant)
A semi-field study was undertaken to determine the potential exposure of honeybee colonies to glyphosate by quantifying residues in relevant food matrices, i.e. pollen and nectar, when the formulation MON 52276 was applied to flowering *Phacelia* grown in two large (180 m²) glasshouses. Following treatment of nominal 8 L/ha, equivalent to 2.88 kg a.e./ha, two honeybee colonies per glasshouse were exposed. Foraging activity in the crop and activity at each hive was assessed daily for 7 days. On days 0, 1, 2, 3, 4 and 7, forager bees were taken to get hold of the nectar from the honey stomach of the bees after foraging in the treated crop. On days -1, 1, 2, 3, 4 and 7, samples of pollen were collected from the pollen traps fitted to each hive. Samples of nectar were also collected from the combs in each hive on day 7. Furthermore, samples of larvae were collected from the combs in each hive on days 4 and 7. Daily assessments were made of the percentage of plants with wilted leaves or flowers.
The authors concluded the following:
Foraging assessment showed foraging activity on the crop from start of study throughout the exposure period in glasshouse 1 with a peak on day 4. The lowest foraging activity was observed on day 5 at 38% of the mean pre-spray activity. In glasshouse 2 the activity declined throughout the assessment period to reach less than 10% of mean spray activity on days 5-7. In line with the decreased foraging activity in glasshouse 2, the crop started to show significant effects of the treatment from day 4 onwards.
Residues in nectar samples taken from forager bees at various time points after application ranged from 2.78 to 31.3 mg a.e./kg; residues in nectar samples taken from the colonies ranged from below LOQ (1.0 mg a.e./kg) to 1.30 mg a.e./kg. Residues in pollen samples taken from the pollen trap at various time points after application ranged from 87.2 to 629 mg a.e./kg. Residues in larvae samples ranged from 1.23 to 19.50 mg a.e./kg.
The residue data can be used to assess the approximate exposure level of brood within colonies exposed under worst-case conditions.
The maximum pollen collected per colony was 2.9 g on day 0 and the traps are estimated to be about 50% efficient so about 6 g of pollen per day was returned to the hive (the colony is using about 4.5 g of this based on the Rortais *et al.* 2005).
The nectar can be assessed using a mean of 18 foragers returning to the hive per 30 seconds and approximately 50 µL per load (max), which gives 18 trips/30 sec * 60 sec/min * 60 min/hour * 12 hours max foraging/day, equal to 25,920 trips/day * 0.050 mL, resulting in 1296 mL/day (of which the colony is using 135 g based on Rortais *et al.* 2005).
As a worst-case example considering the colony size of the present study, a honey bee colony collects 6 g pollen and 1296 mL nectar and of this the brood consumes 4.5 g pollen and 135 g nectar, which allows the excess to be stored for later consumption. As simulated in this study, for honeybee colonies foraging on the model crop *Phacelia* treated with 8 L MON 52276/ha, a total daily intake of glyphosate residues of 44.0 mg a.e. (based on day 1 maximum mean residues) and of 22 mg a.e. (based on mean residues over days 1-3) can be estimated.

For RMS conclusion, please refer to the commenting box .

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276 (Soluble concentrate)
Active substance:	Glyphosate acid 360 g glyphosate acid equivalents/L (nominal)
Active substance content:	358.8 g glyphosate acid equivalents/L (according to the Certificate of Analysis)
Proposed use:	Herbicide
Description:	Clear brown liquid

Lot/Batch #:	A9K0106104
Density:	1.1693 g/mL at 20°C (according to the Certificate of Analysis)
Test organism:	
Species:	*Apis mellifera* L.
	4 honeybee colonies containing 4 – 6 frames of brood, containing 6000 – 12000 adult bees
Age:	Not stated
Source:	UK national Bee Unit
Acclimatisation:	3 days
Test system:	Two 180 m² glasshouses at Stockbridge Technology Centre, Selby, North Yorkshire, U.K.
Crop cultivated:	*Phacelia* (sown directly into soil of the glasshouse, no pesticide use during cultivation)
Replication:	2 glasshouses, each containing 2 bee colonies
Environmental conditions:	
Temperature:	Glasshouse 1: 7.7 – 39.9°C, temperatures of >35 °C were recorded on day 6 and 7 for 10 and 30 min.
	Glasshouse 2: 8.3– 47.4°C, temperatures of >35 °C were recorded on days -1, 1, 2, 4, 6 and 7 for up to 30 min until day 4, for 1.5 h on day 4, 50 min on day 6 and 40 min on day 7.
	High temperatures occurred primarily between 11:30 and 14:00 and exhibited no obvious effects on crop or foraging bees
Humidity:	Glasshouse 1: 19.5 to 93.4 %
	Glasshouse 2: 13.9 to 100 %
Experimental dates:	12 May – 22 June 2011

B. STUDY DESIGN

Experimental treatments

Study site: The study was conducted in two 180 m² glasshouses situated at Stockbridge Technology Centre, Cawood, Selby, North Yorkshire. The glasshouses were well ventilated (but equipped with insect proof) to be as representative as possible of the outdoor situation but without direct precipitation. *Phacelia* was planted directly into the soil inside the glasshouse and no pesticides were applied during cultivation. The timing of the start of test i.e. transfer of colonies into the glasshouse was determined by the flowering of the crops. Temperature and humidity in the glasshouses were recorded continuously.

Experimental design: Four colonies of bees and brood comprising each of 4 to 6 frames of brood and containing 6000 to 12000 adult bees were used. Hives were fitted with a pollen trap. Three days prior to application two colonies each were located on opposite sides of each glasshouse and allowed to fly freely within the glasshouse. Colonies A and B were placed in glasshouse 1, colonies C and D were placed in glasshouse 2.

Test item application: The test item MON 52276 (nominal content: 360 g glyphosate acid equivalent/L) was applied onto the crop grown in the glasshouse on day 0 during a period when bees were actively foraging using a 3 nozzle lunch box sprayer unit with a hand-held boom fitted with Lurmark 03 F110 nozzles. The sprayer was pre-calibrated to deliver a known application rate of 400 L/ha. The colonies were protected from direct overspray and spray drift during the application.

Observations

Foraging assessments were performed each day during times peak foraging activity. The assessments were performed by counting the number of bees foraging in a marked area (5 m by 1 m transects) during a 1 minute period during peak activity. In addition, the number of bees returning to each hive and the number carrying pollen loads were counted during a 30 second period.

Visual assessment of the crop was performed daily by determination of the proportion of plants with wilted flowers and wilted leaves.

The contents of the pollen traps were collected on days -1, 1, 2, 3, 4 and 7 after application. Samples of forager bees were collected on days 0, 1, 2, 3, 4 and 7 after application. The nectar was collected from the bees honey stomachs. On days 4 and 7 samples of ten 4-5 day old larvae were taken from each colony, on day 7 an additional sample of nectar was collected from the combs of each colony.

Residues analysis

Analysis of glyphosate acid in samples was conducted following extraction with acetonitrile:water (1:4, v/v), clean up by solid phase extraction on C18 and derivatisation as FMOC-glyphosate and a second clean up (solid phase extraction on Oasis HLB, methanolic elution) by HPLC-MS/MS. Limit of quantification (LoQ) and limit of detection (LoD) were 1.0 and 0.3 mg/kg, respectively.

Data analysis

Considering residue levels determined in nectar and pollen after treatment of a model crop, possible exposure scenarios of honeybee brood are estimated based on information available from literature and the present study.

II. RESULTS AND DISCUSSION

The results and discussion as proposed by the applicant contains some RMS comments that should be considered together with the conclusion of the RMS given below.

A. FINDINGS

Verification of test item application: The actual application rates were 8.19 L MON 52276/ha (2.94 kg a.e./ha) in glasshouse 1 and , 8.30 L MON 52276/ha (2.98 kg a.e./ha) in glasshouse 2. The application rate was 102 – 104% of the nominal application rate of 8 L MON 52276/ha and 102-103% of the nominal application rate of 2.88 kg a.e./ha.

Residue analysis: Residues in nectar samples taken from forager bees at various time points after application ranged from 2.78 to 31.3 mg a.e./kg; residues in nectar samples taken from the colonies ranged from below LOQ (1.0 mg a.e./kg) to 1.30 mg a.e./kg. Residues in pollen samples taken from the pollen trap various times after application ranged from 87.2 to 629 mg a.e./kg. Residues in larvae samples ranged from 1.23 to 19.50 mg a.e./kg.

Table B.9.5-5: Summary of residue analysis of pollen, nectar and larvae samples

		Days after treatment [mg glyphosate acid equivalent/kg]					
	Hive	**-1**	**1**	**2**	**3**	**4**	**7**
Nectar (honey stomachs)	A+B	n.d.	25.5	9.24	4.90 (samples combined DAT 3, 4, 7)		
	C+D	n.d.	31.3	15.2	7.18 (samples combined DAT 3, 4)		2.78

	Overall mean	**n.d.**	**28.4**	**12.2**	**6.0**		
Nectar (hive)	A	-	-	-	-	-	<LOQ
	B	-	-	-	-	-	1.30
	C	-	-	-	-	-	1.06
	D	-	-	-	-	-	1.00
Mean							**0.99**
Larvae (comb)	A	-	-	-	-	8.32	2.54
	B	-	-	-	-	16.70	10.6
	C	-	-	-	-	19.50	6.72
	D	-	-	-	-	2.88	1.23
Mean						**11.9**	**5.3**
Pollen (pollen trap)	A	n.d.	325	255	119 (samples combined)	134 (samples combined)	87.2 (samples combined)
	B	n.d.	405	213			
	Mean A&B	**n.d.**	**365**	**234**	**119**	**134**	**87.2**
	C	n.d.	518	333	181	176	130 (samples combined)
	D	n.d.	629	477	147	180	
	Mean C&D	**n.d.**	**574**	**405**	**164**	**178**	**130**
	Overall mean	**n.d.**	**469**	**320**	**142**	**156**	**109**

DAT day after treatment
n.d. not detected
<LOQ 0.6 mg/kg
LOD 0.3 mg/kg
LOQ 1.0 mg/kg

B. OBSERVATIONS

Foraging activity: Foraging assessment showed foraging activity on the crop from start of study throughout the exposure period in glasshouse 1 with a peak on day 4. The lowest foraging activity was observed on day 5 at 38% of the mean pre-spray activity. In glasshouse 2 the activity declined throughout the assessment period to reach less than 10% of mean spray activity on days 5-7. In line with the decreased foraging activity in glasshouse 2, the crop started to show significant effects of the treatment from day 4 onwards.

Data analysis: The residue data can be used to assess the approximate exposure level of brood within colonies exposed under worst-case conditions.

Table B.9.5-6: Assessment of possible exposure of honey bee colonies to glyphosate residues under two scenarios is depicted below.

Scenario	Daily intake of glyphosate residues in nectar (1296 g nectar/d) [mg]	Daily intake of glyphosate residues in pollen (6 g pollen/d) [mg]	Total daily intake of glyphosate residues [mg a.e.]
Day 1 maximum mean residues (31.3 µg a.e./g in nectar, 574 µg a.e./g in pollen, glasshouse 2)	40.6	3.4	44.0
Mean residues over days 1-3 (15.5 µg a.e./g in nectar, 310 µg a.e./g in pollen, both glasshouses)	20.1	1.9	22.0

RMS comment:

- No residue measurement is available at day 0 (immediately after spray). First measurements were made at day 1. Residues decreased from 28.4 mg/kg nectar at day 1 to 12.2 mg/kg nectar at day 2 and 6.0 mg/kg nectar at day 3 (i.e. by a factor 2 approximately between each measurement). RMS believes that it could be reasonably assumed that the residues at day 0 may be approximately at least twice higher than those measured at day 1.
- Initial residue values may then be underestimated by a (maximum) factor of 2 approximately in nectar. The residues also decrease in pollen but at slower rate (underestimation by less than 2 in pollen).

Two approaches can be made to assessing exposure - one based on generic published data on the requirements for nectar and pollen by larvae (generic data) and the other based on the observations made in this study (study data).

The nectar and pollen consumption were estimated by the study authors:
Generic data: The calculations are based on a daily brood requirement of 30 mg nectar (based on 40% sugar in nectar) and 1 mg pollen for worker brood (Rortais et al. 2005). Based on a brood frame being 3600 cells and 25% of the time is as unsealed brood (hatch day 3 to sealed day 8 with emergence day 21) then five frames of brood (4-6 were used in this study) is 18,000 brood cells therefore for 4500 larvae with a requirement of 135 g/day nectar and 4.5 g/day pollen for the colony.

RMS comment:
The consumption data from Rortais et al, 2005 are relevant for the risk assessment (at individual bee level). The sugar content of the nectar should have been measured but it is RMS opinion that 40% sugar can reasonably be assumed for this plant species.
RMS highlights that the calculations above only represent the food that is consumed by larvae. Calculations are in agreement with the data consumption available for larvae in Rortais et al, 2005). The foragers and in-hive bees also consume nectar but are not considered in the calculation above. Then the results obtained here are not relevant for a whole colony. Anyway, this has no consequence on the outcome of the study as these estimates were not used (measured data were used instead, see below).

Study data: The second approach is to assess the amount of pollen and nectar returning to the hive over the time course of exposure using the data on the numbers of returning foragers in the study and the amounts of pollen and nectar collected from bees by using the pollen trap and individual bee samples. The maximum pollen collected per colony was 2.9 g on day 1 and the traps are estimated to be about 50% efficient so about 6 g of pollen per day was returned to the hive (the colony is using about 4.5 g of this based on the Rortais *et al.* 2005).

RMS comment:
The efficiency of the pollen traps cannot be verified with the available information. It is RMS opinion that the quantity of pollen brought back to hive every day may have been underestimated as it would be equivalent to 2.19 kg pollen/year at best (assuming a constant foraging throughout the year that is unrealistic). Rortais et al, 2005 provides rough estimates of 10-20 kg pollen during the only flowering periods of sunflower and maize. The amount of pollen collected per colony and per year is in the range of a few tens of kilos to about 55 kg (Rortais et al, 2005). RMS considers average value of 35-40 kg relevant (found in french literature, Traité Rustica de l'apiculture edition 2011). RMS also notes that pollen collection is not steady during the whole year. RMS also questions the potential effect of the enclosure in glasshouse on pollen collection. Assuming that bee colony will forage pollen 8 months/year (assuming no pollen collection in winter) and an assumed pollen collection of 40 kg/colony, the quantity of pollen brought back to the hive could be 27 times higher (i.e. 164 g pollen per day).

The nectar can be assessed using a mean of 18 foragers returning to the hive per 30 seconds and approximately 50 µL per load (max), which gives 18 trips/30 sec * 60 sec/min * 60 min/hour * 12 hours max foraging/day, equal to 25,920 trips/day * 0.050 mL, resulting in 1296 mL/day (of which the colony is using 135 g based on Rortais *et al.* 2005).

RMS comment:
RMS cannot verify the reliability of the load of 50 µL (source not provided) but seems appropriate to RMS. The estimated quantity of 1296 mL/day seems realistic.

III. CONCLUSION

Assessment and conclusion by applicant:

As a worst case example considering the colony size of the present study, a honey bee colony collects 6 g pollen and 1296 mL nectar and of this the brood consumes 4.5 g pollen and 135 g nectar, which allows the excess to be stored for later consumption. As simulated in this study, for honeybee colonies foraging on the model crop *Phacelia* treated with 8 L MON 52276/ha, a total daily intake of glyphosate residues of 44.0 mg a.e. (based on day 1 maximum mean residues) and of 22 mg a.e. (based on mean residues over days 1-3) can be estimated.

This study is considered valid and suitable for risk assessment purposes.

Assessment and conclusion by RMS:

The study is well designed. However some of the assumptions are not sufficiently supported and uncertainties remain of the actual exposure of the bees.

The RMS analysis on each assumption is given in the summary above for clarity (see RMS comment in the study summary above).

Overall, the initial residue levels may have been underestimated but this uncertainty is estimated factor 2 at most.

The quantity of pollen brought back to the hive seems underestimated and could be approx.27 times higher (i.e. 164 g pollen per day).

The estimated quantity of 1296 mL/day seems realistic.

Overall conclusion by RMS:

Considering the colony size of the present study, and based on the assumptions described above, a honey bee colony may collect up to 164 g pollen and 1296 mL nectar per day.

As simulated in this study, for honeybee colonies foraging on the model crop Phacelia treated with 8 L MON 52276/ha, a total daily intake of glyphosate residues of 269.3 mg a.e. (based on day 1 maximum mean residues) and of 141.8 mg a.e. (based on mean residues over days 1-3) can be estimated.

Scenario	**Daily intake of glyphosate residues in nectar (1296 g nectar/d) [mg]**	**Daily intake of glyphosate residues in pollen (164 g pollen/d) [mg]**	**Total daily intake of glyphosate residues [mg a.e.]**
Day 1 maximum mean residues* (62.6 µg a.e./g in nectar, 1148 µg a.e./g in pollen, glasshouse 2)	81.1	188.2	269.3
Mean residues over days 1-3* (31 µg a.e./g in nectar, 620 µg a.e./g in pollen, both glasshouses)	40.1	101.7	141.8

*considering a conservative factor of 2

Data point:	CA 8.3.1.3 CP 10.3.1.5/002
Report author	Thompson, H.M., Levine, S.L. *et al.*
Report year	2014
Report title	Evaluating Exposure and Potential Effects on Honeybee Brood (*Apis mellifera*) Development Using Glyphosate as an Example
Document No	DOI: 10.1002/ieam.1529 E-ISSN: 1551-3793
Guidelines followed in study	Oomen *et al*. 1992
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Not applicable*
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (literature publication)
Acceptability/Reliability (RMS):	- see CP 10.3.1.5/001, [redacted] 2011 and CA 8.3.1.4/001, [redacted] 2012

The first stage (on exposure) of this publication actually corresponds to the study summarized above and already assessed by RMS (CP 10.3.1.5/001, [redacted] 2011, Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions. Ref V7YH1002).

The second part (for effect) of this publication actually corresponds to the study summarized in Volume 3CA and already assessed by RMS (CA 8.3.1.4/001, [redacted] 2012, Glyphosate: Evaluating potential effects on honeybee brood (Apis mellifera) development, V7YH1001).

Thus the summary of this publication as proposed by the applicant was not reported here. Only assessment and conclusion part of the applicant and RMS are reported.

Assessment and conclusion by applicant:

The Oomen et al. (1992) approach was used to quantify at residues in relevant matrices (pollen, nectar, and larvae) following application of glyphosate at 2.88 kg a.e./ha (400 L water/ha) to flowering *Phacelia tenacetifolia* in large glasshouses. Then brood feeding tests following the Oomen approach, were conducted by feeding 1 L treated sucrose solution at 75 / 150 and 301 mg glyphosate a.e./L directly to honeybee colonies.

The study is adequately described and all information to evaluate the study are available. At the time the study was conducted, there were no field level test guidelines adopted for use in the EU. The test did follow a recognised approach and is considered fit for purpose. The study is considered as reliable.

Assessment and conclusion by RMS:

This paper develops a 2 stages-approach to evaluate potential effects of plant protection products on honeybee brood with colonies.
In a first stage (exposure assessment), honeybee colonies were exposed to a commercial formulation of glyphosate applied to flowering *Phacelia tanacetifolia* with glyphosate residues quantified in relevant matrices (pollen and nectar). Residue data along with foraging rates and food requirements of the colony were then used to set dose rates in the effects study (second stage).

In the second stage, the toxicity of technical glyphosate to developing honeybee larvae and pupae, and residues in larvae, were determined by feeding treated sucrose directly to honeybee colonies at the dose rates that were assumed to reflect worst-case exposure scenarios (based on first stage).

The first stage (on exposure) of this publication actually corresponds to the study summarized above and already assessed by RMS (CP 10.3.1.5/001, [redacted], Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions. Ref V7YH1002).

The second part (for effect) of this publication actually corresponds to the study summarized in Volume 3CA and already assessed by RMS (CA 8.3.1.4/001, [redacted] 2012, Glyphosate: Evaluating potential effects on honeybee brood (Apis mellifera) development, V7YH1001).

Thus the summary of this publication as proposed by the applicant was not reported here. Only assessment and conclusion part of the applicant and RMS are reported.

Therefore, these sections were not reassessed by RMS.

RMS however notes the following proposals made in this publication:
Considering that bee colonies used in the brood study were up to 50% bigger than those used in the residue study, an additional calculation for the expected total daily intake of glyphosate residues was undertaken assuming that such colonies would collect 9 g pollen and 1944 mL nectar. Furthermore, the determined residue content based on a worst-case application rate of 2.88 kg a.e./ha for spot treatments in orchards and vines and was adjusted to reflect the more realistic maximum application rate of 2.16 kg a.e./ha for preplanting, preemergence of crops, and preharvest applications.

The recalculation for bigger colonies makes sense. However RMS expressed concerns on the reliability of the actual exposure of the colony during the exposure assessment phase (initial residues, quantity of pollen brought back to the hive). RMS estimated that 164 g pollen could be collected per day. This RMS proposal was based on empirical data available for "natural" colonies i.e. bigger than those used for the exposure assessment (stage 1). Then no recalculation is considered needed for pollen. Recalculation remains relevant for nectar (nectar intake was deemed acceptable by RMS).

The recalculation for lower application rates is another issue linked to the product GAPs. So it will be required for each application rate intended. For instance, recalculation proposed for 2.16 kg a.e./ha, is reported below:

Scenario	**Daily intake of glyphosate residues in nectar (1296 g nectar/d) [mg]**	**Daily intake of glyphosate residues in pollen (164 g pollen/d) [mg]**	**Total daily intake of glyphosate residues [mg a.e.]**
	Adjusted for : colony size, application rate	**Adjusted for : application rate**	
Day 1 maximum mean residues* (62.6 µg a.e./g in nectar, 1148 µg a.e./g in pollen, glasshouse 2)	81.1	141.15	222.2

Mean residues over days 1-3* (31 µg a.e./g in nectar, 620 µg a.e./g in pollen, both glasshouses)	45.1	76.3	121.4

*considering a conservative factor of 2

For honeybee colonies foraging on the model crop Phacelia treated with 6 L MON 52276/ha, a total daily intake of glyphosate residues of 222.2 mg a.e. (based on day 1 maximum mean residues) and of 121.4 mg a.e. (based on mean residues over days 1-3) can be estimated.

B.9.5.1.1.7. Field tests with honeybees

In view of the available information and the outcome of the risk assessment, field studies with honeybees for the representative EU formulation MON 52276 are not considered required.

B.9.5.2. Effects on non-target arthropods other than bees

B.9.5.2.1. Standard laboratory testing for non-target arthropods

Data point:	CP 10.3.2.1/001
Report author	[redacted]
Report year	1995
Report title	Testing toxicity to beneficial arthropods. Cereal aphid parasitoid - *Aphidius rhopalosiphi* (De Stefani-Perez) / Imagines according to IOBC Guideline (Mead-Briggs 1992). Roundup Ultra
Report No	95 10 48 054
Document No	-
Guidelines followed in study	IOBC Guideline (Mead-Briggs 1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current guideline IOBC (2000):* *Major:* - *For mortality phase, 3 replicates were used in test item treatment groups and 1 in reference item, instead of 4* *Minor:* - *none*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
The toxicity of MON 52276 to the parasitic wasp, *Aphidius rhopalosiphi* was tested with two day old wasps exposed to the equivalent of 10 L MON 52276/ha applied in 200 L/ha water on glass plates. A control was prepared in parallel (deionized water only) and dimethoate product was used as a reference item 0.2 L/ha in 200 L/ha water.
Three replicate cages, each containing 10 wasps, (30 wasps per treatment in total) were used for the test item treatment and the control group, with a single replicate used for the reference item. Mortality and sublethal effects were recorded at 0.5, 2, 24 and 48 hours after application, following application and then drying of the test substance onto glass plates.
After 24 hours, 100% of the wasps died after treatment with MON 52276 after 24 h of exposure. Therefore, the parasitisation efficiency of the exposed wasps was not evaluated. All validity criteria were met. As there was 100% mortality during the exposure phase, a full set of endpoints for the study could not be determined.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276 (Product name: Roundup Ultra)
Description:	Not stated
Lot/Batch #:	080694
Purity:	Glyphosate (isopropylamine salt) 360 g/L (31.0% according to certificate)
Density:	1.1694 g/cm^3
Reference item:	Dimethoate product (dimethoate: 411.14 g/L)
Test organisms:	
Species:	Cereal aphid parasitoid (*Aphidius rhopalosiphi*)
Age:	Approximately 2 days
Source:	PK Nützlingszuchten, Welzheim, Germany
Diet/Food:	Honey + water (1 : 2)
Acclimatisation:	Not stated
Environmental conditions:	
Temperature:	20 – 23°C
Relative humidity:	58 – 77% in the testing room
Photoperiod:	16 hours light / 8 hours darkness
Experimental dates:	September 18th, 1995 to September 20th, 1995

B. STUDY DESIGN AND METHODS

Experimental treatments:

The test solutions were sprayed onto the surface of glass plates using an automatic application cabin, in water volumes equivalent to spraying 200 L/ha deionized water as control, 10 L MON 52276/ha in 200 L/ha water (equivalent to 3.6 kg a.e./ha) and 0.2 L Dimethoate product/ha in 200 L water/ha (reference substance). Plates were air dried in the laboratory for 2 - 3 hours and then with the sprayed surfaces inner-most, 2 plates were put together with a square aluminium frame. Then 5 females and 5 males *Aphidius* wasps were introduced into each cage through holes in the frame sides which were closed after insect insertion. The honey solution was offered to the parasitoids with at cotton wool stopper in one hole of the frame. The test cages were set up in a climatic test room and connected over a water bottle with an aquarian pump for ventilation with humid air.

In the test, three replicate cages, each containing 10 wasps, were used for the test item treatment and the control. The reference item was tested in one replicate. Because of high mortality (100%) of the parasitoids in the treated variant the experiment was finished 48 h after application.

Observations: Mortality and sublethal effects were recorded 0.5, 2, 24 and 48 hours after application.

Statistical calculations: descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality:

Table B.9.5-7: Toxicity of MON 52276 to parasitic wasps (*Aphidius rhopalosiphi*) in a 48 h laboratory test

Test solutions	Replicates	2 h	24 h		48 h	
		Surviving wasps	Surviving wasps	Mortality %	Surviving wasps	Mortality %
Control: 200 L/ha deionized	1	10	10	3.3	9	6.7
	2	10	9		9	
	3	10	10		10	
	Σ	30	29	-	28	-
Test substance: 10 L/ha MON 52276	1	3	0	100	-	-
	2	3	0		-	
	3	4	0		-	
	Σ	10	0	-	-	-
Reference substance: 0.2 L Dimethoate /ha	1	3	0	100	-	-
	Σ	3	0	-	-	-

B. OBSERVATIONS

The mortality in the control treatments did not exceed 10% for 48 hours, the corrected mortality in the reference treatment was >50%. The test was stopped after 24 hours for test item treatment and no evaluation of reproduction was conducted for the control treatments.
The applicant notes that the study is not reliable to be used in risk assessment (as the study pre-dates the Mead-Briggs approach: the control was conducted using 30 instead of 40 wasps and no reproduction assessment was included).
RMS is of the opinion that any observed effect should be considered in depth even if the study design is not completely in adequation with current recommendations (see commenting box below).

III. CONCLUSIONS

Assessment and conclusion by applicant:
There was 100% mortality during the exposure phase at the rate tested (10 L MON 52276/ha) and therefore, no parasitisation efficiency data generated. Highly likely that the findings in the study may have been confounded by the wet sticky layer on the treated glass plates in the MON 52276 treatment group.
This study is therefore considered supportive and unreliable for use in the risk assessment.

Assessment and conclusion by RMS:
RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.

The study author noted that the 5 % v/v test solution of ROUNDUP ULTRA used in the test produced a wet sticky layer on the treated glass plates that resulted in alterations of the moving behaviour of the wasps to the point of sticking. RMS cannot quantify the impact of this sticky layer on mortality.

RMS considers this study as informative only.

Aphidius rhopalosiphi exposed via treated glass plates: 100% mortality at the rate of 10 L MON 52276/ha (i.e. $LC_{100} \leq$ 10 L MON 52276/ha) (to be used as supportive data)

Data point:	CP 10.3.2.1/002
Report author	[redacted]
Report year	1995
Report title	Testing toxicity to beneficial arthropods. Predacious mite - *Typhlodromus pyri* (Scheuten) according to IOBC Guideline (Overmeer 1988 and Louis 1994). Roundup Ultra
Report No	95 10 48 056
Document No	-
Guidelines followed in study	*IOBC Guideline (Overmeer 1988 and Louis 1994).*
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations compared to current IOBC guidelines (2000):* *Major:* - *60 mites were used instead of 100* *Minor:* - *none*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
In the laboratory study the toxicity of MON 52276 to the predatory mites, *Typhlodromus pyri* was tested. Freshly hatched mites were exposed to 10 L MON 52276/ha in 200 L/ha water on dried glass plates. In addition, an undosed control was tested (200 L/ha deionized water). Kelthane 50 (480 g dicofol/L) was used as a reference item 0.1 L/ha in 200 L/ha water.
The test was conducted with 6 replicates per test concentration; control and reference control each containing 10 mites. Mortality was recorded 1 and 4 days after application.
100% of the wasps died in treatment with MON 52276 after 4 days of exposure. Validity criteria were met. However due to 100% mortality, endpoints could not be properly determined. Therefore, study does not provide relevant endpoints.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Description:	Not stated
Lot/Batch #:	080694
Purity:	Glyphosate (isopropylamine salt) 360 g/L (31.0% according to certificate)
Density:	1.1694 g/cm^3

2. Reference item: Kelthane 50 (dicofol: 480 g/L)

3. Test organisms:

Species:	Predacious mite (*Typhlodromus pyri*)
Age:	Approximately 1 day
Source:	PK Nützlingszuchten, Welzheim, Germany
Diet/Food:	spider mites (*Tetranychus urticae*) and during the test pollen
Acclimatisation:	Not stated

4. Environmental conditions:

Temperature:	25 - 27°C
Relative humidity:	72 - 78%
Photoperiod:	16 hours light / 8 hours darkness

5. Experimental dates: August 17th, 1995 to August 21st, 1995

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Glass plates were sprayed with the deionised water, test substance or reference substance. Test concentrations used were 200 L/ha deionised water (control), 10 L MON 52276/ha in 200 L/ha water (test substance treatment) and 0.1 L Kelthane 50/ha in 200 L water/ha (reference substance). After air-drying at room temperature (about 60 minutes), glass plates were infested with young freshly hatched predacious mites together with pollen for food supply. The test was conducted with 6 replicates for control, test item and reference item, each replicate containing 10 mites.

2. Observations: Mortality was recorded 1 and 4 days after application

3. Statistical calculations: No statistical calculations performed.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-8: Toxicity of MON 52276 to predatory mites (*Typhlodromus pyri*) in a 4 day laboratory test

Test concentration	Mortality [%]	
	1 d	4 d

Control: 200 L/ha deionised	5	10
Test substance: 10 L/ha MON 52276 in 200 L/ha water	90	100
Reference substance: 0.1 L Kelthane 50 /ha in 200 L water/ha	100	--

B. OBSERVATIONS

The final assessment was performed 4 days after the application, because of total mortality of the predacious mites in the test variant.

III. CONCLUSIONS

Assessment and conclusion by applicant:
Under the conditions of the present test, MON 52276 applied at 10 L/ha in 200 L/ha water resulted in 100% mortality of the predatory mites after 4 days of exposure.
The study is considered supportive and not sufficiently reliable to be used in risk assessment (as the study pre-dates the Blümel approach and the control was conducted using 60 instead of 100 mites and no reproduction assessment was included).

Assessment and conclusion by RMS:
RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.

The applicant notes that the study pre-dates the Blümel approach and the control was conducted using 60 instead of 100 mites and no reproduction assessment was included.
Indeed mortality was assessed at 4 days (instead of 7) as 100% mortality was already observed then. 60 mites were used instead of 100. RMS considers the study not robust enough to derive an endpoint. RMS nevertheless considers this study indicative of a strong effect at the dose of 10 L MON 52276/ha (i.e. LC100 ≤ 10 L MON 52276/ha).

Typhlodromus pyri exposed via treated glass plates: 100% mortality at the rate of 10 L MON 52276/ha (i.e. LC100 ≤ 10 L MON 52276/ha) (to be used as supportive data)

Data point:	CP 10.3.2.1/003
Report author	[redacted]
Report year	1995
Report title	Testing toxicity to beneficial arthropods - Carabid beetle - *Poecilus cupreus* L. according to BBA Guideline VI, 23-2.1.8 (1991) ROUNDUP ULTRA
Report No	95 10 48 055
Document No	-
Guidelines followed in study	BBA Guideline VI, 23-2.1.8 (1991)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current guideline Heimbach et al. (2000):* *Major:* - *none* *Minor:* - *Beetles should be kept at least 7 days before application in the lab (no indication).* - *One pupa per beetle and per feeding occasion is recommended (2 were provided in this study)*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
In the laboratory study, the toxicity of MON 52276 to the carabid beetle - *Poecilus cupreus* was tested. Adult carabid beetle were exposed to 10 L MON 52276/ha in 400 L/ha water on moistened quartz sand. In addition, an untreated control was tested (400 L/ha deionized water). Afugan was used as a toxic reference item (0.8 L/ha in 400 L/ha water).
In the test, five replicate cages, each containing 6 carabid beetles (3 females + 3 males) were used for each treatment group. Feeding, mortality and sublethal effects were recorded 2, 4 and 6 hours after application. Then 1, 2, 4, 7, 9, 11 and 14 days after application.
The mortalities in the control and in the MON 52276 treatments were 0%. Consequently, the test fulfilled the validity criterion (mortality in the control <10 %) and the LC_{50} was higher than 10 L MON 52276/ha. The feeding rate showed no differences in comparison with the control variant. No behavioural anomalies were observed.
The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON (1982) was E = 1 %.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276 (ROUNDUP ULTRA SL)
Active substance	Glyphosate
Lot/Batch #:	080694
Purity:	31% (Glyphosate (isopropylamine salt) 360 g/L)
Density:	1.1694 g/cm^3
Toxic reference:	Afugan (pyrazophos 294 g/L)
Test organism:	
Species:	Carabid beetle - *Poecilus cupreus* L.
Age:	Adults (7 weeks old)
Source:	laboratory rearing of BBA Braunschweig
Food:	Onion fly (*Delia antiqua*)
Acclimatisation:	3 days under laboratory conditions without food
Environmental conditions:	
Temperature:	18 - 21°C
Photoperiod:	16 h
Light intensity	approx. 1000 lux
Relative humidity:	Test units: 54 - 82%
Experimental dates:	7 August - 21 August 1995

B. STUDY DESIGN AND METHODS

Experimental treatments

The test carabid beetles were kept for 3 days under laboratory conditions for acclimatisation. Three females and three males were placed into each test cage (cages of plastics: 18.3 cm × 13.6 cm × 6.4 cm) with moistened sand (250 g) covering the bottom without food. Immediately before the treatment the beetles were inspected, the ones which appear damaged were replaced by animals of the same sex. Then the sand was moistened with deionized water and fly pupae were added as food supply. The treatments were applied to the cages with the beetles in an automatic application cabin. The control treatment was sprayed with deionized untreated water, the test item treatment was sprayed with 10 L MON52276/ha solution and the toxic reference item was sprayed with 0.8 L Afugan/ha (equivalent to 235 g a.s./ha). After application the cages were incubated in an air condition room (20°C, 16/8 h light/dark) for 14 days. After 1, 2, 4, 7 and 11 days food was changed (2 pupae/beetle) and sand was moistened.

Observations

The sex of the adults was determined before the beginning of the test. The number of dead beetles, the number of fed pupae and any behavioural effects were assessed after 2, 4 and 6 hours, as well as 1, 2, 4, 7, 11 and 14 days after application.

Calculations

The mortality of beetles was corrected following the formula of SCHNEIDER-ORELLI. The relative decrease of the beneficial effectivity was assessed by the formula of OVERMEER & VAN ZON. For evaluating the influence of the test substance on the test animals the results of the tests were rated

according to the four categories selected by the IOBC Working Group "Pesticides and beneficial organisms":
- 1 = harmless: E <30% reduction of beneficial effectivity
- 2 = slightly harmful: E = 30 – 79% reduction of beneficial effectivity
- 3 = moderately harmful: E = 80 - 99% reduction of beneficial effectivity
- 4 = harmful: E >99% reduction of beneficial effectivity

II. RESULTS AND DISCUSSION

A. FINDINGS

The results of the test are given in the following tables.

Table B.9.5-9: Effects of the MON 52276 on adult mortality

Time after application	Control (untreated deionized water)		Test item (10 L MON 52276/ha)		Toxic reference (0.8 L Afugan/ha)	
	No. of dead females	No. of dead males	No. of dead females	No. of dead males	No. of dead females	No. of dead males
2 hours	0	0	0	0	0	0
4 hours	0	0	0	0	0	0
6 hours	0	0	0	0	0	0
Day 1	0	0	0	0	0	0
Day 2	0	0	0	0	15	15
Day 4	0	0	0	0	0	0
Day 7	0	0	0	0	0	0
Day 11	0	0	0	0	0	0
Day 14	0	0	0	0	0	0
Total	0	0	0	0	15	15
Total in percentage	0		0		100	

Initial number of female and male beetles: 15

No behavioural effects were observed in the control and test item groups. Stilted legs, troubles of locomotion and dorsal position symptoms were recorded in the toxic reference group.

Table B.9.5-10: Effects of the MON52276 on the feeding rate

Time after application	**Control (untreated deionized water)**	**Test item (10 L MON 52276/ha)**	**Toxic reference (0.8 L Afugan/ha)**
	females + males	females + males	females + males
Day 1	50	50	25
Day 2	28	27	0
Day 4	33	32	0
Day 7	36	41	0
Day 11	47	49	0
Day 14	38	31	0
Total	232	230	25
Fed pupae/beetle	7.7	7.7	0.8
Fed pupae/group	232	230	25

Initial number of female and male beetles: 15

B. OBSERVATIONS

The mortality in the control was 0%. The test item MON 52276 was tested at a dose of 10 L/ha in 400 L/ha of water and caused 0% mortality.
The corrected mortality according to SCHNEIDER-ORELLI was 0%. The feeding rate showed no differences in comparison with the control variant. No behavioural anomalies were observed.

The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON (1982) was E = 1%.

According to the study protocol based on BBA Guideline VI, 23-2.1.8 (1991), for the study to be valid, mortality in the control group should not exceed 10%. Consequently, the test accomplished the validity criterion (mortality in the control <10%).

The following validity criteria according to the current laboratory method to test effects of plant protection products on the carabid beetle *Poecilus cupreus* (Coleoptera: Carabidae) (Heimbach, 2000) were fulfilled:

- The control mortality must be <6.7% taking into account 5 replicates × 6 beetles (actual value: 0%).
- The mortality in the toxic reference item should be 65 ± 35% after 2 weeks (actual value: 100%).

The applicant also noted that the following points deviated from the guideline:

- Beetles should be kept at least 7 days before application in the lab (no indication).
- One pupa per beetle and per feeding occasion is recommended (2 were provided in this study)

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a laboratory test to determine the effects of MON 52276 on the carabid beetles, *Poecilus cupreus* L., the LC_{50} was higher than 10 L MON 52276/ha. MON 52276, applied at the rate of 10 L/ha, had no adverse effects on the feeding performance.

The study fulfilled the IOBC guideline validity criteria and is therefore considered valid and suitable to be used in the risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that *due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.*

Here the study has been checked by mean of the current guideline Heimbach et al. (2000) and can thus be considered for risk assessment.

Deviations from the current guideline Heimbach et al. (2000) are noted:
Beetles should be kept at least 7 days before application in the lab (no indication).
One pupa per beetle and per feeding occasion is recommended (2 were provided in this study)
RMS agrees that these deviations are minor.

The study is valid (validity criteria fulfilled).

Poecilus cupreus L. exposed under laboratory conditions: LD50 > 10 L MON 52276/ha (no mortality at 10 L MON 52276/ha)
MON 52276, applied at the rate of 10 L/ha, had no adverse effects on the feeding performance.

Data point	CP 10.3.2.1/004
Report author	[redacted]
Report year	1995
Report title	Testing toxicity to beneficial arthropods - Spider - *Pardosa spp*. According to BBA Guideline (Proposal 1994) ROUNDUP ULTRA
Report No	95 10 48 053
Document No	-
Guidelines followed in study	BBA Guideline (Proposal 1994)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current guideline Heimbach et al. (2000):* *Major:* - *none* *Minor:* - *Spiders should be kept at least 7 days before application in the lab (5 days in the study)* - *Spiders should be weighed before test start (no indication)* - *Minimum number of spider is 26 (20 in this study)* - *5 flies per feeding occasion for each spider is recommended (1 or 2 were provided in this study)* - *Temperature rose above 20 ± 2°C (23°C in the study)*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid (reliable for beginning august and onwards application)

Summary
In the laboratory study, the toxicity of MON 52276 to the spider *Pardosa spp* was tested. Adult spiders were exposed to 10 L MON 52276/ha in 400 L/ha water on moistened quartz sand. In addition, an undosed control was tested (400 L/ha deionized water). Thiodan 35 EC was used as a reference item 0.085 L/ha in 400L/ha water.
In the test, twenty replicate cages, each containing 1 spider (10 females + 10 males per treatment in total) were used for all the treatment groups. Feeding, mortality and sublethal effects were recorded 2, 4 and 6 hours after application. Then 1, 2, 3, 4, 7, 9, 11 and 14 days after application.
There was 0% spider mortality in the control and in the test item treatments. Consequently, the test fulfilled the validity criterion (mortality in the control < 10%) and the LC_{50} was higher than 10 L MON 52276/ha. The feeding rate showed a low increase in comparison with the control variant. No behavioural anomalies were observed.
The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON (1982) was E = -4.5%.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: MON 52276 (ROUNDUP ULTRA SL)
Active substance Glyphosate

Lot/Batch #:	080694
Purity:	31% (Glyphosate (isopropylamine salt) 360 g/L)
Density:	1.1694 g/cm^3
Positive control:	Thiodan 35 EC (endosulfan 34.4% w/w)
Test organism:	
Species:	Linyphiid spider - *Pardosa spp*
Age:	Adults
Source:	field population (Cunnersdorf/Paitzsch) - June 1995
Food:	Onion fly (*Delia antiqua*), reared in the laboratory
Acclimatisation:	5 days under laboratory conditions (20 ± 2°C)
Environmental conditions:	
Temperature:	20 - 23 °C
Photoperiod:	16 h
Light intensity	approx. 1000 lux
Relative humidity:	Test units: 74 – 85%
Experimental dates:	3 July - 17 July 1995

B. STUDY DESIGN AND METHODS

Experimental treatments

The test spiders were kept for 5 days under laboratory conditions at (20 ± 2°C) for acclimatisation. Three days before treatment one female or one male was placed into each test cage (cages of plastics: 11.5 cm × 11.5 cm × 6.0 cm) with moistened sand (148 ± 2 g) covering the bottom without food. The following species have been collected and identified: *Pardosa Agricola, Pardosa agrestis and Pardosa lugubris*. Immediately before the treatment the spiders were inspected, the ones which appear damaged were replaced by animals of the same sex and the sand was moistened with deionized water. The treatments were applied to the cages with the spiders in an automatic application cabin. The control treatment was sprayed with deionized, the test item treatment was sprayed with 10 L MON 52276/ha solution and the toxic reference item was sprayed with 0.085 L Thiodan 35 EC/ha (equivalent to 30 g a.s./ha). Immediately after application two onion flies (*Delia antiqua*) were added as food supply to each spider and the cages were closed with gauze covers. After a waiting period of 2 hours the cages were incubated in an air condition room (20°C, 16/8 h light/dark) for 14 days. Every 1, 2 or 3 days food was changed and every 3 or 4 days the sand was moistened.

Observations

The sex of the adults was determined before the beginning of the test. The species of the collected spider was determined on ten females and ten males for each treatment group. The number of dead spiders, the number of fed flies and any behavioural effects were assessed after 2, 4 and 6 hours, as well as 1, 2, 3, 4, 7, 9, 11 and 14 days after application.

Calculations

The mortality of spiders was corrected following the formula of SCHNEIDER-ORELLI. The relative decrease of the beneficial effectivity was assessed by the formula of OVERMEER & VAN ZON. For evaluating the influence of the test substance on the test animals the results of the tests were rated according to the four categories selected by the IOBC Working Group "Pesticides and beneficial organisms":

- 1 = harmless: E< 30% reduction of beneficial effectivity
- 2 = slightly harmful: E = 30-79% reduction of beneficial effectivity

- 3 = moderately harmful: E = 80-99% reduction of beneficial effectivity
- 4 = harmful: E> 99% reduction of beneficial effectivity

II. RESULTS AND DISCUSSION

A. FINDINGS

The results of the test are given in the following tables.

Table B.9.5-11: Effects of the MON 52276 on adult mortality

Time after application	Control (untreated deionized water)		Test item (10 L MON 52276/ha)		Toxic reference (0.085 L Thiodan 35 EC/ha)	
	No. of dead females	No. of dead males	No. of dead females	No. of dead males	No. of dead females	No. of dead males
2 hours	0	0	0	0	0	0
4 hours	0	0	0	0	2	0
6 hours	0	0	0	0	1	1
Day 1	0	0	0	0	3	9
Day 2	0	0	0	0	3	0
Day 3	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 7	0	0	0	0	0	0
Day 9	0	0	0	0	0	0
Day 11	0	0	0	0	0	0
Day 14	0	0	0	0	0	0
Total	0	0	0	0	9	10
Total in percentage	0		0		95	

Number of tested spiders: 10

No behavioural effects were assessed in the control and test item groups. Stilted legs, troubles of locomotion and dorsal position symptoms were recorded in toxic reference group.

Table B.9.5-12: Effects of the MON52276 on the feeding rate

Time after application	Control (untreated deionized water)		Test item (10 L MON 52276/ha)		Toxic reference (0.085 L Thiodan 35 EC/ha)	
	females	males	females	males	females	males

Day 1	16	12	17	15	1	0
Day 2	8	8	10	8	0	0
Day 3	7	7	10	9	0	0
Day 4	8	9	8	9	1	0
Day 7	11	16	11	10	2	0
Day 9	8	10	9	10	1	0
Day 11	10	9	10	9	1	0
Day 14	8	10	9	9	1	0
Total	75	81	84	79	7	0
Fed flies / spider	7.8		8.2		0.4	
Fed flies / group	156		163		7	

Number of tested spiders: 10

B. OBSERVATIONS

The mortality in the control was 0%. The test item MON 52276 was tested at a dose of 10 L/ha in 400 L/ha of water and caused 0% mortality.

The corrected mortality according to SCHNEIDER-ORELLI was 0%. The feeding rate showed a low increase in comparison with the control variant. No behavioural anomalies were observed.

The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON (1982) was E = -4.5%.

Consequently, the test accomplished the validity criterion (mortality in the control < 10%).
According to the study protocol based on BBA Guideline (Proposal 1994), for the study to be valid, mortality in the control group should not exceed 10%. This criterion was satisfied.

The following validity criteria according to the current laboratory method to test effects of plant protection products on spiders of the genus *Pardosa* (Aranea: Lycosidae) (Heimbach, 2000) were fulfilled:

- The control mortality must be <3.9% taking into account 20 replicates (actual value: 0%),
- The mortality in the toxic reference item should be 65 ± 35% after 2 weeks (actual value: 95%)

The applicant notes that the following points deviated from the guideline (Heimbach, 2000):

- Spiders should be kept at least 7 days before application in the lab (5 days in the study)
- Spiders should be weighed before test start (no indication)
- Minimum number of spiders is 26 in guideline (20 in this study)
- 5 flies per feeding occasion for each spider is recommended (1 or 2 were provided in this study)
- Temperature rose above 20 ± 2°C (23°C in the study)

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a laboratory test to determine the effects of MON 52276 on the spiders, *Pardosa*, the LC_{50} was higher than 10 L MON 52276/ha. MON 52276, applied at the rate of 10 L/ha, had no adverse effects on the feeding performance.
The study fulfilled the IOBC guideline validity criteria and is therefore considered valid and suitable to be used in the risk assessment.

Assessment and conclusion by RMS:
RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that *due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.*

Here the study has been checked by mean of the current guideline Heimbach et al. (2000) and can thus be considered for risk assessment.

The applicant noted the following points deviated from the guideline:
- Spiders should be kept at least 7 days before application in the lab (5 days in the study)

This precaution aims to reduce mortality so no impact in this study.
- *Spiders should be weighed before test start (no indication)*

RMS believes the spiders may widely differ in size. Large spiders tend to be less sensitive than smaller ones, This may lower the reliability of the endpoint. However as toxic reference performed well, RMS considers the deviation acceptable.
- *Minimum number of spiders is 26 in guideline (20 in this study)*

As no effect was observed on the 20 individuals, RMS considers the deviation minor and acceptable.
- *5 flies per feeding occasion for each spider is recommended (1 or 2 were provided in this study)*

The deviation is considered minor by RMS.
- *Temperature rose above 20 ± 2°C (23°C in the study)*

RMS considers the impact minor.

RMS notes that the spiders were collected in the field in June (i.e. in summer). In the case of a test item intended to be used without temporal restrictions (i.e. throughout the year), over-wintered animals are preferred as these are more sensitive to plant protection products than the new generation of spiders that can be collected in the autumn. So the individuals collected for this test may be less sensitive than those exposed in the field in spring and early summer.

The study is considered informative as sensitivity of the collected spiders may be lesser than for over-wintered individuals (potentially at risk when test item is sprayed in spring or early summer).
It may be considered valid for application from the beginning of August onwards.

Pardosa sp. exposed under laboratory conditions: LD50 > 10 L MON 52276/ha (no mortality at 10 L MON 52276/ha)
MON 52276, applied at the rate of 10 L/ha, had no adverse effects on the feeding performance.

B.9.5.2.2. Extended laboratory testing, aged residue studies with non-target arthropods

Data point:	CP 10.3.2.2/001
Report author	[redacted]
Report year	2010
Report title	An extended laboratory bioassay of the effects of fresh residues of MON 52276 on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae)
Report No	MON-09-3
Document No	MT-2009-404
Guidelines followed in study	Blümel et al. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current guideline Blümel et al. (2000):* *Major:* *- None* *Minor:* *- None*
Previous evaluation	Yes, accepted RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
The aim of this study was to determine the effects of fresh dry residues of MON 52276 on the predatory mite, *Typhlodromus pyri*, under extended laboratory test conditions. The test was conducted with 3 replicates per test concentration, control and reference control each containing 20 mites. The 60 mites were exposed to 3, 6, 8, 12 and 16 L product/ha in 200 L water/ha on leaf discs of French beans (equivalent to 1080, 2160, 2880, 4320 and 5760 g a.e./ha). Afterwards, their survival was assessed after a 7-day period. A check was then made for sub-lethal effects on reproduction. For this, mites were left *in situ* and the numbers of eggs produced per female were recorded over a further 7 day period. The mean number of eggs produced per female between 7-14 days after treatment (DAT), and the overall mean number of eggs produced per female over the 7-day period of assessment was calculated for each treatment. In addition, a control and a toxic reference substance (Dimethoate) were tested.
The 7-day LR_{50} (median lethal rate) was higher than 16000 mL formulation/ha (nominally 5760 g a.e./ha). MON 52276 had no adverse effects on the reproductive performance of surviving mites up to and including a treatment rate of 8000 mL formulation/ha (nominally 2880 g a.e./ha). 16 L/ha > ER50 > 12 L/ha (reduction in no. of egg/female 44.9 % at 12 L/ha).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: MON 52276 (SL)
Description: Yellow/amber fluid
Lot/Batch #: A9B1207115

Purity:	360 g/L glyphosate acid equivalent, nominal 372.9 ± 2.1g/L glyphosate acid equivalent, measured
Density:	1.1683 g/mL
Positive control:	BASF Perfektion EC (400 g/L dimethoate)
Test organisms:	
Species:	Predatory mite (*Typhlodromus pyri*)
Age:	less than 24 h old
Source:	In-house originally from PK. Niitzlingszuchten, Welzheim, Germany (pre-1995).
Diet/Food:	Mix of 3 pollen sources.
Acclimatisation:	culture maintained at 24-26°C one week prior bioassay.
Environmental conditions:	
Temperature:	Mortality test: 25-26 °C Reproductive test: 25-27 °C
Relative humidity:	Mortality test 49.6-79% Reproductive test: 63-79%
Photoperiod:	16 hours light / 8 hours darkness
Light intensity	660-1230 lux
Experimental work dates:	19 October 2009 to 24 November 2009

B. STUDY DESIGN AND METHODS

Experimental treatments: Leaf discs of French beans were treated with 3, 6, 8, 12 and 16 L product/ha in 200 L water/ha (equivalent to 1080, 2160, 2880, 4320 and 5760 g a.e./ha), a water control and toxic reference item. After the leaf discs had dried, they were placed into arenas with their treated surface facing upwards. Twenty proto-nymphal *T. pyri* were placed into each replicate arena, with three replicates (i.e. 60 mites) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed after a 7-day period, by which time mites in the control treatment were adult. A check was then made for sub-lethal treatment effects on reproduction. For this, mites were left *in situ* and the numbers of eggs produced per female were recorded over a further 7-day period. Temperature and humidity measurements were taken at hourly intervals throughout the bioassay using an electronic data logger. Light intensities were recorded at the start of assessments. Although the relative humidity fell below the intended range, this was for a period of less than two hours so was not therefore considered a deviation.

Observations: Mortality was recorded 1 and 7 days after application. The numbers of any *drowned, stuck* or *missing* mites were added to the number of dead mites found in each treatment to derive the overall mortality. Assessments of oviposition activities were carried out at 10, 13 and 14 DAT. Any eggs and nymphs present were recorded and then removed. The mean number of eggs produced per female between 7-14 days after treatment (DAT), and the overall mean number of eggs produced per female over the 7-day period of assessment was calculated for each treatment group.

During the mortality phase, the temperatures ranged between 25 and 26°C and the relative humidity ranged from 49.6 to 79%. During the reproduction phase, the temperatures ranged between 25 and 27°C and the relative humidity ranged from 63 to 79%. The photoperiod was 16 hours light per day between 600 and 1230 lux.

Statistical calculations: The percentage mortality was compared to the control using Fisher's Exact Test (error rate of $\alpha = 0.05$). For reproduction, the results were compared by one-way ANOVA and Dunnett's Test.

Validity criteria according to Blümel et al.,(2000):

- The mortality in control group should not exceed 20% on day 7 after test start.
- The cumulative mean number of eggs per female from day 7 – 14 was ≥ 4 eggs/female
- The cumulative mortality of the reference item on day 7 should be between 50 and 100%.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality

Table B.9.5-13: Toxicity of MON 52276 to predatory mites (*Typhlodromus pyri*)

Test concentration [L/ha]	Mortality after 7 days[(a)] [%]	Abbott corrected mortality [%]	Mean number of eggs per female [(b)]	Effects on reproduction [(c)] [%]
Control	15	-	6.9	-
3	13	0	8.1	-17.4
6	18	4	4.2	39.1
8	23	9	5.9	14.5
12	32	20	3.8*	44.9
16	40*	29	3.0*	56.5

(a) Mortality in the individual test item treatments at 7 DAT was compared to that in the control using Fisher's Exact Test.

(b) Results for reproduction compared by one-way ANOVA and Dunnett's Test.

* Significantly different from the control.

(c) Change in numbers of eggs per female, relative to control (after Blümel et al., 2000). A positive value indicates a decrease and a negative value indicates an increase

B. OBSERVATIONS

The 7-day LR_{50} is found to be higher than the maximum rate tested >16 L MON 52276 /ha (nominally 5760 g a.s./ha). The mean number of eggs produced per female was calculated to be 6.9 in the control. There were no significant effects in reproduction, compared to the control, at treatment rates up to and including 8 L MON 52275/ha (ANOVA, P > 0.05).

Reference test: Treatment with the reference item BASF Perfektion resulted in significant effects on reproduction (85% Abbott corrected mortality).

Validity criteria according to Blümel et al.,(2000) were fulfilled; as mortality in control group not exceeded 20% on day 7 after test start (actual value: 15%). The cumulative mean number of eggs per female from day 7 – 14 was ≥ 4 eggs/female (actual value: 6.9) and the cumulative mortality of the reference item on day 7 was between 50 and 100% (actual value: 85%).

The following points deviated from the guideline recommendations:

- The application for toxic reference was 30 mL product/ha instead of 9-15 mL/ ha recommended.
- The application substrate was plant instead of glass.

The applicant argues that these deviations are due to the extended test design and are not expected to have any negative impact on the study validity.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In an extended laboratory test to determine the effects of MON 52276 on the predatory mite, *Typhlodromus pyri*, the 7-day LR_{50} (median lethal rate) was higher than 16 L formulation/ha (nominally 5760 g a.e./ha) and the surrogate endpoint for reproduction was set to be $ER_{50} \geq 12$ L MON 52276 /ha. MON 52276 had no adverse effects on the reproductive performance of surviving mites up to and including a treatment rate of 8000 mL formulation/ha (nominally 2880 g a.e./ha).

The study is considered to be valid and suitable to be used for the risk assessment.

Assessment and conclusion by RMS:

This study was already assessed and accepted in previous RAR.

MON 52276 was applied to leaf discs cut from French bean plants (Phaseolus vulgaris), i.e. a 2-dimensional foliar substrate.

The following points deviated from the guideline recommendations:

- The application for toxic reference was 30 mL product/ha instead of 9-15 mL/ ha recommended.
- The application substrate was plant instead of glass.

The applicant argues that these deviations are due to the extended test design and are not expected to have any negative impact on the study validity.
RMS notes that toxic reference induced targeted mortality (i.e. between 50-100%) but at rate more than twice higher than recommended in the guideline for study design with glass plates. It is RMS opinion that this is likely due to lesser bioavailability of the substance via treated leaves. Overall the deviations are considered acceptable by RMS.

RMS notes that reproduction was affected at highest dose but no clear dose relationship was observed. Then RMS does not consider that a calculation of an ED50 is necessary.

The study is valid (validity criteria of Blümel et al.,(2000) fulfilled)

Typhlodromus pyri exposed under extended laboratory conditions: 7-day LD50 > 16 L MON 52276/ha
MON 52276, applied at the rate of 8 L/ha, had no adverse effects on reproduction.
16 L/ha > ER50 > 12 L/ha (reduction in no. of egg/female 44.9 % at 12 L/ha)

Data point:	CP 10.3.2.2/002
Report author	[redacted]
Report year	1999
Report title	An extended laboratory test to determine the effects of MON 52276 on the predatory mite, *Typhlodromus pyri* (Phytoseiidae)
Report No	MON-99-2
Document No	US-99-092
Guidelines followed in study	Barrett et al. (1994): Guidance document on regulatory testing procedures for pesticides with non-target arthropods.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations compared to current guideline Blümel et al. (2000):* *Major:* - *Control mortality exceeded the trigger of 20% (24%)* - *Reproduction assessment conducted on untreated glass plates* - *Assessments of fecundity between 7 and 14 days have not been conducted (3 times)* *Minor:* - *none*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid.

Summary
In the laboratory study the toxicity of MON 52276 to the predatory mites, *Typhlodromus pyri* was tested. 100 mites were exposed to 0.6, 3, 6 and 12 L product/ha in 200 L water/ha on leaves of potted French beans. Afterwards, the surviving females were put on untreated glass plates for the fecundity test, where the number of laid eggs was counted after another 7 days. In addition, a control and a toxic reference substance (Dimethoate 40) were tested.
The test was conducted with 5 replicates per test concentration, control and reference control each containing 20 mites. Mortality was recorded 7 days after application and the eggs counted 14 days after application.
At the concentration of 12 L test item/ha, 30% and higher mortality was observed for lower concentration (6 L test item/ha), while no effects on fecundity were noticed.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276 (EC)
Description:	Not stated
Lot/Batch #:	290598
Purity:	31% w/w glyphosate acid, nominal 30.9% w/w glyphosate acid, measured

2. positive control: BASF Dimethoate 40 (400 g/L dimethoate)

3. Test organisms:

Species:	Predacious mite (*Typhlodromus pyri*)
Age:	Approximately 4 days after eggs laying
Source:	In-house originally from PK. Niitzlingszuchten, Welzheim, Germany (pre-1998).
Diet/Food:	Untreated broad bean pollen
Acclimatisation:	Not stated

4. Environmental conditions:

Temperature:	Mortality test: 21 – 26 °C Reproductive test: 22- 26 °C
Relative humidity:	Mortality test 43 - 61% Reproductive test: 41-75%
Photoperiod:	16 hours light / 8 hours darkness
Light intensity	Mortality test: 2600 – 3400 lux Reproductive test: ~2600 lux
5. Experimental dates:	May 27th, 1999 to June 16th, 1999

B. STUDY DESIGN AND METHODS

1. Experimental treatments: 20 protonymphal mites (*Typhlodromus pyri*) were placed on leaves of potted French bean plants (*Phaseolus vulgaris*) which were treated with 0.6, 3, 6 and 12 L product/ha. The leaf petioles were surrounded with a sticky gel barrier to prevent the mites from escaping. Also, a control and a toxic reference were tested. The test was conducted with 5 replicates per test concentration, control and reference treatment each containing 20 mites. Surviving mites were transferred to untreated glass surfaces and the fecundity of these mites was assessed up to 14 days after treatment (thus, additional 7 days) by counting the produced eggs.

2. Observations: Mortality was recorded 7 days after application. Eggs were counted 14 days after treatment.

3. Statistical calculations: The mortality was corrected with the control mortality using Abbott's correction (1925).

I. RESULTS AND DISCUSSION

A. FINDINGS

Mortality

Table B.9.5-14: Toxicity of MON 52267 to predatory mites (*Typhlodromus pyri*) in a 7 day laboratory test

Test concentration [L/ha]	Mortality after 7 days [%]	Abbott corrected mortality [%]
Control	24	-
0.6	19	0
3	40	21
6	51	36
12	47	30

Fecundity

Table B.9.5-15: Toxicity of MON 52267 to predatory mites (*Typhlodromus pyri*) in the following 7 day fecundity test

Test concentration [L/ha]	Number females transferred 7 days after treatment	Number eggs/nymphs produced 14 days after treatment	Mean egg number/ female after 14 days
Control	42	174	4.1
0.6	52	246	4.7
3	41	194	4.7
6	33	136	4.1
12	28	136	4.9

B. OBSERVATIONS

The test item resulted in ≥ 30% mortality of *Typhlodromus pyri* when applied at concentration of 6 L/ha and higher. In the fecundity assessment, no dose-response relationship was observed.

Reference test: Treatment with the reference item BASF Dimethoate 40 resulted in significant effects on reproduction (100%). (RMS notes that dose rate was inappropriate, see commenting box).

Validity criteria according to Blümel et al. (2000) were not fulfilled; as mortality in control group slightly exceeded 20% on day 7 after test start (24%). The cumulative mean number of eggs per female from day 7 – 14 was ≥ 4 eggs/female and the cumulative mortality of the reference item on day 7 was between 50 and 100% (but , as noted by RMS, considering a dose far exceeding the recommendations).

III. CONCLUSIONS

Assessment and conclusion by applicant:
Under the conditions of the present test, MON 52276 applied at concentrations of 6 L/ha in 200 L/ha water resulted in 30% and more mortality of the predatory mites after 7 days of exposure. In the fecundity assessment, no dose-response relationship was observed.

The study is considered supportive and is not used in the risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that *due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.*

The test design is unusual (whole leaf). MON 52276 was applied to individually-potted French bean plants at a spray volume rate of 200 L water/ha. The plants were prepared for spraying by removing all of the leaves, except one, from each plant. Each leaf was presented horizontally to ensure maximal exposure to the spray. A gel was placed around the petiole of each leaf and 20 protonymphal mites from the stock culture were placed on the upper leaf surface using a fine brush. RMS then assumes that protonymphal mites were free to move to both sides of the leaf.

Deviations compared to current guideline Blümel et al. (2000):

- Control mortality exceeded the trigger of 20% (24%)

RMS notes that most of the mites were actually "missing". Nevertheless these missing individuals are counted as "dead" for the interpretation of lethal effect. This percentage of 24% exceeds the validity criteria (and decreases statistical power of the test). RMS considers the study unreliable.

Table 1. A summary of the fate of the mites 7 days after treatment.

The data presented are the total number of *T. pyri* (n = 100 per treatment) recorded as being *alive* or *dead*. The numbers of dead mites are divided into those that were either *moribund/dead* on the plant leaves (D), *stuck* in the sticky barrier (S) or *missing* (Mi). The Abbott corrected percentage mortality in each treatment is also presented.

	Live	Dead			Abbott-corrected % mortality
		D	S	Mi	
control	76	2	0	22	-
MON 52276 (12 L/ha)	53	15	2	30	30
MON 52276 (6 L/ha)	49	12	1	38	36
MON 52276 (3 L/ha)	60	2	1	37	21
MON 52276 (0.6 L/ha)	81	3	0	16	0
dimethoate	0	47	0	53	100

- Reproduction assessment conducted on untreated glass plates

This change to an inert surface was required since it was not practical to continue to use a treated plant as the test substance was a herbicide. This change is considered acceptable by RMS.

- Assessments of fecundity between 7 and 14 days have not been conducted (3 times)

RMS considers that this deviation is minor.

RMS also notes that application rate for toxic reference was 850 mL product/ha instead of 9-15 mL/ ha recommended. Such dose is inappropriate.

RMS notes that no dose relationship was observed. Then RMS does not consider that a calculation of an LD50/ED50 is necessary.

The study is not valid (validity criteria of Blümel et al.,(2000) not fulfilled)

Data point:	CP 10.3.2.2/003
Report author	[redacted]
Report year	1998

Report title	Testing toxicity to beneficial arthropods - Predatory mite – *Typhlodomus pyri* (SCHEUTEN) (extended laboratory test) according to IOBC Guideline (Oomen 1988)
Report No	95 10 48 065
Document No	-
Guidelines followed in study	IOBC Guideline (Oomen 1988), ESCORT Guidance Document (1994)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current guideline Blümel et al. (2000):* *Major:* *- none* *Minor:* - *Temperature and humidity in the test room were for short periods of time above the (25 ± 2°C) range (21 - 28°C) and the 60-80% range (53 -100%), respectively.* - *100 mites per treatment are recommended (60 were used)* - *Test lasted 18 days long (14 days is required)* - *Dimethoate rate recommended between 9 and 15 mL/ha (100 mL/ha was used).*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid but no reliable endpoint / informative of effects between 6 and 12 L product/ha

Summary
In the laboratory study, the toxicity of MON 52276 to the predatory mite *Typhlodromus pyri* (SCHEUTEN) was tested. MON 52276 was evaluated in a test with three spray application rates of 3, 6 and 12 L test item/ha. Leaves of potted vine plants, cultivated under field conditions without pesticide treatments were sprayed in an automatic application cabin once with untreated water, the test or reference substance at the stated concentrations. The test comprised 6 replicates per control, test item treatment and reference treatment with 10 predatory mites each. The number of living predatory mites were counted 1, 4, 8, 11, 13, 15 and 18 days after the application (from 8th day onward separated according to the sex), also behaviour recorded on days 8, 11, 13, 15 and 18. The number of laid eggs (with the exception of the 1st and 4th day) and the hatching rate of the mites as of day 10 were determined. The final assessment were performed 18 days after treatment. Three days later the last mites hatched were counted.
Exposure to dried spray deposits of MON 52276 on vine leaves resulted in low mortality at the dose of 3 L/ha and high mortality at 6 and 12 L/ha. There was no significant difference with controls in fecundity or fertility at 3 L/ha. At higher doses, the number of eggs produced by surviving female was either strongly reduced or not measured due to mortality.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276
Active substance	Glyphosate
Lot/Batch #:	270198
Purity:	31% (Glyphosate (isopropylamine salt)
Density:	1.166 g/cm^3
Positive control:	Dimethoate EC 400
Test organism:	
Species:	*Typhlodromus pyri* (SCHEUTEN)
Age:	Approx. 1 day old protonymphs
Source:	MITOX Consultants (Kruislaan 320, 1098 Amsterdam, Netherland) – July 1998
Food:	Pollen (pine, birch) at each assessment day or more often if required
Environmental conditions:	
Temperature:	21 - 28 °C
Photoperiod:	16 h
Light intensity	approx. 1000 lux
Relative humidity:	Test units: 52 – 100%
Experimental dates:	16 July – 6 August 1998

B. STUDY DESIGN AND METHODS

Experimental treatments

The test item MON 52276 was evaluated in a test with three spray application rates of 3, 6 and 12 L test item/ha. Leaves of potted vine plants, cultivated under field conditions without pesticide treatments were sprayed in an automatic application cabin once with untreated water, the test or reference substance at the stated concentrations. The test comprised 6 replicates per control, test item treatment and reference treatment with 10 predatory mites each. After air-drying of the spray deposits at room temperature (about 1 hour and 2 hours at 12 L/ha, respectively) leaf discs (Ø ~4 cm) of the treated leaves were placed with the treated surface upwards in petri dishes (Ø 9 cm) on moistened cotton wool. Each leaf disc was lined with insect glue and infested with 10 protonymphs. Pollen was added as food supply. The test units were then placed in a climatic test room.

Observations

The number of living predatory mites were counted 1, 4, 8, 11, 13, 15 and 18 days after the application (from 8th day onward separated according to the sex), also behaviour recorded on days 8, 11, 13, 15 and 18. The number of laid eggs (with the exception of the 1st and 4th day) and the hatching rate of the eggs as of day 10 were determined. The final assessment were performed 18 days after treatment. Three days later the last mites hatched were counted.

Statistical calculations

In order to detect any significant differences the STUDENT-t-test was used (RATTE 1998).

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of MON 52276 were tested at nominal rates equivalent to 3, 6 and 12 L/ha in 200 L/ha of water. The results are summarised in the following table.

Table B.9.5-16: Findings *Typhlodromus pyri* (SCHEUTEN), extended laboratory test

	***Typhlodromus pyri* (SCHEUTEN)**			
Exposure	**Spray treatment**			
Test formulation/reference	**MON 52276**			**Dimethoate EC 400**
Application	**3 L/ha**	**6 L/ha**	**12 L/ha**	**100 mL/ha**
Corrected mortality (%) until day 8 until day 18	 18 36	 84 86	 89 88	 100 100
Fecundity (% relative to controls)	113	10	0	-
Egg fertility (hatching rate) (% relative to controls)	97	(53)*	-	-
Total effect E (%) according to OVERMEER & VAN ZON	8	98	100	100

B. OBSERVATIONS

Table B.9.5-17: Surviving predatory mites

	Number of surviving predatory mites																
Days after application	**1**	**4**	**8**			**11**			**13**			**15**			**18**		
	mites	mites	♀	♂	∑	♀	♂	∑	♀	♂	∑	♀	♂	∑	♀	♂	∑
Control	60	58	37	18	55	36	18	54	35	17	52	34	17	51	33	17	50
MON 52276																	
3 L/ha	60	55	27	18	45	27	18	45	27	16	43	26	15	41	20	12	32
6 L/ha	60	18	6	3	9	5	2	7	5	2	7	5	2	7	5	2	7
12 L/ha	56	14	5	1	6	5	1	6	5	1	6	5	1	6	5	1	6
Dimethoate 400 EC																	
100 mL/ha	0																

Table B.9.5-18: Egg production of surviving females

	Number of eggs				
Days after application	**11**	**13**	**15**	**18**	**total**
Control	32	49	81	45	207
MON 52276					
3 L/ha	35	54	63	23	175
6 L/ha	2	1	0	0	3

In the reference variant and the highest dose test substance variant (12 L/ha), no eggs were laid.

Table B.9.5-19: Hatching rate of the eggs

	Number of larvae				
Days after application	**13**	**15**	**18**	**21**	**total**
Control	24	35	47	23	129
MON 52276					
3 L/ha	24	42	29	14	109
6 L/ha	1	0	0	0	1

Exposure to dried spray deposits of MON 52276 on vine leaves resulted in low mortality at the dose of 3 L/ha and high mortality at 6 and 12 L/ha. There was no significant difference with controls in fecundity or fertility at 3 L/ha. RMS however notes that only 60 individuals were used for each dose and control, and therefore, the statistical power of the test decreases. At higher doses, the number of eggs produced by surviving female was either strongly reduced or not measured due to mortality.

In all treatments the vine leaves showed (since day 3) damages as a result of herbicide effects. The leaves of the 12 L/ha treatment showed low damages at the leaf-edges already after 2 hours air-drying.

The applicant notes that the following point deviated from the guideline:

- Temperature and humidity in the test room were for short periods of time above the (25 ± 2°C) range (21 - 28°C) and the 60-80% range (53 -100%), respectively.
- Less than 100 mites per treatment were used (actual number: 60)
- Test lasted 18 days long (14 days is required)
- Dimethoate rate recommended between 9 and 15 mL/ha (100 mL/ha was used).

The validity criteria of the current guideline were fulfilled as:

- The control mortality did not exceed 20% after 7 days of exposure (actual values: 8% after 8 days)
- The mean mortality in the toxic reference item ranged between 50% and 100% after 7 days of exposure (actual value: 100% after 1 day). Nevertheless, the rate of toxic reference item was about 10 times the current recommended rate.
- The mean number of eggs per female at the end of the test in control is ≥4 eggs/female (actual value: 6.15)

III. CONCLUSIONS

Assessment and conclusion by applicant:

The laboratory test to determine the effects of MON 52276 on the predatory mite *Typhlodromus pyri*, resulted in low mortality at the dose of 3 L/ha and high mortality at 6 and 12 L/ha. There was no significant difference with controls in fecundity or fertility at 3 L/ha. All of the current validity criteria for this study design were satisfied in this test.

The study was considered supplemental in the 2015 RAR, due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001. This study is no longer considered appropriate for a quantitative risk assessment according to current standards. Therefore, it is considered supportive.

Assessment and conclusion by RMS:

RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that *due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.*

MON 52276 was applied to potted vine plants under field conditions and leaf discs were prepared afterwards.

Deviations from current guideline Blümel et al. (2000):

- Temperature and humidity in the test room were for short periods of time above the (25 ± 2°C) range (21 - 28°C) and the 60-80% range (53 -100%), respectively.

The study auhor considers these deviations did not affect the study. RMS agrees.

- 100 mites per treatment are recommended (60 were used)

This lowers the reliability of the measurements and endpoints.

- Test lasted 18 days long (14 days is required)

This is not major deviation as measurements (for egg production) were already available at 15 days.

- Dimethoate rate recommended between 9 and 15 mL/ha (100 mL/ha was used).

This dose rate is inappropriate. The sensitivity of the protonymphal mites is then not clearly addressed as such dose rate is expected to kill even most resistant individuals.

RMS notes that high mortality was observed at 6 and 12 L/ha but no dose relationship was observed (84 and 89 % mortality respectively). All mortality actually occurred during first day. No distinction was made between dead and missing (no record available for missing individuals). It is also not known if they were found dead in the glue barrier. Then RMS considers that robust endpoint cannot be derived from this study.

The study is of low reliability and is not acceptable for the risk assessment. It seems indicative of an effect on mortality at 6 and 12 L/ha but the impact of the treatments (if any) is highly uncertain. Informative only.

Data point:	CP 10.3.2.2/004
Report author	[redacted]
Report year	2010
Report title	A rate-response extended laboratory test to determine the effects of MON 52276 on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)
Report No	MON-09-2
Document No	MT-2009-405
Guidelines followed in study	Mead-Briggs *et al.* (in press). An extended laboratory test for evaluating the effects of plant protection product on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations compared to current Mead-Briggs et al. (in press):* *Major:* *- none* *Minor:* *- none*
Previous evaluation	Yes, accepted RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
In the extended laboratory study the toxicity of MON 52276 to the parasitic wasp, *Aphidius rhopalosiphi* was tested. Adult parasitic wasps approximately 48 h old were exposed in a definitive rate-response test to 4000, 6000, 8000, 12000 and 16000 mL product/ha. In addition, a water control and a toxic reference (Perfekthion, 400 g/L dimethoate) were tested.
Five female wasps were exposed per replicate, with six replicates (i.e. a total of 30 wasps) prepared for each treatment. Mortality and repellence effects were recorded within the 3 first hours, 24 and 48 hours after application. The parasitisation efficiency of surviving insects in the control and in treatment groups with ≤60% corrected mortality, was studied by confining wasps individually over pots of untreated cereal plants, previously infested with cereal aphids. After 24 hours, wasps were removed and after a further 10 days, the number of mummies (parasitized aphids containing wasp pupae) that had developed was recorded.
The 48-h LR_{50} was higher than 16000 mL product/ha. MON 52276 had no adverse effects on the reproductive performance of surviving wasps up to and including a treatment rate of 16000 mL product/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Appearance:	Yellow/amber fluid
Lot/Batch #:	A9B1207115
Purity:	Glyphosate (isopropylamine salt) 360 g/L
Density:	1.1683 g/cm^3 (at 20 °C ± 0.5 °C)

2. positive control: Perfekthion - BAS 152 11 I (dimethoate: 400 g/L)

3. Test organisms:

Species:	Parasitic wasp (*Aphidius rhopalosiphi*)
Age:	Adults approximately 48 h old
Source:	In-house culture originally obtained from PK Nützlingszuchten, Welzheim, Germany
Diet/Food:	Solution of honey in water (1 : 3 v/v)

4. Environmental conditions:

Temperature:	Mortality phase: 20 °C Reproduction phase: 18 - 20°C
Relative humidity:	69 – 72%
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	Mortality phase: 2030 lux Reproduction phase: 4290 lux

5. Experimental work dates: 14 October 2009 to 09 November 2009

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Following a preliminary range-finding test, MON 52276 was evaluated in a definitive rate-response test at five application rates, equivalent to 16000, 12000, 8000, 6000 and 4000 mL product/ha. These variants were compared to a control treatment of purified water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 10 mL product/ha (nominally 4 g a.s./ha). Treatments were applied at a volume rate equivalent to 400 L spray solution/ha to pots of seedling barley. Once dry, the barley plants were enclosed within cylindrical, ventilated collars (clear acrylic cylinders with fine gauge mesh netting secured over the open end. Five female wasps were then confined in each arena, with six replicates (i.e. a total of 30 wasps) prepared for each treatment. To determine any significant sub-lethal effects on wasp reproduction, assessments were then carried out using the surviving insects from the control and the three highest treatment rates of the test item that resulted in < 60% corrected mortality. Fifteen wasps from each treatment were confined individually over pots of untreated barley plants that had previously been infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed from the plants after 24 h and the aphids and plants left for a further 10 days before the number of 'mummies' (parasitized aphids containing wasp pupae) that had developed was recorded.

2. Observations: Mortality of the wasps was recorded approximately 2, 24 and 48 h after treatment. The behaviour of the wasps was assessed during the first 3 h after treatment and also at 24 and 48 h after treatment, to determine whether there was any apparent repellence from the treated plants. The percentage mortality of the test insects over 48 h was calculated. For the reproduction assessments, the

number of mummies produced per female found alive after the 24 h parasitisation period was determined.
The temperature and relative humidity were recorded at hourly intervals using an electronic data logger for mortality phase. For reproduction phase, the temperature in the room was recorded using a minimum-maximum mercury thermometer. Light levels were recorded at the start of each bioassay using an ELE Single Channel Light Measuring System. For the mortality-assessment phase of the definitive test, the room was maintained at 20 ºC and 69-72% RH, with lighting of 2030 lux provided for a 16 h photoperiod. For the reproduction-assessment phase the pots of seedlings and parasitoids were maintained at 18-20 ºC, with a 16 h photoperiod (4290 lux).

3. Statistical calculations: Fisher's Exact test ($\alpha = 0.05$) for mortality. One-way ANOVA and Dunnett's Test as post hoc ($\alpha = 0.05$) for reproduction. Angularly transformation (square root arcsine), then ANOVA and Dunnett's test (Fowler & Cohen, 1990; SPSS, 2008) for repellence.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-20: Toxicity of MON 52276 to parasitic wasps (*Aphidius rhopalosiphi*) in a 48 h extended laboratory test

Test rate [mL/ha]	Mortality [%]	Corrected mortality [%] [1)]
Control	0	--
4000	0	0
6000	0	0
8000	0	0
12000	3.3	3.3
16000	0	0

[1)] Derived using Abbott's formula.

Reference test: Treatment with the reference item Perfekthion at a concentration of 10 mL/ha resulted in 90% mortality after 48 h of exposure.

Table B.9.5-21: Sublethal effects of MON 52276 to parasitic wasps (*Aphidius rhopalosiphi*) in a 48 h extended laboratory test (summary of wasp repellence assessments)

Test rate [mL/ha]	% observations where wasps recorded to be settled on the treated plants	
	Initial 3 h [1)]	24 h & 48 h [2)]
Control	32.7	40.0
4000	22.0	28.3
6000	24.7	28.3
8000	26.0	25.0
12000	20.7 *	27.5
16000	20.0 *	28.3

[1)] Data from assessments made during the initial 3 h after wasp introduction. Results for the individual test item treatments were compared by one-way ANOVA and Dunnett's Test. Values marked with asterisks differed significantly from the control (* $P < 0.05$).
[2)] Data from assessments made at 24 h and 48 h after wasp introduction. Results for the individual test item treatments were compared by one-way ANOVA ($\alpha = 0.05$), but values for the test item treatments did not differ significantly from the control.

Reference test: Treatment with the reference item Perfekthion at a concentration of 10 mL/ha resulted in significant effects on reproduction after 48 h of exposure.

Table B.9.5-22: Toxicity of MON 52276 to the parasitisation capacity of *Aphidius rhopalosiphi*

Test rate [mL/ha]	n [a)]	Means number of mummies per female [b)]	% change in reproduction, relative to control [c)]
Control	14	21.4	-
8000	14	28.4*	-32.3
12000	14	30.6**	-43.0
16000	15	31.5**	-46.8

a) n = number of female wasps successfully assessed for their reproductive capacity.

b) The results for the test items treatments were compared to the control by one-way ANOVA and Dunnett's Test ($\alpha = 0.05$). Results that differed significantly from the control are indicated with asterisks; however, these were due to a significant increase in the number of mummies produced (* $P < 0.05$; ** $P < 0.01$).

c) Percentage effect on reproduction. A negative value indicates an increase, relative to the control

B. OBSERVATIONS

The following point deviated from the Mead-Briggs *et al.* (2010):

- Light intensity during mortality phase was 2030 lux, compared to 400 to 1200 lux requested by guideline.

The mortality in the control treatments did not exceed 10%, the corrected mortality in the reference treatment was >50%. In the control treatments, more than a minimum mean value of 5.0 mummies was produced per female. No more than two of the surviving wasps of the control treatments did not reproduce. Therefore, the test is considered valid according to validity criteria (Mead-Briggs *et al.* , 2010).

III. CONCLUSIONS

Assessment and conclusion by applicant:

In an extended laboratory test to determine the effects of MON 52276 on the parasitic wasp, *Aphidius rhopalosiphi*, the 48-h LR_{50} was higher than 16000 mL product/ha. MON 52276 had no adverse effects on the reproductive performance of surviving wasps up to and including a treatment rate of 16000 mL product/ha.

This study is considered to be valid and relevant for use in risk assessment.

Assessment and conclusion by RMS:

This study was part of 2015 RAR.

MON 52276 was applied to pots of seedling barley (3D)
The study was well conducted.
Validity criteria are fulfilled.

The following point deviated from the Mead-Briggs et al. (2010):

- Light intensity during mortality phase was 2030 lux, compared to 400 to 1200 lux requested by guideline.

The possible consequence of a high light intensity is a reduced wasp settling rates. However as reported in Appendix 3 of the guideline Mead-Briggs et al. (2010), even an intensity of 4000 lux

might be regarded as satisfactory (even if lower levels of 400-1200 are advantageous to maximize exposure to residues. Then RMS considers this deviation as minor. During reproduction phase, light intensity was of 4290 lux and in accordance with the recommendations (for reproduction phase).
Settling rate (on leaves) was satisfactory.
The toxic reference performed well.

Aphidius rhopalosiphi exposed under extended laboratory conditions: 48-h LR50 > 16 L MON 52276/ha
MON 52276, applied at the rate of 16 L/ha, had no adverse effects on reproduction (reproduction was stimulated).

Data point:	CP 10.3.2.2/005, CP 10.3.2.2/006 (Amendment)
Report author	[redacted]
Report year	1999
Report title	Testing toxicity to beneficial arthropods Cereal aphid parasitoid - *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) (extended laboratory test) following the IOBC Guideline proposal (MEAD-BRIGGS 1994) MON 52276
Report No	98 10 48 066
Document No	-
Guidelines followed in study	IOBC Guideline (Proposal 1994). An extended laboratory test to evaluate the side-effects of pesticides applied to plant material on adults of the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations compared to current Mead-Briggs et al. (2010):* *Major:* *- none* *Minor:* *- For mortality phase, 4 replicates (5 wasps each) were used in test item treatment groups and 1 in reference item, instead of 6 replicates*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
In the extended laboratory study the toxicity of MON 52276 to the parasitic wasps *Aphidius rhopalosiphi* was tested. Adult parasitic wasps approximately 48 h old were exposed to 3, 6 and 12 L test item/ha sprayed onto potted cereal plants and mortality and reproduction were assessed. In addition, a water control was tested and a toxic reference (Dimethoate EC 400 (0.85 mL/ha)) were tested.
Five female wasps were then confined in each of four arenas (i.e. a total of 20 wasps) prepared for each treatment. Mortality and sub-lethal effects were recorded 1, 2, 4, 24 and 48 hours after application. After 48 h, 14 surviving females from the control and the test item treated variants were confined in glass cylinders containing untreated potted wheat plants, infested with ~ 100 aphids (*Rhopalosiphum padi* L.) to assess the parasitisation capacity. The authors concluded that reduction of the beneficial effectivity of *Aphidius rhopalosiphi* was < 30% in all variants and that the behaviour of the wasps treated with the test item did not differ from the control. The number of mummies developed was recorded.
RMS concluded that no reliable endpoint could be derived.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Description:	Liquid, yellowish to brown
Lot/Batch #:	270198
Purity:	31% Glyphosate acid
Density:	1.166 g/cm^3 (at 20 °C ± 0.5 °C)

2. Positive control: Dimethoate EC 400 (0.85 mL/ha)

3. Test organisms:

Species:	*Aphidius rhopalosiphi* (DESTAFANI-PEREZ), cereal aphid parasitoid
Age:	Adults approximately 48 h old
Source:	PK Nützlingszuchten, 73642 Welzheim, Germany
Diet/Food:	Solution of honey in water (1 : 2 v/v), the wasps were not fed for 12 – 18 h prior to exposure.
Acclimatisation:	Not stated

4. Environmental conditions:

Temperature:	19 - 22 °C
Relative humidity:	65 - 84%
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	~ 1000 lux

5. Experimental dates:

B. STUDY DESIGN AND METHODS

1. Experimental treatments: MON 52276 was evaluated in a test at three application rates of 3, 6 and 12 L test item/ha. These treatments were compared to a control treatment of deionised water and a toxic reference treatment of Dimethoate EC 400 applied at a rate of 0.85 mL product/ha. Potted wheat plants were sprayed with 25% aqueous fructose and left to dry for 1 h, followed by application of the test items, applied in final water volumes equivalent to ~200 L spray solution/ha onto the plants surface. Once dry, the treated plants were put in glass cylinders and five female wasps were then confined in each arena, with 4 replicates (i.e. a total of 20 wasps) prepared for control and test item treatment. After 48 h, 14 surviving females from the control and the test item treated variants were confined in glass cylinders containing untreated potted wheat plants, infested with ~ 100 aphids (*Rhopalosiphum padi* L.) to assess the parasitisation capacity. The wasps were then removed from the plants after 24 h and the aphids and plants left for a further 10 days before the number of mummies (parasitized aphids containing wasp pupae) that had developed, was recorded.

2. Observations: Mortality and behaviour of the wasps were recorded 1, 2, 4, 24 and 48 h after treatment.

The number of parasitized aphids (aphid mummies) was recorded 10 days after the wasps were able to lay eggs.

3. Statistics: The parasitisation rate was calculated using Mead-Briggs (1992). According to Overmeer

& Van Zon (1982) the total effect "E" was calculated.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality

Table B.9.5-23: Toxicity of MON 52276 to parasitic wasps (*Aphidius rhopalosiphi*) in a 48 h extended laboratory test

Test rate [L/ha]	Mortality [%]		
	4 h	24 h	48 h
Control	0	0	0
3	0	0	15
6	0	0	15
12	0	0	25

Effects on parasitisation capacity

Table B.9.5-24: Toxicity of MON 52276 to the parasitisation capacity of *Aphidius rhopalosiphi*

Test rate [L/ha]	∑ no. of females examined	Average no. of parasitized aphids per female after 11 days	Parasitisation rate relative to control [%]
Control	14	11.6	-
3	14	11.1	96
6	14	11.7	101
12	14	10.9	94

The total effect "E" is 18.7% for 3 L test item/ha, 14.3% for 6 L test item/ha and 29.5% for 12 L test item/ha

Reference test: Treatment with the reference item Dimethoate EC 400 at a concentration of 0.85 mL product/ha resulted in 80% mortality after 48 h of exposure.

B. OBSERVATIONS

The reduction of the beneficial effectivity of *Aphidius rhopalosiphi* was < 30% in all variants. The behaviour of the wasps treated with the test item did not differ from the control.
Reference test: Treatment with the reference item Dimethoate EC 400 at a concentration of 0.85 mL product/ha resulted in significant effects on reproduction after 48 h of exposure. Nevertheless, the rate of toxic reference item was below the current recommended rate (5-20 mL product/ha).

All validity criteria according to Mead-Briggs et al. (2010): "An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (*Hymenoptera*, Braconidae) were fulfilled, as there was no mortality in control group and the mortality in the toxic reference was >50% (RMS notes that the rate of toxic reference item was below the current recommended rate however uncertainty remains on the actual dose of toxic reference due to typing errors in the study summary, see RMS comment), the number of mummies/female in the control was at least 5 and no more than 2 wasps produced no mummies.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In conclusion, no significant mortality of *Aphidius rhopalosiphi* was observed after treatment with the maximum test rate of 12 L MON 52276/ha (< 30%). The parasitisation rate showed no significant changes compared to the control and the total effect was between 14.3 and 29.5%.
This study is considered to be valid and relevant for use in risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that *due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.*

MON 52276 was applied to potted wheat plants. (3D)

Deviations from current guideline Mead-Briggs et al. (2010):

- - For mortality phase, 4 replicates (5 wasps each) were used in test item treatment groups and 1 in reference item, instead of 6 replicates

This lowers the reliability of the measurements and endpoints.

- Dimethoate rate recommended between 5 and 20 mL/ha. Due to typing errors in the study summary, uncertainty remains on the actual dose of toxic reference that was applied. Doses of 0.85 mL or 0.85 L product/ha are reported throughout the report.

In both cases the dose rate is inappropriate. The sensitivity of the wasps is then not clearly addressed. In case 0.85 L product/ha was applied such dose rate is expected to kill even most resistant individuals. Yet, “only” 80% mortality was observed. The sentitivity of the test system is doubtful.

Plant size is unknown.

The study is of low reliability and is not acceptable for the risk assessment.

This study provides information that *Aphidius rhopalosiphi* exposed up to 12 L product/ha under extended laboratory conditions should not lead to more than 50% effects on mortality and no adverse effects on reproduction.
No reliable endpoint could be derived.

Data point:	CP 10.3.2.2/007
Report author	[redacted]
Report year	2010
Report title	An extended laboratory test to determine the effects of MON 52276 on the ground-active beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae)
Report No	MON-09-4
Document No	MT-2009-403
Guidelines followed in study	Grimm *et al.* A test for evaluating the chronic effects of plant protection products on the rove beetle, *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae), under laboratory and extended laboratory conditions
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations compared to current guideline IOBC (2000):* *Major:* - *none* *Minor:* - *none*
Previous evaluation	Yes, accepted RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
In the extended laboratory study the toxicity of MON 52276 to the rove beetle, *Aleochara bilineata* was tested. Adult rove beetles (3 - 4 days old) were exposed in the definitive rate-response test to 6000, 8000 and 12000 mL product/ha. In addition, a water control and a toxic reference (Cyren, 480 g/L chlorpyrifos) were tested.
Ten female and ten male beetles (i.e. a total of 20 beetles) were introduced in each testing arena, with four replicates prepared for each treatment. Assessments of the condition of the beetles were made at 1, 7 and 28 days after treatment (DAT). The parasitic success of their larval offspring was assessed by the provision of ca. 500 onion fly pupae (*Delia antiqua*) in each replicate box on three weekly occasions, i.e. at 7, 14 and 21 DAT. The original adult beetles were removed from the arenas at 28 DAT and the number of new adults (F1 progeny) that subsequently developed from the parasitized fly pupae was recorded over a further 46-day period. The validity criteria according to Grimm *et al.* (2000) are fulfilled. No significant effects on the parasitation success of the beetles were observed up to and including the highest treatment rate of 12000 mL/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Description:	Yellow/amber-coloured liquid appearance
Lot/Batch #:	A9B1207115
Purity:	Glyphosate (glyphosate acid equivalent) 360 g/L
Density:	1.1683 g/cm^3 (at 20 °C ± 0.5 °C)

2. Positive control:	Reference item: Cyren (chlorpyrifos: 480 g/L)
3. Test organisms:	
Species:	Rove beetle (*Aleochara bilineata*)
Age:	Physiologically 3 - 4 days old
Source:	Commercial supplier (De Groene Vlieg, Nieuwe Tonge, The Netherlands)
Diet/Food:	Pellets (approximately 0.2-0.5 g) of raw minced beef for food every 1-3 days, until the adult beetles were removed 28 days after treatment (DAT)
Acclimatisation:	Not stated
4. Environmental conditions:	
Temperature:	19 – 21 °C
Relative humidity:	51 – 86%
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	340-700 lux
5. Experimental work dates:	02 October 2009 to 02 January 2010

B. STUDY DESIGN AND METHODS

1. Experimental treatments: MON 52276 was evaluated at three treatment rates, equivalent to 6000, 8000 and 12000 mL product/ha. These were compared to a water-treated control and a toxic reference treatment of chlorpyrifos (a 480 g/L EC formulation applied at a rate equivalent to 240 g a.s./ha). All treatments were applied to boxes (17.1 cm x 11.3 cm in area (= 193.2 cm surface area) by 6 cm deep) of a standard sandy soil (LUFA 2.1), using a track sprayer calibrated to deliver the equivalent of 400 L spray solution/ha. Applications were made to four replicate arenas per treatment and, immediately following spraying, twenty adult *Aleochara bilineata* (10 males: 10 females) were introduced into each replicate. Beetles were fed with pellets of raw minced beef one hour after treatment and then every 1 to 3 days thereafter. The parasitic success of their larval offspring was assessed by the provision of ca. 500 onion fly pupae (*Delia antiqua*) in each replicate box on three weekly occasions, i.e. at 7, 14 and 21 DAT. The original adult beetles were removed from the arenas at 28 DAT and the number of new adults (F1 progeny) that subsequently developed from the parasitized fly pupae was recorded over a further 46-day period.

2. Observations: Assessments of the condition of the beetles were made at 1, 7 and 28 days after treatment (DAT). Assessment of reproduction was conducted from 28 DAT for 46 days.

The temperature and relative humidity conditions were recorded at hourly intervals using an electronic data logger. Light intensities were recorded at the start of the assessments using an ELE Single Channel Light Measuring System. During the bioassay the temperature range recorded was 19-21ºC and the relative humidity range recorded was 51-86%, with a 16 h photoperiod of 340-700 lux

3. Statistical calculations: Fisher's Exact test (α = 0.05) for mortality, ANOVA (α = 0.05) for reproduction.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality

Table B.9.5-25: Toxicity of MON 52276 to rove beetles (*Aleochara bilineata*) after 28 days in an extended laboratory test

Test rate [mL/ha]	Mortality [%]	Corrected mortality [%] [1)]
Control	32.5	--
6000	38.8	9.3
8000	47.5	22.2
12000	35.0	37.0 (as reported in the study report)

[1)] Derived using Abbott's formula

Reference test: Treatment with the reference item Cyren at a concentration of 240 g a.s./ha resulted in 100% mortality after 28 d of exposure.

Reproduction effect

Table B.9.5-26: Sublethal effects of MON 52276 to rove beetles (*Aleochara bilineata*) in an extended laboratory test (mean number of F_1 progeny)

Test rate [mL/ha]	Mean number of F_1 progeny per arena [1)]	Standard deviation	Effect on reproduction [%] [2)]
Control	862.5	66.8	--
6000	706.3	84.6	18.1
8000	846.0	109.5	1.9
12000	778.0	102.6	9.7

[1)] The numbers of progeny emerging in the control and test item treatments were compared by ANOVA, but treatment means did not differ significantly ($P > 0.05$). For the toxic reference treatment (where all values were zero), no statistical comparisons were made.

[2)] The percentage change in numbers of F1 progeny, relative to the control was calculated using the formula: $R = (1-(Rt/Rc)) \times 100$, where Rt and Rc are the numbers of offspring observed in the treatment and control groups, respectively. Positive values indicate a decrease, relative to the control.

Reference test: Treatment with the reference item Cyren at a concentration of 240 g a.s./ha resulted in 100% effects on reproduction.

B. OBSERVATIONS

The following point deviated from the IOBC guideline:

- Minor deviations to the required range of 60-90% relative humidity (actual values: 51-86%). No impact on the study validity

The average number of beetles emerging from parasitized fly pupae in the control treatment was >400 per replicate, and a minimum reduction of 50% reproductive capacity was achieved in the reference item treatment when compared to the control. The validity criteria according to Grimm *et al.* (2000) are therefore fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In an extended laboratory test to determine the effects of MON 52276 on the rove beetle (*Aleochara bilineata*), no significant effect on the parasitisation success of the beetles were observed up to and including the highest treatment rate of 12000 mL/ha.

This study is considered valid and relevant for use in the risk assessment.

Assessment and conclusion by RMS:
The study was part of 2015 RAR.

The aim of this study was to determine whether the test item had harmful effects on adults of the rove beetle, *Aleochara bilineata* (Gyll.) (Coleoptera: Staphylinidae), or their larval offspring, under extended-laboratory test conditions.

MON 52276 was applied to boxes of a standard sandy soil (LUFA 2.1)

Validity criteria are fulfilled.

The following point deviated from the IOBC guideline:

- Relative humidity was below the minimum threshold of the required range of 60-90% during the last week (actual values: 51-86%). RMS agrees that this has no impact on the study validity.

The toxic reference performed well (100% effect). RMS notes that no dose rate (and range of effect) is recommended for chlorpyrifos in the guideline. RMS nevertheless considers the results of the toxic reference satisfactory.

RMS does not agree with the corrected mortality of 37% proposed in the study report at the rate of 12L/ha (mortality being very similar to control).
At 28 days, mortality in the control was 32.5 %, compared with 35.0 %, 47.5 % and 38.8 % in the 12000, 80000 and 6000 mL/ha treatment rates of MON 52276, respectively. No dose relationship was observed.

The study is valid.

In the extended laboratory test to determine the effects of MON 52276 on the rove beetle (*Aleochara bilineata*), no significant effects on the parasitation success of the beetles were observed up to and including the highest treatment rate of 12000 mL/ha.

Data point:	CP 10.3.2.2/008
Report author	[redacted]
Report year	1999
Report title	A Laboratory Evaluation of the Effects of MON 52276 on the Green Lacewing, *Chrysoperla carne*a
Report No	MON-99-3
Document No	US-99-093

Guidelines followed in study	Bigler (1988)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the current guideline IOBC (2000):* *Major:* *- The mean number of eggs per female/day was 7.9 (guideline: > 15)* *- The toxic reference item was applied at 0.255 L product/ha (guideline: 0.04 L product /ha).* *Minor:* *- none*
Previous evaluation	Yes, only the endpoint was reported in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
The effects of MON 52276 (nominally 31% w/w glyphosate acid) on the development and fecundity of *Chrysoperla carnea* were evaluated. The toxicity test was performed using three concentrations, 0.6, 6 and 12 L MON 52276/ha. A negative control group (tap water only) and a positive control (dimethoate only) were included in the test design. Exposure arenas were 7.5 cm^2 glass plates, sprayed with product using a Potter tower applicator and left to air-dry for approximately 1 h, before a single larva (2-3 days old) was added to each plate, contained within a cylinder (44 mm internal diameter x approx. 25 mm tall) covered in a mesh netting to prevent escape of the developing larva. UV sterilised *Sitotroga* sp. eggs were added *ad libitum* each day until larval pupation. There were 50 test units per treatment. After pupation, pupae were transferred into ventilated plastic boxes. Once hatched, the adult lacewings were counted and transferred to oviposition boxes. Pre-imaginal mortality was recorded daily. For the following 21 days, the fecundity was assessed by observing the number of eggs laid, the viability of the eggs and the numbers of hatched juveniles.

During the larval development stage, there was no significant mortality of *Chrysoperla carnea* observed at rates up to 6 L MON 52275/ha. A significant pre-imaginal mortality was observed at 12 L MON 52275/ha. During the fecundity assessment no evidence of a dose-response relationship was found.
The authors concluded this study as supportive and unreliable for use in risk assessment.
RMS proposed to calculate an LR50 (using RegTox EV7.0.6, based on Bootstrap calculation) that may be used as weight of evidence: LR50 = 10.34 L MON 52276/ha (95% CI: 8.3-10.4). No reliable endpoint could be set for reproduction.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276 (EC)
Active substance	Glyphosate acid
Active substance content	31.0% w/w glyphosate acid (nominal) 30.9% w/w glyphosate acid (measured)
Proposed use:	Herbicide
Lot/Batch #:	290598

2. Positive control:	BASF Dimethoate 40 (EC)
3. Test organism:	
Species:	*Chrysoperla carnea* Steph. (Neuoptera, Chrysopidae)
Age:	Larvae, 2-3 day-old larvae
Source:	Eggs: Bioplanet, Cesena, Italy (commercial supplier)
Egg treatment:	After delivery, the eggs were cooled to 0 – 4 °C to delay hatching. To encourage hatching, the eggs were placed for one day in warmer conditions (14 – 19 °C) with a 16 h photoperiod of 640 lux. Afterwards the temperature was brought to 22-24 °C with 16 h light of 3180 lux in ventilated plastic boxes lined with a fibrous tissue.
Diet/ Food:	Larvae: UV-killed eggs of *Sitotroga cerealella ad libitum* Adults: artificial diet (powdered yeast mixed 1:1 with honey and made into a paste with water, a 1:2 – 1:3 honey/water solution on a cotton wool pad, fresh water on a cotton wool pad
4. Environmental conditions:	
Temperature:	Test units: 21 - 25 °C Adult maturation: 22 – 24°C Oviposition boxes: 20 – 26 °C
Photoperiod:	16 h
Light intensity	Test units: 3100 – 3140 lux Adult maturation: 6690 lux Oviposition boxes: 6690 lux
Relative humidity:	Test units: 63 - 75% Adult maturation: 65 - 88% Oviposition boxes: 57 - 99%
5. Experimental dates:	May 25th, 1999 to July 22nd, 1999

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The study encompassed three concentrations of 0.6, 6 and 12 L MON 52275/ha. In addition, *Chrysoperla carnea* were exposed to a toxic reference and a water control.

The test item, as well as the toxic reference and the water control, were applied to square glass plates using a Potter Laboratory Spray Tower with a delivery rate equivalent to 200 L/ha at a spray pressure of 0.7 bar.

One 2-3-day-old larva was put into a test arena along with a sufficient amount of *Sitotroga* eggs. The test arena is a treated glass plates covered with a perspex sheet with a 50-mm-diameter hole and an exact fitting acrylic cylinder. The cylinder was treated with polytetrafluoroethylene. A mesh with 0.5 x 0.5 mm netting was placed over each cylinder.

After pupation they were transferred on the treated glass plate into ventilated plastic boxes. After hatching, the adult *Chrysoperla* were counted and transferred to oviposition boxes. Once a week a sheet of fibrous material was placed under the lid of each box as a site for oviposition. The egg sheets were removed after 24 h for a period of 21 days and put into ventilated plastic pots were the eggs were assessed for viability and number of emerged larvae. Emerging larvae were removed daily.

2. Observations: The larvae were assessed daily for mortality, sub-lethal effects and pupation. The emerging 2nd generation larvae were counted daily. The sex of the adults was determined on dead individuals and at test end.

3. Calculations: The mortality of larval insects was corrected with the losses in the control using Abbott's formula. The pre-imaginal mortality at each test concentration and the control were compared by Chi-square test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The results of the test are depicted in the following tables.

Table B.9.5-27: Mortality during the development of the test insects

Concentration [L MON 52276/ha]	Number of Larvae tested	Insects pupating [%]	Emerging as adults [%]	Pre-imaginal mortality [%]	Abbot-corrected pre-imaginal mortality [%]
0 (control)	48	83	81	19	-
0.6	50	76	72	28	11
6	50	66	64	36	21
12	48	35	33	67	59*
Dimethoate 40	48	0	0	100	100*

*significant difference compared to the blank control

Table B.9.5-28: Egg production and viability assessment

Concentration [L MON 52276/ha]	Mean number eggs/ female/ day	Mean percentage viability	Mean no. viable eggs/ female/ day	Change relative to control [%]
0 (control)	7.9	89	7.0	-
0.6	6.3	84	5.3	-24
6	9.6	85	8.2	+17
12	6.3	89	5.6	-20
Dimethoate 40	-	-	-	-

B. OBSERVATIONS

During the development no significant mortality of *Chrysoperla carnea* was observed up to and including 6 L MON 52275/ha. A significant pre-imaginal mortality was noticed at 12 L MON 52275/ha. During the fecundity assessment no evidence of a dose-response relationship was found.

According to the study protocol based on the method by Bigler (1988), for the study to be valid, pre-imaginal mortality in the control group would not exceed 30% and would be greater than 80% in the positive control. These criteria were satisfied.

The validity criteria according to the current laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera:Chrysopidae) (Vogt, 2000) state that maximum

cumulative mortality in the control group (dead larvae, pupae and adults) must be ≤ 20%, fecundity (mean number of eggs per female per day) must be ≥15, fertility (mean hatching rate) must be ≥70% and the mortality in the positive control group should be ≥50%. Compared to these current criteria, two of the four criteria were satisfied. For control group fecundity, the mean number of eggs per female per day, was lower than 15 (7.9). RMS considers the dose rate used for toxic reference inappropriate and the effects on mortality may potentially have been underestimated.

III. CONCLUSIONS

Assessment and conclusion by applicant:

MON 52276 did not affect the survival or fecundity of the green lacewing, *Chrysoperla carnea*, when applied at rates of 0.6 or 6 L MON 52276/ha. At the maximum rate of 12 L/MON 52276/ha, corrected mortality was 59%, which exceeds the threshold of 30% currently accepted for indicating a harmful treatment effect. However, the fecundity of the surviving insects at this dose rate was only reduced by 20%, relative to the control. There was no apparent dose-response effect on the fecundity of surviving lacewings, so it was considered unlikely that the slight reduction in fecundity seen in the 12 L MON 52276/ha treatment rate was of biological significance.

This study is therefore, considered to be supportive and unreliable for use in risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance). Former RMS indicated that *due to the significant developments and changes in the risk assessment approach and strategy for terrestrial non-target arthropods since the evaluation of glyphosate in 2001, the old studies are no longer considered appropriate for a quantitative risk assessment according to current standards. Nevertheless, the results from the laboratory tests on inert substrates are useful as additional information, also due to the fact that the spectrum of tested species partly differs from that of the newly submitted studies.*

MON 52276 was applied to treated glass plates.

Deviations from current guideline Vogt et al. (2000):

- Mean number of eggs per female/day recommended is >15, actual value : 7.9

This is a validity criteria, RMS considers the results on reproduction unreliable. Besides, Vogt et al 2000 recommends that eggs (laid within 24 hrs) are collected twice within a week. This was done only once a week in the study.

- Temperature of 25 ± 2 °C is recommended, actual values in oviposition boxes 20-26°C

RMS considers the impact minor.

- Relative humidity 60-90% is recommended, actual values in oviposition boxes: 57 - 99%

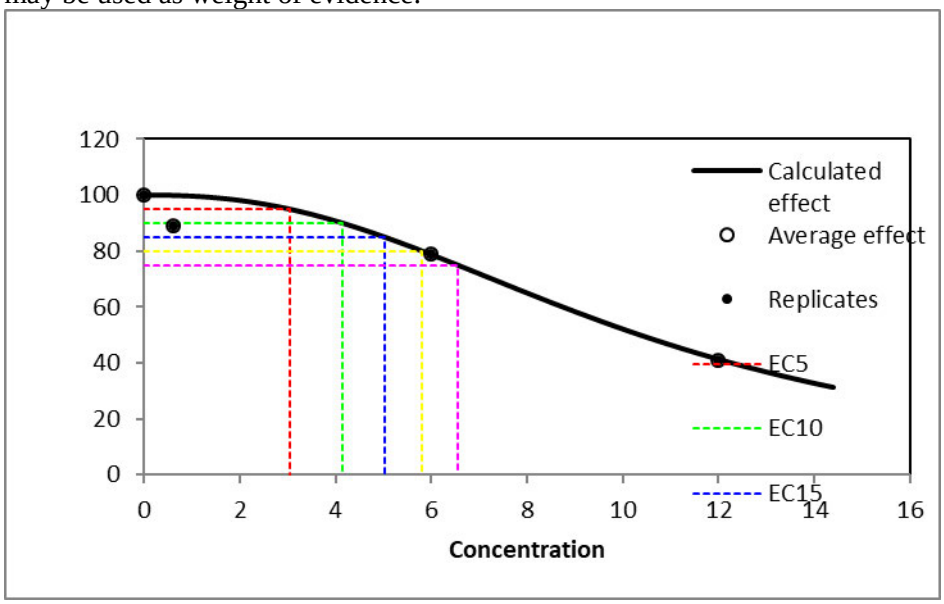
RMS considers the impact minor.

- Dimethoate rate recommended between 30-45 mL/ha, actual value 255 mL/ha was used.

This dose rate is inappropriate. The sensitivity of the larvae is then not clearly addressed as such dose rate may have cause detrimental effects even most resistant individuals. The sentitivity of the test system is doubtful. Any potential may have been underestimated.

The study is of low reliability for reproduction parameters and these are not acceptable for the risk assessment.

However a significant effect on mortality (on larvae) was observed at 12 L/ha and this effect seems dose-dependent. A LR50 should have been derived.
RMS proposes to calculate an LR50 (using RegTox EV7.0.6, based on Bootstrap calculation) that may be used as weight of evidence.



Chrysoperla carnea (larvae) exposed under laboratory conditions:
LR50 = 10.34 L MON 52276/ha (95% CI: 8.3-10.4)

No reliable endpoint could be set for reproduction

B.9.5.2.3. Semi-field studies with non-target arthropods

No semi-field studies with non-target arthropods are required since the risk assessment indicates an acceptable risk for non-target arthropods for the intended uses of MON 52276.

B.9.5.2.4. Field studies with non-target arthropods

No field studies with non-target arthropods are required since the risk assessment indicates an acceptable risk for non-target arthropods for the intended uses of MON 52276.

B.9.5.2.5. Other routes of exposure for non-target arthropods

Testing conducted in accordance with points 10.3.1 and 10.3.2.1 to 10.3.2.4 of Regulation (EU) No 284/2013 is appropriate. Thus additional specific testing is not required.

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for bees

Toxicity studies available for honey bees, bumble bees and solitary bees, covering exposure to the different life stages of these *Apis* and non-*Apis* bee species from the active substance glyphosate and MON 52276 formulation are summarised in the tables below. For study summaries of toxicity tests performed with the active substance and the product, please refer to Vol.3 CA B.9.3.1 and Vo.3 CP B.9.6.1.

Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made by the acid equivalent purity of the test item as stated in the report. By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table B.9.6-1: Endpoints and effect values of glyphosate relevant for the risk assessment for honey bees, bumble bees and solitary bees

Annex point	Study	Test species	Substance(s)	Study type	LD_{50} (µg a.e./bee)	NOED (µg a.e./bee)	Status	Remark
Acute toxicity								
CA 8.3.1.1.1/001	[redacted] 2003	*Apis mellifera L.*	Glyphosate K-salt	Acute oral	>104	-	Valid	-
CA 8.3.1.1.1/002	[redacted] 1998	*Apis mellifera L.*	Glyphosate acid	Acute oral	>182	182	Valid	-
CA 8.3.1.1.1/003	[redacted]1996	*Apis mellifera L.*	Glyphosate	Acute oral	>40	-	Valid	-
CA 8.3.1.1.1/004	[redacted] 1995	*Apis mellifera L.*	Glyphosate acid	Acute oral	>200	-	Valid	-
CA 8.3.1.1.1/005	[redacted] 1995	*Apis mellifera L.*	Glyphosate	Acute oral	116.67	-	Valid	-
CA 8.3.1.1.1/006	[redacted] [redacted]1972	*Apis mellifera L.*	Glyphosate technical and IPA-salt	Acute oral	-	-	Invalid	Cf RMS comment in study summary
CA 8.3.1.1.1/007	[redacted] 2017a	*Bombus terrestris*	Glyphosate IPA-salt (in MON 0139)	Acute oral	>412	412	Valid	-
CA 8.3.1.1.2/001	[redacted] 2003	*Apis mellifera L.*	Glyphosate K-salt	Acute contact	>100	-	Valid	-
CA 8.3.1.1.2/002	[redacted] 2000	*Apis mellifera L.*	Glyphosate isopropylamine salt	Acute contact	>61.3 (IPA salt equivalent)*	-	Valid	-
CA 8.3.1.1.2/003	[redacted] 1998	*Apis mellifera L.*	Glyphosate acid	Acute contact	>103	-	Valid	-
CA 8.3.1.1.2/004	[redacted] 1996	*Apis mellifera L.*	Glyphosate	Acute contact	>20	-	Valid	-
CA 8.3.1.1.2/005	[redacted] 1995	*Apis mellifera L.*	Glyphosate acid	Acute contact	>200	-	Valid	-
CA 8.3.1.1.2/006	[redacted] 1995	*Apis mellifera L.*	Glyphosate	Acute contact	>100	-	Valid	-
CA 8.3.1.1.2/007	[redacted] [redacted] 1972	*Apis mellifera L.*	Glyphosate technical and IPA-salt	Acute contact	-	-	Invalid	Cf RMS comment in

								study summary
CA 8.3.1.1.2/008	[redacted] 2017a	*Bombus terrestris*	Glyphosate IPA-salt	Acute contact	>461	461	Valid	-
CA 8.3.1.1.2/009	[redacted] 2017b	*Osmia bicornis*	Glyphosate IPA-salt	Acute contact	>461	461	Valid	-
CP 10.3.1.1.1/001	[redacted] 2001	*Apis mellifera*	MON 52276	Acute oral, 48 h	**> 77**	-	Valid	-
CP 10.3.1.1.2/001	[redacted] 2001	*Apis mellifera*	MON 52276	Acute contact, 48 h	**> 100**	-	Valid	-
Chronic toxicity								
Annex point	**Study**	**Test species**	**Substance(s)**	**Study type**	LDD_{50} (µg a.e./bee/d)	NOEDD (µg a.e./bee/d)	**Status**	**Remark**
CA 8.3.1.2/001	[redacted] 2017	*Apis mellifera*	Glyphosate IPA-salt (in MON 0139)	Chronic, Adult 10 days	>179.9	**179.9**	Valid	-
Honey bee development and other honey bee life stages toxicity								
Annex point	**Study**	**Test species**	**Substance(s)**	**Study type**	LD_{50} (µg a.e./larva)	NOED (µg a.e./larva)	**Status**	**Remark**
CA 8.3.1.3/001	[redacted], 2020	*Apis mellifera*	Glyphosate IPA-salt (in MON 0139)	Chronic larvae, 22-day	-	80 ED10 = **75.6**	Valid	-
Sub-lethal toxicity								
Annex point	**Study**	**Test species**	**Substance(s)**	**Study type**	LD_{50} (µg a.e./L)	NOAEL (µg a.e./L)	**Status**	**Remark**
CA 8.3.1.4/001	[redacted] 2012	*Apis mellifera*	Glyphosate IPA-salt (in MON 0139)	Bee brood feeding test. Field study	-	**301 mg/L (nominal), 266 mg/kg, (measured).**	Valid	-
Other studies								
Annex point	**Study**	**Test species**	**Substance(s)**	**Study type**	Magnitude of residues in mg a.e./kg		**Status**	**Remark**

CP 10.3.1.5/001	[redacted] 2011	*Apis mellifera*	MON 52276	Residues in honeybee colony - Phacelia semi-field application at 8 L product/ha (2.88 g a.e./ha) during flowering and in the presence of foraging bees	Total daily intake of glyphosate residues (via nectar + pollen) of: - 269.3 mg a.e. (based on day 1 maximum mean residues), - 141.8 mg a.e. (based on mean residues over days 1-3).	Valid	-

a.e.: acid equivalents
Endpoints in **bold** is used for risk assessment
*acid equivalent purity not provided

Literature data on bees

Studies related to indirect effects are considered in the assessment of risk biodiversity via indirect effects and trophic interactions. For bees, please refer to Volume 3 CP B.9 under B.9.14.1.3.

None of the articles that were assessed by RMS was deemed reliable enough for use in the risk assessment. Please refer to the Table B.9.11.1.4-2 of Volume 3 CA B.9 and the appendix to Volume 3 CA B.9 on literature data related to ecotoxicology. Please note that RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

Dai, P. et al., 2018[9] evaluated the effects of glyphosate on survival, developmental rate, larval weight, and midgut bacterial diversity of *Apis mell*ifera in the laboratory. Larvae were reared in vitro and fed diet containing glyphosate 0.8, 4, and 20 mg/L. Brood survival decreased in 4 or 20 mg/L glyphosate treatments but not in 0.8 mg/L, and larval weight decreased in 0.8 or 4 mg/L glyphosate treatments. Exposure to three concentrations did not affect the developmental rate.
The intestinal bacterial communities showed significant changes in the species diversity and richness in 20 mg/L glyphosate group. However these results (and those of other microbiota related studies available, see Table B.9.11.1.4-2 of Volume 3 CA B.9 and the appendix to Volume 3 CA B.9 on literature data related to ecotoxicology.) are considered unreliable and not reported here.
RMS notes that the concentrations inducing adverse effects on brood survival (4 or 20 mg/L glyphosate) were below those issued from regulatory studies. In an other study ([redacted] 2020, CA 8.3.1.3/001) NOEC was 505 mg a.s./kg diet (NOED was 80 µg a.s./larva). The reason of such difference is not clear but RMS cannot discard a difference of toxicity between the test items that were tested. Indeed, in this study glyphosate was tested, although the other study ([redacted] 2020, CA 8.3.1.3/001) used an IPA salt of glyphosate. The results of the study of Dai, P. et al., 2018 (brood survival) are considered reliable with restrictions by RMS. However the magnitude of effects was low, survival of ~85 and ~75% can be inferred for 4 or 20 mg/L groups, respectively. Although the effects were statistically significant, they may be caused by natural variation. Indeed, one of the validity criteria for the test (OECD Series on Testing and Assessment No. 239) is a minimum 70% emergence (i.e. survival) by day 22. Since the survival in all treatments was above 70%, the significancy of the effects should be considered with caution. Overall these results may entail a need to consider the glyphosate forms in the products undergoing a marketing procedure but overall, no evidence of adverse effects on survival is found in this study.

Overall there is no studies that may impact the outcome the risk assessment of direct effects. This may be reconsider as RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and data gap in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents.

Consideration of metabolites

Applicant's proposal:
The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Most of the parent glyphosate is remained unchanged and only a small amount (less than 1% of the applied dose) is transformed to aminomethylphosphonic acid (AMPA).

[9] Dai P. et al., 2018. The Herbicide Glyphosate Negatively Affects Midgut Bacterial Communities and Survival of Honey Bee during Larvae Reared in Vitro. Journal of agricultural and food chemistry (2018), Vol. 66, No. 29, pp. 7786

Following application to plant tissues, unchanged glyphosate was the only residue detected in significant amounts. In presence of soil as a substrate and rotational crops glyphosate degrades quickly and AMPA was found at rates comparable or even higher than the parent glyphosate. However, the uptake via roots and translocation in the plants was very low, resulting in not significant residue levels as confirmed by several plant metabolism and confined rotational crop studies (e.g. lettuce, cabbage, peas, barley, wheat, carrot, beets and radishes) involving application rates to bare soil equivalent to 3.87 - 6.5 kg ae/ha (exceeding the application rates according to the recent GAP). Neither glyphosate nor AMPA show a potential uptake into crops, as a major part of the glyphosate is degraded into CO_2. See Vol. 3 CA Section 6, for details.

Therefore, studies with the metabolites are not considered necessary since the exposure to bees is covered by the assessment conducted with the parent glyphosate.

RMS assessment and conclusion:

In the metabolism studies on primary crops, AMPA is indeed found at lower levels compared to the parent compound glyphosate. However, in the metabolism studies for rotational crops, most of the time it is the opposite (levels of AMPA are greater than ones of glyphosate). The residue section therefore concluded that the metabolism studies for rotational crops are not sufficient to predict the residue level as they do not cover the maximum PEC soil of AMPA. Therefore, a data gap has been set for residue level in rotational crops (see Volume 1, point 2.7.7). Therefore the conclusions proposed by the applicant cannot be confirmed at the moment.

In relation to the data gap set for rotational crops in the residue section, further consideration of the relevance of metabolites for bees will have to be provided (data gap).

Risk assessment for bees

All intended uses presented in the GAP are covered by the risk assessment strategy for pollinators that is summarised in the table below.

Table B.9.6-2: Risk assessment strategy for Pollinators

GAP number and summary of use	Maximum single application rate (g a.e./ha)				
	540	**720**	**1080**	**1440**	**1800**
Uses 1 a-c: Applied to weeds; pre-sowing, pre-planting, pre emergence of **field crops**		X	X	X	
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of **field crops**		X	X	X	
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of **field crops**	X				
Uses 4 a-c, 5a-c: Applied to weeds (post emergence) below trees in **orchards** and **vineyards**		X	X	X	
Use 6 a-b: Applied to weeds (post emergence) in **field crops BBCH < 20**		X	X		
Use 7 a-b: Applied to weeds (post emergence) around **rail tracks**					X
Use 8 and 9: Applied to invasive species (post emergence) in **agricultural and non-agricultural areas**					X
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of **field crops**		X	X	X	

X = this use is covered by the application rate indicated and a risk assessment in provided.

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev.2 (final), October 17, 2002).
In addition, a risk assessment according to the "EFSA Guidance Document on the risk assessment of plant protection products on bees *(Apis mellifera*, *Bombus* spp. and solitary bee)" (2013) is presented to address the data requirements of the Regulation (EU) No. 284/2013, chronic risk to adult honey bees and honey bee brood. In consideration of the recommendations of the "Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology"[10] currently no risk assessment for bumble bees and solitary bees is required, given that the EFSA Bee Guidance has not yet been noted. Furthermore, EFSA stated that it is not recommended to routinely perform a risk assessment for bumble bees and solitary bees. Nevertheless, acute studies for bumble bees and solitary bees are available and the results are presented.

Risk assessment according to SANCO/10329/2002 rev 2 final

The hazard quotients for oral and contact exposure of honey bees are based on the recommended field use rates and are presented in the table below.

[10]Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, provided by EFSA, published December 22, 2015

Table B.9.6-3: Assessment of the risk of glyphosate for honey bees due to the use of MON 52276

Intended use	All uses (Uses: 1a-10c)		
Active substance	Glyphosate		
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha		
Test design	**LD_{50} (lab.) (µg a.e./bee)**	**Single max. application rate (g a.e./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>77	1800	< 23.4
		1440	< 18.7
		1080	< 14.0
		720	< 9.4
		540	< 7.0
Contact toxicity	>100	1800	<18.0
		1440	<14.4
		1080	<11.0
		720	<7.2
		540	<5.4

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.

According to the risk assessment conducted according to SANCO/10329/2002 rev 2 final , the oral and contact hazard quotients (Q_{HO}, Q_{HC}) are below the trigger value of 50. An acceptable risk to honey bees is concluded for all intended use patterns.

Further considerations regarding the chronic risk to bees

The applicant provided a chronic risk assessment based results of [redacted] (2011, Vol.3 CP 10.3.1.5/001). RMS presented thereafter the risk assessment as proposed by the applicant with correction of the residues in nectar and pollen that RMS has recalculated (please refer to the study summary of [redacted] (2011, Vol.3 CP 10.3.1.5/001) above.

[redacted] (2011, Vol.3 CP 10.3.1.5/001) provides measurements of the levels of exposure in nectar and honey following an application at 2.88 kg a.e./ha, which exceeds the maximum single application rate of the proposed uses in the GAP. Residues in nectar samples taken from forager bees at various time points after application were up to 62.6 mg a.e./kg (based on RMS recalculation). Residues in pollen samples taken from the pollen trap (higher than from pollen taken from foragers) at various times after application were up to 1148 mg a.e./kg (based on RMS estimation). Using this information, a risk assessment may be conducted in line with the recommendations of Reg (EU) No 283/2013 section 8(10) which states: "*Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of relevant sub-lethal effects in the risk assessment*". Furthermore, under section 8.3.1. Effects on bees of the same Regulation it states that: "[…] *risk assessment shall be based on a comparison of the relevant endpoint with those residue concentrations. If this comparison indicates that an exposure to toxic levels cannot be excluded, effects shall be investigated with higher tier tests.*"

A comparison can be made between the chronic and larval endpoint based on concentration in test diets and the maximum concentrations of glyphosate measured in nectar and pollen. In the chronic adult study the NOEC and NOEDD values (10 days) were 10000 mg a.e./kg feeding solution and 179.9 µg a.e./bee/day, respectively. As forager bees consume a diet which is virtually 100% nectar this endpoint can be compared to the maximum measured residues in nectar of 62.6 mg a.e./kg demonstrating a margin of safety of 16.

In the larval toxicity study the EC10 and ED10 values (over the larval development period) were 477 mg a.e./kg diet and 75.6 µg a.e./larva. Because larvae consume a mix of nectar and pollen it is necessary to consider the proportion of nectar and pollen in the diet and the contribution towards the exposure concentration. According to Rortais *et al*. (2015)[11] a single larva consumes 59.4 mg sugar and 5.4 mg pollen over 5 days. Assuming the nectar is foraged from treated weeds with a sugar content of 30% (w/w) this means that the larval diet consists of 198 mg nectar and 5.4 mg of pollen, i.e. a ratio of 0.973:0.027 (nectar:pollen). As the maximum concentration in nectar was 62.6 mg a.e./kg and in pollen 1148 mg a.e./kg the diet would have a concentration of:

Nectar: 0.973 x 62.6 mg a.e./kg = 60.9 mg a.e./kg + Pollen: 0.027 x 1148 mg a.e./kg = 31 mg a.e./kg diet

Concentration of glyphosate in the larval diet = 91.9 mg a.e./kg (based on nectar and pollen)

Comparing the larval endpoint to the maximum measured residues in the larval diet of 91.9 mg a.e./kg a margin of safety of 5.2 is calculated. Note: This is considered a worst-case estimate of exposure as honey bee larvae are fed with royal jelly for the first two days of their development period.

Overall, a margin of safety between 16 and 5.2 is demonstrated for chronic exposure to adult honey bees and honey bee larvae. This approach indicates that the risk to honey bees is acceptable.

In addition, a honey bee brood feeding test ([redacted] 2012, KCA 8.3.1.4/001) was conducted to evaluate the potential risk to honey bee brood when they are directly exposed to glyphosate (tested as IPA salt). This study provides further information regarding the chronic risk to honey bees and honey bee brood. The dose levels of the test item were based on the residues characterised in the glasshouse study ([redacted] 2011, CP 10.3.1.5/001). The highest dose was of 1 L syrup at 301 mg a.e./L. This dose covers the total intake of glyphosate residues (via nectar + pollen) measured in the glassouse study i.e. 269.3 mg a.e. (based on day 1 maximum mean residues), Mortality of adult honey bees as well as honey bee brood was assessed over a period of 7 days. Overall, no treatment related effects were observed. The NOAEL for adult mortality and brood development was the highest dose tested; 301 mg a.e./L nominal (equivalent to 266 mg/kg, measured concentration).

Consequently, the presented risk assessment for honey bees according to SANCO10329/2002 and taking into account the provisions in Reg (EU) No 283/2013 demonstrate that the risk to honey bees for glyphosate and for all uses of MON 52276 could be considered acceptable.

Risk assessment according to the EFSA GD on the Risk Assessment on Bees (2013)

In addition, the risk assessment for honey bees is performed in accordance with the recommendations of the "Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees)" (EFSA Journal 2013;11(7):3295 doi: 10.2903/j.efsa.2013.3295, July 04, 2014).

[11] Rortais *et al*. (2015) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36 (2005) 71–83

The risk assessment presented here considers also the consumption of contaminated water (guttation water, surface water and puddles).

The screening step was conducted considering all recommended application rates according to the proposed use pattern (downwards spray).

Table B.9.6-4: Screening assessment of the risk of glyphosate for honey bees due to the use of MON 52276

Intended use	All uses (Uses: 1a-10c)
Application method	downward spraying
Active substance	Glyphosate
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha

Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (g a.e./ha)**	**$HQ_{contact}$ criterion**	**Trigger**
Adult acute contact toxicity	>100	1800	<18.0	42
		1440	<14.4	
		1080	<10.8	
		720	<7.2	
		540	<5.4	

Type design	**Endpoint**	**Max. single application rate (kg a.e./ha)**	**E_f × SV**	**ETR**	**Trigger**
Adult acute oral toxicity	LD_{50} = 77 µg a.e./bee	1.80	7.6	0.18	≤ 0.2
		1.44		0.14	
		1.08		0.11	
		0.72		0.07	
		0.54		0.05	
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	1.80	7.6	**<0.076**	≤ 0.03
		1.44		**<0.06**	
		1.08		**<0.04**	
		0.72		**<0.0304**	
		0.54		<0.023	
Larval toxicity	ED10 = 75.6 µg a.e./larva	1.80	4.4	0.10	≤ 0.2
		1.44		0.08	
		1.08		0.06	
		0.72		0.04	
		0.54		0.03	

Ef: exposure factor; SV: shortcut value; $HQ_{contact}$: Hazard quotient for contact exposure; ETR: Exposure toxicity ratio; ETR values shown in **bold** breach the relevant trigger.

The exposure toxicity ratio (ETR) for adult chronic toxicity is above the respective trigger value for application rates of 720 g a.e./ha, 1080 g a.e./ha, 1440 g a.e./ha and 1800 g a.e./ha. Therefore, a Tier 1 risk assessment is required for these use patterns. An acceptable risk is indicated at the screening step for the use rate of 540 g a.e./ha.

For the Tier 1 risk assessment calculations considering application of MON 52276 in crops planted in wide rows (i.e. orchards and vines) the "under crop application" scenario is used. The crop itself will not be over-sprayed as the application is done only to the area under the crop. Thus, no treated crop scenario is included in the following tables. Only weeds, field margin, adjacent crop and next crop scenarios are considered.

Table B.9.6-5: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 in orchard crops and vines at 1440 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4a, 5a)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	**Endpoint (lab.)**	**Scenario**	**BBCH**	**E_f**	**SV**	**ETR**	**Trigger**
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.01	0.03
			weed ≥10	1	2.9	<0.02	
		field margin	weed <10	0.0092	2.9	<0.01	
			weed ≥10	0.0092	2.9	<0.01	
		adjacent crop	weed <10	0.0033	5.8	<0.01	
			weed ≥10	0.0033	5.8	<0.01	
		next crop	weed <10	1	0.54	<0.01	
			weed ≥10	1	0.54	<0.01	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation

Table B.9.6-6: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 in orchard crops and vines at 1080 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4a, 4b, 5a, 5b)
Application method	downward spraying
Crop category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-3 x 1080 g a.e./ha

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.001	0.03
			weed ≥10	1	2.9	<0.013	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed ≥10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed ≥10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.002	
			weed ≥10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1080 g a.e./ha considered for risk calculation

Table B.9.6-7: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 in orchard crops and vines at 720 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4b, 4c, 5b, 5c)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-3 x 720 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.001	0.03
			weed ≥10	1	2.9	<0.008	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed ≥10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed ≥10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.002	
			weed ≥10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 720 g a.e./ha considered for risk calculation

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating acceptable risk to honey bees following application of MON 52276 in orchard crops and vines according to the proposed use pattern.

The recommended use pattern for MON 52276 includes also application on railroad tracks. Application is done by spray trains (spraying tanks, pumps and nozzles are mounted on special trains). As no definite crop scenario for railroad tracks is provided by EFSA, the under-crop application scenario was considered to address uses on railroad tracks.

Table B.9.6-8: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – railroad tracks at 1800 g a.e./ha

Intended use	Railroad tracks (Uses: 7a, 7b)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1800 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.002	0.03
			weed ≥10	1	2.9	<0.021	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed ≥10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed ≥10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.004	
			weed ≥10	1	0.54	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for railroad tracks is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, the under crop application scenario was considered to address uses on railroad tracks
[2] Max. single application rate of 1800 g a.e./ha considered for risk calculation

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating acceptable risk to honey bees following application of MON 52276 on railroad tracks according to GAP.

Besides uses in agricultural areas and railroad tracks a proposed use of MON 52276 is also to control invasive weeds.

MON 52276 is applied by spot application with a maximum single application rate of 1800 g a.s/ha in a 12 month period. Nevertheless, bees can be exposed while they are foraging by direct overspray or dried residues on plants and by oral uptake of contaminated pollen and nectar. Thus, an appropriate assessment is presented here to address risk from the use of MON 52276 on invasive weeds in agricultural and non-agricultural areas.

Table B.9.6-9: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – invasive plant species in agricultural and non-agricultural areas at 1800 g a.e./ha

Intended use	invasive plant species in agricultural and non-agricultural areas (Uses: 8, 9)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1 x 1800 g a.e./ha

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.002	0.03
			weed >10	1	2.9	<0.021	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed >10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed >10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.004	
			weed >10	1	0.54	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] As no definite scenario for invasive weeds is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, under crop application: giant hogweed (*Heracleum* spp.) and Japanese knotweed (*Reynoutria japonica*)

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating acceptable risk to honey bees following application of MON 52276 on invasive species in agricultural and non-agricultural areas according to GAP.

For the Tier 1 risk assessment calculations considering the pre-sowing, pre-planting and post-harvest uses the “bare soil application” scenario is selected.

Table B.9.6-10: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – pre-sowing, pre-planting and post-harvest uses at 1440 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet (Uses: 1a, 2a)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	<10	1	0.54	<0.003	0.03
		Weeds	<10	1	0.27	<0.002	
		field margin	<10	0.0092	2.9	<0.001	
		adjacent crop	<10	0.0033	5.8	<0.001	
		next crop	<10	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation

Table B.9.6-11: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - pre-sowing, pre-planting and post-harvest uses at 1080 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet, Legume vegetables (Uses: 1b, 2a, 2b, 2c, 6a, 10a)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-3 x 1080 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	<10	1	0.54	<0.002	0.03
		Weeds	<10	1	0.27	<0.001	
		field margin	<10	0.0092	2.9	<0.001	
		adjacent crop	<10	0.0033	5.8	<0.001	
		next crop	<10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1080 g a.e./ha considered for risk calculation

Table B.9.6-12: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - pre-sowing, pre-planting and post-harvest uses at 720 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet, Legume vegetables (Uses: 1c, 2b, 6b, 10b, 10c)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-3 x 720 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	<10	1	0.54	<0.002	0.03
		Weeds	<10	1	0.27	<0.001	
		field margin	<10	0.0092	2.9	<0.001	
		adjacent crop	<10	0.0033	5.8	<0.001	
		next crop	<10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category in the first tier oral assessment according to the EFSA GD on the Risk Assessment on Bees (2013)
[2] Max. single application rate of 720 g a.e./ha considered for risk calculation

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating acceptable risk to honey bees following application of MON 52276 pre-sowing, pre-planting and post-harvest.

For the Tier 1 risk assessment calculations, considering ground directed inter-row applications in vegetables the following crop categories are selected:

Crop according to GAP	Crop Category[1]
Root vegetables	Root vegetables
Tuber vegetables	Potatoes
Bulb vegetables	Bulb vegetables
Fruiting vegetables	Fruiting vegetables 1, fruiting vegetables 2
Brassica	Leafy vegetables
Leafy vegetables	Leafy vegetables, lettuce
Stem vegetables	Leafy vegetables
Sugar beet	Sugar beet
Legume vegetables	Pulses

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator

The Tier 1 risk assessment is presented only for the highest intended application rate per crop category as it covers the lower application rates.

Table B.9.6-13: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – fruiting vegetables

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Fruiting vegetables 1							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	<0.003	0.03
			10 - 49[3]	1	5.8	**<0.033**	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 49[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 49[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 49[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 - 49[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	
Fruiting vegetables 2							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.012	<0.000	0.03
			10 - 49[3]	1	0.92	<0.005	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 49[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 49[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 49[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
			10 - 49[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-14: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - root vegetables

Intended use	Root vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	Root vegetables[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	<0.003	0.03
			10 - 39[3]	1	5.8	**<0.033**	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 39[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 39[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 39[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 - 39[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, e.g. fruiting vegetables 2 = tomatoes, eggplants
[2] Max. single application rate of 1080 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-15: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 –tuber vegetables

Intended use	Tuber vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	potatoes[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.012	<0.000	0.03
			10 - 39[3]	1	0.92	<0.005	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 39[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 39[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 39[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 - 39[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, e.g. fruiting vegetables 2 = tomatoes, eggplants
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-16: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – Bulb vegetables

Intended use	Bulb vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	bulb vegetables[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	<0.003	0.03
			10 - 39[3]	1	5.8	**<0.033**	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 39[3]	1	2.9	<0.017	
			≥ 70	0.6	2.9	<0.010	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 39[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 39[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 - 39[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-17: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - Brassica, leafy and stem vegetables

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Leafy vegetables							
		treated crop	< 10	1	0.54	<0.003	0.03

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day		10 - 49[3]	1	5.8	**<0.033**	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 49[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 49[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 49[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 - 49[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	
Lettuce							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.012	<0.000	0.03
			10 - 49[3]	1	0.92	<0.005	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 49[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 49[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 49[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 – 49[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-18: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - Sugar beet

Intended use	Sugar beet (Uses: 1, 2, 3, 10)
Application method	downward spraying
Crop category	sugar beet[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	**Endpoint (lab.)**	**Scenario**	**BBCH**	**Ef**	**SV**	**ETR**	**Trigger**
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	<0.003	0.03
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			≥ 70	0.25	2.9	<0.004	
		field margin	< 10	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

Table B.9.6-19: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - legume vegetables

Intended use	Legume vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	pulses[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	<0.003	0.03
			10 49[3]	1	5.8	**<0.033**	
			≥ 70	1	0	<0.000	
		Weeds	< 10	1	2.9	<0.017	
			10 - 49[3]	1	2.9	<0.017	
			≥ 70	0.3	2.9	<0.005	
		field margin	< 10	0.0092	2.9	<0.000	
			10 - 49[3]	0.0092	2.9	<0.000	
			≥ 70	0.0092	2.9	<0.000	
		adjacent crop	< 10	0.0033	5.8	<0.000	
			10 - 49[3]	0.0033	5.8	<0.000	
			≥ 70	0.0033	5.8	<0.000	
		next crop	< 10	1	0.54	<0.003	
			10 - 49[3]	1	0.54	<0.003	
			≥ 70	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value except for the "treated crop" scenario at BBCH 10-49 or BBCH 10-39 for fruiting vegetables, root vegetables, bulb vegetables, leafy vegetables and legume vegetables at the highest intended rate of 1440 g a.e./ha. Nevertheless, these scenarios are only relevant for uses 6a and b for which the highest intended application rate is 1080 g a.s./ha. Therefore, a risk assessment considering the lower intended application rate is presented below:

Table B.9.6-20: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 on fruiting, root, bulb and leafy vegetables and pulses for "treated crop" scenario at all application rates for uses 6a and 6b

Crop	Fruiting vegetables 1, Root vegetables, Bulb vegetables, Leafy vegetables, Pulses (uses 6a and 6b)					
Application method	downward spraying					
Active substance	Glyphosate					
Toxicity value	LDD_{50} > 179.9 µg a.e./bee/day					
Scenario	**BBCH stage**	**Max. single application rate (kg a.e./ha)**	**E_f**	**SV**	**ETR**	**Trigger**
Treated crop	BBCH 10-39 or BBCH 10-49	1.08	1	5.8	<0.025	0.03
		0.72	1	5.8	<0.017	

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating an acceptable risk to honey bees following application of MON 52276 for all intended uses.

Overall, an acceptable risk to honey bees has been demonstrated in the risk assessment above for all uses according to proposed GAP.

In addition, a honey bee brood feeding test ([redacted] 2012, KCA 8.3.1.4/001) was conducted to evaluate the potential risk to honey bee brood when they are directly exposed to glyphosate (tested as IPA salt). This study provides further information regarding the chronic risk to honey bees and honey bee brood. The dose levels of the test item were based on the residues characterised in the glasshouse study ([redacted] 2011, CP 10.3.1.5/001). The highest dose was of 1 L syrup at 301 mg a.e./L. This dose covers the total intake of glyphosate residues (via nectar + pollen) measured in the glasshouse study i.e. 269.3 mg a.e. (based on day 1 maximum mean residues, and covering all application rates intended), Mortality of adult honey bees as well as honey bee brood was assessed over a period of 7 days. Overall, no treatment related effects were observed. The NOAEL for adult mortality and brood development was the highest dose tested; 301 mg a.e./L nominal (equivalent to 266 mg/kg, measured concentration).

Assessment of risk according to EFSA GD on bees (2013) from exposure to contaminated water

An assessment of the risk to bees from contaminated water is provided in the table below.

Table B.9.6-21: Assessment of the risk for bees due to the use of MON 52276 considering exposure to contaminated water

Intended use	All uses (Uses: 1a-10c)
Application method	downward spraying
Active substance	Glyphosate
Use pattern	2 x 1440 g a.e./ha (worst-case identified for PECsw see B.9.4)
Water solubility	100000 mg/L (see Volume 1, [redacted] (2020a), KCA 2.5/001)
PEC_{sw}	worst case Step 2 of 69.95 µg/L
PEC_{puddle}	worst case Step 2 of 65.47 µg/L

Surface water[1] (provisional, data gap related to e-fate data gap on new PECsw**)**				
Test design	**Endpoint (lab.)**	**water consumption (µl)**	**ETR[1]**	**Trigger**
Acute	77 µg a.e./bee	11.4	0.00	0.2
Chronic	>179.9 µg a.e./bee/day	11.4	0.000	0.03
Larvae	75.6 µg a.e./larva	111	0.00	0.2
Puddle water[1,2] (provisional, data gap related to e-fate data gap on new PECsw**)**				
Test design	**Endpoint (lab.)**	**water consumption (µl)**	**ETR[2]**	**Trigger**
Acute	77 µg a.e./bee	11.4	0.00	0.2
Chronic	>179.9 µg a.e./bee/day	11.4	0.000	0.03
Larvae	75.6 µg a.e./larva	111	0.00	0.2
Guttation water				
Test design	**Endpoint (lab.)**	**water consumption (µl)**	**ETR**	**Trigger**
Acute	77 µg a.e./bee	11.4	**14.8**	0.2
Chronic	>179.9 µg a.e./bee/day	11.4	**<3.3**	0.03
Larvae	75.6 µg a.e./larva	111	**105.7**	0.2

ETR: exposure toxicity ratio.
Values shown in **bold** breach the relevant trigger.

The refinement of potential risk due to guttation proposed by the applicant is based on the following assumptions:

- MON 52276 applications are made to a potentially diverse assemblage of weeds to be controlled. Several species of weed at different growth stages may be present and will not necessarily all be producing guttation fluid.
- In [redacted] (2011) treated plants start to wilt soon after treatment and honey bee foraging was greatly reduced after 4 – 5 days.
- Root pressure and cell turgor are required for a plant to produce guttation fluid and wilted plants will rapidly stop producing guttation fluid.
- The EFSA bee guidance assumes that the whole water consumption is based on guttation, surface or puddle water. However, honey bees also use different sources and is most likely a mixture of available water resources.
- The NOAEL (301 mg a.e./L) obtained from the bee brood feeding test ([redacted] 2012) is based on the measured residues after an application of 2880 g a.e./ha. The highest maximum single application rate according to proposed GAP is 1800 g a.e./ha on grasses and 1440 g a.e/ha on field crops.

Overall, based on the above, RMS considers that the occurrence of glyphosate secretion via guttation should be limited. Guttation events that may be observed in some crops are expected less important on weed communities as not all species produce guttation water. Besides these weeds will be present at different growth stages, RMS therefore believes the availability of guttation water at the time of spray should be limited. Taking into account that root pressure and cell turgor are required for a plant to produce guttation fluid, in the case of glyphosate, a chronic exposure is unlikely (plants wilt soon after treatment). Overall, it seems unlikely that guttation water will represent a significant (major) source of water in more than 10% of cases (i.e. hives at the edge of the fields).
Also considering the absence of effects from the bee brood feeding test ([redacted] 2012), RMS considers that potential for adverse effects on colonies via guttation water is unlikely.

The applicant also provided the following statements (not considered necessary by RMS but reported for completeness):

Applicant's proposal:

The assumption that guttation fluid will contain the active substance at its limit of solubility is a huge over estimate of exposure for substances of higher water solubility such as glyphosate. There are technical considerations regarding this point to consider in relation to the risk assessment. Assuming a guttation droplet contains glyphosate at the limit of water solubility, ca. 12000 mg/L, and the daily water intake of 11.4 µl/bee/day (EFSA bee GD 2013) this is equivalent to a forager daily intake of 136.8 µg a.e./bee. In the 10-day chronic study honey bees were observed to consume 179.9 µg a.e./bee/day without any observed mortality or other adverse effects. Given that the chronic risk assessment requires a trigger equivalent to approximately 34x the endpoint this would mean in order to pass the risk assessment the endpoint would need to be > 4651.2 µg a.e./bee/day which is almost 5% of the average body weight of a honey bee of 100 mg. This level of consumption would not be achievable in a standard laboratory test with ad libitum feeding and is not likely to occur under field conditions. Currently it is not possible to gavage honey bees to achieve higher doses. Even so the 10-day chronic endpoint, which is a NOEDD, is higher than the worst-case unrealistic daily dose via guttation fluid which gives a good indication that there is an acceptable risk.

For larvae the exposure to water is considered a moot point. For the first 3 days they are fed exclusively on worker jelly which is a secretion from the glands of nurse bees. After that on days 4 and 5 they are still fed with jelly but also receive some pollen and nectar from hive stores. Larval

water needs are met from the liquid food they receive but some dilution of stored honey may occur and fed to the larvae on days 4 and 5 of their development if these coincide with periods of cool wet weather and the colony needs to use some of the stored honey. Overall of the 111 µl water required by larval bees (EFSA bee GD 2013) only a minor proportion would come from extraneously collected water and of that only a fraction would be derived from guttation fluid. The real-life exposure of larvae to guttation water is probably negligible and the level of exposure to a low toxicity substances such as glyphosate arising from this is unlikely to pose a risk to honey bee brood.

The water exposure route and in particular via consumption of guttation fluid, is not considered as a major exposure route compared to nectar and pollen. The presented higher-tier assessment for honey bees based on the worst-case exposure via nectar and pollen should be sufficiently protective for the risk from exposure via contaminated water.

Risk assessment for bumble bees

EFSA stated that it cannot be recommended to routinely perform a risk assessment for bumble bees. Nevertheless, an acute oral and contact study for bumble bees is available and a corresponding risk assessment is presented.

The risk assessment for the proposed uses of MON 52276 and the effects on bumble bees is provided below.

Table B.9.6-22: Screening assessment of the risk of glyphosate for bumble bees due to the use of MON 52276

Intended use	All uses (Uses: 1a to 10c)				
Application method	downward spraying				
Active substance	Glyphosate				
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha				
Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (g a.e./ha)**		**$HQ_{contact}$ criterion**	**Trigger**
Acute contact toxicity	>461	1800		<3.9	**7**
		1440		<3.1	
		1080		<2.3	
		720		<1.6	
		540		<1.2	
Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (kg a.e./ha)**	**E_f × SV**	**ETR**	**Trigger**
Acute oral toxicity	>412	1.80	11.2	**<0.05**	**0.036**
		1.44		**<0.04**	
		1.08		<0.03	
		0.72		<0.02	
		0.54		<0.01	

Ef: exposure factor; SV: shortcut value; $HQ_{contact}$: Hazard quotient for contact exposure; ETR: Exposure toxicity ratio; ETR values shown in **bold** breach the relevant trigger.

The exposure toxicity ratio (ETR) for acute oral toxicity is above the respective trigger value for the application rates of 1440 g a.e./ha and 1800 g a.e./ha. Therefore, Tier 1 risk assessment is required for these use patterns. No risk is indicated at the screening step for the use rate of 540 g a.e./ha, 720 g a.e./ha and 1080 g a.e./ha.

For the Tier 1 risk assessment calculations considering application of MON 52276 in crops planted in wide rows (i.e. orchards and vines) the “under crop application” scenario is used. The crop itself will

not be over-sprayed as the application is done only to the area under the crop. Thus, no treated crop scenario is included in the following assessment. Only weeds, field margin, adjacent crop and next crop scenarios are considered.

Table B.9.6-23: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 in orchard crops and vines at 1440 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4a, 5a)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	weeds	weed <10	1	0.46	<0.01	0.036
			weed ≥10	1	6.5	<0.023	
		field margin	weed <10	0.0092	6.5	<0.01	
			weed ≥10	0.0092	6.5	<0.01	
		adjacent crop	weed <10	0.0033	11.2	<0.01	
			weed ≥10	0.0033	11.2	<0.01	
		next crop	weed <10	1	0.9	<0.01	
			weed ≥10	1	0.9	<0.01	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation

All exposure toxicity ratios (ETRs) for acute oral toxicity are below the respective trigger value, indicating acceptable risk to bumble bees following application of MON 52276 in orchard crops and vines according to the proposed use pattern.

The recommended use pattern for MON 52276 includes also application on railroad tracks. Application is done by spray trains (spraying tanks, pumps and nozzles are mounted on special trains). As no definite crop scenario for railroad tracks is provided by EFSA, the under crop application scenario was considered to address uses on railroad tracks as well.

Table B.9.6-24: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 – railroad tracks at 1800 g a.e./ha

Intended use	Railroad tracks (Uses: 7a, 7b)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1800 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	weeds	weed <10	1	0.46	<0.002	0.036
			weed ≥10	1	6.5	<0.028	
		field margin	weed <10	0.0092	6.5	<0.001	
			weed ≥10	0.0092	6.5	<0.001	
		adjacent crop	weed <10	0.0033	11.2	<0.001	
			weed ≥10	0.0033	11.2	<0.001	
		next crop	weed <10	1	0.9	<0.004	
			weed ≥10	1	0.9	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for railroad tracks is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, the under crop application was considered to address uses on railroad tracks
[2] Max. single application rate of 1800 g a.e./ha considered for risk calculation

All exposure toxicity ratios (ETRs) for acute oral toxicity are below the respective trigger value, indicating acceptable risk to bumble bees following application of MON 52276 on railroad tracks.

Besides uses in agricultural areas and railroad tracks MON 52276 is also used to control invasive weeds. MON 52276 is applied by spot application with a maximum single application rate of 1800 g a.s/ha in a 12 month period. Nevertheless, bees can be exposed while they are foraging by direct overspray or dried residues on plants and by oral uptake of contaminated pollen and nectar. Thus, an appropriate risk assessment is presented in the following to address risk from the use of MON 52276 on invasive weeds.

Table B.9.6-25: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 – invasive plant species in agricultural and non-agricultural areas at 1800 g a.e./ha

Intended use	invasive plant species in agricultural and non-agricultural areas (Uses: 8, 9)
Application method	downward spraying
Crop Category	under crop application [1]
Active substance	Glyphosate
Use pattern	1 x 1800 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	weeds	weed <10	1	0.46	<0.002	0.036
			weed >10	1	6.5	<0.028	
		field margin	weed <10	0.0092	6.5	<0.001	
			weed >10	0.0092	6.5	<0.001	
		adjacent crop	weed <10	0.0033	11.2	<0.001	
			weed >10	0.0033	11.2	<0.001	
		next crop	weed <10	1	0.9	<0.004	
			weed >10	1	0.9	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for invasive weeds is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, under crop application: giant hogweed (*Heracleum* spp.), Japanese knotweed (*Reynoutria japonica*)
[2] Max. single application rate of 1800 g a.e./ha considered for risk calculation

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating acceptable risk to honey bees following application of MON 52276 on invasive species in agricultural and non-agricultural areas according to proposed GAP.

For the Tier 1 risk assessment calculations considering the pre-sowing, pre-planting and post-harvest uses the “bare soil application” scenario is selected.

Table B.9.6-26: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 –pre-sowing, pre-planting and post-harvest uses at 1440 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet (Uses: 1a, 2a)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	<10	1	0.9	<0.004	0.036
		weeds	<10	1	0.46	<0.002	
		field margin	<10	0.0092	6.5	<0.001	
		adjacent crop	<10	0.0033	11.2	<0.001	
		next crop	<10	1	0.9	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower application rates.

All exposure toxicity ratios (ETRs) for acute oral toxicity are below the respective trigger value, indicating acceptable risk to bumble bees following application of MON 52276 pre-sowing, pre-planting and post-harvest.

For the Tier 1 risk assessment calculations considering ground directed inter-row applications at a rate of 1440 g a.e./ha in vegetables the following crop categories are selected:

Crop according to GAP	Crop Category[1]
Root vegetables	Root vegetables
Tuber vegetables	Potatoes
Bulb vegetables	Bulb vegetables
Fruiting vegetables	Fruiting vegetables 1, fruiting vegetables 2
Brassica	Leafy vegetables
Leafy vegetables	Leafy vegetables, lettuce
Stem vegetables	Leafy vegetables
Sugar beet	Sugar beet

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator

The Tier 1 risk assessment is presented only for the highest intended application rate per crop category as it covers the lower application rates.

Table B.9.6-27: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 – fruiting vegetables

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Fruiting vegetables 1							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 49[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	
Fruiting vegetables 2							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.03	0.0001	0.036
			10 - 49[3]	1	2.3	0.0080	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-28: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 –rootvegetables

Intended use	Root vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	Root vegetables[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 39[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 39[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 39[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 39[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 39[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-29: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - tuber vegetables

Intended use	Tuber vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	potatoes[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.03	0.0001	0.036
			10 - 39[3]	1	2.3	0.0080	
			≥ 70	1	0	0.0000	

Intended use	Tuber vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	potatoes[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
		Weeds	< 10	1	6.5	0.0227	
			10 - 39[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 39[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 39[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 39[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-30: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - Bulb vegetables

Intended use	Bulb vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	bulb vegetables[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 39[3]	1	11.2	0.0391	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 39[3]	1	6.5	0.0227	
			≥ 70	0.6	6.5	0.0136	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 39[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 39[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 39[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-31: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - Brassica, leafy and stem vegetables

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Leafy vegetables							
Acute oral toxicity		treated crop	< 10	1	0.9	0.0031	0.036

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
	LD_{50} > 412 µg a.e./bee		10 - 49[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	
Lettuce							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.03	0.0001	0.036
			10 - 49[3]	1	2.3	0.0080	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 – 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table B.9.6-32: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - Sugar beet

Intended use	Sugar beet (Uses: 1, 2, 3, 10)
Application method	downward spraying
Crop category	sugar beet[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			≥ 70	0.25	6.5	0.0057	
		field margin	< 10	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

Table B.9.6-33: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - legume vegetables

Intended use	Legume vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	pulses[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.03
			10 49[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	

Intended use	Legume vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	pulses[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

All exposure toxicity ratios (ETRs) for acute oral toxicity are below the respective trigger value, except for the "treated crop" scenario at BBCH 10-49 or BBCH 10-39 for fruiting vegetables, root vegetables, bulb vegetables, leafy vegetables and legume vegetables at the highest intended rate of 1440 g a.e./ha. Nevertheless, these scenarios are only relevant for uses 6a and b for which the highest intended application rate is 1080 g a.s./ha. As this application rate presented an acceptable risk at screening step, an acceptable risk to bumble bees following application of MON 52276 can be concluded for all uses.

Solitary bees

In consideration of the recommendations of the "Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology"[12] currently no risk assessment for solitary bees is required, given that the EFSA Guidance Document on the risk assessment of plant protection products on bees has not yet been noted. Furthermore, EFSA stated that it cannot be recommended to routinely perform a risk assessment for solitary bees. Nevertheless, an acute contact study for solitary bees is available and a corresponding risk assessment is presented.

Details of the studies with *Osmia bicornis* and glyphosate are summarised in Vol.3 CA, B.9.3.1.1.2 and relevant endpoints for the risk assessment are provided in the table below.

[12]Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, provided by EFSA, published December 22, 2015

Table B.9.6-34: Endpoints and effect values of glyphosate relevant for the risk assessment for bees

Acute toxicity					
Reference	**Test item**	**Species**	**Test design/ GLP**	**LD_{50} (µg a.e./bee)**	**NOED (µg a.e./bee)**
[redacted] 2017b CA 8.3.1.1.2/009	Glyphosate K-salt	*Osmia bicornis*	Acute contact, 48 h	**>461**	≥461

Further testing with the representative product MON 52276 and the toxicity to *Osmia bicornis* was not considered necessary and the risk assessment will be conducted on the active substance data.

Risk assessment for solitary bees

The risk assessment for the proposed uses of MON 52276 and the effects on solitary bees is provided below.

Table B.9.6-35: Screening assessment of the risk of glyphosate for solitary bees due to the use of MON 52276

Intended use	All uses (Uses: 1a-10c)			
Application method	downward spraying			
Active substance	Glyphosate			
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha			
Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (g a.e./ha)**	**$HQ_{contact}$ criterion**	**Trigger**
Adult acute contact toxicity	>461	1800	<3.9	8
		1440	<3.1	
		1080	<2.3	
		720	<1.6	
		540	<1.2	

$HQ_{contact}$: Hazard quotient for contact exposure

The hazard quotients (HQ) for acute contact toxicity are below the respective trigger value for the application rates of 540 g a.e./ha, 720 g a.e./ha, 1080 g a.e./ha, 1440 g a.e./ha and 1800 g a.e./ha. Therefore, no Tier 1 risk assessment is required.

Currently no official OECD test guideline considering oral toxicity to solitary bees is available. Thus, no study was conducted.

B.9.6.2. Risk assessment for non-target arthropods

Studies on effects of the representative formulation MON 52276 on non-target arthropods to fulfill the data requirements according to EU Regulation No 284/2013 are presented in the following. The validity of all studies (newly submitted as well as already submitted) have been checked based on latest guidelines available at time of assessment. The table below summarised the information available on non target arthropods.

Endpoints of all available studies with the representative product MON 52276 are shown in the table below. Although no NTA studies with the active substance are available, the endpoints for MON 52276 have been converted to acid equivalents (a.e.) to be consistent with the other organism groups. This conversion has been made by the acid equivalent purity of the test item stated in the reports.

Table B.9.6-36: Endpoints: studies on toxicity of MON 52276 to non-target arthropods other than bees

Reference	Test item	Species	Test design	Status	Mortality LR_{50}	Effects on reproduction
Tier 1 – laboratory studies						
[redacted] 1995 CP 10.3.2.1/003	MON 52276	*Poecilus cupreus*	Laboratory	Valid	> 10 L/ha (3600 g a.e./ha)	-
[redacted] 1995 CP 10.3.2.1/004	MON 52276	*Pardosa sp.*	Laboratory	Valid	> 10 L/ha (3600 g a.e./ha)	-
[redacted] 1995CP 10.3.2.1/001	MON 52276	*Aphidius rhopalosiphi*	Laboratory	Supportive	10 L MON 52276/ha (3.6 kg a.e/ha) = 100% mortality at 24 hrs.	No reproduction endpoints available.
[redacted] 1995 CP 10.3.2.1/002	MON 52276	*Typhlodromus pyri*	Laboratory	Supportive	10 L MON 52276/ha (3.6 kg a.e/ha) = 100% mortality at day 4.	No reproduction endpoints.
Tier 2 – extended laboratory and aged residue						
[redacted] 2010 CP 10.3.2.2/001	MON 52276	*Typhlodromus pyri*	Extended laboratory 2D	Valid	> 16.0 L/ha (5760 g a.e./ha)	**ER_{50} ≥ 12 L/ha (4320 g a.e./ha) Reduction in no. of egg/female 44.9 % at 12 L/ha** NOER = 8 L/ha (2880 g a.e./ha)

Reference	Test item	Species	Test design	Status	Mortality LR_{50}	Effects on reproduction
Tier 1 – laboratory studies						
[redacted] 2010 CP 10.3.2.2/004	MON 52276	*Aphidius rhopalosiphi*	Extended laboratory 3D	Valid	> 16.0 L/ha (5760 g a.e./ha)	**ER_{50} > 16 L/ha (5760 g a.e./ha)** NOER ≥ 16 L/ha (5760 g a.e./ha)
[redacted]2010 CP 10.3.2.2/007	MON 52276	*Aleochara bilineata*	Extended laboratory	Valid	> 12.0 L/ha (4320 g a.e./ha)	ER_{50} > 12 L/ha (4320 g a.e./ha) NOER ≥ 12 L/ha (4320 g a.e./ha)
[redacted] 1999 CP 10.3.2.2/002	MON 52276	*Typhlodromus pyri*	Extended laboratory	Not valid	-	-
[redacted]1998 CP 10.3.2.2/003	MON 52276	*Typhlodromus pyri*	Extended laboratory	Supportive	Indicative of an effect on mortality at 6 L/ha (84%) and 12 L/ha (89%)	-
[redacted] 1999 CP 10.3.2.2/005	MON 52276	*Aphidius rhopalosiphi*	Extended laboratory	Supportive (sensitivity of species questionable and low robustness)	Effects on mortality: less than 50% expected up to 12 L/ha	No adverse effects on reproduction expected up to 12L/ha
[redacted] 1999 CP 10.3.2.2/008	MON 52276	*Chrysoperla carnea*	Extended laboratory	Supportive (Control eggs < 15. (actual 7.9). Sensitivity of species questionable.	LR50 = 10.34 L MON 52276/ha (supportive):	No reliable endpoint could be set for reproduction.

a.e. glyphosate acid equivalents
Endpoints in **bold** are used for risk assessment

Literature data on non-target arthropods

The scientific literature review conducted for the last Annex I renewal (submitted in 2012) that appears in the RAR (2015) contains an extensive review of ecotoxicological papers considered relevant but supplementary to the Annex I renewal.

Studies related to indirect effects are not considered here. The previous conclusion of RMS 2015 on indirect effects are reported in the assessment of risk biodiversity via indirect effects and trophic interactions. For NTA, please refer to Volume 3 CP B.9 under B.9.14.1.4. Below are reported information related to direct effects.

The RMS (UBA) stated that effects on various developmental stages of arthropods, physiology, and behavior or prey consumption are not given consideration in traditional risk assessment. Bueno et al., (2008) could show that glyphosate containing products can be harmfull towards egg stages of *Trichogramma*, whereas at other parasitoid stages the same product was harmless. Sublethal effects of glyphosate were assessed in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of a web weaver spider (*Alpaida veniliae*) in Argentina (Benamu et al., 2010) and on wolf spiders in north America (Evans et al., 2010). The authors concluded that the exposure to glyphosate containing products affects the behavior of the animals and their capacity to grow and persist in agroecosystems. In contrast, short term exposures (2h and one-day residues) of spiders and carabid beetles, respectively *Pardosa agricola* and *Poecilus cupreus*, did not affect mating or avoidance of the arthropods, but (only) slightly slower movement (Michalkova et al., 2009).

Concerning the current literature review, there were no literature articles considered relevant for the ecotoxicological risk assessment. A number of papers were identified by the applicant as relevant but supplementary but were not included in the list of data retained as supportive for risk assessment (see criteria for assessment of literature review proposed by applicant and discussed by RMS under Volume 3 CA B.9 point B.9.11). These papers discuss the effects of glyphosate based herbicides on a range of non-target arthropods such as Culicidae (Bara et al., 2014[13], Mohamed et al., 2016[14]), *Chrysoperla externa* (Castilhos et al., 2011[15], Pasini et al., 2018[16]), Colorado potato beetle (Rainio et al., 2019[17]), rose-grain aphids (Saska et al., (2016)[18], Hymenopterans (Stecca et al., 2016)[19], *Bombyx mori* (You et al., 2010[20] and Zhang et al., 2011[21]). Tahir H. M. et al., 2019 investigated the effect of glyphosate on the mortality, avoidance behavior, foraging activity, and activity of acetylcholine esterase (AChE) and carboxylesterase (CarE) in *Neoscona theisi* (Araneae: Araneidae). RMS agreed with applicant that these studies are not relevant and/or reliable and was not considered further for weight of evidence (please refer to Table B.9.11.1.4-2 of Volume 3 CA B.9 for more details about the studies exclusion).

In Mirande L. et al., 2010[22], the authors evaluated the side-effects of glyphosate on larvae (third instar) and adults of *Eriopis connexa* Germar (Coleoptera: Coccinelidae). GlifoGlex 48® (48% glyphosate) was used an the study is therefore considered of limited relevance for the assessment of glyphosate itself as

[13] Bara J. J. et al. 2014. Sublethal effects of atrazine and glyphosate on life history traits of Aedes aegypti and Aedes albopictus (Diptera: Culicidae).

[14] Mohamed I. A-w. et al.,2016. Unique efficacy of certain novel herbicides against Culex pipiens (Diptera: Culicidae) mosquito under laboratory conditions

[15] Castilhos R. V. et al., 2011.Selectivity of pesticides used in peach orchard on adults of Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae). Original title: Seletividade de agrotoxicos utilizados em pomares de pessego a adultos do predador Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae).

[16] Pasini R. A. et al., 2018. Comparative selectivity of herbicides used in wheat crop on the predators Chrysoperla externa and Eriopis connexa. Planta Daninha (2018), Vol. 36,pp. E018179968

[17] Rainio M. J. et al., 2019. Effects of a glyphosate-based herbicide on survival and oxidative status of a non-target herbivore, the Colorado potato beetle (Leptinotarsa decemlineata). Comparative biochemistry and physiology. Toxicology & pharmacology (2019), Vol. 215, pp. 47

[18] Saska P. et al., 2016. Treatment by glyphosate-based herbicide alters life history parameters of the rose- grain aphid Metopolophium dirhodum. Scientific reports (2016), Vol. 6, pp. 27801

[19] Stecca C. S. et al., 2016. Side-Effects of Glyphosate to the Parasitoid Telenomus remus Nixon (Hymenoptera: Platygastridae). Neotropical entomology (2016), Vol. 45, No. 2, pp. 192

[20] You W-y. et al., 2010. Toxicity Evaluation of Sixteen Herbicides to Bombyx mori. Asian Journal of Ecotoxicology (2010), Vol. 5, No. 1, pp. 91

[21] Zhang Q. et al., 2011. An evaluation on acute toxicity of 29 pesticides to Bombyx mori. Canye Kexue (2011), Vol. 37, No. 2, pp. 343

[22] Mirande L. et al., 2010. Side effects of glyphosate on the life parameters of Eriopis connexa (Coleoptera: Coccinelidae) in Argentina. Communications in Agricultural and Applied Biological Sciences, (2010) Vol. 75, No. 3, pp. 367 72

no information was provided on the surfactants present in the formulation. The concentration of 192 mg a.i./litre is considered realistic and even below the concentration that may be sprayed under realistic conditions of use. The exposure was by ingestion through the treated prey (*Rophalosiphum padi*) or by drinking treated water during 48 h for treatment of the adult. Larvae from glyphosate treatment molted earlier than controls. In addition, the weight of pupae, longevity, fecundity and fertility were drastically reduced in treated organisms. The reductions were more drastic when the treatments were performed at the third larval stage than as adult. The reproduction capacity of the predator was the most affected parameter and it was deemed to be related to a hormonal disruption by glyphosate in the treated organisms. No raw data is available, only graphics are presented. The study is considered reliable with restrictions (please refer to the appendix to Volume 3 CA B.9 related to literature data on ecotoxicology for the detailed summary and assessment).

Overall there is no studies that may impact the outcome the risk assessment of direct effects. This may be reconsider as RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and data gap in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents.

Risk assessment for other non-target arthropods

The table below summarises the GAP of MON52276.
RMS considered a risk envelop approach[23] by presenting a risk assessment for the uses leading to the worst case in-field PER. This was obtained with the uses around railroad tracks. Railroad track uses may use specific equipment which may not be considered realistically covered by standard risk assessment according to ESCORT 2. However given the drift percentage used of 2.77% is considered to be suitable for all intended uses. Overall, RMS is of the opinion that the risk assessment according to ESCORT 2 is suitable to cover this use.

Table B.9.6-37: Overview of GAP of MON52276

GAP number and summary of use	Application rate of glyphosate considered g/ha (28 day interval unless otherwise stated)									
	1 × 540	**1 × 720**	**1 × 1080**	**2 × 720**	**1 × 1440**	**3 × 720**	**1 × 1800**	**2 × 1080**	**2 × 1440**	**2 × 1800 (90 days apart)**
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre-emergence of **field crops.**		X	X		X					
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of **field crops.**		X	X	X	X	X		X		
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of **field crops.**	X									
Use 4 a-c: Applied to weeds (post-emergence)		X	X	X	X	X		X	X	

[23] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach" SANCO/11244/2011 rev. 5, 14 March 2011

below trees in **orchards.**										
Use 5 a-c: Applied to weeds (post-emergence) below vines in **vineyards**			X	X	X	X		X	X	
Use 6 a-b: Applied to weeds (post-emergence) in **field crops BBCH < 20**		X	X							
Use 7 a-b: Applied to weeds (post-emergence) around **railroad tracks**							X			X
Use 8 and 9: Applied to invasive species (post-emergence) in **agricultural and non-agricultural areas**							X			
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of **field crops**		X	X							

X = this use is covered by the application rate indicated
Grey shaded cells: risk assessment presented.

NB : The applicant precised that applications using high boom or blast sprayer applicators associated with for example, 'over the top' applications in perennial crops, are not a use on the proposed GAP table. The intended uses are for low boom – ground directed applications.

The evaluation of the risk for non-target arthropods was performed according to the "Guidance Document on Terrestrial Ecotoxicology"(SANCO/10329/2002 rev.2 (final), October 17, 2002), and the guidance document ESCORT 2 [24].

According to ESCORT 2 and Rautmann (2001) the estimated spray drift deposition for two applications on field crops (% of in-field target deposition) downwind of a sprayed (ground directed application) to a bare soil surface (without interception by vegetation) representing a field crop situation at distances of 1 meters from the target area, is 2.38.

The stated percentage drift values are for field crop drift values used for all crops according to recommendations of the Guidance Document on Terrestrial Ecotoxicology (2002) and are based on Rautmann (2001).

In-field risk assessment

The in-field risk assessment is presented below for the use of MON 52276 in field crops, orchards, vineyards, railroad tracks and agricultural/non-agricultural areas for the control of invasive species and considers the worst case application rate for railroad tracks (2 x 1800 g a.e./ha) that covers all intended uses. As a worst-case, a MAF factor of 2 is used in order to cover all intended intervals between applications for all uses.

[24] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet MC, Lewis G, Oomen PA, Schmuck R and Vogt H (eds) (2001): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop. SETAC, Pensacola, 46 p

Table B.9.6-38: In-field HQs for non-target arthropods (Tier 2) exposed to MON 52276 in field crops, orchards, vineyards, railroad tracks, agricultural and non-agricultural areas– considering downward ground-directed spray

Intended use	All uses
Active substance/product	Glyphosate/ MON52276
Application rate (g/ha)	2 × 1800 (90 d)
MAF	2 (foliar and/or soil))

Crop scenario	**Test species Tier II**	**LR_{50}/ER_{50} (ext. lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
All uses	*T. pyri*	>4320	3600	Yes
	A. rhopalosiphi	>5760		Yes
	Poecilus cupreus	> 3600		Yes
	Pardosa sp.	> 3600		Yes
	Aleochara bilineata	>4320		Yes

a.e. glyphosate acid equivalents
PER: Predicted environmental rate

Off-field risk assessment

The off-field risk assessment is presented below for the use of MON 52276 in field crops, orchards, vineyards, railroad tracks and agricultural/non-agricultural areas. As for in-field risk assessment, the off-field risk assessment considers the worst case application rate for railroad tracks (2 x 1800 g a.e./ha) that covers all intended uses. As a worst-case, a MAF factor of 2 is used in order to cover all intended intervals between applications for all uses. The risk assessment presented below also covers the highest single application rates, which would have considered a higher drift value (2.77% for 1 application at 1 m for ground-directed applications).

Table B.9.6-39: Off-field HQs for non-target arthropods exposed to MON 52276 in field crops, orchards, vineyards, railroad tracks, agricultural and non-agricultural areas – considering downward ground-directed spray

Intended use	All uses
Active substance/product	Glyphosate/MON52276
Application rate (g a.e./ha)	1800
MAF	2 (foliar and/or soil)
Drift rate (%)	2.38 (1 m)
vdf	5 (Tier I) / 5 (Tier II, 2D test design) * or 1 (Tier II, 3D test design)

Crop scenario	**Test species Tier II**	**LR_{50}/ER_{50} (ext. lab.) (g/ha)**	**MAF (foliar/soil)**	**VDF**	**Correction factor**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
All uses	*T. pyri* (2D)	>4320	2	5*	5	85.68	yes
	A. rhopalosiphi (3D)	>5760		1	5	428.4	yes
	Poecilus cupreus (2D)	> 3600		1**	5	428.4	yes
	Pardosa sp. (2D)	> 3600		1**	5	428.4	yes
	Aleochara bilineata (2D)	>4320		1**	5	428.4	yes

a.e. glyphosate acid equivalents
PER: Predicted environmental rate, vdf: vegetation distribution factor; CF: correction factor
* as recommended in the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA Supporting publication 2019:EN-1673)
**A VDF of 1 has been considered since these species are considered to be soil-dwelling arthropods.

An acceptable risk can be expected for non-target arthropods other than bees from the proposed uses of MON 52276 considering in-field or off-field habitats of field crops, orchards, vineyards, railroad tracks and agricultural/non-agricultural areas for the control of invasive species.

A summary of the risk assessment regarding non-target arthropods biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

B.9.7.1.1. Earthworms – sub-lethal effects

Data point	CP 10.4.1.1/001
Report author	[redacted]
Report year	2020
Report title	MON 52276: Effects on survival, growth and reproduction of the earthworm *Eisenia andrei* tested in artificial soil
Report No	20 48 TEC 0028
Document No	BI-2019-0632
Guidelines followed in study	OECD 222 (2016), ISO 11268-2 (2012)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 222 (2016):* *Major:* *- none* *Minor:* *- none*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of MON 52276 (360 g/L glyphosate acid equivalent) on *Eisenia andrei* were tested in a 56-days sublethal laboratory test (according to OECD 222) with regard to the parameters mortality, behavioural and pathological symptoms, body weight change and reproduction in OECD soil containing 10% sphagnum peat. The test was conducted with nominal test concentrations of 11.7, 16.3, 22.9, 32.0, 44.8, 62.8, 87.9, 123 mg test item/kg soil dry weight, equivalent to 3.6, 5.0, 7.1, 9.9, 14, 19, 27, 38 mg a.e./kg soil dry weight, respectively. In addition, a control group was exposed to soil mixed with deionised water only.

After 56 days, the test item caused no mortality at any tested concentrations and control. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass and in number of juveniles when compared to the control group. Therefore, No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 38 mg a.e./kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 38 mg a.e./kg soil dry weight. All validity criteria according to the OECD guideline 222 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Description:	Yellow liquid
Lot/Batch #:	11511167 (manufacturing lot AZE200810A)

Purity:	360 g/L glyphosate acid equivalent (nominal)
	362 g/L glyphosate acid equivalent (analysed)
2. positive control:	Maypon Flow (carbendazim, SC 500), tested in a separate study
3. Test organism:	
Species:	Earthworm (*Eisenia andrei* (BOUCHÉ, 1972))
Age:	Adults, approx. 4 months old with clitellum
Weight:	270 - 423 mg/worm
Source:	In-house rearing (originally from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Food:	Air-dried and finely ground horse manure
Acclimation period:	Approx. 24 hours in the artificial substrate
4. Environmental conditions:	
Temperature:	19.9 – 21.8 ºC
Photoperiod:	16 h light (630 Lux)/ 8 h dark
Soil pH:	5.99 - 6.06 (test start); 5.74 - 5.83 (test termination)
Soil moisture content:	test start: 34.9 – 35.0 (equivalent to 56.0 – 56.2 % of WHC)
	test end: 34.3 – 34.8 (equivalent to 55.1 – 55.9 % of WHC)
	(difference between start and end of the test: max. 2.0 %)
5. Experimental work dates:	2020-02-26 to 2020-04-22

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A sublethal test was conducted with nominal test concentrations of 11.7, 16.3, 22.9, 32.0, 44.8, 62.8, 87.9, 123 mg test item/kg soil dry weight, equivalent to 3.6, 5.0, 7.1, 9.9, 14, 19, 27, 38 mg a.e./kg soil dry weight, respectively. In addition, a control group was exposed to soil mixed with deionised water only. The test concentrations were prepared by dispersing an exact weighed amount of the test item in deionised water (stock solutions) and thereafter diluted to obtain different test concentrations, which were thoroughly mixed with the artificial soil, achieving desired test concentrations with a final nominal water content of 40 - 60% of WHC. The artificial soil substrate was composed of 10% sphagnum peat, 20% kaolin clay, 69.5% industrial quartz sand and 0.5% calcium carbonate. Four replicate test containers (test item) and 8 replicate test containers (control) with 675 g soil (wet weight) were prepared for each treatment group. 10 adult earthworms were exposed per replicate for 56 days.

As a toxic reference, earthworms were exposed in a separate study to Maypon Flow (carbendazim, SC 500). The results are in line with the OECD requirements (53 and 99% of reduction in the number of juveniles at concentrations of 5 and 10 mg product/ kg dry soil respectively).

2. Observations: At test initiation, individual fresh weight, behavioural responses of earthworms and physico-chemical parameters of the artificial soil were recorded. Behavioural and pathological symptoms including feeding activity were observed on a weekly basis. Four weeks after test initiation, number of surviving adult earthworms and fresh weight of surviving adult earthworms per replicate were recorded. At test termination (8 weeks after test initiation), number of surviving juveniles per replicate, observation of behavioural/pathological symptoms and determination of physico-chemical parameters of the artificial soil were observed.

3. Statistical calculations: The Williams-t-test was used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used. The statistical analysis was performed with the software ToxRat Professional 3.2.1 (Ratte 2015).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.7-1 : Sublethal effects of MON 52276 on earthworms

MON 52276 [mg a.e./kg soil d.w.]	**Control**	**3.6**	**5.0**	**7.1**	**9.9**	**14**	**19**	**27**	**38**
Mortality of adult worms after 4 weeks (%)	0	0	0	0	0	0	0	0	0
Mean biomass change (%)	27.9	26.2	28.2	29.1	27.7	28.9	25.6	28.4	26.6
Mean number of juveniles per replicate after 8 weeks	222.9	225.5	218.5	232.3	223.5	214.5	211.3	227.3	221.0
CV %	12.8	26.8	17.0	8.3	18.0	13.0	20.0	16.7	23.5
Change of reproduction compared to control (%)	-	101.2	98.0	104.2	100.3	96.2	94.8	102.0	99.2
EC_{10} / EC_{20}	Not determined								
LOEC	> 38 mg a.e./kg soil d.w.								
NOEC	≥ 38 mg a.e./kg soil d.w.								

a.e.= acid equivalent

B. OBSERVATIONS

Mortality rates of 0 % were recorded in the test item treatment groups and in the control. No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test. The weight change of adult worms ranged between 25.6 and 29.1 % in the treated groups and 27.9 % in the control group. The test item caused no statistically significant change in biomass compared to the control groups at any concentration tested. No statistically significant effects on the number of juveniles compared to the control group were found at any concentration tested.

The validity criteria according to guideline OECD 222 are fulfilled as each replicate (containing 10 adults) has produced ≥ 30 juveniles by the end of the test in the control (actual value: 181-267 juveniles),the coefficient of variation of reproduction was ≤ 30% in the control (actual value: 12.8%) and adult mortality over the initial 4 weeks of the test was ≤ 10% in the control (actual value: 0%).

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate on mortality and reproduction of earthworms (*Eisenia andrei*) were assessed following application of MON 52276 under laboratory conditions and according to OECD 222.

The EC_{10} / EC_{20} of MON 52276 for earthworm reproduction could not be calculated due to lack of effects. The overall NOEC was determined to be ≥ 38 mg a.e./kg dry soil, equivalent to 123 mg test item/kg dry soil. The overall LOEC was determined to be > 38 mg a.e./kg soil d.w.

The study is considered valid and is suitable for risk assessment purposes.

Assessment and conclusion by RMS:
This is a new study.

Test item: MON 52276
Artificial soil containing 10% peat
Test item was mixed into the soil.

This study is valid.

NOEC for earthworms = 123 mg MON 52276/kg dry soil, equivalent to 38 mg glyphosate acid equivalent/kg dry soil.

B.9.7.1.2. Earthworms – field studies

No field studies with earthworms are required since the risk assessment indicates an acceptable risk for earthworms following the application of MON 52276 when applied in accordance with the proposed GAP for uses in field crops, orchards, vineyards, railroad tracks and in agricultural/non-agricultural areas for the control of invasive species.

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

B.9.7.2.1. Species level testing

B.9.7.2.1.1. *Folsomia candida* – sub-lethal effects

Data point	CP 10.4.2.1/001
Report author	[redacted]
Report year	2020
Report title	MON 52276: Effects on reproduction of the collembolan *Folsomia candida*
Report No	20 48 TCC 0037
Document No	BI-2020-0179
Guidelines followed in study	OECD 232 (2016)
Deviations from current test guideline identified by the applicant:	Deviation from guideline OECD 232 (2016): None.

See RMS analysis in RMS comment box	
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of MON 52276 (360 g/L glyphosate acid equivalent) on *Folsomia candida* were tested in a 28- days laboratory test (according to OECD 232) with regard to the parameters mortality, behavioural symptoms and reproduction in OECD soil containing 5% sphagnum peat. The test was conducted with nominal test concentrations of 95.3, 171.5, 308.6, 555.6, 1000, 1800, 3240, 5832 mg test item/kg soil dry weight, equivalent to 29.4, 53.0, 95.4, 172, 309, 556, 1001, 1802 mg a.e./kg soil dry weight, respectively. In addition, a control group was exposed to soil mixed with deionised water only. After 28 days, the test item caused no statistically significant effects on mortality and reproduction at any tested concentrations and control. No effects on behaviour of the collembolans were observed during the test at the end of the test.

The test item caused no statistically significant change in mortality and in number of juveniles when compared to the control group. Therefore, No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 1802 mg a.e./kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1802 mg a.e./kg soil dry weight. All validity criteria according to the OECD guideline 232 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Description:	Yellow liquid
Lot/Batch #:	11511167 (manufacturing lot AZE200810A)
Purity:	360 g/L glyphosate acid equivalent (nominal)
	362 g/L glyphosate acid equivalent (analysed)

2. positive control: Boric acid, tested in a separate study

3. Test organism:

Species:	*Folsomia candida*
Age:	Juvenile collembolans, 9-12 days old
Source:	originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem. reared under ambient laboratory conditions in the test facility
Food:	2 mg granulated dry yeast at start of the test and after 14 days

4. Environmental conditions:

Temperature:	19.0 – 21.8 ºC

Photoperiod:	16 h light (580 Lux)/ 8 h dark
Soil pH:	5.51 – 6.06 (test start); 5.56 – 5.58 (test termination)
Soil moisture content:	test start: 24.9 – 25.0 (equivalent to 58.2 – 58.4 % of WHC) test end: 24.3 – 24.6 (equivalent to 56.8 – 57.5 % of WHC)
5. Experimental work dates:	2020-06-26 to 2020-07-24

B. STUDY DESIGN AND METHODS

1. Experimental treatments: In a 28-day collembolan reproduction study, soil was treated with nominal test concentrations of 95.3, 171.5, 308.6, 555.6, 1000, 1800, 3240, 5832 mg test item/kg soil dry weight, equivalent to 29.4, 53.0, 95.4, 172, 309, 556, 1001, 1802 mg a.e./kg soil dry weight, respectively. In addition, a control group was exposed to soil mixed with deionised water only. The test concentrations were prepared by dispersing an exact weighed amount of the test item in deionised water (stock solution) and thereafter diluting to obtain different test concentrations. The test solutions were thoroughly mixed with the artificial soil, achieving desired test concentrations with a final nominal water content of 40 - 60% of WHC. The artificial soil substrate was composed of 5% sphagnum peat, 20% kaolin clay, 74.7% industrial quartz sand and 0.3% calcium carbonate. Four replicate test containers (test item) and 8 replicate test containers (control) with 30 g soil (dry weight) were prepared for each treatment group. 10 juveniles Collembola (9 - 12 days) were exposed per replicate for 28 days.

As a toxic reference, collembolans were exposed to Boric acid in a separate study. The results are in line with the OECD. The EC_{50} was determined to be 103 mg/kg soil dry weight. The LC_{50} was determined to be 161 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 44 mg/kg soil dry weight.

2. Observations: Assessment of adult mortality, reproduction and behavioural effects was carried out after 28 days.

3. Statistical calculations: For reproduction data, the Williams-t-test was used to compare the control with the independent test item groups, and for the mortality data, the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm was used to compare the control with the independent test item groups. For statistical evaluation the percentage mortality of the springtails was calculated for each treatment. The reproductive output for each test item treatment group was calculated in % compared to control. The statistical analysis was performed with the software ToxRat Professional 3.3.0 (Ratte 2018).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.7-2 Effects on mortality and reproduction of MON 52276 on the Collembolans

MON 52276 [mg a.e./kg soil d.w.]	**Control**	**29.4**	**53.0**	**95.4**	**172**	**309**	**556**	**1001**	**1802**
Mortality of parental collembolans after 4 weeks (%)	5.0	2.5	0.0	2.5	2.5	2.5	0.0	2.5	5.0
Mean number of juveniles after 4 weeks	676	672	679	653	651	691	667	703	603
CV %	12.3	14.2	5.7	15.7	8.1	13.2	15.0	10.8	9.3
Reproduction in (%) of control	100	99	100	97	96	102	99	104	89
EC_{10} / EC_{20}	Not determined								
LOEC	> 1802 mg a.e./kg soil d.w.								
NOEC	1802 mg a.e./kg soil d.w.								

a.e.= acid equivalent

B. OBSERVATIONS

Mortality rates of 0.0 % - 5.0 % were recorded in the test item treatment groups. 5.0 % parental mortality was observed in the control. No effects on behaviour of the collembolans were observed during the test. The mean number of juvenile Collembolans counted four weeks after introduction of the parental Collembolans into the test vessels was 676 in the control and 672, 679, 653, 651, 691, 667, 703 and 603 at concentrations of 29.4, 53.0, 95.4, 172, 309, 556, 1001 and 1802 mg a.e./kg soil d.w., respectively.
No statistically significant effects on parental mortality and on the number of juveniles compared to the control group were found at any concentration tested. Due to the lack of a concentration-response relationship, no reliable EC_X-calculation was possible. Therefore, no EC_{10} / EC_{20}-value can be reported. The NOEC for reproduction was determined to be 1802 mg a.e./kg soil dry weight, and the LOEC was determined to be > 1802 mg a.e./kg soil dry weight.

The validity criteria according to guideline OECD 232 are fulfilled as each replicate has produced ≥ 100 juveniles by the end of the test in the control (actual value: 676 juveniles), the coefficient of variation of reproduction was < 30% in the control (actual value: 12.3%) and adult mortality after 28 days of the test was ≤ 20% in the control (actual value: 5.0%).

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of glyphosate on mortality and reproduction of collembolan (*Folsomia candida)* were assessed following application of MON 52276 under laboratory conditions and according to OECD 232.

The EC_{10} / EC_{20} of MON 52276 for Collembola reproduction was not determined due to lack of effects.
The overall NOEC was determined to be ≥ 1802 mg a.e./kg dry soil, equivalent to ≥ 5832 mg test item/kg dry soil. The overall LOEC was determined to be > 1802 mg a.e./kg soil d.w.

The study is considered valid and is suitable for risk assessment purposes.

Assessment and conclusion by RMS:

This is a new study.

Test item: MON 52276
Artificial soil containing 5% peat
Test item was mixed into the soil.

For *Folsomia candida,* only females should be used in the test. The sex of the collembolans used in this study was not specified.
The soil depth within the test vessel was not specified (it should be 2-4 cm).
OECD 232 guidance document indicates that "A reference substance should be tested at its EC_{50} concentration for the chosen test soil type either at regular intervals or possibly included in each test run to verify that the response of the test organisms in the test system are responding within the normal level." The type of soil used in the toxic reference test (BioChem project No. 19 48 TCC 0057, dated 2019-08-19) is not specified.
RMS considers these deviations acceptable.

The validity criteria according to the current guideline OECD 232 are fulfilled. This study is considered valid.

NOEC reproduction = 1802 mg a.e./kg dry soil, equivalent to ≥ 5832 mg test item/kg dry soil.
LOEC > 1802 mg a.e./kg dry soil.
The EC_{10} / EC_{20} of MON 52276 for Collembola reproduction was not determined due to lack of effects.

B.9.7.2.1.2. *Hypoaspis aculeifer* – sub-lethal effects

Data point	CP 10.4.2.1/002
Report author	[redacted]
Report year	2020
Report title	MON 52276: Effects on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* tested in artificial soil
Report No	20 48 THC 0031
Document No	BI-2020-0183

Guidelines followed in study	OECD 226 (2016)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation from guideline OECD 226 (2016): None.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The purpose of this study was to determine potential effects of the test item on mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative species of soil micro-arthropods during a test period of 14 days. The test was conducted at the treatment rates of 95.3, 171.5, 308.6, 555.6, 1000, 1800, 3240, 5832 mg test item/kg soil dry weight, equivalent to 29.4, 53.0, 95.4, 172, 309, 556, 1001, and 1802 mg a.e./kg soil dry weight. An untreated control was also conducted.

The test item MON 52276 showed no statistically significant adverse effects on adult mortality of the predatory mite *Hypoaspis aculeifer* in artificial soil at any tested concentration. The test item showed no statistically significant adverse effects on reproduction of *Hypoaspis aculeifer* up to and including 1001 mg a.e./kg soil dry weight. At a concentration of 1802 mg a.e./kg soil dry weight a statistically significant reduction of reproduction was observed.

Therefore, the No-Observed-Effect-Concentration (NOEC) and the Lowest-Observed-Effect-Concentration (LOEC) for mortality were determined to be =1802 mg and > 1802 mg a.e./kg soil d.w., respectively. The NOEC and LOEC for reproduction were determined to be 1001 mg and 1802 mg a.e./kg soil d.w., respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 52276
Description:	Yellow liquid
Lot/Batch #:	11511167 (manufacturing lot AZE200810A)
Purity:	30.9% wt 360 g/L glyphosate acid equivalent (nominal) 362 g/L glyphosate acid equivalent (analysed)

2. positive control: Dimethoate, tested in a separate study

3. Test organism:

Species:	*Hypoaspis aculeifer* (CANESTRINI)
Age:	Adults from a synchronised culture with an age difference of 2 days

Source:	Purchased from "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany, on 2020- 06-19 and kept in the test facility under ambient laboratory conditions until test start
Food:	During the test, the predatory mites were fed every 2 - 3 days with *Tyrophagus putrescentiae* (SCHRANK), originally obtained from "Bayer CropScience AG", Monheim am Rhein, Germany, reared in the test facility

4. Environmental conditions:

Temperature:	19.5 – 21.4 ºC
Photoperiod:	light : dark = 16 hours : 8 hours (497 lux)
Soil pH:	5.6 - 6.3 (test start); 5.7 - 6.0 (test end)
Soil moisture content:	test start: 20.22 - 21.12, equivalent to 46.8 - 48.9 % of WHC test end: 20.16 - 20.96, equivalent to 46.7 - 48.5 % of WHC

5. Experimental work dates: 2020-06-22 to 2020-07-13

B. STUDY DESIGN AND METHODS

1. Experimental treatments: In a 14-day *Hypoaspis aculeifer* reproduction study, soil was treated with nominal test concentrations of 95.3, 171.5, 308.6, 555.6, 1000, 1800, 3240, 5832 mg test item/kg soil dry weight, equivalent to 29.4, 53.0, 95.4, 172, 309, 556, 1001, 1802 mg a.e./kg soil dry weight, respectively. In addition, a control group was exposed to soil mixed with deionised water only. The test concentrations were prepared by dispersing an exact weighed amount of the test item in deionised water (stock solution) and thereafter diluting to obtain different test concentrations, which were thoroughly mixed with the artificial soil, achieving desired test concentrations with a final nominal water content of 40 - 60% of WHC. The artificial soil substrate was composed of 5.0% sphagnum peat, 20.0% kaolin clay, 74.75% industrial quartz sand and 0.25% calcium carbonate. Four replicate test containers (test item) and 8 replicate test containers (control) with 20 g soil (dry weight) were prepared for each treatment group. Ten adult female *Hypoaspis* (2 days old) were exposed per replicate for 14 days.

As a toxic reference, *Hypoaspis* were exposed to Dimethoate in a separate study. The EC_{50} reproduction of the reference item dimethoate (98.8 % ± 0.5 %, analysed) was calculated to be 6.3 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

2. Observations: Assessment of juvenile mites for reproduction and adult mortality was carried out after 14 days.

3. Statistical calculations: The statistical analysis was performed with ToxRat Professional 3.3.0 (2018). Statistical tests included the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Williams Multiple Seq control with the independent test item groups.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.7-3 : Effects on mortality and reproduction of MON 52276 on *Hypoaspis aculeifer*

MON 52276 [mg a.e./kg soil d.w.]	**Control**	**29.4**	**53.0**	**95.4**	**172**	**309**	**556**	**1001**	**1802**
Mean mortality of soil mites after 14 days (%)	2.5	2.5	5.0	0.0	2.5	2.5	2.5	2.5	0.0
Mean number of juveniles after 14 days	259.1	243.0	259.8	258.5	243.8	247.8	250.0	251.5	223.8*
CV %	4.7	4.3	8.2	5.3	5.4	4.7	9.9	5.2	8.2
Reproduction in (%) of control	100	94	100	100	94	96	96	97	86
EC_{10} / EC_{20}	Not determined								
LOEC	>1802 mg a.e./kg soil d.w. mortality 1802 mg a.e./kg soil d.w. reproduction								
NOEC	1802 mg a.e./kg soil d.w. mortality 1001 mg a.e./kg soil d.w. reproduction								

a.e.= acid equivalent

* Statistically significantly different compared to the control (Williams Multiple Sequential t-test Procedure for reproduction, $\alpha = 0.05$, one-sided smaller)

B. OBSERVATIONS

In the control group, a parental mortality of 2.5 % was observed. The mortality in the test item treatment groups was 0.0 % - 5.0 %. Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 259.1 in the control and 243.0, 259.8, 258.5, 243.8, 247.8, 250.0, 251.5 and 223.8 at concentrations of 95.3, 171.5, 308.6, 555.6, 1000, 1800, 3240 and 5832 mg test item/kg soil d.w., respectively.

The validity criteria according to guideline OECD 226 was fulfilled as the mean mortality of females was ≤ 20 % by the end of the test in the control (actual value: 2.5 %), the mean number of juveniles per replicate was ≥ 50 (actual value: 259.1), and the coefficient of variation number of juveniles per replicate) was <30% in the control (actual value: 4.7%).

III. CONCLUSIONS

Assessment and conclusion by applicant:

The test item MON 52276 showed no statistically significant adverse effects on adult mortality of the predatory mite *Hypoaspis aculeifer* in artificial soil at any tested concentration. The test item showed no statistically significant adverse effects on reproduction of *Hypoaspis aculeifer* up to and including 1001 mg a.e./kg soil dry weight. At a concentration of 1802 mg a.e./kg soil dry weight a statistically significant reduction of reproduction was observed.

Therefore, the No-Observed-Effect-Concentration (NOEC) and the Lowest-Observed-Effect-Concentration (LOEC) for mortality were determined to be ≥ 1802 mg and 1802 mg a.e. item/kg soil d.w., respectively. The NOEC and LOEC for reproduction were determined to be 1001 mg and 1802 mg a.e./kg soil d.w., respectively. The EC_{10} / EC_{20} of MON 52276 for reproduction was not determined due to lack of effects.

The study is considered valid and is suitable for risk assessment purposes.

Assessment and conclusion by RMS:

This is a new study.

Test item: MON 52276
Artificial soil containing 5% peat
Test item was mixed into the soil.

Water Holding Capacity was measured in the test according to DIN ISO 11465 (1996) while the ISO 11268-2 is recommended in the GD OECD 226. This is considered as a minor deviation.

The validity criteria according to the current guideline OECD 226 are fulfilled. This study is considered valid.

NOEC mortality = 1802 mg a.e./kg soil d.w.
LOEC mortality > 1802 mg a.e./kg soil d.w.
NOEC reproduction = 1001 mg a.e./kg soil d.w.
LOEC reproduction = 1802 mg a.e./kg soil d.w.
The EC_{10} / EC_{20} of MON 52276 for Hypoaspis reproduction was not determined due to lack of effects above 14%.

B.9.7.2.2. Higher tier testing

Pending the outcome of the risk assessment (studies with on *Folsomia cand*ida and *Hypoaspis aculeifer* were ongoing).

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

Relevant and reliable studies for the risk assessment of glyphosate and relevant metabolites are summarised in the tables below. Details of the studies are summarised in Volume 3 CA B.9.4.

Table B.9.8-1: Endpoints and effect values for glyphosate relevant for the risk assessment for soil organisms

Reference	Test item	Species	Test design/ GLP	Status	NOEC (mg a.e./kg dry soil)
[redacted] 2009 CA 8.4.1/001	Glyphosate IPA-salt (in MON 0139)	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	valid	473
[redacted] 2000 CA 8.4.1/002	Glyphosate IPA salt (in MON 0139)	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	Supportive*	21.31
[redacted] 2009 CA 8.4.2.1/002	Glyphosate IPA-salt (in MON 0139)	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% peat content	valid	473
[redacted] 2010 CA 8.4.2.1/001	Glyphosate IPA-salt (in MON 0139)	*Folsomia candida*	Mixed into substrate 28 d, chronic 10% peat content	valid	587

a.e. glyphosate acid equivalents
*not in line with latest guideline (assimilation to limit-test possible but will have required higher number of replicates)

Table B.9.8-2: Endpoints and effect values for AMPA relevant for the risk assessment for soil organisms

Reference	Test item	Species	Test design/ GLP	Status	NOEC (mg/kg dry soil)
[redacted] 2000 CA 8.4.1/002	AMPA	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	Supportive*	28.12
[redacted] 2003 CA 8.4.1/003	AMPA	*Eisenia fetida fetida*	Mixed into substrate 56 d, chronic 10% peat content	valid	131.9
[redacted] 2002 CA 8.4.1/004	AMPA	*Eisenia fetida fetida*	Mixed into substrate 56 d, chronic 10% peat content	Supportive**	19.7
[redacted] 2010 CA 8.4.2.1/004	AMPA	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% peat content	valid	320
[redacted] 2010 CA 8.4.2.1/003	AMPA	*Folsomia candida*	Mixed into substrate	valid	315

Reference	Test item	Species	Test design/ GLP	Status	NOEC (mg/kg dry soil)
			28 d, chronic 5% peat content		

*not in line with latest guideline (assimilation to limit-test as 2 concentrations instead of 5 will require 8 replicates instead of 4)

**design not in line with latest guideline. One validaity criteria not met (CV<30%, actual 38%) (assimilation to limit-test possible but will have required higher number of replicates)

A study with the representative product MON 52276 is available and has also been assessed for validity to current and relevant guidelines and is summarised in the following table.

Table B.9.8-3: Endpoints: studies on toxicity of MON 52276 to soil organisms

Reference	Test item	Species	Test design/ GLP	Status	NOEC (mg a.e./kg dry soil)
[redacted] 2020 CP 10.4.1.1/001	MON 52276	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	Valid	38
[redacted] 2020 CP 10.4.2.1/001	MON 52276	*Folsomia candida*	Mixed into substrate 28 d, chronic 5% peat content	Valid	1802
[redacted] 2020 CP 10.4.2.1/002	MON 52276	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% peat content	Valid	1802

a.e. glyphosate acid equivalents

Considering that the NOEC values of earthworms were both 'greater than' endpoint (> 38 mg a.e./kg dw soil for the product and >473 mg a.e./kg dw soil for the active substance), the applicant proposed to perform the risk assessment on the higher endpoint for the active substance glyphosate of 473 mg a.e./kg dw soil, as there is no significant difference in the toxicity exhibited by the product compared to the active substance to earthworms. RMS disagrees with this proposal. Indeed, even if the percent of effects on reproduction is low at the highest tested dose for both the active substance and the product (6% reduction of reproduction at 473 mg a.e./kg soil for glyphosate and less than 1% at 38 mg a.e./kg soil for the product), it could not be affirmed that the NOEC of the product will be equivalent to the one of the active substance (i.e. 12 times higher than the highest tested dose of the test performed with the product).

Literature data on soil organisms

The scientific literature review conducted for the last Annex I renewal (submitted in 2012) that appears in the RAR (2015) contains an extensive review of ecotoxicological papers considered relevant but supplementary to the Annex I renewal.

Studies related to indirect effects are not considered here. The previous conclusion of RMS 2015 on indirect effects are reported in the assessment of risk biodiversity via indirect effects and trophic interactions. For soil macro organisms, please refer to Volume 3 CP B.9 under B.9.14.1.5.

RMS (UBA in 2015) noted that for acute effects on soil organisms, behaviour is not included as a sensitive endpoint. However, these responses might also have negative consequences, e.g. –when worms move to the surface of contaminated soil- exposure to predators or to detrimental light. It could be shown that the activity of worms was influenced by the exposure to environmentally relevant concentration of commercial formulation of glyphosate (Verrel and Buskirk, 2004). The worms emerged onto the surface within 2 h after exposure. Nevertheless, after 48 h animals were found to be buried in the soil again. Authors concluded that acute exposure to the glyphosate containing plant protection product may compromise the survival of earthworms even though its direct toxicity appears low (Verrel & Buskirk, 2004). Nevertheless it seems important to assess not only the active ingredients, but also of the different formulations (Piola et al. 2013). The study of Piola et al (2013) includes earthworm toxicity data. According to the summary available in the RAR 2015, results of this study highlight the importance of ecotoxicological assessment not only of the active ingredients, but also of the different formulations. Median lethal concentration (LC50) showed that glyphosate-A was 4.5-fold more toxic than glyphosate-B. Sublethal concentrations caused a concentration-dependent weight loss, consistent with the reported effect of glyphosate as uncoupler of oxidative phosphorylation. Glyphosate- A showed deleterious effects on DNA and lysosomal damage at concentrations close to the applied environmental concentrations (14.4 lg ae cm ^{2}). With glyphosate-B toxic effects were observed at higher doses, close to its LC50, suggesting that the higher toxicity of formulate A could be attributed to the effects of some of the so-called ‘‘inert ingredients’’, either due to a direct intrinsic toxicity, or to an enhancement in the bioavailability and/or bioaccumulation of the active ingredient. For aquatic organsims it was also demonstrated that commercial formulations can be more toxic than the active substance itself because of the adjuvants present in the formulations.
A data gap is set for the applicant to provide the full text of Piola et al 2013 together with a summary and assessment in light with both direct and indirect effects risk assessment related to glyphosate based products.

It was also reported by RMS 2015 (UBA) that effects on reproduction were examined by Casabé et al. (2007), Kaneda et al. (2009) and Yasmin et al. (2006) using commercial formulations with the recommended application rates. It was concluded that the observed responses will not impact the population of earthworm in nature. Santos et al. (2012) could also confirm that glyphosate application to agricultural fields did not seem to affect either earthworms or collembolans in the recommended field dose. Consistently Zhou (2012 and 2013) confirmed that glyphosate has very low toxicity to the earthworms. However, it could not be excluded that with repeated appplications of glyphosate containing plant protection products during the season or year by year will have negative effects on the biotic soil community. It was considered that herbicide application did not directly affect the mortality or reproduction but instead the biological activity of the animals.
In a reproduction test with *Eisenia fetida*, which was conducted with the active substance glyphosate itself (Correia et al., 2012), earthworms were kept in treated soil and were classified as alive after the evaluation period, but showed significant reduction in mean weight at all test concentrations. Moreover morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg). Further behavioural abnormalities were described in terms of reduced casting production (Kaneda et al., 2009), reduced cocoon viability, a reduction in the feeding activity (Casabé et al., 2007) or reduced body weight

(Yasmin et al., 2006). However, the test rates were similar or above the one tested in the offically submitted studies, so that the outcome of the risk assessment for earthworm did not change.

Concerning the current literature review, there were no literature articles that were considered relevant and reliable by the applicant. There were 9 peer reviewed papers considered relevant but supplementary by the applicant for the risk assessment for soil meso-organisms (Correia et al., 2010[25], Dominguez et al., 2016[26], Gaupp-Berghausen et al., 2015[27], Jarmul-Pietraszczyk et al., 2012[28], Nathan et al., 2019[29], Pochron et al., 2019[30], Santos et al., 2012[31], Sihtmaee et al., 2013[32] and Stellin et al., 2017[33]). The RMS agrees with applicant justification (see Table Table B.9.11.1.4-2 in Volume 3 CA B.9) except for Correia et al., 2010 and Santos et al., 2012 (see appendix to Volume 3 CA B.9 related to literature data on ecotoxicology). Moreover RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

Correia et al (2010) was reassessed and showed (despite absence of mortality) significant reduction in mean weight (50%) at all test concentrations (i.e. including lowest concentration of 10 mg/kg). All the tested concentrations however exceed the estimated PEC soil. Morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens.

Santos et al., 2012 investigated the impact of glyphosate on the avoidance behaviour and reproduction of the earthworm *Eisenia andrei* and the collembolan *Folsomia candida*. The study authors determined an LC50 for *Folsomia* of 1.13 mg/kg soil and an EC50 of 0.54 mg/kg soil. The reliability of these LC50 and EC50 values cannot be assessed (no data presented). The effects on *F. candida* are notably different than those obtained in regulatory study. However these results are of limited value for other formulations than Montana, as toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product. No adverse effects on earthworms were noted.

Overall there is no studies that may impact the outcome the risk assessment of direct effects. This may be reconsider as RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and data gap in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents.

[25] Correia F. V. et al., 2010. Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests. Bulletin of environmental contamination and toxicology (2010), Vol. 85, No. 3, pp. 264

[26] Dominguez A. et al., 2016. Toxicity of AMPA to the earthworm Eisenia andrei Bouche, 1972 in tropical artificial soil. Scientific reports (2016), Vol. 6, pp. 19731

[27] Gaupp- Berghausen M. et al., 2015. Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. Scientific reports (2015), Vol. 5, pp. 12886

[28] Jarmul- Pietraszczyk J. et al., 2012. Herbicide toxicity to the California earthworms Eisenia fetida Sav. and Dendrobaena veneta Rosa. Ecological Chemistry and Engineering A (2012), Vol. 19, No. 9, pp. 1133

[29] Nathan V. K. et al, 2020. Pesticide application inhibit the microbial carbonic anhydrase-mediated carbon sequestration in a soil microcosm. Environmental science and pollution research international (2020), Vol. 27, pp. 4468

[30] Pochron S. et al., 2019. Temperature and body mass drive earthworm (Eisenia fetida) sensitivity to a popular glyphosate-based herbicide. Applied soil ecology (2019), Vol. 139, pp. 32-39

[31] Santos M. J. G. et al., 2012. Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of Folsomia candida and Eisenia andrei. Ecotoxicology (2012), Vol. 21, No. 8, pp. 2113

[32] Sihtmaee M. et al. 2013.Ecotoxicological effects of different glyphosate formulations Applied soil ecology (2013), Vol. 72, pp. 215

[33] Stellin F. et al., 2017. Effects of different concentrations of glyphosate (Roundup 360A®) on earthworms (Octodrilus complanatus, Lumbricus terrestris and Aporrectodea caliginosa) in vineyards in the North-East of Italy. Applied soil ecology (2018), Vol. 123, pp 802

B.9.8.1. Risk assessment for earthworms

Chronic earthworm toxicity studies have been conducted with glyphosate, the main metabolite AMPA and the product MON 52276 (Tables B.9.8-1, 9.8-2 and B.9.8-3) and are considered in the risk assessment.
The risk assessment is performed in accordance with the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev.2 (final), October 17, 2002).

RMS considered a risk envelop approach[34] by presenting a risk assessment for the uses leading to the worst case PECsoil and thus covering all intended uses. A detailed description of PEC_{soil} calculations for glyphosate and its metabolite AMPA is provided in the Vol.3 CP B.8.2.

The studies conducted with glyphosate, AMPA and MON 52276 were conducted in soils with 5% or 10% organic matter. As the log P_{ow} values for glyphosate and AMPA are less than 2, it is not necessary to correct the endpoints by a factor of 2 in order to account for the organic matter content of the artificial test soil.

The resulting TER values are shown in the tables below.

Table B.9.8-4: First-tier assessment of the chronic risk for earthworms due to the use of MON 52276 (covering all representative uses)

Chronic effects on earthworms			
Intended use	All uses		
Product/active substance	**NOEC (mg/kg dw)**	**$PEC_{soil,\ accu}$ (mg/kg)**	**TER_{lt}***
Glyphosate	473	5.123	92.3
AMPA	131.9	6.845	19.3
MON 52276	38	5.123	7.4

* TER: toxicity to exposure ratio = Endpoint / PEC_{soil} given in mg glyphosate acid equivalents/kg dw.

The TER values calculated using worst-case accumulation $PEC_{soil\ accu}$ values for glyphosate and its metabolite AMPA exceed the relevant trigger values of 5, indicating that the risk to earthworms is acceptable following the proposed uses of MON 52276.

A summary of the risk assessment regarding soil macroorganisms biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

B.9.8.2. Risk assessment for soil meso- and macrofauna (other than earthworms)

Chronic toxicity studies have been conducted with MON 52276, glyphosate and the main metabolite AMPA, to assess the toxicity to *Hypoaspis aculeifer* and *Folsomia candida.* The relevant and reliable endpoints for use in risk assessment are summarised in Tables B.9.8-1 and B.9.8-2.

[34] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach" SANCO/11244/2011 rev. 5, 14 March 2011

The risk assessment is based on the approach as described for earthworms above in Section B.9.8.1, using the $PEC_{soil,accu}$ values for glyphosate and its main metabolite AMPA in a risk envelop approach[35], as provided in the Vol.3 CP B.8.2. The resulting TER values are presented below for the proposed uses of MON 52276.

Table B.9.8-5: First-tier assessment of the chronic risk to *Hypoaspis aculeifer* from glyphosate and AMPA (covering all representative uses)

Chronic effects on *Hypoaspis aculeifer*			
Intended use	All uses		
Product/active substance	**NOEC (mg/kg dw)**	**$PEC_{soil,\ accu}$ (mg/kg)**	**TER_{lt}***
Glyphosate	473	5.123	92.3
AMPA	320	6.845	46.7
MON52276	1802	5.123	351.7

* TER: toxicity to exposure ratio = Endpoint / PEC_{soil} given in mg glyphosate acid equivalents/kg dw.

Table B.9.8-6: First-tier assessment of the chronic risk to *Folsomia candida* from glyphosate and AMPA (covering all representative uses)

Chronic effects on *Folsomia candida*			
Intended use	All uses		
Product/active substance	**NOEC (mg/kg dw)**	**$PEC_{soil.\ accu}$ (mg/kg)**	**TER_{lt}***
Glyphosate	587	5.123	114.6
AMPA	315	6.845	46.0
MON52276	1802	5.123	351.7

* TER: toxicity to exposure ratio = Endpoint / PEC_{soil} given in mg glyphosate acid equivalents/kg dw.

The TER values calculated using worst-case $PEC_{soil,accu}$ values for glyphosate and its metabolite AMPA, and the representative formulation MON52276 exceed the relevant trigger value of 5, indicating that the risk to other non-target soil organisms is acceptable following the proposed uses of MON 52276.

A summary of the risk assessment regarding soil macroorganisms biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

[35] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach" SANCO/11244/2011 rev. 5, 14 March 2011

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Data point:	CP 10.5/001
Report author	[redacted]
Report year	2012
Report title	MON 52276: Effect on Soil Microbial Activity, Carbon and Nitrogen Transformations
Report No	CEMR-5259
Document No	CE-2011-0537
Guidelines followed in study	OECD Guidelines 217 (2000) and 216 (2000)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current guidelines OECD 216 (2000) and OECD 217 (2000):* *Major:* *- none* *Minor:* *- The changes in nitrate production was determined between each time point and not on the whole test from 0-28 days.* *- The temperature dropped under 18°C for 4 hours.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
The effects of MON 52276 on the carbon and nitrogen transformation pathways were assessed in a LUFA standard soil type 2.3. The transformation rates were determined in replicate soil samples treated with MON 52276 at rates of 18.8 and 94 mg MON 52276/kg dry soil (equivalent to 1 and 5 × the initial Predicted Environmental Concentration for a rate of 12 L MON 52276/ha) and compared to a control treatment of deionised water. The concentrations of 18.8 and 94 mg MON 52276/kg dry soil are equivalent to 5.768 and 28.84 mg glyphosate acid equivalent/kg dry soil. Substrate-induced (glucose) respiration measurements were made on Day 0, 7, 14 and 28 by measuring the carbon dioxide evolution over a 12-hour period. The products of the process of nitrification were extracted from the soil on Day 0, 7, 14 and 28 after treatment.
As the difference in respiration rates between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil, equivalent to initial predicted environmental concentrations of 12 L/ha and 60 L/ha, respectively) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on carbon transformation in soils. As the average rate of production of nitrate (mg/kg/day) from Day 14 to Day 28 between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long term influence on nitrogen transformation in soils.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 52276
Formulation type:	Soluble concentrate (SL)
Description:	Not reported
Lot/Batch #:	A9K0106104
Purity:	30.68% or 358.8 g/L a.e. glyphosate
positive control:	Deionised water control

Test system:

Soil	Sandy loam soil "LUFA standard soil 2.3" (Batch number F2.34011)
Source:	LUFA-Speyer, Obere Langgasse 40, 67346 Speyer, Germany
Water holding capacity	35.6% (g water/100 g dry soil)
pH:	7.5
Org. Carbon:	0.94%
Microbial biomass:	1.91% to C_{org}.
Clay (< 0.002 mm):	8.7%
Silt 0.002 - 0.050 mm):	27.6%
Sand (0.050 – 2.0 mm):	63.7%
Acclimation:	35% (± 5 %) of MWHC at 20 ± 2 °C for 5 days

Environmental conditions:

Temperature:	20 ± 2 °C (except during 4 hours dropping to 17.93 °C)
pH:	7.5 - 7.9
Water content:	40% (± 5 %) of MWHC (actual achieved values: 38.9%)
Photoperiod:	24 hours darkness
Experimental Dates:	November 11 – December 15, 2011

B. STUDY DESIGN AND METHODS

Experimental treatments

Soil samples were bulk dosed with MON 52276 at nominal rates equivalent to 1 and 5 × $PEC_{plateau}$ (18.8 and 94 mg MON 52276/kg dry soil, respectively). The concentrations of 18.8 and 94 mg MON 52276/kg dry soil are equivalent to 5.768 and 28.84 mg glyphosate acid equivalent/kg dry soil.

Five days before the start of the exposure phase, the soil moisture content was nominally adjusted to 35% (± 5%) of the MWHC. The soil was placed in the test cabinet in the dark at 20 ± 2 ºC. On the day of dosing, the moisture of the soil was adjusted to 40% (± 5%) of the MWHC with deionised water with the appropriate dose of test item. Three replicates were prepared for the control treatment (deionised water) and the test item treatments. For the nitrogen test each replicate contained 500 g (dry weight equivalent) of soil. For the carbon test each replicate contained 1000 g (dry weight equivalent) of soil. Each replicate of soil was transferred to plastic test vessels (2 L). The test soil used in the carbon transformation test was amended with glucose at each sampling time point, to elicit a maximum respiratory response (8.0 mg glucose/g dry weight of soil). The test soil used in the nitrate transformation test was amended with lucerne (2.5 g of lucerne/500 g of soil) to the control and treatment groups on

Day 0. The moisture content of soil samples was maintained during the test at 40 % of the maximum water holding capacity of the soil with a range of ± 5%.

Observations
Soil microbial carbon respiration was measured for the individual respirometers from the Day 0 to Day 28. The mean concentrations of CO_2 (mg CO_2/kg/hour) were monitored over the 12-hour period and the mean respiration rates for the 12-hour period for each treatment at each time point were defined.
Concentrations of nitrate (as TON) and ammonium were measured (mg/kg dry soil) from Day 0 to Day 28. The nitrite values determined were not reported as the detected nitrite-N levels were all below 0.5 mg/L, and therefore considered not to have nitrite present in any of the extracted soil solutions. Changes in concentration of nitrate and nitrate transformation rates (mg/kg/day) over the duration of the study were measured. The changes in nitrate production from 0-7, 7-14 and 14-28 days were also determined.

Statistical calculations
Results were evaluated using Dunnett's two-tail test, $p \leq 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.9-1: Effects of MON 52276 on soil nitrogen transformation

		Nitrogen concentration [mg/kg soil]		% deviation from control	
Concentration in MON 52276	**Control**	**18.8 mg/kg dws**	**94 mg/kg dws**	**18.8 mg/kg dws**	**94 mg/kg dws**
Concentrations in glyphosate a.e.	Control	5.768 mg/kg dws	28.84 mg/kg dws	5.768 mg/kg dws	28.84 mg/kg dws
Nitrate (NO_3^-)					
Day 0	22.4	24.4	25.1	+8.93	+12.05
Day 7	0	0	0	-	-
From Day 0-7	-3.20	-3.48	-3.59	+8.84	+12.24
Day 14	25.8	32.8	42.3	+27.13	+63.95
From Day 7-14	3.69	4.69	6.04	+27.14	+63.72
Day 28	75.3	84.5	95.7	+12.22	+27.09
From Day 14-28	3.54	3.69	3.81*	+4.31	+7.85
Ammonium (NH_4^+)					
Day 0	10.3	10.7	11.2	+3.88	+8.74
Day 7	3.0	2.9	2.8	-3.33	-6.67
Day 14	1.6	1.6	1.6	0	0
Day 28	1.1	1.1	1.0	0	-9.09

dws: dry weight soil
* = Significantly different from control ($\alpha = 0.05$)
- = inhibition, + = stimulation

Table B.9.9-2: Effects of MON 52276 on soil microflora respiration (carbon cycle)

	CO_2 [mg CO_2/kg soil/h]			% deviation from control	
Concentration in MON 52276	**Control**	**18.8 mg/kg dws**	**94 mg/kg dws**	**18.8 mg/kg dws**	**94 mg/kg dws**
Concentrations in glyphosate a.e.	Control	5.768 mg/kg dws	28.84 mg/kg dws	5.768 mg/kg dws	28.84 mg/kg dws
Day 0	16.08	16.16	17.24	+0.47	+7.19
Day 7	15.42	16.64	18.73	+7.97	+21.52
Day 14	15.42	16.93	18.77	+9.78	+21.71
Day 28	16.49	17.15	18.90*	+3.96	+14.57

dws: dry weight soil
* = Significantly different from control ($\alpha = 0.05$)
- = inhibition, + = stimulation

B. OBSERVATIONS

Statistical analysis showed there was a significant difference ($p<0.05$) between the treatment rate of 94 mg MON 52276/kg dry soil and the control treatment for nitrate production from Day 14 to 28.

As the average rate of production of nitrate (mg/kg/day) from Day 14 to Day 28 between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil, equivalent to 5.768 and 28.84 mg glyphosate acid equivalent/kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long term influence on nitrogen transformation in soils.
Statistical analysis showed there was a significant difference ($p<0.05$) between the treatment rate of 94 mg MON 52276/kg dry soil and the control treatment for soil carbon transformations at Day 28.

As the difference in respiration rates between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on carbon transformation in soils.

Validity criteria

All validity criteria for the study were met for the study as the variation between replicate control treatments did not vary by more than ± 15 % at each sampling time point for nitrogen concentrations (actual values from -7.0 to 7.1%) and for carbon transformation (actual values from -5.7 to 6.2%).

III. CONCLUSIONS

Assessment and conclusion by applicant:

At soil concentrations of 18.8 and 94 mg MON 52276/kg dry soil (equivalent to 5.768 and 28.84 mg glyphosate acid equivalent/kg dry soil), there were < 25% effect at Day 28 in nitrogen and carbon transformation, so MON 52276 is expected to have no long-term influence on the nitrogen and carbon transformation pathways in soils up to and including a test concentration 94 mg MON 52276 /kg dry soil.

The study is considered valid and is suitable for risk assessment purposes.

Assessment and conclusion by RMS:

Deviations from OECD 216 and 217 was noted:
- increase in temperature for 4 hours during study (minimum 17.93 °C).

This deviation is considered minor.

The conditions of the test and the soil used were adequate.

It seems that no nitrate was measured at day 7 in none of the treatments including control. The study do not report any malfunction nor comments the absence of nitrate at this time point. Since this happened at an intermediate point, RMS is not concerned since further time points showed normal behaviour. The applicant is requested to provide clarification on this point (data gap).

The study is valid and reliable for risk assessment.

At soil concentrations of 18.8 and 94 mg MON 57726/kg dry soil (5.76 and 28.8 mg a.s./kg dry soil), equivalent to 12 and 60 L MON 52276/ha, respectively, there were <25% effect at Day 28 in nitrogen transformation.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

Relevant and reliable studies for the risk assessment of soil microflora from the active substance glyphosate and relevant metabolites are summarised in the tables below, presenting the most sensitive endpoints. Details of the studies are summarised in Vol.3 CA B.9.5.

Table B.9.10-1: Endpoints and effect values for glyphosate and AMPA for soil microflora

Reference	Test item	Species	Test design	Status	Effect
[redacted], 2014 CA 8.5/001	Glyphosate acid	N-mineralisation	28 d, aerobic	Valid Data gap *	**< 25% effect at Day 28 at 33.1 mg/kg dry soil**
[redacted] 2000 CA 8.5/002	Glyphosate technical	N-mineralisation	28 d, aerobic	invalid	-
[redacted] 1995 CA 8.5/003	Glyphosate	N-mineralisation	-	Not assessed	-
[redacted] 2010 CA 8.5/004	AMPA	N-mineralisation	28 d, aerobic	Supportive Data gap **	**< 25% effect at Day 28 at 160 mg/kg dry soil (**

a.e. glyphosate acid equivalents

Endpoint in **bold** is used for risk assessment.

* Data gap: No nitrate was measured at day 7 in none of the treatments including control. The study do not report any malfunction nor comments the absence of nitrate at this time point. The applicant is requested to provide clarification on this point (see study summary)

** Data gap: applicant to submit soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day (see study summary)

Studies on effects of the representative formulation MON 52276 on soil microflora to fulfil the data requirements according to EU Regulation No 284/2013 are presented in the following.

Endpoints of studies considered valid with the representative product MON 52276 are shown in the table below. In order to make a direct comparison of toxicity between studies conducted with MON 52276 and those conducted with IPA salt, glyphosate technical and glyphosate acid, the endpoints from all these studies have been converted to acid equivalents (a.e.). This conversion has been made by the acid equivalent purity of the test item stated in the reports.

Table B.9.10-2: Endpoints: studies on toxicity of MON 52276 to soil microflora

Reference	Test item	Test design	Status	NOEC (mg a.e./kg dry soil)
[redacted] 2012 CP 10.5/001	MON 52276	N- mineralisation, 28 d	Valid *	< 25% effect at Day 28 at 28.8 mg a.e./kg dry soil

a.e. glyphosate acid equivalents

*datagap: calculation of soil nitrogen daily transformation rates (please refer to study summary)

The study with MON 52276 shows effects less than 25% compared to control up to 21.63 mg a.e./kg dry soil. The endpoint with the active substance glyphosate also indicated less than 25% deviation from the control up to 33.1 mg a.e./kg dry soil. The percents of deviation from control at 28 days were similar. Therefore, the risk assessment could be based on the higher endpoint : 33.1 mg a.e./kg dry soil.

Literature data on soil microflora

Studies related to indirect effects are considered in the assessment of risk biodiversity via indirect effects and trophic interactions. The conclusions of RAR 2015 and the current dossier on those issues for soil micro organisms are reported under Volume 3 CP B.9 under B.9.14.1.6. Here below are reported the studies that provide data on parameters of direct relevance for the risk assessment (i.e. soil functions).

The scientific literature review conducted for the last Annex I renewal (submitted in 2012) that appears in the RAR (2015) contained only one publication from Cycon & Kaczynska (2004) that has been classified as "UBA1" (critical data, high weight of evidence in risk assessment). In this study, performed according to the OECD guidelines 216 and 217, the authors applied glyphosate at the field rate of 4.5 mg/ kg of soil (PEC) as well as at a 5-fold higher concentration (22.5 mg/ kg of soil). After 1, 7, 14 and 28 days of incubation, soil respiration rates (SIR – Substrate Induced Respiration) and the amounts of nitrate did not significantly differ from control soil.

It was stated in RAR 2015, that in the case of glyphosate, only few studies failed to detect significant effect on soil functional diversity after application of the herbicide (e.g. Liphadzi, et al. 2005). Zabaloy, et al. (2008) reported that "the addition of glyphosate at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness". In rare cases, inhibitory effects have also been reported. In a land set-aside in the western part of Prague (Czech Republic), Ruzkova et al. (2011) found that repeated application of Roundup desiccation caused a significant increase of microbial biomass (+69 %), but also strongly decreased the immobilization of nitrates by the plants (nitrate-nitrogen ratio +86%) as well as the arylsulfatase activity (–28 %).
In some studies, differences in microbial parameters were more a function of time and site quality than pesticides doses. For example, Gomez et al. (2009) detected significant differences in microbial

respiration over the time but not between doses of applied glyphosate. In Hart et al. (2009), seasonality was a significant determinant of denitrifier and fungal abundance. Parallelly, Busse et al. (2001) found that variation in microbial community size, activity and metabolic diversity depended more of time of year and land-use that herbicide treatment.
Nevertheless, as stated in RAR 2015, glyphosate is an organophosphonate herbicide that can be easily used as a source of P, C or N by either by gram-positive or gram-negative bacteria (van Eerd et al., 2003). Therefore, in most studies, the application of glyphosate at expected or higher field concentration rates was correlated with an immediate and significant increase in soil respiration (Accinelli et al., 2002), microbial biomass (Lupwayi et al., 2004), C- and N-mineralizations (Lancaster et al., 2006; Haney et al., 2002a, 2002b). This stimulation of soil principal functional parameters is assumed to be linked to a rapid use of glyphosate as source of nutrients (Mijangos et al., 2009) usually correlated with a metabolisation of the pesticide. Araujo et al. (2003) demonstrated in two Brazilian soils a rapid biodegradation of glyphosate by soil microorganisms with the formation the metabolite AMPA, resulting in short- and long-term positive effect of the herbicide on the soil microbial activity (increase of 10–15 % in the CO^2 evolved and a 9–19 % increase in FDA hydrolyses in the presence of glyphosate). This potential use of glyphosate as a source of P, C or N by soil non-target micro-organisms is likely to induce a shift in their community structures.

The papers submitted for the previous Annex I submission were not reassessed by RMS. None of them impacted the outcome of the risk assessment (RAR 2015).

Concerning the literature review for the current dossier:
RMS retained the following studies after detailed assessment: Rose M. T. et al., 2018[36] (see summary in appendix to Volume 3 (AS) on general literature on ecotoxicology). In this study, the authors investigated the effect of glyphosate (acid), on microbial activities and N-transformation in 5 different Australian broadacre (cereal) cropping soils. Glyphosate was applied at a recommended and 5 times recommended rate. Mineral N-levels were monitored over a 28 day period. Experiments were established as per OECD Guideline 216 with minor modifications. The soils used were not always as recommended in the OECD Guideline 216 (sand content, pH, total carbon content). RMS cannot ensure that adsorption of the test chemical was sufficiently minimized and its availability to the microflora sufficiently high. No significant effects on NO3− formation and NH4+ levels were determined for the applications rates of glyphosate at 1 kg/ha (corresponding to 1.33 mg/kg) and 5 kg/ha (corresponding to 6.67 mg/kg) compared to the control.

Overall there is no studies that may impact the outcome the risk assessment of direct effects. This may be reconsider as RMS identified some studies in Volume 3 CA B.9 under the table “List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant” and data gap in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents.

Risk assessment for Soil Nitrogen Transformation

The risk assessment is performed in accordance with the “Guidance Document on Terrestrial Ecotoxicology”.

RMS considered a risk envelop approach[37] by presenting a risk assessment for the uses leading to the worst case PECsoil and thus covering all intended uses. A detailed description of PEC_{soil} calculations for glyphosate and its metabolite AMPA is provided in Vol.3 CP B.8.2.

[36] Rose M. T. et al., 2018. Minor effects of herbicides on microbial activity in agricultural soils are detected by N-transformation but not enzyme activity assays. European journal of soil biology (2018), Vol. 87, pp. 72
[37] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the “risk envelope approach” SANCO/11244/2011 rev. 5, 14 March 2011

The resulting assessment of the risk for nitrogen transformation is shown in the tables below.

Table B.9.10-3: Assessment of the risk for effects on nitrogen transformation due to the use of MON 52276 (covering all representative uses)

Nitrogen transformation			
Intended use	All uses		
Product/active substance	**Max. conc. with effects ≤ 25% (mg/kg)**	**$PEC_{soil, accu}$ (mg/kg)**	**Risk acceptable?**
Glyphosate	≥ 33.1	5.123	yes
AMPA	≥ 160	6.845	yes

No effects on nitrogen transformation were observed from the maximum expected concentrations of glyphosate and AMPA to the soil. It can be concluded that proposed uses of MON 52276 will pose an acceptable risk to soil microflora.

A summary of the risk assessment regarding soil microorganisms biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1. Summary of screening data

No data. Not required as seedling emergence and vegetative vigour tests are available with MON 52276.

B.9.11.2. Testing on non-target plants

Summaries of studies conducted with MON 52276 are reported thereafter.

Data point	CP 10.6.2/001
Report author	[redacted]
Report year	2019
Report title	MON52276: Effects on the Seedling Emergence and Growth of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions
Report No	S19-03634
Document No	EUR-2019-0233
Guidelines followed in study	OECD Guideline 208 (2006)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current test guideline OECD 208 (2006):* *Major:* *- none* *Minor:* *- No reference substance or historical data were mentioned in the report.*
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

A seedling emergence study was conducted exposing six dicotyledonous (cucumber, oilseed rape, radish, soybean, sunflower and tomato) and four monocotyledonous (corn, oat, wheat and onion) plant species to five nominal test concentrations of 0.12, 0.37, 1.11, 3.33 and 10.00 L MON52276/ha (equivalent to 0.045, 0.134, 0.401, 1.203, and 3.610 kg glyphosate acid/ha). In addition, one negative control group (tap water) was tested. For each of the ten species, there were twenty seeds tested per treatment group.

Plants were assessed for seedling emergence, plant survival, growth stage, and phytotoxicity symptoms on days 7, 14 and 21 after 50 % of the seeds in the control had emerged in each species. The effects on plant shoot height and shoot dry weight were determined on day 21.

Compared to the control group, exposure of 10 plant species to MON52276, resulted in no statistically significant differences in seedling emergence, mortality (survival), shoot heights and shoot dry weight, in any of the plant species tested. Therefore, the NOER is considered to be = 10.00 L MON52276/ha, with the corresponding LOER, ER_{25} and ER_{50} for all parameters considered to be >10.00 L MON52276/ha.

The validity of the present study according to OECD guideline 208 was achieved.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item::	MON52276 (formulated product)
Description:	Yellowish to brown liquid
Lot/Batch #:	AZE200810A
Purity:	Glyphosate acid (361 g/L); glyphosate IPA salt (487 g/L)

Test organism:

Species:	6 Dicotyledons: *Cucumis sativus* (cucumber), *Brassica napus* (oilseed rape), *Raphanus sativus* (radish), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Lycopersicon esculentum* (tomato) 4 Monocotyledons: *Zea mays* (maize), *Triticum aestivum* (wheat), *Avena sativa* (oat), *Allium cepa* (onion)
Source:	Battle: cucumber, maize, wheat and onion KWS: oilseed rape Hild: radish Baywa: soybean Bringenheimer: sunflower Monsanto: tomato Intersemillas: oat
Environmental conditions:	
Temperature:	17.5 – 36.2°C
Relative humidity:	41 - 82 %
Photoperiod:	16 hours light/8 hours dark
Light intensity:	596 µEs/m^2
Soil textural class:	Sandy Loam (field collected) 67.28 % sand, 14.0 % silt, 18.72 % clay
Soil pH:	8.48
Soil organic content:	0.80 %
Soil conductivity:	0.351 mS/cm
Experimental work dates:	17 May - 22 August 2019

B. STUDY DESIGN

Experimental treatments

Twenty seeds per treatment group and per species were sown into plastic pots (diameter of 15 cm and capacity 1.5 L). Seeds of six dicotyledonous and four monocotyledonous species were sown into sandy-loam soil, with a pH of 8.48 and an organic carbon content of 0.80 %. For cucumber, oilseed rape, radish, soybean, sunflower, tomato and maize, ten replicates (including 2 seeds each) were set up. For wheat, oat and onion, five replicates (including 4 seeds each) were set up. MON52276 was applied on the soil surface with a track-sprayer (Company Schachtner, Ludwigsburg, Germany) at the rates of control (0), 0.12, 0.37, 1.11, 3.33, and 10.00 L test item/ha (equivalent to 0.045, 0.134, 0.401, 1.203, and 3.610 kg glyphosate acid/ha). The track-sprayer was calibrated before the application to provide an output of 200 L with a tolerance of 10 % per ha.

Observations

Following the application, seedling emergence assessment was carried out daily (until no more emergence) and mortality, phytotoxicity and growth stage were assessed at 7, 14 and 21 days after 50 % of the seedlings in the control had emerged. At test termination, assessment of shoot height and dry weight were carried out. Results were compared to the tap water treated control. Analysis of the fortified and test item rate solution (10.00 L test item/ha) were analysed by HPLC. Phytotoxicity assessments were made with a gradual rating (ranging from 0 to 100%) to describe necrosis, chlorosis and other characteristics that could be treatment related. Shoot heights of above-ground vegetation was measured for each surviving plant from the soil surface to the apical tip (oilseed rape, radish, maize, wheat, oat and onion), or highest aerial part (cucumber, soybean, sunflower and tomato). Surviving plants were clipped at soil level on the last assessment day and dried at 60°C for at least 48 hours. The shoot dry weight was determined per replicate.

Test solutions were analysed for the concentrations of glyphosate, the active ingredient in MON 52276

using a liquid chromatography tandem mass spectrometry (LC-MS/MS) system. The samples were collected from each test solution and control at application to the test systems for the definitive test.

Statistical calculations

Statistical analysis of data was performed using the ToxRat Solutions program (ToxRat® Professional Version 3.2.1). For determination of significant difference to the control, the significance level was set to $\alpha = 0.05$ for all tests. For seedling emergence and mortality data, when the monotonic rate-response is not evident a Bonferroni-Fisher-Test was performed. Shoot height and shoot dry weight data was tested for normality of data with the Shapiro-Wilk's test and for homoscedasticity with the Levene's test before performing the appropriate statistical test. Comparison between each rate of the test item assayed, with at least three replicates with surviving individuals and the relative control, was performed for all the plant species. For shoot height and shoot dry weight data, when normal distribution and homogeneity of variance of the data was obtained, and a monotonic rate-response was evident, Williams test (α=0.05) was performed. With the same conditions, where a monotonic rate-response was not evident, a Dunnett's test (α=0.05) was performed. When normal distribution of the data was not obtained, Step-down Jonckheere-Tepstra (α=0.05) or Multiple Sequentially Rejective U test after Bonferroni Holm (α=0.05) was performed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The highest test item application solution served as a stock solution. For all lower application rates aliquots were taken and diluted in water. The stock solution was analysed and details are given below:

Table B.9.11-1: Analytical verification of the stock solution concentrations

	Nominal concentration [L test item/ha]	**Nominal concentration** [g glyphosate acid/ha]	**Nominal concentration** [g glyphosate acid/L]	**Determined concentration** [g glyphosate acid/L]	**% of the nominal**
Control	0	0	<LOD	<LOD	-
Test item solution	10	3610	18.05	15.3	85
Fortified solution	10	3610	18.0	13.4	74

LOD = 0.00300 g glyphosate/L = 30 % of the LOQ

LOQ = 0.0324 g test item/L (=0.0100 g glyphosate/L)

Table B.9.11-2: Effects of MON52276 after 21 days

Crops	MON52276 [L test item/ha]					
	Control	**0.12**	**0.37**	**1.11**	**3.33**	**10**
	Glyphosate acid [kg a.s./ha]					
	Control	**0.045**	**0.134**	**0.401**	**1.203**	**3.610**
Mean seedling emergence [%]						
Cucumber	95	95	95	100	95	95
Oilseed rape	85	95	90	95	95	100
Radish	85	80	75	75	75	90
Soybean	75	90	80	80	80	80
Sunflower	85	85	85	85	90	85
Tomato	100	100	95	100	95	100
Maize	100	100	95	95	100	100
Wheat	95	95	85	85	95	100
Oat	100	100	95	100	95	100
Onion	85	85	100	95	100	90
Mean mortality						
Cucumber	0	0	0	0	0	0
Oilseed rape	0	0	0	0	0	0
Radish	0	0	0	0	0	0
Soybean	0	0	0	0	0	0
Sunflower	0	0	0	0	0	0
Tomato	0	0	0	0	0	0
Maize	0	0	0	0	0	0
Wheat	0	0	0	0	0	0
Oat	0	0	0	0	0	0
Onion	0	0	0	0	0	0
Phytotoxicity						
Cucumber	0	0	0	0	0	0
Oilseed rape	0	0	0	0	0	0
Radish	0	0	0	0	0	0
Soybean	0	0	0	0	0	0
Sunflower	0	0	0	0	0	0
Tomato	0	0	0	0	0	0
Maize	0	0	0	0	0	0
Wheat	0	0	0	0	0	0
Oat	0	0	0	0	0	0
Onion	0	0	0	0	0	0

Table B.9.11-2: Effects of MON52276 after 21 days

Crops	MON52276 [L test item/ha]					
	Control	**0.12**	**0.37**	**1.11**	**3.33**	**10**
	Glyphosate acid [kg a.s./ha]					
	Control	**0.045**	**0.134**	**0.401**	**1.203**	**3.610**
Inhibition on shoot length [%][a]						
Cucumber	--	-9.04	-33.05	-35.31	-22.93	-26.96
Oilseed rape	--	9.77	-6.54	-0.56	-0.08	-2.50
Radish	--	-0.45	4.23	2.0	6.68	7.57
Soybean	--	-12.46	-1.15	-10.2	-13.46	-11.49
Sunflower	--	5.47	-1.56	0.7	-0.31	0.20
Tomato	--	-2.34	-10.23	-11.8	-6.68	14.14
Maize	--	3.45	1.67	0.11	-1.0	-1.02
Wheat	--	1.94	-5.69	1.75	0.79	4.4
Oat	--	9.38	7.21	4.38	0.02	-5.94
Onion	--	2.5	8.94	5.33	-11.01	12.55
Inhibition on dry weight [%][a]						
Cucumber	--	-0.78	-8.68	1.5	-12.91	-9.67
Oilseed rape	--	2.86	9.99	3.69	6.59	9.19
Radish	--	-2.3	-2.86	-9.33	4.13	10.75
Soybean	--	-17.05	-13.11	-34.62	-16.92	-5.30
Sunflower	--	-5.66	-13.15	-23.09	-28.28	-20.63
Tomato	--	13.54	12.52	13.43	-6.84	-1.51
Maize	--	3.14	-0.77	3.15	-2.63	3.91
Wheat	--	-1.91	-3.90	-7.32	9.14	10.38
Oat	--	13.26	1.11	15.08	8.12	-15.62
Onion	--	-13.54	-25.02	-21.70	-24.49	1.05

* = significantly different when compared to the control ($\alpha = 0.05$)
NA = not applicable
[a] compare to the control

Table B.9.11-3: 21-day NOER, LOER, ER_{25} and ER_{50} values for all parameter

Crop	Endpoints [L MON57226/ha]		
	Seedling emergence/Mortality/Phytoxicity/Length/Dry weight		
	NOER	LOER	EC_{25}/EC_{50}
Cucumber	≥10	>10	>10
Oilseed rape	≥10	>10	>10
Radish	≥10	>10	>10
Soybean	≥10	>10	>10
Sunflower	≥10	>10	>10
Tomato	≥10	>10	>10
Maize	≥10	>10	>10
Wheat	≥10	>10	>10
Oat	≥10	>10	>10
Onion	≥10	>10	>10

B. OBSERVATIONS

Analytical data: Correct rate preparation and application was confirmed both by analysis of the stock solution, with recoveries of 85 % of glyphosate and via calibration of the spray equipment.

Mortality results: None of the tested rates of the test item MON52276 significantly affected the survivorship of the tested species.

Seedling emergence results: None of the tested rates of the test item MON52276 significantly affected the emergence of the tested species.

Phytotoxicity results: None of the tested rates of the test item MON52276 showed phytotoxicity symptoms for any of the tested species.

Growth stage results: No differences in growth stage could be detected between the test item groups and the control for the ten tested species at any of the rates tested.

Dry weight results: No statistically significant reductions on shoot dry weight were observed for the tested treatment rates of the test item MON52276 for all tested species.

Shoot height results: No statistically significant reductions on shoot height were observed for the tested treatment rates of the test item MON52276 for all tested species.

The following point deviated from the current guideline recommendations:

- No reference substance or historical data were mentioned in the report.

Validity criteria according to OECD 208 were fulfilled for all species tested:

- Seedling emergence: The control seedling emergence was ≥70 % (actually: 75 % to 100 %).
- Phytotoxicity: The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibited only normal variation in growth and morphology for that particular species.
- Mean survival: The mean survival of emerged control seedlings was ≥90 % (actually: 95 % to 100 %).
- Cultivation Conditions: The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Compared to the control group, exposure of 10 plant species to MON52276, resulted in no statistically significant differences in seedling emergence, mortality (survival), shoot heights and shoot dry weight, in any of the plant species tested. Therefore, the NOER is considered to be ≥10.00 L MON52276/ha (equivalent to ≥3.610 kg glyphosate acid/ha), with the corresponding LOER, ER_{25} and ER_{50} for all parameters considered to be >10.00 L MON52276/ha (>3.610 kg glyphosate acid/ha).

Therefore, the study was classified as valid

Assessment and conclusion by RMS:

Test item: MON52276 (applied on the soil surface)
Natural soil was used.

The following deviation was noted by the applicant:
- *No reference substance or historical data were mentioned in the report.*
RMS agrees with the applicant that this deviation is acceptable.

RMS also notes that temperature rose above 22±10°C (actual max value 36.2°C), the light intensity was above recommended 350 ± 50 µE/m²/s (actual value [min]: 596 µE/m²/s) and the hygrometry dropped under 70±25% (actual min value 41). RMS considers these deviations acceptable.
The validity criteria according to the current guideline OECD 208 are fulfilled. This study is considered valid.

For all species tested: NOER = 10.00 L MON52276/ha (equivalent to 3.610 kg glyphosate acid/ha), ER25 and ER50 for all parameters >10.00 L MON52276/ha (>3.610 kg glyphosate acid/ha).

Data point	CP 10.6.2/002
Report author	[redacted]
Report year	2014
Report title	MON 52276: Effects on the Vegetative Vigor of Non-Target Terrestrial Plants (Tier II)
Report No	80477
Document No	-
Guidelines followed in study	OECD Guideline 227 (2006)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current test guideline OECD 227 (2006):* *Major: none* *Minor:* *- No reference substance or historical data were mentioned in the report.* *- Light intensity was lower than 350 µE/m^2/s (mean values 170/173 µEs^{-1}m^{-2})*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary

A vegetative vigour study was conducted exposing six dicotyledonous (cucumber, oilseed rape, radish, soybean, sunflower and tomato) and four monocotyledonous (corn, oat, wheat and onion) plant species to seven nominal test concentrations of 20, 40, 80, 160, 320, 640, and 1280 g MON 52276 a.e./ha. In addition, one negative control group (deionized water) was tested. The test was replicated four times for all species. At test initiation, each pot contained five plants per pot, except for cucumber which contained three plants per pot.

Following the application, plant damage and phytotoxic effects were recorded weekly until the test termination at 21 days after application. At test termination, the numbers of live and dead plants were recorded along with the visual assessments. Shoots were composited by replicate and fresh weights were measured and recorded.

The most sensitive monocotyledonous plant species was wheat with an ER_{50} value of 38.2 g a.e./ha for shoot fresh weight. Cucumber was the most sensitive dicotyledonous plant species with an ER_{50} value of 28.4 g a.e./ha for shoot fresh weight.

The validity of the present study according to OECD guideline 227 was achieved.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:: MON 52276 (formulated product)
Description: Amber liquid
Lot/Batch #: GLP-1308-22862-F
Purity: 30.45 % glyphosate acid

Test organism:

Species:	6 Dicotyledons: (cucumber, oilseed rape, radish, soybean, sunflower and tomato) 4 Monocotyledons: (corn, oat, wheat and onion)
Source:	Syngenta Seed: corn, sunflower Ohio Foundation Seeds: oat Park Seed Co.: onion L.A. Hearne company: wheat NE Seed: cucumber, tomato Johnny's Selected Seeds: oilseed rape Sustainable Seed Company: radish Missouri Foundation Seeds: soybean
Environmental conditions:	
Temperature:	17.0 - 28.3°C: corn, oat, onion, wheat, soybean, sunflower 21.4 - 29.4°C: cucumber, oilseed rape, radish, tomato
Relative humidity:	32 – 92 %: corn, oat, onion, wheat, soybean, sunflower 27 – 73 %: cucumber, oilseed rape, radish, tomato
Photoperiod:	16 hours light/8 hours dark 170 µEs/m^2 (daily accumulated PAR was 10 E/m^2) for corn, oat, onion, wheat, soybean, sunflower 173 µEs/m^2 (daily accumulated PAR was 10 E/m^2) for cucumber, oilseed rape, radish, tomato
Soil textural class:	Sandy Loam (72 % sand; 18 % silt; 10 % clay)
Soil pH:	5.9
Soil organic content:	1.5 % (equivalent to 2.5 % organic matter)
Experimental work dates:	5 November - 26 November 2013

B. STUDY DESIGN

Experimental treatments

Prior to treatment, seedlings were grown (in 16.5 cm- diameter plastic pots containing 11.5 cm depth of soil) to the 2 to 3 - 4 true leaf stage from untreated seed in a sandy Loam soil (1.5 % organic matter, pH 5.9) in a greenhouse. The test was replicated four times for all species. Because the test species are different in their size and growth requirements, numbers of test plants per pot and pots per replicate were adjusted accordingly. Applications of the formulated product were made using a calibrated overhead track sprayer (De Vries Manufacturing). The single nozzle sprayer was equipped with a TeeJet 4001 E nozzle and operated at 40 psi. The target application volume was 100 L of water per hectare (L/ha). The application started with the controls and then progressed upward in treatment rates. The applications produced target application rates of 0 (control), 20, 40, 80, 160, 320, 640, and 1280 g a.e./ha.

Observations

Observations of survival (numbers of live plants present and cumulative mortality) and phytotoxicity ratings (i.e., visual injury assessments) were performed on a weekly basis for all species. Visual injury assessments were made on a scale of 0 to 100. The range and severity of effects as compared to the control plants are as follows: 0 to 10, no effect; 20 to 30, slight effect; 40 to 60, moderate effect; 70 to 90, severe effect; with 100 meaning all plants dead. Visually observed phytotoxic effects were stunting, chlorosis, wilting, leaf wrinkling, necrosis, and damping off, though not all manifested on all species.
Shoot lengths were measured from the base of the stem to the tip of the longest leaf for bulb or leaf rosette plants and from the base of the stem to the apical bud for other plants. The in-life phase was terminated 21 days after application of the test substance. At test termination, the numbers of live and dead plants were recorded along with the visual assessments. Plants were watered prior to taking fresh weights.

Test solutions were analysed for the concentrations of glyphosate, the active ingredient in MON 52276 using a liquid chromatography tandem mass spectrometry (LC-MS/MS) system. The samples were collected prior to and after application to the test systems for the definitive test.

Statistical calculations

All statistical computations were performed using SAS Version 9.3 software. Continuous data (length, weight) was analysed using analysis of variance (ANOVA) and Jonckheere-Terpstra test if monotonous. The NOEC for quantal data (survival) for species less than 100% was determined by Cochran-Armitage. If monotonicity was not determined then pair-wise testing was performed using Dunnett's or Dunn's test for continuous data, after Shapiro-Wilk and Levene testing for normality and homogeneity of variance respectively, and Fisher's Exact test for quantal data.

Estimates for continuous data (length, weight) were calculated by Bruce and Versteeg weighted Probit or other appropriate regression models, fit using the Marquardt method. Estimates for Quantal data (survival) were calculated using Probit when possible or Moving Average Angle or Binomial analysis when appropriate.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.11-4: Analytical verification of the concentrations

Parameter	**Nominal concentration of glyphosate acid equivalent [g/ha]**						
	0	320		640		1280	
	Nominal concentration of glyphosate acid equivalent [mg/L]						
	0	**3.20**		**6.40**		**12.8**	
	Measured concentration of glyphosate acid equivalent [mg/L]						
Pre-application concentration	< MQL[a]	3.10	3.08	6.0	5.88	12.8	13.3
Pre-application % of nominal	-	97	96	94	92	100	104
Post-application concentration	<MQL[a]	2.92	2.98	6.0	5.8	12.3	12.5
Post-application % of nominal	-	91	93	94	91	96	98

[a] MQL = 0.0200 mg/mL

Table B.9.11-5: Effects of MON 52276 after 21 days

Crops	Glyphosate acid equivalent g/ha							
	Control	**20**	**40**	**80**	**160**	**320**	**640**	**1280**
Survival [%]								
Corn	100	100	100	100	100	95	50*	37*
Oat	100	100	100	100	92	22*	0*	0*
Onion	100	100	100	100	100	87*	67*	35*
Wheat	100	100	100	57*	2*	0*	0*	0*
Cucumber	100	100	100	42*	0*	0*	0*	0*
Oilseed rape	100	100	100	100	100	100	47*	0*
Radish	100	100	100	100	97	72*	20*	2*
Soybean	100	100	100	95	65*	7*	0*	0*
Sunflower	100	100	100	85*	20*	0*	0*	0*
Tomato	100	100	100	90	5*	0*	0*	0*
Phytotoxic Effects rating								
Corn	0	0	10	18	43	70	80	80
Oat	0	5	10	15	60	93	100	100
Onion	0	18	10	15	30	45	73	90
Wheat	0	0	25	45	98	100	100	100
Cucumber	0	10	28	63	100	100	100	100
Oilseed rape	0	0	3	8	38	60	85	100
Radish	0	5	10	23	43	65	90	98
Soybean	0	0	18	53	70	93	100	100
Sunflower	0	0	33	50	83	100	100	100
Tomato	0	0	33	55	95	100	100	100
Mean plant fresh weight [g/treatment replicate]								
Corn	74.329	69.113	68.585	60.905*	28.846*	2.448*	0.498*	0.454*
Oat	57.927	61.129	55.825	53.543	12.260*	0.814*	NA	NA
Onion	66.191	49.072	60.458	57.666	28.238*	8.926*	1.458*	0.218*
Wheat	31.373	27.120	14.170*	0.865*	0.059*	NA	NA	NA
Cucumber	157.47	122.01*	76.04*	10.298*	NA	NA	NA	NA
Oilseed rape	129.14	126.62	133.98	125.62	56.161*	17.283*	4.168*	NA
Radish	95.009	82.301	83.568	57.897*	19.982*	6.095*	0.919*	0.956*
Soybean	88.13	76.772	62.966*	20.617*	2.522*	2.175*	NA	NA
Sunflower	133.33	107.05*	42.117*	7.855*	1.017*	NA	NA	NA
Tomato	210.403	155.438*	60.455*	11.604*	0.291*	NA	NA	NA

Table B.9.11-5: Effects of MON 52276 after 21 days

Crops	Glyphosate acid equivalent g/ha							
	Control	**20**	**40**	**80**	**160**	**320**	**640**	**1280**
Mean shoot length [mm]								
Corn	691	667	670	608*	361*	207*	188*	187*
Oat	720	690	687	709	367*	245*	NA	NA
Onion	417	398	386*	388*	289*	191*	157*	143*
Wheat	478	449	319*	260*	286*	NA	NA	NA
Cucumber	591	419*	151*	55*	NA	NA	NA	NA
Oilseed rape	264	261	266	268	189	175*	148*	NA
Radish	183	167	174	158*	134*	109*	94*	151*
Soybean	548	533	454*	226*	138*	146*	NA	NA
Sunflower	498	445	284*	146*	118*	NA	NA	NA
Tomato	302	314	158*	73*	71*	NA	NA	NA

* = significantly different when compared to the control determined by Cochran-Armitage test ($\alpha = 0.05$)
NA = not applicable

Table B.9.11-6: 21-day NOER, ER_{25} and ER_{50} values

Crop	Endpoints [g acid equivalent/ha]		
	% Survival		
	NOER	ER_{25} (95 % CI)	ER_{50} (95 % CI)
Corn	320	522 (414 - 626)	854 (714 - 1069)
Oat	160	204 (175 - 228)	252 (225 - 281)
Onion	160	536 (424 - 650)	916 (752 - 1194)
Wheat	40	70.2 (59.6 - 78.3)	85.9 (76.8 - 96.0)
Cucumber	40	NC	76.7 (65.1 - 92.7)
Oilseed rape	320	NC	632 (558 - 728)
Radish	160	305 (252 - 353)	431 (374 - 497)
Soybean	80	134 (112 - 153)	179 (157 - 204)
Sunflower	40	92.2 (78.3 - 104)	117 (104 - 132)
Tomato	80	92.4 (81.8 - 102)	108 (98.1 - 120)
Crop	**Fresh weight**		
	NOER	ER_{25} (95 % CI)	ER_{50} (95 % CI)
Corn	40	87.1 (58.3 - 130)	131 (99.0 - 174)
Oat	80	91.7 (79.3 - 106)	120 (109 - 132)
Onion	80	103 (77.7 - 137)	163 (133 - 199)
Wheat	20	29.1 (24.6 - 34.5)	38.2 (33.9 - 43.1)
Cucumber	<20	28.4 (22.6 - 35.7)	39.2 (33.7 - 45.7)
Oilseed rape	80	96.0 (81.7 - 113)	153 (137 - 171)
Radish	40	55.3 (42.0 - 72.9)	94.9 (78.1 - 115)
Soybean	20	34.7 (26.7 - 45.0)	52.9 (44.1 - 63.5)
Sunflower	<20	21.9 (19.1 - 25.2)	31.1 (27.7 - 34.8)
Tomato	<20	19.5 (15.7 - 23.3)	30.0 (26.2 - 33.8)
Crop	**Shoot length**		
	NOER	ER_{25} (95 % CI)	ER_{50} (95 % CI)
Corn	40	55.8 (27.7 - 112)	207 (133 - 323)
Oat	80	112 (85.9 - 147)	204 (174 - 238)
Onion	20	99.7 (60.9 - 136)	387 (291 - 514)
Wheat	20	32.5 (16.7 - 63.2)	120 (73.4 - 197)
Cucumber	<20	18.3 (14.0 - 22.5)	28.4 (24.1 - 32.7)
Oilseed rape	160	202 (131 - 313)	689 (525 - 902)
Radish	40	130 (42.8 - 392)	1144 (526 - 2487)
Soybean	20	33.0 (19.8 - 54.8)	75.3 (54.7 - 103)
Sunflower	20	21.4 (13.8 - 32.3)	50.9 (38.7 - 67.1)
Tomato	20	22.9 (12.7 - 41.0)	46.7 (32.1 - 67.9)

CI = confidence interval
NC= not calculated

B. OBSERVATIONS

Analytical data:
Chemical analyses were performed on samples of the three highest test solutions to quantify glyphosate in the test solution. The mean measured concentrations ranged from 92 to 104 % in the pre-application samples and ranged from 91 to 98 % to the post-application samples. The measured content of the test item always ranged between 80 and 120 % of nominal, so the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Survival and phytotoxicity results:

There were no phytotoxic effects and the survival was 100 % in the control for all species. There was significant ($p = 0.05$) reduction in survival compared to the control in all species tested. After 21 days, treatment level mean phytotoxicity ratings ranged from 0 to 100 for all species and progressed toward moderate or severe with increasing test substance concentration. The lowest NOEC values was 40 g a.e./ha for wheat, cucumber and sunflower. The most sensitive species based on survival EC_{50} values was cucumber with an EC_{50} of 76.7 g a.e./ha.

Fresh weight results:

Shoot fresh weight was significantly reduced in all species. The most sensitive species based on shoot fresh weight EC_{50} values was tomato, with an EC_{50} of 30.0 g a.e./ha

Shoot length results:

Shoot length was significantly reduced in all species. The most sensitive species based on shoot length EC_{50} values was cucumber with an EC_{50} of 28.4 g a.e./ha.

The most sensitive monocotyledonous plant species was wheat with an EC_{50} value of 38.2 g a.e./ha (for shoot fresh weight). Cucumber was the most sensitive dicotyledonous plant species with an EC_{50} value of 28.4 g a.e./ha (for shoot fresh weight).

The applicant noted that the following points deviated from the current guideline recommendations:

- No reference substance or historical data were mentioned in the report.
- Light intensity was lower than 350 $\mu E/m^2/s$ (means values 170/173 $\mu Es^{-1}\ m^{-2}$)

The applicant considers these deviations minor and provided the following justification:

"*However, there were no phytotoxic effects observed in the controls for any of the species tested, meaning that the growing conditions were appropriate for the species. In addition any competition for light was minimized considering that due to the test species being different in their size and growth requirements, numbers of test plants per pot and pots per replicate were adjusted accordingly*." RMS opinion is given below in the commenting box.

According to the study report, the validity criteria according to the OECD 227 were fulfilled. The seedling emergence was at least 70 % (actual values from 85 to 99 %). In the control, the plants did not exhibit visible phytotoxic effects; the mean plant survival is at least 90 % for the duration of the study (actual value 100%); environmental conditions for a particular species were identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The lowest EC_{50} value for MON 52276 was observed with cucumber and was calculated to be 28.4 g acid equivalent/ha for shoot fresh weight. The lowest NOEC values were observed with cucumber, sunflower and tomato for fresh weight parameter and with cucumber for shoot length parameter and were calculated to be <20 g acid equivalent/ha.

RMS conclusion in the RAR 2015:
Despite the assumption that the study was considered to be valid as criteria according to OECD 227 were fulfilled, RMS questioned the reliability of the endpoints from the study with half the recommended light intensity. RMS could not exclude the possibility that sensitivity of the test species was underestimated under the proposed environmental conditions and with the choice of the endpoint shoot length. RMS considered that uncertainties exist in terms of a reliable exposure of test plants and concerning the full potential of glyphosate action to affect a down regulated plastid localised pathway. Nevertheless, this study displayed the only dataset provided for the representative formulation MON 52276 and therefore, included information about the relevance of the formulants. In general, toxicity studies with the commercial product are more appropriate than studies with the active ingredient only for the assessment of the effects on non-target plants.

Assessment and conclusion by RMS:

The following deviations were noted by the applicant:

- *No reference substance or historical data were mentioned in the report.*

RMS agrees with the applicant that this deviation is acceptable but still increases the uncertainty on sensitivity of the test system (considering the other deviations listed below).

- *Light intensity was lower than 350 µE/m²/s (actual mean values 170 and 173 µE.s⁻¹.m⁻²)*

The applicant argued that no phytotoxic effects observed in the controls for any of the species tested, meaning that the growing conditions were appropriate for the species. The applicant also considers that any competition for light was minimized considering that due to the test species being different in their size and growth requirements, numbers of test plants per pot and pots per replicate were adjusted accordingly.
RMS does not agree. RMS considers the justification not sufficient as such light intensity may not be representative of real conditions. This deviation was already noted in RAR 2015 and former RMS highlighted that the OECD 227 guideline recommends additional lighting to become necessary if intensity decreases below 200 µE.s^{-1}.m^{-2}, wavelength 400-700 nm except for certain species which light requirements need less light intensity. This study was conducted with an average daily photosynthetically active radiation (PAR) of 170 µE.s^{-1}.m^{-2}and 173 µE.s^{-1}.m^{-2} (daily accumulated PAR was 10E/m^2) with an average 16 hours light.
In RAR 2015, former RMS hypothetized that reduced light intensity during plant growth might decrease carbon flow through the shikimic acid pathway and that reduced light might have induced shade avoidance responses, which include increases in plant height, as well as a reduction in biomass and leaf numbers. Major uncertainties were raised in terms of a reliable exposure of test plants and concerning the full potential of glyphosate action to affect a down regulated plastid localized pathway under the used light conditions. This study was used only because it displays the only dataset provided for the representative formulation MON 52276 and therefore, includes information about the relevance of the formulants.

RMS still considers that light may be pivotal factor. The reliability of the study with half the recommended light intensity is questionnable.

The following deviations were also noted by the RMS:

- Five plant per pot instead of one or two for bigger plants as corn, soybean, tomato, cucumber (3 plants per pot).
  Plants were thinned to five plants of uniform height per pot. RMS notes that ideally, after thinning, one single plant should remain for the bigger plant species to avoid overcrowding and shading of plants by each other for the duration of the test. As an example OECD 227 recommends one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15 cm container. This should avoid crowding of the plants that could affect growth and overlapping of leaves that could affect exposure. However, RMS notes that plants were treated at earlier stage (2 leaves) instead of 2-4 leaves (OECD 227). Seedlings were grown to 2 leaves stage for all species except for wheat (3 leaves) and oil seed rape and radish (4 leaves). RMS assumes that crowding was then limited. Overall RMS considers this deviation acceptable.

- Plants were watered prior to taking fresh weights if not fully turgid. RMS questions the impact of watering on fresh weight measurements (dry weight was not measured) and if this may have erased partly the potential effects.

- Temperature was adequate but the hygrometry often (almost every day) dropped under 70±25%.
  RMS considers this deviation acceptable.

- The seedling emergence reported in the study report (actual 85 to 99%) was in fact the germination rate as provided by the seed suppliers. The seedling emergence during the test was not reported in the study report. However, given the germination rates, RMS is of the opinion that this validity criteria could be considered as met.

The validity criteria according to the current guideline OECD 227 are considered to be fulfilled however severe drawbacks were noted.
Overall, RMS considers that the conditions of the test may underestimate the effects, this study is not reliable enough to be used alone in the risk assessment but can still be supportive. The results could be considered as further evidence together with the results of the new vegetative vigour test of [redacted] (2021, CP 10.6.2/005).

Data point	CP 10.6.2/003
Report author	[redacted]
Report year	2005
Report title	Evaluation of the toxicity of glyphosate and paraquat to terrestrial non-target plants
Report No	CEA.104; BX-0928
Document No	-
Guidelines followed in study	OECD 208B (draft, 2000): Terrestrial non-target plant test; Vegetative Vigour Test.
GLP	No, no claims for GLP compliance were made for the study.
Previous evaluation	Yes, evaluated and not accepted: • RAR 2015

Short description of study design and observations	The vegetative vigour test assesses the potential damage to plants following exposure of Roundup (360 g glyphosate/L, EC) on non-target plants (*Beta vulgaris* (Sugar beet); *Raphanus rapistrum* (Rape); *Lepidium sativum* (Garden cress); *Pisum sativum* (Pea); *Lolium perenne* (Perennial ryegrass) and *Triticum aestivum* (Winter wheat)) following deposition on the leaves and above-ground portions of the plants. Seedlings were grown in pots filled with sterilised Kettering loam and Derby Quartz (mixture loam and grit: 5:1). Each treatment/crop combination was replicated four times. Prior to treatment, seedlings were grown to at least 2-4 true leaves. Roundup was applied indoors with a Mardrive pot sprayer at 225 L/ha. The plants were treated with seven nominal concentrations of 0.00004, 0.0004, 0.004, 0.04, 0.4, 2.0 and 4.0 L prod/ha. One negative control group was tested. After treatment plants were kept in a greenhouse at 12 to 18°C. Phytotoxicity ratings, according to a nine point scoring system were recorded for the first 4 days and at approximately 7, 15 and 22 days after the application. All plots were harvested between 20 to 22 days after treatment to determine fresh shoot weight. The weights of plants in one pot were combined. Data for the No Observed Effect Rates (NOER) were analysed using one-way ANOVA and Dunnett's t-test was performed as post-hoc. The highest concentration not significantly different from the control was identified as the NOER.
Short description of results	*B. vulgaris* (Sugar beet) and *R. rapistrum* (Rape) responded most quickly to the application of glyphosate as Roundup, with both species showing significant differences in vegetative vigour from the controls at 50% field application rate (2.0 L/ha) one day after application of the test item. The NOER was 0.4 L/ha (10% field application rate). There was no increase in the sensitivity of either *B. vulgaris* (Sugar beet) or *R. rapistrum* (Rape) for the duration of the study and the fresh shoot weight NOER was also 0.4 L/ha. *L. sativum* (Garden cress) was the most sensitive species according to the vegetative vigour scores with a NOER of 0.04 L/ha (1% field rate) from day 2 to the end of the study. The fresh shoot weight was a less sensitive endpoint, with a NOER of 0.4 L/ha. The NOER calculated for fresh shoot weight was the same for all test species.
Reasons for why the study is not considered relevant/reliable or not considered as key study (applicant) **For RMS see commenting box below**	The study design is not in line with the current guideline OECD 227 requirements. The validity criteria according to the current guideline could not be fulfilled. Therefore, no consistent conclusions could be drawn from the study. Deviations from current guideline: - The mean plant survival was not evaluated - Seedling emergence rate for *Lepidum sativuma and Lolium perenne* is not known. - Analytical verification of the concentrations were not performed. - Soil characteristics were not provided (max: 1.5 % organic carbon acceptable). - Light intensity was not provided.

Reasons why the study report is not available for submission (applicant) For RMS see commenting box below	The study is not considered as relevant because of the various shortcomings.

Assessment and conclusion by RMS:
As previously noted in RAR 2015, no mean plant survival data for the control are available and only NOER are presented. The lowest NOER values of 0.04 L Roundup/ha (equivalent to 14.4 g a.s./ha based on nominal content of active substance) were observed for garden cress and winter wheat (22 days). The lack of raw data including standard deviation values was also noted, no robust ER50 could be recalculated. The study was not considered to be valid in RAR 2015.

The applicant listed several shortcomings and considered the study not relevant. RMS further notes that no reference substance or historical data were mentioned in the report. Hygrometry data are not available. Three plant per pot were used instead of one or two for sugar beet (as recommended in OECD 227 for bigger plants).
As validity criteria cannot be checked (absence plant survival data for the control), RMS considers that the study is not reliable enough to derive an endpoint.

Data point:	CP 10.6.2/004
Report author	[redacted]
Report year	2012
Report title	Comparative Post-Emergence Phytotoxicity of AMPA and Glyphosate to Crop and Annual Weed Species
Report No	MSL0024009
Document No	-
Guidelines followed in study	Not applicable.
GLP	No, this report do not contain any test material and any experimentation.
Previous evaluation	Yes, evaluated and accepted in RAR 2015
Short description of study design and observations:	The purpose of this evaluation was to compare relative post-emergence phytotoxicity between glyphosate and aminomethylphosphonic acid (AMPA) with crop and annual weed species. At planting, containers were packed with sterilized silt loam soil. Seeds were planted between 5 and approximately 30 specimens depending on the species planted. After planting, plants were moved to the greenhouse with supplementary lighting and sufficient tap water was provided. Nominal test concentrations for foliar applications were prepared from a 1 % stock solution for glyphosate acid equivalent and AMPA and applied as needed to achieve the desired rate of application to young plants. Low rates required further dilution of the 1% stock solution to 0.1 % and 0.01 % stock solutions to ensure accuracy in pipetting. To complete the formulation prior to application 0.4 % of emulsifier-L (cyclo-L) was added to each spray bottle and then water was added in sufficient volume to provide a spray volume of 200 gallons/A. The plants were inspected approximately twice per week. Phytotoxicity was recorded as visual percent injury (chlorosis) relative to the untreated control and evaluated two weeks after test initiation. The percent injury

observations were used as the phytotoxicity endpoint to calculate EC_{50} values in this analysis. Glyphosate Isopropylamine (IPA) and AMPA data from studies run in parallel were available from a studies conducted on 12 March and 15 August 1986. The glyphosate levels tested in March 1986 included 0.0625, 0.125, 0.25, 0.5, 1 and 5 lb a.e./A and the glyphosate levels tested on 15 August 1986 were 1, 5, 10 and 20 lb a.e./A. Statistical calculations: EC_{50} values were calculated using a 3-parameter logistic model with the software package GraphPad Prism version 5.04 (GraphPad Software, Inc.). The maximum asymptote was constrained in the logistic model to 100 % to reflect the maximum potential response based on percent injury observations.

Short description of results:

EC_{50} molar ratios were calculated as EC_{50} AMPA/EC_{50} glyphosate acid and ranged from 3.4 for hemp sesbania to 87 for common lambsquarters. All AMPA/EC_{50} glyphosate acid ratios were greater than 2, with an average ratio across the seventeen tested species of 22, indicating that AMPA has significantly lower herbicidal activity compared to glyphosate.

RMS notes that the reports contains two tables, one presenting the EC50 based on kg/ha and the other based on moles/ha. Both are reported here below:

Table 2. Post-emergence EC_{50} values for AMPA and Glyphosate to Crop and Annual Weed Species based on units of moles/ha

Species Common name	Species Scientific Name	Glyphosate Acid EC_{50} (Moles/ha)	AMPA EC_{50} (Moles/ha)	EC_{50} Molar Ratio [1]
BARNYARD GRASS	*Echinochloa crus-galli*	4.202	103.972	24.745
COCKLEBUR	*Xanthium strumarium*	4.123	25.963	6.297
CORN	*Zea mays*	1.710	42.346	24.762
CRABGRASS	*Digitaria ischaemum*	2.320	70.661	30.458
GREEN FOXTAIL	*Setaria veridis*	2.146	42.790	19.937
HEMP SESBANIA	*Sesbania exaltata*	5.933	20.159	3.398
LAMBSQUARTERS	*Chenopodium album*	2.303	199.869	86.773
MORNING GLORY	*Ipomoea sp.*	6.913	128.603	18.602
PROSO MILLET	*Panicum miliaceum*	1.949	43.668	22.401
RICE	*Oryza sativa*	5.537	87.650	15.831
SMARTWEED	*Polygonum pensylvanicum*	4.882	37.682	7.718
SORGHUM	*Sorghum bicolor*	3.310	97.663	29.504
SOYBEAN	*Glycine max*	5.618	92.677	16.496
SUGAR BEET	*Beta vulgaris*	3.291	39.237	11.923
VELVETLEAF	*Abutilon theophrasti*	5.204	142.432	27.370
WHEAT	*Triticum aestivum*	4.703	131.732	28.007
WILD BUCKWHEAT	*Polygonum convolvulus*	3.287	25.821	7.856
AVERAGE		**3.9667**	**78.407**	**22.475**

[1] EC_{50} ratio calculated as EC_{50} AMPA/ EC_{50} glyphosate. Results were calculated with full precision mode in Excel and may differ slightly from hand calculated values.

Table 1. Post-emergence EC_{50} values for AMPA and Glyphosate to Crop and Annual Weed Species based on units of kg/ha

Species Common name	Species Scientific Name	Glyphosate Acid EC_{50} (kg/ha)	AMPA EC_{50} (kg/ha)	EC_{50} Ratio [1]
BARNYARD GRASS	*Echinochloa crus-galli*	0.711	11.545	16.249
COCKLEBUR	*Xanthium strumarium*	0.697	2.883	4.135
CORN	*Zea mays*	0.289	4.702	16.260
CRABGRASS	*Digitaria ischaemum*	0.392	7.846	20.000
GREEN FOXTAIL	*Setaria veridis*	0.363	4.751	13.091
HEMP SESBANIA	*Sesbania exaltata*	1.003	2.238	2.231
LAMBSQUARTERS	*Chenopodium album*	0.389	22.193	56.978
MORNING GLORY	*Ipomoea sp.*	1.169	14.280	12.215
PROSO MILLET	*Panicum miliaceum*	0.330	4.849	14.709
RICE	*Oryza sativa*	0.936	9.732	10.395
SMARTWEED	*Polygonum pensylvanicum*	0.826	4.184	5.068
SORGHUM	*Sorghum bicolor*	0.560	10.844	19.373
SOYBEAN	*Glycine max*	0.950	10.291	10.832
SUGAR BEET	*Beta vulgaris*	0.557	4.357	7.829
VELVETLEAF	*Abutilon theophrasti*	0.880	15.815	17.972
WHEAT	*Triticum aestivum*	0.795	14.627	18.391
WILD BUCKWHEAT	*Polygonum convolvulus*	0.556	2.867	5.158
Average		**0.6701**	**8.706**	**14.758**

[1] EC_{50} ratio calculated as EC_{50} AMPA/EC_{50} glyphosate. Results were calculated with full precision mode in Excel and may differ slightly from hand calculated values.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	This report is a comparison of post-emergence phytotoxicity between glyphosate and AMPA with crop and annual weed species, according to data generated from several screening studies previously performed. Nevertheless the results could be useful as supplemental data.
Reasons why the study report is not available for submission	The report is considered as supportive only.

Assessment and conclusion by RMS:

Relative post-emergence phytotoxicity between glyphosate and aminomethylphosphonic acid (AMPA) were compared for the following species:
9 Dicotyledons: (cocklebur, hemp sesbania, lambsquarters, morning glory, smartweed, soybean, sugar beet, velvetleaf, wild buckwheat)
8 Monocotyledons: (barnyard grass, corn, crabgrass, green foxtail, proso millet, rice, sorghum, wheat)

EC_{50} molar ratios were calculated as EC_{50} AMPA/ EC_{50} glyphosate acid and ranged from 3.4 for hemp sesbania to 86.8 for common lambsquarters. In all cases, the ratios were greater than two, indicating that AMPA has less than 50% of the herbicidal activity of glyphosate.

The endpoints presented above cannot be used for the risk assessment (no full evaluation of the study was feasible) but the analysis presented above is considered informative.

Data point	CP 10.6.2/005
Report author	[redacted]
Report year	2021
Report title	MON 52276: Effects on the Vegetative Vigour of Ten Non-Target Terrestrial Plant species under Greenhouse conditions
Report No	S20-05300
Document No	-
Guidelines followed in study	OECD Guideline 227 (2006)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviations from current test guideline OECD 227 (2006) Minor: Guideline recommends light intensity of 350 ± 50 µE/m2/s. In this study 300 µE/m2/s was used.
Previous evaluation	New study not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Yes, except results on cucumber not considered reliable

Summary

A vegetative vigour study was conducted exposing six dicotyledonous (cucumber, oilseed rape, radish, soybean, sunflower and tomato) and four monocotyledonous (maize, oat, wheat and onion) plant species to seven nominal test concentrations of 0.022, 0.043, 0.087, 0.173, 0.346, 0.693 and 1.385 L MON52276/ha, equivalent to 7.8, 15.7, 31.3, 62.7, 125.3, 250.7 and 501.4 g glyphosate acid equivalent/ha. In addition, one negative control group (tap water) was tested.

To allow for the test species growth requirements, numbers of test plants per pot and pots per replicate were adjusted accordingly. For the cucumber, oilseed rape, radish, soybean, sunflower, tomato, maize and onion, there were 2 plants per pot and 15 replicates per treatment group. For oat and wheat there were 4 plants per pot and 8 replicates per treatment group.

The test observation period was 21 days following application. During this period, plants were assessed for mortality and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant shoot height and shoot fresh weight were determined for day 21. Results were compared to the water treated control.

According to the study report, the lowest ER50 was 0.193 L test item/ha (equivalent to 69.87 g acid equivalent/ha) for the dicotyledonous species *Lycopersicon esculentum* (tomato) in shoot fresh weight. The lowest ER50 was 0.431 L test item/ha (equivalent to 156.02 g acid equivalent/ha) for the monocotyledonous species *Triticum aestivum* (wheat) in shoot fresh weight.
RMS considered results for cucumber as not reliable (see RMS assessment and conclusion below).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item::	MON 52276 (formulated product)
Description:	Yellow liquid
Lot/Batch #:	1151167 (lot AZE200810A)
Purity:	362 g/L glyphosate acid

Test organism:

Species:	6 Dicotyledons: (cucumber, oilseed rape, radish, soybean, sunflower and tomato) 4 Monocotyledons: (maize, oat, wheat and onion)
Source:	Untreated seeds from commercial suppliers were used.

Environmental conditions:

Temperature:	18.7 – 31.8°C: cucumber, oilseed rape, radish, tomato, soybean, sunflower 15.9 – 31.8°C: maize, oat, onion, wheat
Relative humidity:	50 – 100 %: cucumber, oilseed rape, radish, tomato, soybean, sunflower 47 – 100 %: maize, oat, onion, wheat
Photoperiod:	16 hours light/8 hours dark (min 696 – max 826 µE/m^2/s)
Soil textural class:	Sandy Loam (73 % sand; 14 % silt; 12 % clay)
Soil pH:	8.21
Soil organic content:	0.8 % organic carbon content
Experimental work dates:	02 September 2020 – 01 December 2020

B. STUDY DESIGN

Experimental treatments

Prior to treatment, seedlings were grown in 15.1 cm- diameter pots (capacity of 1.5L) from untreated seeds in a sandy loam soil (0.8 % organic matter, pH 8.2) in a greenhouse. In each treatment group a total of 30 or 32 plants at BBCH growth stage 12 – 14 were used. Because the test species are different in their size and growth requirements, numbers of test plants per pot and number of pots per treatment were adjusted accordingly. For the dicotyledonous species (cucumber, oilseed rape, radish, soybean, sunflower and tomato), maize and onion, there were 2 plants per pot and 15 replicates per treatment group. For Oat and wheat, therewere 4 plants per pot and 8 replicates per treatment group. All plants pots containing soil and seeds, were bottom watered by placing them in watering saucers that were regularly replenished with water during the study. All species were fertilized with a tank mixture of calcium nitrate, potassium nitrate, magnesium sulphate and monopotassium phosphate diluted in tap water once a week with the exception of the last week, in which it was not necessary to fertilize.

Observations

Plants were assessed for mortality and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant shoot height and shoot fresh weight were determined on day 21. Results were compared to the water treated control. Analysis of the stock Solution (1.385 L test item/ha) were analyzed by LC-MS/MS.

Statistical calculations
Mean mortality, mean final shoot height and mean final shoot fresh weight of the surviving plants were determined for each test rate and the control. Statistical analysis of data was performed using the ToxRat Solutions program (ToxRat® Professional Version 3.3.0). Mean mortality, mean final heights and mean final shoot fresh weight were compared using a suitable statistical test in order to obtain the NOER, LOER ER25, 50 and LR25,50 values.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analysis of the stock solution, highest test solution concentration, was performed and is summarised below.

Table B.9.11-7: Analysis of stock solution

Application rate L/ha	Spray volume (L/ha)	Nominal concentration of glyphosate (g/L)	Determination of glyphosate (g/L)	% of nominal
1.385	100	5.01	5.10	102
1.385	100	5.01	4.92	98

The LOQ was determined to be 107.7 mg test item/L (33.3 mg glyphosate/L), the LOD was determined to be 50.05 mg glyphosate/L = 15% of the LOQ.

Significant effects on mortality were observed for all the species.

Table B.9.11-8: Effects of MON 52276 on survival after 21 days

Treatment		**Mean mortality (%)**									
ID	L test item/ha	*Cucumis sativus #*	*Brassica napus*	*Raphanus sativus*	*Glycine max*	*Helianthus annuus*	*L. esculentum*	*Zea mays*	*Triticum aestivum*	*Avena sativa*	*Allium cepa*
C	0.000	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T1	0.022	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T2	0.043	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	0.087	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	0.173	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T5	0.346	*3.33*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	0.693	*43.33**	3.33	16.67*	0.00	30.00*	16.67*	13.33*	71.88*	31.25*	10.00*
T7	1.385	*90.00**	60.00*	93.33*	43.33*	100.00*	86.67*	100.00*	100.00*	100.00*	60.00*

* *significant difference from the control at the 0.05 probability level*

data for cucumber (*cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).

Injury symptoms were observed for all the plant species.

Table B.9.11-9: Phytotoxic effects of MON 52276 after 21 days

Application rate (L test item/ha)	**% Phytotoxicity**				
	Cucumis sativus (cucumber) $	*Brassica napus* (oilseed rape)	*Raphanus sativus* (radish)	*Glycine max* (soybean)	*Helianthus annuus (sunflower)*
0.022	*0*	0	0	0	0
0.043	*0*	0	0	0	0
0.087	*0*	0	10	0	0
0.173	*0*	0	20	0	0
0.346	~~*20*~~ *10#*	20-40	40	10	30-50
0.693	~~*40 50*~~ *30-50#*	50-60	50	30-50	50-60
1.385	*40-50*	60	50-60	40-50	-
Application rate (L test item/ha)	**% Phytotoxicity**				
	Lycopersicon esculentum (tomato)	*Zea mays* (maize)	*Triticum aestivum* (wheat)	*Avena sativa* (oat)	*Allium cepa* (onion)
0.022	0	0	0	0	0
0.043	0	0	0	0	0
0.087	0	0	0	0	0
0.173	0	0	0	0	0
0.346	30-40	0	10	0-10	10
0.693	50	50-60	50	40-50	30-50#
1.385	60	-	-	~~40~~ -#	50-60

$ data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).
values modified by RMS to reflect the information from raw data tables of the study report.

Significant effects on shoot height were observed at one or more treatment rates of MON 52276 for all the tested species.

Table B.9.11-10: Effects of MON 52276 on shoot height after 21 days

Application rate (L test item/ha)	**Mean shoot length [cm] (% inhibition compared to control)**				
	Cucumis sativus (cucumber) #	*Brassica napus* (oilseed rape)	*Raphanus sativus* (radish)	*Glycine max* (soybean)	*Helianthus annuus (sunflower)*
Control	*131.97*	54.27	13.63	76.18	26.1
0.022	*130.77(0.91)*	49.60 (8.60)*	14.83 (-8.80%)	74.27 (2.52)	27.18 (-4.15)
0.043	*125.25 (5.09)*	52.17 (3.87)	13.2 (3.18)	72.92 (4.29)	25.1 (3.83)
0.087	*29.87 (1.59)*	49.72 (8.38)*	13.02 (4.52)	75.5 (0.90)	25.85 (0.96)
0.173	*123.52 (6.40)*	46.22 (14.83)*	13.52 (0.86)	76.22 (0.04)	26.23 (-0.51)
0.346	*78.73 (40.34)**	34.32 (36.76)*	10.93 (19.80)*	65.10 (14.55)*	14.90 (42.91)*
0.693	*17.60 (86.66)**	17.02 (68.64)*	9.43 (30.81)*	27.37 (64.08)*	4.68 (82.07)*
1.385	*12.00 (90.91)**	15.88 (70.75)*	10.50 (22.98)*	12.50 (83.59)*	--
Application rate (L test item/ha)	**Mean shoot length [cm] (% inhibition compared to control)**				
	Lycopersicon esculentum (tomato)	*Zea mays* (maize)	*Triticum aestivum* (wheat)	*Avena sativa* (oat)	*Allium cepa* (onion)
Control	46.66	182.1	52.58	49.06	43.33
0.022	48.3 (-3.51)	185.48 (-1.86)	49.5 (5.85)	47.03 (4.14)	41.97 (3.15)
0.043	46.13 (1.13)	184.68 (-1.42)	52.94 (-0.68)	44.75 (8.79)	41.13 (5.08)
0.087	49.48 (-6.05)	176.68 (2.97)	52.31 (0.51)	46.84 (4.52)	44.47 (-2.62)
0.173	42.60 (8.70)*	175.63 (3.55)	49.69 (5.50)	47.75 (2.68)	44.13 (-1.85)
0.346	21.40 (54.14)*	180.07 (1.12)	45.00 (14.41)*	45.3 (7.68)	37.43 (13.62)*
0.693	8.45 (81.90)*	42.25 (76.80)*	18.17 (65.45)*	23.29 (52.53)*	22.63 (47.77)*
1.385	8.08 (82.68)*	--	--	--	18.55 (57.19)*

* = significantly different when compared to the control (P = 0.05)
# data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).

Significant effects on shoot fresh weight were observed at one or more treatment rates of MON 52276 for all the tested species.

Table B.9.11-11: Effects of MON 52276 on shoot fresh weight after 21 days

Application rate (L test item/ha)	**Mean shoot fresh weight [g] (% inhibition compared to control)**				
	Cucumis sativus (cucumber) #	*Brassica napus* (oilseed rape)	*Raphanus sativus* (radish)	*Glycine max* (soybean)	*Helianthus annuus (sunflower)*
Control	*116.12*	102.3	9.13	54.39	62.63
0.022	*116.59 (-0.41)*	99.48 (2.76)	9.83 (-7.69)	54.83 (-0.81)	66.57 (-6.29)
0.043	*106.66 (8.14)*	95.26 (6.88)	8.53 (6.54)	51.28 (5.71)	63.93 (-2.08)
0.087	*109.45 (5.74)*	95.71 (6.45)	8.51 (6.74)	47.47 (12.73)*	63.23 (-0.95)
0.173	*99.67 (14.16)**	106.62 (-4.22)	6.43 (29.58)*	48.92 (10.06)*	53.79 (14.12)*
0.346	*76.62 (34.01)**	44.45 (56.55)*	4.13 (54.76)*	40.01 (26.44)*	26.57 (57.58)*
0.693	*15.72 (86.46)**	3.21 (96.86)*	1.60 (82.51)*	15.91 (70.75)*	3.54 (94.35)*
1.385	*13.62 (88.27)**	1.58 (98.46)*	0.96 (89.48)*	4.75 (91.27)*	--
Application rate (L test item/ha)	**Mean shoot fresh weight [g] (% inhibition compared to control)**				
	Lycopersicon esculentum (tomato)	*Zea mays* (maize)	*Triticum aestivum* (wheat)	*Avena sativa* (oat)	*Allium cepa* (onion)
Control	45.74	135.83	11.76	9.39	19.63
0.022	46.47 (-1.58)	134.28 (1.15)	9.68 (17.67)	9.11 (2.97)	19.94 (-1.60)
0.043	43.65 (4.58)	142.68 (-5.04)	11.29 (3.96)	9.33 (0.57)	17.51 (10.82)
0.087	47.8 (-4.49)	148.87 (-9.60)	11.85 (-.078)	8.37 (10.81)	19.72 (-0.48)
0.173	22.34 (51.16)*	145.92 (-7.43)	9.40 (20.08)*	9.17 (2.29)	20.8 (-5.97)
0.346	10.41 (77.23)*	140.71 (-3.59)	7.91 (32.71)*	8.15 (13.14)	15.16 (22.79)*
0.693	2.43 (94.70)*	5.55 (95.91)*	1.02 (91.29)*	1.76 (81.25)*	3.83 (80.47)*
1.385	1.35 (97.04)*	--	--	--	1.24 (93.70)*

* = significantly different when compared to the control (P = 0.05)
# data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).

The species *Glycine max* (soybean) showed significant effects on mortality at the highest treatment rate but reduction on mortality did not reach 50 %. Rate that cause 25 % of mortality was 1.297 L test item/ha (equivalent to 469.51 g acid equivalent/ha) but this was not statistically reliable for the lack of confident limits. Lethal rates (25 % and 50 % effect) were estimated for all the species with exception of *Glycine max* (soybean). The rates that cause 50 % of mortality with respect to the control group were ranged from
0.648 L test item/ha (equivalent to 234.58 g acid equivalent/ha) for *Triticum aestivum* (wheat) to 1.273 L test item/ha (equivalent to 460.83 g acid equivalent/ha) for *Brassica napus* (oilseed rape).

Table B.9.11-12: 21-day NOER, LOER, LR25 and LR50 values for survival

Family	Species	Common Name	MON52276 [L test item/ha]			
			NOER	LOER	LR25	LR50
Dicotyledonous species						
Cucurbitaceae	*Cucumis sativus #*	Cucumber #	*0.346 #*	*0.693 #*	*0.564 # (0.453; 0.659)*	*0.763 # (0.653; 0.896)*
Brassicaceae	*Brassica napus*	Oilseed rape	0.693	1.385	1.018 (0.817; 1.164)	1.273 (1.110; 1.515)
Brassicaceae	*Raphanus sativus*	Radish	0.346	0.693	0.753 (0.638; 0.849)	0.909 (0.804; 1.034)
Fabaceae	*Glycine max*	Soybean	0.693	1.385	1.297 (n.d.; n.d.)	> 1.385
Asteraceae	*Helianthus annuus*	Sunflower	0.346	0.693	0.673 (0.571; 0.764)	0.797 (0.705; 0.965)
Solanaceae	*Lycopersicon esculentum*	Tomato	0.346	0.693	0.767 (0.634; 0.874)	0.958 (0.837; 1.099)
Monocotyledonous species						
Poaceae	*Zea mays*	Maize	0.346	0.693	0.737 (0.678; 0.805)	0.806 (0.740; 0.883)
Poaceae	*Triticum aestivum*	Wheat	0.346	0.693	0.600 (0.570; 0.633)	0.648 (0.615; 0.685)
Poaceae	*Avena sativa*	Oat	0.346	0.693	0.666 (0.571; 0.752)	0.788 (0.701; 0.949)
Amaryllidaceae	*Allium cepa*	Onion	0.346	0.693	0.984 (0.756; 1.128)	1.271 (1.105; 1.477)

n.d.: not determined
data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).

Injury symptoms in monocotyledoneous species were assessed as modification in colour of leaves, that were either paler green going to almost white or reddish to brownish, finally leaves turned brown and died. There was a delay in the growth rate of the plants and in the plant size.
Injury symptoms in dicotyledoneous plants were assessed as modification in colour and malformation of leaves; the younger leaves of treated plants become chlorotic and growth ceases; leaf chlorosis was followed by necrosis; cupped, crinkled and small leaflets were also observed. No symptoms were observed in the control plants.

Table B.9.11-13: 21-day NOER and LOER values for phytotoxicity

Family	Species	Common Name	MON 52276 [L test item/ha]		
			NOER	LOER	Max phytotoxicity %
Dicotyledonous species					
Cucurbitaceae	*Cucumis sativus #*	Cucumber	0.173	0.346	50
Brassicaceae	*Brassica napus*	Oilseed rape	0.173	0.346	60
Brassicaceae	*Raphanus sativus*	Radish	0.043	0.087	60
Fabaceae	*Glycine max*	Soybean	0.173	0.346	50
Asteraceae	*Helianthus annuus*	Sunflower	0.173	0.346	60
Solanaceae	*Lycopersicon esculentum*	Tomato	0.173	0.346	60
Monocotyledonous species					
Poaceae	*Zea mays*	Maize	0.346	0.693	50
Poaceae	*Triticum aestivum*	Wheat	0.173	0.346	50
Poaceae	*Avena sativa*	Oat	0.173	0.346	50
Amaryllidaceae	*Allium cepa*	Onion	0.173	0.346	60

data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).

Effective rates (25 % and 50 % effect) were estimated for all the tested species. The rates at which mean plant shoot height were reduced by 50 % with respect to the control group were ranged from 0.339 L test item/ha (equivalent to 122.72 g acid equivalent/ha) for *Lycopersicon esculentum* (tomato) to 1.336 L test item/ha (equivalent to 483.63 g acid equivalent/ha) for *Raphanus sativus* (radish).

Table B.9.11-14: 21-day NOER and LOER, ER_{25} and ER_{50} values for shoot height

Family	Species	Common Name	MON 52276 [L test item/ha]			
			NOER	LOER	ER25	ER50
Dicotyledonous species						
Cucurbitaceae	*Cucumis sativus #*	Cucumber #	*0.173*	*0.346*	*0.285 (0.241; 0.318)*	*0.396 (0.360; 0.432)*
Brassicaceae	*Brassica napus*	Oilseed rape	0.043	0.087	0.243 (0.203; 0.279)	0.516 (0.461; 0.573)
Brassicaceae	*Raphanus sativus*	Radish	0.173	0.346	0.484 (0.386; 0.607)	>1.385[$] (0.869; 2.025)
Fabaceae	*Glycine max*	Soybean	0.173	0.346	0.415 (0.390; 0.437)	0.604 (0.579; 0.629)
Asteraceae	*Helianthus annuus*	Sunflower	0.173	0.346	0.276 (0.251; 0.298)	0.392 (0.368; 0.417)
Solanaceae	*Lycopersicon esculentum*	Tomato	0.087	0.173	0.223 (0.200; 0.244)	0.339 (0.315; 0.363)
Monocotyledonous species						
Poaceae	*Zea mays*	Maize	0.346	0.693	0.517 (0.439; 0.610)	0.595 (0.499; 0.716)
Poaceae	*Triticum aestivum*	Wheat	0.173	0.346	0.427 (0.392; 0.465)	0.583 (0.529; 0.644)
Poaceae	*Avena sativa*	Oat	0.346	0.693	0.531 (0.459; 0.614)	0.689 (0.572; 0.828)
Amaryllidaceae	*Allium cepa*	Onion	0.173	0.346	0.465 (0.357; 0.557)	0.912 (0.786; 1.042)

data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).
[$] estimated value in the study report : ER50 = 1.336 L/ha (95% CI: 0.869; 2.025)

Effective rates (25 % and 50 % effect) were estimated for all the species. The rates at which mean plant shoot fresh weight were reduced by 50 % with respect to the control group were ranged from 0.193 L test item/ha (equivalent to 69.87 g acid equivalent/ha) for *Lycopersicon esculentum* (tomato) to 0.531 L test item/ha (equivalent to 192.22 g acid equivalent/ha) for *Zea mays* (maize).

Table B.9.11-15: 21-day NOER, LOER, ER_{25} and ER_{50} values for shoot fresh weight

Family	Species	Common Name	MON 52276 [L test item/ha]			
			NOER	LOER	ER25	ER50
Dicotyledonous species						
Cucurbitaceae	*Cucumis sativus #*	Cucumber #	*0.087*	*0.173*	*0.298 (0.248; 0.358)*	*0.427 (0.347; 0.531)*
Brassicaceae	*Brassica napus*	Oilseed rape	0.173	0.346	0.241 (0.207; 0.308)	0.322 (0.284; 0.374)
Brassicaceae	*Raphanus sativus*	Radish	0.087	0.173	0.152 (0.110; 0.187)	0.288 (0.237; 0.341)
Fabaceae	*Glycine max*	Soybean	0.043	0.087	0.341 (0.300; 0.388)	0.524 (0.453; 0.610)
Asteraceae	*Helianthus annuus*	Sunflower	0.087	0.173	0.211 (0.175; 0.241)	0.303 (0.270; 0.338)
Solanaceae	*Lycopersicon esculentum*	Tomato	0.087	0.173	0.130 (0.106; 0.159)	0.193 (0.154; 0.247)
Monocotyledonous species						
Poaceae	*Zea mays*	Maize	0.346	0.693	0.485 (0.257; 0.797)	0.531 (0.356; 0.711)
Poaceae	*Triticum aestivum*	Wheat	0.087	0.173	0.328 (0.261; 0.411)	0.431 (0.333; 0.562)
Poaceae	*Avena sativa*	Oat	0.346	0.693	0.420 (0.328; 0.539)	0.523 (0.402; 0.691)
Amaryllidaceae	*Allium cepa*	Onion	0.173	0.346	0.363 (0.291; 0.453)	0.483 (0.377; 0.625)

data for cucumber (*Cucumis sativus*) are not considered reliable by RMS (see RMS commenting box below).

B. OBSERVATIONS

Chemical analysis was performed on the stock solution at the highest concentration of 1.385 L test item/ha.
The concentrations ranged from 98 to 102 % of nominal, so the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

It can be concluded that MON 52276 had significant effects on mortality, shoot height and on shoot fresh weight for all the species at the tested rates. The most sensitive endpoint proved to be shoot height and shoot fresh weight, where numeric endpoints (ER50 values) could be estimated for all the tested plant species. The overall lowest ER50 was 0.193 L test item/ha (equivalent to 69.87 g acid equivalent/ha) for the species *Lycopersicon esculentum* (tomato) in shoot fresh weight. The overall lowest NOER was estimated to be 0.043 L test item/ha (equivalent to 15.57 g equivalent/ha), based on nominal treatment levels for shoot height of *Brassica napus* (oilseed rape) and *Glycine max* (soybean) for shoot fresh weight.

The following points deviated from the current guideline recommendations:

- Light intensity was lower than 350 µE/m^2/s (300 µE/m2/s was used)

However, there were no phytotoxic effects observed in the controls for any of the species tested, meaning that the growing conditions were appropriate for the species. In addition any competition for light was minimized considering that due to the test species being different in their size and growth requirements, numbers of test plants per pot and pots per replicate were adjusted accordingly.

According to the study authors, the validity criteria according to the OECD 227 were fulfilled. The seedling emergence was at least 70 % (actual values from 74.6 to 99.6 %). In the control, the plants did not exhibit visible phytotoxic effects; the mean plant survival is at least 90 % for the duration of the study (actual value 100%); environmental conditions for a particular species were identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The lowest ER50 was 0.193 L test item/ha (equivalent to 69.87 g acid equivalent/ha) for the dicotyledonous species *Lycopersicon esculentum* (tomato) in shoot fresh weight. The lowest ER50 was 0.431 L test item/ha (equivalent to 156.02 g acid equivalent/ha) for the monocotyledonous species *Triticum aestivum* (wheat) in shoot fresh weight.

The validity criteria were met and the study is classified as valid.

Assessment and conclusion by RMS:

Test item: MON52276 (applied on the soil surface)
Natural soil was used.

The applicant indicates that the validity criteria for seedling emergence is fulfilled as the actual values for emergence ranged from 74.6 % to 99.6%. The raw data of the seedling emergence during the test was not available in the study report and the actual values of seedling emergence cannot be checked. However, the germination rates obtained in germination trials performed when the seed batches were acquired are reported to be between 95 and 97.5%. In addition in the seedling emergence study performed in the same laboratory and with same seed batches, the seedling emergence of control was found to fulfill the validity criteria (control seedling emergence ranged from 75 to 100% in [redacted] 2019, CP 10.6.2/001). RMS just noted that for *Glycine max* and *Triticum* the germination rates of the seed batches were not the same but still in same range). Overall, RMS considered that this validity criteria could be considered as met.

The validity criteria according to the current guideline OECD 227 are considered to be fulfilled.

Minor deviations were noted:
- Light intensity ranged from 696 to 826 µE/m2/s in test 1 and from 726 to 745 µE/m2/s in test 2. OECD 227 recommendation is 350 ± 50 µE/m2/s. However, RMS agrees with the applicant that this deviation does not have an influence on the study outcome.
- The relative humidity was out of the recommended ranges for short-term periods (< 2 hours). However, RMS agrees with the applicant that this deviation does not have an influence on the study outcome.
- No reference substance or historical data were mentioned in the report. RMS considers that this deviation is acceptable but increases the uncertainty on sensitivity of the test system.
- Some inconsistencies were noted in phytotoxicity results between table 4 and tables 16 (*Cucunus sativus*), 22 (*Glycine max*), 30 (*Triticum aestivum*), 32 (*Avena sativa*), 34 (*Allium cepa*) of the study report. However, these are minor differences and they have no impact on the outcome of the study.

Values presented by applicant in table 9.11-9 of this summary were corrected by RMS according to raw data tables of the study report.

Notes :
- In table "4.4 Test Species, Replicates and Treatment Groups" of the study report, the note "** 5 test item treatment groups and 1 control group " seems incorrect, as there was not 5 but 7 test item treatment groups and 1 control group.
- In table 25 of the study report, mean mortality for treatment 6 for Tomato should be 16.67% instead of 16.17%. However, the correct value was reported in summary table 1 "mean mortality data" of the study report. The correct value is also presented in this study summary by the applicant.

For tomato, BBCH of plants in the control group and in groups T1 to T4 vary from 18 to 62 at the end of the study (i.e. from end of leaf development stage to flowering stage with already open flower). Given than no major difference in height was observed, and that it was observed both in the control and treated groups (T1 to T4), RMS considers that this is not likely to influence the results reported for tomato.

For the species *Brassica napus* (oilseed rape), statistically significant differences on shoot height was found at treatment T1 and T3 while no statistically significant reduction was observed in treatment T2. RMS agrees with the applicant that the reduction found at treatment T1 was most probably due to a biological variability as no statistically significant reduction was observed in treatment T2 and reported the NOER as T3.

Mean mortality values for cucumber differ in raw data table 16 (*Cucumis sativus* mortality results) and raw data table 17 (*Cucumis sativus* individual results) of the study report for treatments 5 at D21, treatment 6 at D14 and D21, and treatment 7 at D7, D14, D21. Moreover, height and weight values and phytotoxicity of raw data table 17 are not consistent with mortality values of raw data table 16 (for example, height, weight and phytotoxicity data are available at day 21 in table 17 for plants who were already dead at day 14 according to table 16). Therefore, data for cucumber are not considered reliable and endpoints for cucumber presented by applicant in this study summary should not be taken into account as basis for risk assessment.

The applicant did not calculate EC50 for phytotoxicity. However, for all species except *Helianthus annuus,* less than 40% effects were observed at 0.346 L test item/ha. Therefore EC50 for phytotoxicity > 0.346 L test item/ha for all species except *Helianthus annuus.*
For Helianthus annuus, 30 to 50% effects were observed at 0.346 L test item/ha and 0% effects were observed at 0.173 L test item/ha. Therefore, EC50 for *Helianthus annuus* should be close to 0.346 L test item/ha. For this species, it is proposed to set the EC50 on phytotoxicty to ≥ 0.346 L test item/ha. Therefore, EC50 is close to or higher than 0.346 L test item/ha (equivalent to 125.3 g glyphosate acid/ha) for all species, which is higher than the lowest ER50 for *Lycopersicon esculentum*.

The lowest reliable ER50 value is 0.193 L MON52276/ha (equivalent to 69.87 g glyphosate acid/ha) based on shoot fresh weight of *Lycopersicon esculentum* (tomato).
The lowest reliable NOER value is 0.043 L MON52276/ha (equivalent to 15.7 g glyphosate acid/ha) based on shoot fresh weight of *Glycine max* (soybean) and shoot height of *Brassicus napus* (oilseed rape).

B.9.11.3. Extended laboratory studies on non-target plants

No data available.

B.9.11.4. Semi-field and field tests on non-target plants

No data available.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

Toxicity tests for effects on terrestrial non-target plants have been performed with the active substance and the representative formulation MON 52276. Endpoints of the studies are presented in the table below.

Table B.9.12-1 : Studies on toxicity of the active substance glyphosate and the representative formulation MON52276 to terrestrial non-target higher plants

Annex point	Study	Study type	Test species	Substance (s)	Status	ER_{50} (g a.e./ha)	NOER (g a.e./ha)	Remark
CA 8.6.2/001	[redacted] 1994	Vegetative vigour, 21d	Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, Onion	Glyphosate	Valid	145.7 (tomato, dry weight)		ER50 is provisional Data gap set for ECx values for phytotoxicity
CA 8.6.2/002	[redacted] 1994		Onion, Field corn, Oat, Wheat, Soybean, Radish, Cucumber, Sunflower, Tomato, Carrot	Glyphosate	Invalid			already invalid in RAR 2015
CP 10.6.2/001	[redacted], 2019	Seedling emergence, 21d	*Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esculentum* *Zea mays* *Triticum aestivum* *Avena sativa* *Allium cepa*	MON 52276	Valid	> 3610 (all tested species and all parameters)	≥ 3610 (all tested species and all parameters)	-
CP 10.6.2/002	[redacted] 2013	Vegetative vigour, 21d	*Zea mays* *Avena sativa* *Allium cepa* *Triticum aestivum* *Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esulentum*	MON 52276	supportive	28.4 (cucumber, shoot length)	< 20 (cucumber: shoot length, shoot weight; sunflower, tomato: shoot weight)	potential under estimation of effects
CP 10.6.2/003	[redacted] 2005	Vegetative vigour, 21d	*Beta vulgaris* *Raphanus rapistrum* *Lepidium sativum* *Pisum sativum*	MON 52276	Invalid	-	-	already invalid in RAR 2015

			Lolium perenne *Triticum aestivum*					
CP 10.6.2/ 004	[redacted] 2012	Comparison of Post-Emergence Phytotoxicity	*Echinochloa crus-galli* *Xanthium strumarium* *Zea mays* *Digitaria ischaemum* *Setaria veridis* *Chenopodium album* *Ipomoea sp.* *Panicum miliaceum* *Oryza sativa* *Polygonum pensylvanicum* *Sorghum bicolor* *Glycine max* *Beta vulgaris* *Abutilon theophrasti* *Triticum aestivum* *Polygonum convolvulus*	MON 52276 and AMPA	Supportive	-	-	Full evaluation of study not feasible
CP 10.6.2/ 005	[redacted] 2021	Vegetative Vigour test, 21d	*Zea mays* *Avena sativa* *Allium cepa* *Triticum aestivum* *Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esulentum*	MON 52276	Valid but result of cucumber unreliable	69.87 (shoot fresh weight of *Lycopersicon esculentum* (tomato)	15.7 (shoot fresh weight of *Glycine max* (soybean) and shoot height of *Brassicus napus* (oilseed rape).	Results for cucumber are not reliable.

Literature data on non-target terrestrial plants

Studies related to indirect effects/biodiversity are not considered here. The conclusions of RAR 2015 and the current dossier on those issues are reported under Volume 3 CP B.9 point B.9.14.1.7.
Here below are reported the studies that provide data on parameters of relevance for the risk assessment.

The scientific literature review conducted for the last Annex I renewal (submitted in 2012) that appears in the RAR (2015) did not provide relevant/reliable endpoints for use in the risk assessment.

From the current literature review, among the studies from which full-text and summaries were submitted, RMS identified one study relevant for the risk assessment of glyphosate for direct effects assessment. Please note that RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents. Therefore the consideration of literature studies in weight of evidence will have to be reconsider.

Rogacz D. et al., (2020) investigated the ecotoxicological and herbicidal effect of glyphosate on oat and radish based on OECD 208 guideline. The following endpoints were derived from this study:

- Shoot height: Glyphosate gave the EC50 value 373.7 mg/kg s.d.w. for oat shoots, and 357.8 mg/kg s.d.w. for radish shoots.
- Root length: Glyphosate was almost twice more toxic to radish roots (EC50 = 269.3 mg/kg) than to oat roots (EC50 = 556.9 mg/kg).
- Fresh mass: Radish: EC50 = 333.2 mg/kg, oat: EC50 = 418 mg/kg.

It was noted that glyphosate caused accumulation of carotenoids in leaves of both tested plants.
Effects of glyphosate were observed even at its lowest applied concentration 100 mg/kg of soil dry weight but RMS notes that the concentrations tested are above those expected in real conditions of use. RMS considers that critical validity criteria are lacking (as highlighted by the applicant). No information on the study methodology and environmental conditions were reported in this article. These informations are reported in a supplementary document.
Thus the study is considered only supportive.

Overall there is no studies that may impact the outcome the risk assessment of direct effects. This may be reconsider as RMS identified some studies in Volume 3 CA B.9 under the table "List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant" and data gap in table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents.

Risk assessment for Terrestrial Non-Target Higher Plants

The table below summarises the intended uses and the risk envelop proposed. The risk assessment is presented for the application rates of the grey shaded cells in the table, which represent the worst case exposure to non-target plants for each crop type for the proposed uses of MON 52276. The conclusions of the risk assessment are protective of the other uses.

Table B.9.12-2 : Risk assessment strategy for terrestrial non-target plants

GAP number and summary of use	Application rate considered (28 day internal unless otherwise stated)								
	1 × 540 g/ha	1 × 720 g/ha	2 × 720 g/ha	3 × 720 g/ha	1 × 1080 g/ha	2 × 1080 g/ha[A]	1 × 1440 g/ha	2 × 1440 g/ha	1 × 1800 g/ha	2 × 1800 g/ha (90 days apart)
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre-emergence of **field crops.**		X			X		X*			
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of **field crops.**		X	X	X	X	X	X*			
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of **field crops.**	X									
Use 6 a-b: Applied to weeds (post-emergence) in **field crops BBCH < 20**		X			X					
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of **field crops**		X			X					
Use 4 a-c: Applied to weeds (post-emergence) below trees in **orchards.**		X	X	X**	X	X	X	X		
Use 5 a-c: Applied to weeds (post-emergence) below vines in **vineyards**		X	X	X**	X	X	X	X		
Use 7 a-b: Applied to weeds (post-emergence) around **railroad tracks**									X	X
Use 8 and 9: Applied to invasive species (post-emergence) in **agricultural and non-agricultural areas**									X	

X = this use is covered by the application rate indicated in the grey cell.
Grey shaded cells: risk assessment presented below, representing worst case exposure
* the applicant proposed a risk assessment at 2 x 1080 g/ha, therefore the use field crops at 1 x 1440 g/ha would not be covered. RMS proposed a risk assessment at 1x1440 g as/ha.
** risk assessment at 3 x 720 g /ha added by RMS.
[A] Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.s./ha plus 1 x 720 g a.s./ha (28 day interval between each application)

The risk assessment for non-target terrestrial plants is presented according to the uses described in the table above and grouped as follows:

- in field crops; covering GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c.
- in orchards/vineyards; covering GAP uses 4 a-c, 5 a-c.
- around railroad tracks; covering GAP uses 7 a-b.
- in agricultural and non-agricultural areas to control invasive species; covering GAP uses 8 and 9.

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

The amount of spray drift reaching off-crop habitats is based on the spray-drift predictions (90th percentile) of Rautmann (2001) to calculate maximum off-field predicted environmental rates (PER). The estimated spray drift deposition for one application for field crops at distances of 1, 5 and 10 meters from the target area, are 2.77, 0.57 and 0.29%.

Sideward and upaward applications are no intended uses as it is an herbicide. Therefore, only downward applications were considered for the risk assessment.

According to the SANCO/10329/2002 guidance document on terrestrial organisms, no MAF is needed for calculation of exposure estimate. For information, a foliage DT50 of 2.8 days is available (see Volume 3 B.9 (PPP) B.9.2).The risk assessment for effects on non-target plants is performed in a step-wise approach, first using a deterministic approach and then a probabilistic approach.

The "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002) states in paragraph "Tier 2: Quantitative risk assessment" that "Probabilistic methods that make use of the species sensitivity distribution would be straightforward in this assessment step as data from 6-10 species are available. […] This approach requires that log-normal or another defined type of distribution has been shown to fit the data adequately." If the ER_{50} for less than 5% of the species is above the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable.

Deterministic Risk Assessment for Non-target Terrestrial Plants

The deterministic approach is performed using the most sensitive endpoint from the vegetative vigour and seedling emergence studies.
For seedling emergence, ER_{50} values for all tested species and all parameters are found to be greater than 3.610 kg glyphosate acid/ha(>10.00 L MON52276/ha) ([redacted], 2019, CP 10.6.2/001).

For vegetative vigour, two studies with the representative formulation are available.
The first study ([redacted] 2013, CP 10.6.2/002) is considered as supportive.The lowest endpoint obtained in this study was 28.4 g glyphosate acid/ha) based on shoot height of *Cucumis sativus* (cucumber).
The second study ([redacted] 2021, CP 10.6.2/005) is considered valid, except for cucumber, for which the results are not reliable. The lowest reliable ER_{50} value in this study is 69.87 g glyphosate acid/ha (equivalent to 0.193 L MON52276/ha) based on shoot fresh weight of *Lycopersicon esculentum* (tomato).
In the supportive study by [redacted] (2013), *Cucumis sativus* (cucumber) was the most sensitive species to MON52276 for mortality and height and provided the smallest endpoint for all parameters and all species tested. In the recent study of [redacted] (2021), this species also exhibit a sensitivity to MON52276 but the results are not considered reliable. Considering the information from [redacted] (2013), RMS considered that there is some uncertainty that the endpoint on tomato obtained in [redacted] (2021) will be conservative enough to cover the potential effects on cucumber. Therefore, the results of both studies were considered together and the smallest endpoint of 28.4 g glyphosate acid/ha was used in the risk assessment for vegetative vigor.

Field Crops

Table B.9.12-3 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – field crops considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Field Crops – GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, & 10 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	2.77	19.9	**1.42**
	1 x 1440			39.9	**0.71**
Seedling emergence					
All uses considering downward ground directed spray	3 x 720	>3610	2.77	19.9	>181
	1 x 1440			39.9	>90.5

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Orchards

Table B.9.12-4 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – orchards considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Orchards / vineyards – GAP uses 4 a-c & 5 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	2.77	19.9	**1.42**
	2 x 1440			39.9	**0.71**
Seedling emergence					
All uses considering downward ground directed spray	3 x 720	>3610	2.77	19.9	>181
	2 x 1440			39.9	>90.5

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Railroad tracks

Table B.9.12-5 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – railroad tracks considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Railroad tracks GAP uses 7 a-b					
Vegetative vigour					
All uses considering downward ground directed spray	2 x 1800	28.4	2.77	49.86	**0.57**
Seedling emergence					
All uses considering downward ground directed spray	2 x 1800	>3610	2.77	49.86	>72.4

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Agricultural and non-agricultural area – Invasive species

Table B.9.12-6 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – Agricultural and non-agricultural area – Invasive species considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Agricultural and non-agricultural area – Invasive species – uses 8 & 9					
Vegetative vigour					
All uses considering downward ground directed spray	1 x 1800	28.4	2.77	49.86	**0.57**
Seedling emergence					
All uses considering downward ground directed spray	1 x 1800	>3610	2.77	49.86	>72.4

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Based on seedling emergence data, all TER values are above the trigger value of 5, based on PER at 1m from the application area. Therefore, no refinement is required for seedling emergence.
Based on vegetative vigor data, all TER values are below the trigger value of 5, based on PER at 1m from the application area. Therefore, further refinement is required.

A refined deterministic risk assessment based on the vegetative vigour endpoint is presented below based on PER calculated considering drift rates (%) at 5 and 10 m from the application area.

Field Crops

Table B.9.12-7 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – field crops considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Field Crops – GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, & 10 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	0.57 – at 5 m	4.10	6.92
	1 x 1440			8.21	**3.46**
All uses considering downward ground directed spray	1 x 1440		0.29 – at 10 m	4.18	6.80

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Orchards

Table B.9.12-8 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – orchards considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Orchards / vineyards – GAP uses 4 a-c & 5 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	0.57 – at 5 m	4.10	6.92
	2 x 1440			8.21	**3.46**
All uses considering downward ground directed spray	2 x 1440		0.29 – at 10 m	4.18	6.80

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Railroad tracks

Table B.9.12-9 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – railroad tracks considering downward ground directed spray

Crop scenario	**Appl. Rate [g a.e./ha]**	**ER_{50} [g a.e./ha]**	**Drift [%]**	**PER [2] [g a.e./ha]**	**TER (criterion: TER ≥5)**
Railroad tracks – use 7 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	2 x 1800	28.4	0.57 – at 5 m	10.26	**2.77**
			0.29 – at 10 m	5.22	5.44

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Agricultural and non-agricultural area – Invasive species

Table B.9.12-10 : Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – Agricultural and non-agricultural area – Invasive species considering downward ground directed spray

Crop scenario	**Appl. Rate [g a.e./ha]**	**ER_{50} [g a.e./ha]**	**Drift [%]**	**PER [2] [g a.e./ha]**	**TER (criterion: TER ≥5)**
Agricultural and non-agricultural area – Invasive species – uses 8 & 9					
Vegetative vigour					
All uses considering downward ground directed spray	1 x 1800	28.4	0.57 – at 5 m	10.26	**2.77**
			0.29 – at 10 m	5.22	5.44

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

An acceptable risk for non-target terrestrial plants is demonstrated at 2L MON52276/ha (equivalent to 720 g a.e./ha) when considering deposition via drift at 5m for field crop uses and orchards/vineyards.
An acceptable risk for non-target terrestrial plants is demonstrated at 3, 4 and 5 L MON52276/ha (equivalent to 1080, 1440 and 1800 g a.e./ha respectively) when considering deposition via drift at 10m.

Risk reduction

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The applicant proposed to use drift-reducing nozzles as mitigation mesures, but did not provide calculations. Therefore, RMS provided a risk assessment using these mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %), which issummarised in the following tables.

Field crops

Table B.9.12-11 : Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in field crops (3 x 720 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones and drift-reducing nozzles)

Intended use		Field crops		
Application rate (g a.e./ha)		3 x 720		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	9.97	4.99	-
5	0.57	2.05	-	-
10	0.29	-	-	-
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**2.85**	5.70	-
5		13.84	-	-
10		-	-	-

Table B.9.12-12 : Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in field crops (1 x 1440 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Field crops		
Application rate (g a.e./ha)		1 x 1440		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	19.94	9.97	3.99
5	0.57	4.10	2.05	0.82
10	0.29	2.09	1.04	0.42
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.42**	**2.85**	7.12
5		6.92	13.84	34.60
10		13.60	27.20	68.01

Orchards

Table B.9.12-13 : Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in orchards (3 x 720 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones and drift-reducing nozzles)

Intended use		Orchards		
Application rate (g a.e./ha)		3 x 720		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	9.97	4.99	-
5	0.57	2.05	-	-
10	0.29	-	-	-
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**2.85**	5.70	-
5		13.84	-	-
10		-	-	-

Table B.9.12-14 : Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in orchards (2 x 1440 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Orchards		
Application rate (g a.e./ha)		2 x 1440		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	19.94	9.97	3.99
5	0.57	4.10	2.05	0.82
10	0.29	2.09	1.04	0.42
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.42**	**2.85**	7.12
5		6.92	13.84	34.60
10		13.60	27.20	68.01

Railroad tracks

Table B.9.12-15 : Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 on railroad tracks (2 x 1800 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Railroad tracks		
Application rate (g a.e./ha)		2 x 1800		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	24.93	12.47	4.99
5	0.57	5.13	2.57	1.03
10	0.29	2.61	1.31	0.52
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.14**	**2.28**	5.70
5		5.54	11.07	27.68
10		10.88	21.76	54.41

Agricultural and non-agricultural area – Invasive species

Table B.9.12-16 : Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in agricultural and non-agricultural area – invasive species (1 x 1800 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Agricultural and non-agricultural area – Invasive species		
Application rate (g a.e./ha)		1 x 1800		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	24.93	12.47	4.99
5	0.57	5.13	2.57	1.03
10	0.29	2.61	1.31	0.52
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.14**	**2.28**	5.70
5		5.54	11.07	27.68
10		10.88	21.76	54.41

Probabilistic Risk Assessment

The applicant proposed a probabilistic risk assessment as additional refinement for risk assessment based on vegetative vigor.

For that purpose, the applicant proposed to derive an HC_5 value (hazardous concentration for 5% of the population) considering the results of the supportive study of [redacted] (2013, CP 10.6.2/002).

RMS has considered the use of this supportive data for purpose of tier 1 risk assessment. For higher tier risk assessment, RMS is of the opinion that the validity and reliability of the data used to derive an HC5 should be established. Considering that the results on cucumber, the most sensitive plants in [redacted] (2013), is not reliable in the new vegetative vigour study ([redacted] 2021, CP 10.6.2/005), a HC5 based on [redacted] (2021) may not be suitable. Therefore, no robust probabilistic risk assessment is considered based on the avaible information.

Conclusion of RMS

The risk to non target plants can be considered acceptable when risk mitigations to protect non target terrestrial plants at the edge of the field are implemented. The risk mitigations are reported in the table below.

Table B.9.12-17 : Risk mitigation measures for terrestrial non-target plants

GAP number and summary of use	Application rate considered (28 day internal unless otherwise stated)									
	1 × 540 g/ha	**1 × 720 g/ha**	**2 × 720 g/ha**	**3 × 720 g/ha**	**1 × 1080 g/ha**	**2 × 1080 g/ha[A]**	**1 × 1440 g/ha**	**2 × 1440 g/ha**	**1 × 1800 g/ha**	**2 × 1800 g/ha (90 days apart)**
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre-emergence of **field crops.**		5m BS Or 75% drif-reducting nozzles			10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles		10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles			
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of **field crops.**		5m BS Or 75% drif-reducting nozzles			10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles		10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles			
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting	5m BS Or 75% drif-reducting nozzles									

of **field crops.**										
Use 6 a-b: Applied to weeds (post-emergence) in **field crops BBCH < 20**		5m BS Or 75% drif-reducting nozzles			10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles					
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of **field crops**		5m BS Or 75% drif-reducting nozzles			10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles					
Use 4 a-c: Applied to weeds (post-emergence) below trees in **orchards.**		5m BS Or 75% drif-reducting nozzles			10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles					
Use 5 a-c: Applied to weeds (post-emergence) below vines in **vineyards**		5m BS Or 75% drif-reducting nozzles			10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles					
Use 7 a-b: Applied to weeds (post-emergence) around **railroad tracks**									10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles	
Use 8 and 9: Applied to invasive species (post-emergence) in **agricultural and non-agricultural areas**									10m BS Or 5m BS and 50% drif-reducting nozzles Or 90% drif-reducting nozzles	

BS = Untreated buffer strip

[A] Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.s./ha plus 1 x 720 g a.s./ha (28 day interval between each application)

A summary of the risk assessment regarding non-target plants biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

The regulation (EU) 2017/2324 related to the approval of glyphosate stated that "Member States shall pay particular attention (…) to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions". Currently, there is no validated tools nor methodology for a European harmonized risk assessment of biodiversity and consideration of indirect effects via trophic interactions available.
RMS recommended to the applicant to have a broader consideration of potential effects on non target organisms by exploring the current state of the art in order to identify potential new data/information or new approach or tools that may help to provide some quantitative information to address this specific concern[38]. RMS also advises to use monitoring data to address the point. Furthermore, given the magnitude of use of glyphosate based herbicides, glyphosate is frequently observed in the environment. Even if biodiversity is not affected by glyphosate alone, its effects on biodiversity should be addressed. Indeed, a loss of vegetation/plant biodiversity following the application of plant protection products may affect on the entire food web. It could affect the presence of adequate habitats for arthropods, as well as for birds and mammals. Moreover the presence of appropriate range of plants as food sources is vital to the survival of foliage eating arthropods, birds and mammals, as well as nectar and pollen sources for bees.

The applicant has provided a report entitled "Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment" [redacted] 2020, CA 8.7/001).

Data point	CA 8.7/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment
Report No	TRR0000305
Document No	-
Guidelines followed in study	Not relevant
Deviations from current test guideline	Not relevant.
Previous evaluation	No, submitted for the purpose of renewal
GLP/Officially recognised testing facilities	Not relevant.
Acceptability/Reliability (RMS)	Accepatble

The report of [redacted] (2020) presents the applicant's approach for assessing the risk to biodiversity by informing on potential indirect effects and trophic interactions. This report also provides information for risk managers aiming to provide additional risk mitigations options to protect aquatic and terrestrial biodiversity. The introduction part of the report given general consideration related to the use of glyphosate has been summarized at the beginning of this Volume 3 CP B.9.

[38] Minutes pre-submission meeting GTF2-RMS Fate & Behaviour, Ecotoxicology, Endocrine Disruption of 17/10/2019

In summary, the applicant made an attempt to assess biodiversity via an assessment of indirect effects and trophic interactions. RMS noted that even if indirect effects and trophic interactions are linked to biodiversity, there is much more to consider to protect biodiversity and the providing ecosystem services in Europe in adequacy with the various EU and national legislations. The approach of the applicant mainly focus on definition of Specific Protection Goals that are taken into account in the existing guidance documents. The EFSA guidance on specific protection goals (2016)[39] aims to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services. The method to define SPG follow "three sequential steps: (1) the identification of relevant ecosystem services; (2) the identification of service providing units (SPUs) for these ecosystem services; and (3) the specification of options for the level/parameters of protection of the SPUs using five interrelated dimensions" (ecological entity, attribute to protect, magnitude of effects, temporal scale and spatial scale of the biologically tolerable effects). Definition of SPG require a dialogue between risk assessors and risk managers. RMS considered that it is the most suitable approach available to assess biodiversity in the context of regulatory risk assessment.

The assessment of biodiversity via indirect effects and trophic interactions is presented under B.9.14. It is based on the assessment document of the applicant that considered information of [redacted] 2010 (CA 8.7/001). This assessment is based on literature studies for which summaries have been compiled in the "Appendix to Volume 3 B.9 PPP / Literature data on biodiversity".). The table below provided the main information of each study.

Table B.9.13-1 RMS review of referred papers from public literature related to the biodiversity/indirect effect assessment.

Reference	RMS coment and conclusion	Use of specific information (type of field)	Glyphosate specific information	Direction of glyphosate-induced effect [1]	Relevance for biodiversity assessment
Birds and wild mammals					
Anthony and Morrison 1985	Overall, the results of this experimental study indicate that the abundance, diversity, and biomass of small mammals increased one year post-spray in forestry clearcuts due to increased herbaceous cover and returned to pre-spray levels two years post-spray, possibly due to the recovery of shrubs.	Forestry (clearcuts)	**Yes**	↑↓	Yes
Boatman *et al* 2004	This study illustrates that indirect effects of pesticides in general on birds (e.g. brood reduction, chick condition and number of chicks fledging) are documented for several species, most comprehensive data being on the Grey Partridge. However, the importance of pesticides in relation to	Farmland	No	N/A	General background information

[39] EFSA Scientific Committee, 2016. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 2016;14(6):4499, 50 pp. doi:10.2903/j.efsa.2016.4499

	other stressors affecting farmland birds is still unclear.				
Bright *et al* 2008	This review shows that agricultural intensification has led to large declines in the abundance and range of farmland species over the last four decades. Three main pathways leading to pesticide-induced indirect effects on birds are discussed: direct and indirect reduction of invertebrate abundance and direct reduction in weed seeds.	Farmland	No	N/A	General background information
Burfield 2005	This book illustrates the distribution of birds in steppic (agricultural) habitats. Of the 65 birds identified as priority species for these habitats, 83% had an unfavourable conservation status in Europe.	Farmland	No	N/A	General background information
Campbell and Cooke 1997	This review highlights that identifying the most important factor causing declines in the abundance of bird feed and birds in agricultural areas is not possible due to limited data, with the exception of the grey partridge, whose decline was directly attributed to pesticide use. Nonetheless, short-term declines in farmland bird feed (invertebrates and plants) were linked to pesticide use (% crop area sprayed). The RMS notes that there are other statements in the paper that give a bit more weight to the role of pesticides (pg 9): *"Although the observed long-term declines in invertebrates and plants have taken place during a period of considerable change in agricultural practices and could have been caused by a range of factors, the scale of the short-term effects of*	Farmland	No	N/A	General background information

	pesticides suggest that they are likely to be one of the more important factors influencing the gross abundance of potential bird food items."				
Cunningham *et al* 2005	This study shows that skylarks, granivorous passerines and gamebirds occupied a greater proportion of fields established by non-inversion tillage methods than conventional tillage. Also, more species of granivorous passerines were found in non-inversion tillage fields. Hence, it can be concluded that the intended use of glyphosate in non-inversion tillage systems may influence the species composition in the field.	Farmland (non-inversion tillage)	No	N/A	General background information
D'Anieri *et al* 1987	The results of this experimental study indicate that Southern Red-backed Voles were less abundant in the one-year-old spray area than in the control area, whereas species richness was not affected by glyphosate treatment.	Forestry (five-year-old clearcuts)	**Yes**	↓—	Yes
DEFRA 2005	A large-scale field experiment was carried out for testing Type 1 and 3 indirect effects by altering the food supplies directly (increasing seed densities) and by decreasing arthropod densities using an insecticide. The results show reductions in breeding productivity of yellowhammers due to depletion of arthropod food sources following the use of pesticides. In addition, a model was developed for assessing risks to yellowhammers and pesticide mitigation measures are discussed. No specific results from the use of glyphosate were presented in the report, except for a statement; "…	Farmland (non-inversion tillage)	No	N/A	General background information

	most rotational set-aside is sprayed with glyphosate, in April or May, to prevent weeds from seeding and to clear the ground prior to cultivations for the following crop. The destruction of the vegetation early in the nesting season renders nesting birds vulnerable to predation, and also reduces the density of invertebrates by removing their habitat and food plants."				
Donald *et al* 2006	This study shows that a significant decline in 19 out of the 58 species of farmland birds occurred between 1990–2000, this trend being negatively correlated to indices of agricultural intensity. However, these indices were also positively correlated to 8 out of the 58 species that had positive trends, suggesting that these few species benefit from agricultural intensification.	Farmland	No	N/A	General background information
Easton and Martin 1998	In this experimental study, common bird species increased in abundance, whereas deciduous specialists declined (Warbling vireos) or even disappeared (Nashville warblers) from glyphosate-treated areas. Here also the nesting success of open-cup nesting species was reduced. The observation that the total number of individuals increased in the treated fields might appear as a positive effect, but see discussion on page 9: "the overall abundance of birds increased despite poor nesting success (only 8%). For example, Dusky Flycatchers increased although they had lower nest survival. This implies that trends in abundance	Farmland and forestry	**Yes**	↕	Yes

	may be decoupled from trends in productivity, a characteristic of ‘‘source–sink’’ population regulation (Brawn and Robinson 1996) (…) As the numbers of Dusky Flycatchers was high in the study area, they may have opportunistically inhabited the poorer quality habitat of the herbicide-sprayed areas.”				
Gagné *et al* 1999	In this experimental study, the abundance of the red-backed vole was significantly reduced by glyphosate treatment for two growing seasons.	Forestry	**Yes**	↓	Yes
Guiseppe *et al* 2006	This review identified transient declines and changes in species composition of birds caused indirectly by herbicides. Similarly, indirect effects on mammals were generally short-term.	Forestry	**Yes**	↓—	Yes
Guynn *et al* 2004	This review generally concludes that use of herbicides (glyphosate) in forestry improves productivity, but nonetheless raises societal concerns. However, the response of wildlife to herbicide-induced habitat alteration is highly variable and mostly temporary.	Forestry	**Yes (implied)**	n.d.	Yes
Jahn *et al* 2013	This report reviews many studies on birds, mammals, etc, including glyphosate-specific research. The RMS’ review was limited to the sections dealing with effects of herbicides on biodiversity. Based on expert judgement, the authors classified 30% of the bird and 45% of the mammal species included in this review as being highly negatively impacted by herbicides (including glyphosate).	Farmland	**Yes**	↕ ?	Yes
Marshall *et al* 2001	This is a review report, and the RMS has focused on the sections dealing with	Farmland	No	N/A	General background information

	effects of herbicides on biodiversity. The review shows that herbicides cause changes in vegetation and thus indirectly impact birds and invertebrates. The results are not specific to glyphosate, but to herbicides in general.				
McLaughlin and Mineau 1995	This is a review paper, and the RMS has focused on the sections dealing with effects of herbicides on biodiversity. The results indicate that conservation tillage reduces the risk of accidental mortality of small mammals and promotes greater abundance of waterfowl, compared to ploughed fields. The results are not specific to glyphosate, but to herbicides in general.	Farmland (conservation tillage)	No	N/A	General background information
Santillo *et al* 1989a	This experimental study shows that overall fewer small mammals were found on glyphosate-treated than on untreated clearcuts, in particular insectivores and herbivores being less abundant at least two consecutive years post-treatment.	Forestry	**Yes**	↓	Yes
Santillo *et al* 1989b	This experimental study shows that total number of birds, as well as the abundance of some bird species (common yellowthroats, Lincoln's sparrows and alder flycatchers) are reduced on glyphosate-treated clearcuts. It was also shown that some vegetation management options can compensate for the negative effects of the herbicide.	Forestry	**Yes**	↓	Yes
Sullivan and Sullivan 2000	This book is a compendium of references and abstracts illustrating the available literature on the impact of glyphosate on non-target organisms. No specific examples are discussed.	Farmland and forestry	**Yes**	n.d.	Yes, but only abstracts available

Sullivan and Sullivan 2003	This review showed that overall, the abundance of songbirds which prefer deciduous cover decreased, whereas that of songbirds which prefer 'open' habitat or conifer cover increased after glyphosate treatment, and hence richness and diversity appeared little affected. No effect on the species diversity or richness of small mammals was identified, though reductions in abundance of specific species are documented. Larger mammals were generally less affected by glyphosate treatment; nonetheless, reduced moose activity due to decreased browse availability is reported to last 1-5 years post-treatment. Although the overall the biological significance of the results was considered to be small (magnitude of effect within natural variation), there were several examples of negative effects on birds: "Of the seven published studies reported on avian responses to glyphosate treatment, three reported declines in densities of some songbird species in at least the first posttreatment year." There are also several examples of negative effects of glyphosate on plants, briefly mentioned as 'ephemeral responses': "In a 7-year posttreatment study, glyphosate treatments were found to reduce significantly *Vaccinium* spp. but not species richness or diversity" "herbicide treatments decreasing [woody] cover and affecting the floral	Forestry	**Yes**	↕—	Yes

	community more than manual cutting treatments" "species richness of shrubs and forbs was less on all treated clearcuts compared to untreated clearcuts"				
Traba and Morales 2019	This study showed that annual change rates of bird population indices were positively correlated to the change in fallow surface. In particular, the reduction in the fallow specialist little bustard was strongly and positively related to the reduction in fallow surface.	Fallow	No	N/A	General background information
Amphibians					
Edge *et al* 2011	In this experimental study, the measured application rate of VisionMax™ was negatively correlated to liver somatic index (significant, p=0.0032, r^2=0.75) and fungal infection rates in amphibians (borderline significant p=0.052, r^2=0.41).	Wetlands	**Yes**	↓	Direct effects, included in the standard risk assessment
Edge *et al* 2012	In this experimental study, VisionMAX™ had no negative impact on the survival and growth of green frog larvae, but generally increased the larval abundance. Further studies are needed to investigate the ecological consequences of this amphibian potentially outcompeting other species.	Wetlands	**Yes**	↑—	Direct effects, included in the standard risk assessment
Edge *et al* 2013	In this experimental study, Roundup WeatherMax™ had no effect on juvenile survival, liver somatic index, body condition, or disease incidence in amphibians.	Wetlands	**Yes**	—	Direct effects, included in the standard risk assessment
Edge *et al* 2014	In this experimental study, the survival of the wood frog larvae was lower in *in situ* enclosures treated with high glyphosate concentrations and nutrient enrichment during the first year of the study, and the larvae from all treatments were larger than those in	Wetlands	**Yes**	↕	Yes

	the controls. The abundance of green frog larvae was larger on the treated sides than in the control sides, which may be a concern, since it may result in outcompeting other species (such as the wood frog).				
Edge *et al* 2020	The data presented here were derived from the study by Edge *et al*., 2014, summarised above. The results show that glyphosate may have both a direct negative effect and an indirect positive effect on amphibians and invertebrates.	Wetlands	**Yes**	↕	Yes
Aquatic organisms					
Baker et al. 2016	Replicated split-wetland experiment was conducted to investigate the effects of a nominal concentration of 2.88 mg acid equivalents/L of the glyphosate herbicide Roundup WeatherMax, alone or in combination with nutrient additions, on the changes in the phytoplankton and zooplankton communities. The co-application of herbicide and nutrients resulted in a transient decline in dietary quality of phytoplankton and zooplankton community similarity. However, direct and indirect effects were not evident in wetlands treated only with the formulation.	Wetland	Yes	↓	Yes
Edge et al. 2020	This paper included an investigation of indirect effect on abundance of benthic invertebrates. Indirect effects on the relative abundance (increase) of predatory benthic invertebrates (arose from the direct effects of the herbicide on macrophyte cover. These indirect effects were in the opposite direction to the direct effects of the herbicide, resulting in a compensatory effect and	Wetland	Yes	↑	Yes

	no overall change. The study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift.				
Rolando et al. 2017	This paper presents an international overview of the current use of glyphosate-based herbicides in planted forests and the associated risks. It concludes that glyphosate-based herbicides, as typically employed in planted forest management, do not pose a significant risk to humans and the terrestrial and aquatic environments. This paper also states that subtle, sub-lethal, long-term, indirect effects, or potential interactions of glyphosate-based herbicides with other environmentally relevant stressors (e.g., herbicide mixtures, low dissolved oxygen, pH, excess nutrient inputs, other chemical pollutants) are less well understood as compared to simple direct acute or chronic effects.	Forestry	Yes	—	Yes
Bees					
Burgett and Fisher 1990	The autors concluded that exposure to RoundupR produced no acute or chronic effects on adult honey bees or brood production in the trials. RMS considers these trials poorly described (study design, results) and not relevant/reliable to addresse indirect effects/biodiversity issues. Concerning the removal of the honeybee forage, the potential bee forage that is destroyed by aerial applications of herbicides was considered	Fallow?	Yes	N/A	No

	insignificant by the study authors, this paper is not relevant to address indirect effects/biodiversity issues.				
Chamkasem and Vargo 2017	This paper describes an inter-lab validation of an LCMS /MS method using a negative ion-spray ionization mode for the direct determination of glyphosate, glufosinate, and AMPA in honey.	N/A	Yes	N/A	No
Ferguson 1987 (interim report) Ferguson 1988 (Full report)	Not relevant to address indirect effects/biodiversity issues (this feeding study only aims to investigate direct toxic effects). Study poorly described (study design, environmental conditions, etc…), test item not identified, no results presented (only a statement that glyphosate did not significantly affect the brood and bees).	Farmland	Yes	—	No
Last et al. 2019	If the analysis of Last et al (2019) is used in the purpose to address the impact of weed removal (using glyphosate) on food availability, the results shoults should be re-analysed (with different assumptions). Besides, the drawbacks identified in this analysis may considerably underestimate the relevance of weeds. Morevover, several shortcommings are identified in the study.	Farmland	No	N/A	No
Laberge et al. 1997	No summary nor report are currently available. A datagap has been indicated to the applicant to provide the report.	N/A	N/A	N/A	N/A
Motta et al. 2018	This study states that glyphosate had some effect on honeybee microbiota. However, the absence of clear conceptual link between effects on the honeybee microbiota and the specific protection goals for bees (SPG) has to be noted. It is agreed that it	N/A	Yes	↓	No

	may play a role in the colony/population health, but such link is not immediate in conceptual terms and not quantifiable. This study states that glyphosate reduces the protective effect of the gut microbiota against opportunistic pathogens. The effect of such synergistic effects are however not covered by the current risk assessment scheme.				
Vicini et al. 2020	In this paper, the only data of relevance for the environmental risk assessment is the glyphosate content in honey. This paper contains actually a review of existing published papers related to glyphosate contamination in different matrices. It is RMS opinion that it is not relevant for indirect effects/biodiversity issues.	N/A	Yes	N/A	No
Non-target arthropods					
Guiseppe et al. 2006	This articles reviewed articles related to ecological effects of the herbicide glyphosate used in forested landscapes. In some papers, indirect effects of herbicides on communities of herbivorous arthropods, in most cases, were hypothesized to be a result of reduced floral resources and the effect that this reduction would have on arthropods that require them during at least one phase of their life cycle. Some studies also present correlative relationships that suggest that decreases in flowering plants in agricultural fields results in decreases in the abundance of wasps and bees and often concomitant increases in the density of insect pests.	Forestry	Yes	↓	Yes
Sullivan and Sullivan 2003	This paer concluded that the diversity of terrestrial	Forestry	Yes	— ↑↓	Yes

	invertebrates in glyphosate-treated areas is variable. Abundance and diversity of invertebrates in a given treated area is principally a function of the degree of vegetation control and changes in vegetation structure.				
Warburton and Klimstra 1984	Invertebrate, avian, and small mammal populations in a no-till corn field and a conventionally tilled corn field were compared. This study states (with data) that no-till provides habitat that supports more abundant and stable animal communities.	Farmland	No	N/A	General background information
Soil organisms					
Cerdeira and Duke 2010	This paper provides an overview of GRC (Transgenic glyphosate-resistant crops) related studies and aim to contrast certain risks of GRCs with the risks that the GRCs displace. It states that potential effects of glyphosate on soil and water are minimal, compared to the effects of the herbicides that are replaced when GRCs are adopted. It states that GRCs crops promote the adoption of reduced- or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination. Other studies referenced in this paper concluded that there was still insufficient data to determine whether glyphosate application increases incidence of Fusarium spp. associated diseases in GR crops. Other stated that high doses of glyphosate in soil reduce colonization of pepper (*Capsicum annuum*) plant roots with mycorrhizae. Whether effects were due to a direct effect on the mycorrhizae or to effects on the plant is not known.	Farmland	Yes	↓ ?	Yes

	The doses of glyphosate used also inhibited growth of pepper. However, plants with mycorrhizae were more resistant to the growth-inhibiting effects of glyphosate.				
Duke et al. 2012	This paper evaluates literature on glyphosate-resistant (GR) crops, regarding impact of mineral deficiencies and increased plant disease. This review concludes that: - mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate; - neither the GR transgenes nor glyphosate use in GR crops increases crop disease. However, RMS considers that only data on GS (glyphosate-sensitive) crops are relevant for the purpose of risk assessment. The review states that glyphosate can have effects on mineral nutrition of GS plants through its herbicidal effects on plant roots and other parts of the plant. It also states that treatment of GS plants with glyphosate can result in increased susceptibility to pathogens.	Farmland	Y	↑↓	Yes
Knox et al. 2008	This study indicates that field grown cotton, regardless as to whether it is conventional or GM for either insecticidal or herbicide tolerance or both traits, is mycorrhizal. It does not imply an application of glyphosate (only the genetically modified plant). The paper is not relevant for the assessment of glyphosate. Besides, in Europe, cropping systems are not carried out with	Farmland	N	N/A	No

	glyphosate resistant crops (GMO).				
Silva et al 2018	This study describes a large scale assessment of distribution of glyphosate and its main metabolite AMPA in EU agricultural topsoils (from 11 countries). 300 soil samples were taken from the LUCAS topsoil 2015 survey data base, and 17 soil samples from three independent vineyards in north-central Portugal.	Farmland	Y	N/A	No
Sullivan and Sullivan 2000	This book is a compendium of references and abstracts illustrating the available literature on the impact of glyphosate on non-target organisms. No specific examples are discussed.	Farmland and forestry	Yes	N/A	Yes, but only abstracts available
Powell et al. 2009	Glyphosate applied at recommended field rates had no effect on *Glomus intraradices* or *Bradyrhizobium japonicum* colonization of soybean roots, or on soybean foliar tissue [P]. N_2-fixation was greater for glyphosate-treated soybean plants than for untreated plants in both experiments, but only when glyphosate was applied at the first trifoliate soybean growth stage. These data deviate from previous studies estimating the effect of glyphosate on the rhizobial symbiosis, some of which observed negative effects on rhizobial colonization and/or N_2-fixation. GM soybean was used.	Farmland	Y	↑	No
Lu et al. 2018	Comparative analysis of the soil rhizosphere microbial communities was performed by 16S rRNA gene amplicons sequencing and shotgun metagenome sequencing analysis between the soybean line ZUTS31 foliar sprayed with diluted	Farmland	Y	— ↓	Yes

	glyphosate solution and those sprayed with water only in seed-filling stage. This study indicates that the formulation of glyphosate-isopropylamine salt did not significantly affect the alpha and beta diversity of the rhizobacterial community of the soybean line ZUTS31, whereas it significantly influenced some functional genes involved in PGPT (Plant Growth Promoting Traits) in the rhizosphere during the single growth season. It is RMS opinion that it is not relevant for indirect effects/biodiversity issues.				
Savin et al. 2009	The objective of this study was to determine if dynamics of the rhizosphere microbial community were altered by applications of glyphosate and P fertilizer to glyphosate-tolerant cotton, maize, and soybean growing in low-P soil in the greenhouse. Overall, the study concludes that when the indigenous soil community and potential inoculum was not altered by pasteurization, glyphosate was not inhibitory nor stimulatory to mycorrhizal infection rates after six weeks of plant growth. In contrast, pasteurization, while not reducing the total microbial biomass, did impose a stress on the microorganisms and likely inhibited particular microbes and biochemical functioning in the soil. The potential for glyphosate to alter arbuscular mycorrhizal fungal infection in glyphosate-tolerant plants may depend on whether soil microbial communities are compromised by other factors.	Greenhouse	Y	—	Yes

Non-target terrestrial plants					
Koning et al. 2019	Overall, any method employed influenced the weed composition in some way. Some species were favored over others depending on the weed management method, but the overall biodiversity of the weed community was not more negatively affected by one method compared to another.	Farmland	Yes	↑↓	Yes
Colbach et al. 2018	This study is relevant for assessment of biodiversity and the definition of compensation measures in agricultural landscapes. However RMS considers the output of this simulation of low reliability.	Farmland	No	N/A	General background information

[1] the direction of effect is mathematical and does not necessarily reflect a positive or a negative biological effect (to be judged on a case-by-case basis);
— shows no effect,
↕ indicates that some parameters increased, while others decreased and
↑↓ indicates that the same parameter first increased and then decreased.
↑ indicates that the parameter increased
↓ indicates that the parameter decreased.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

B.9.14.1. Assessment of risk to biodiversity via indirect effects and trophic interactions

Data point	KCA 8.7/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment
Report No.	TRR0000305
Document No.	-
Guidelines followed in study	Not relevant
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	Not relevant
Previous evaluation	No, submitted for the purpose of renewal
GLP/Officially recognised testing facilities	Not relevant
Acceptability/Reliability	Acceptable

For transparency, summary of assessment and conclusion as proposed by the applicant in its document related to risk assessment were reported in grey boxes. When complementary information where found

in [redacted] 2010 (CA 8.7/001), this has been specified. An RMS commenting box followed each part of the applicant's assessment proposal.

B.9.14.1.1. Terrestrial vertebrates – Risk to biodiversity Assessment via Indirect Effects and Trophic Interactions

- **Indirect effects via trophic interactions**

Assessment and conclusion by the applicant

A large regulatory data package exists with acute and long-term studies to inform the avian risk assessments. The results of the avian risk assessment demonstrate that under the intended uses of glyphosate there is negligible risk from direct effects.

An assessment of indirect effects is in part covered by the current EFSA Birds and Mammals assessment guidance through an evaluation of the potential for secondary poisoning (e.g., consumption of earthworms, fish, drinking water) as discussed above.

However, methodology for assessing indirect effects through trophic interaction resulting from in-crop weed control is not addressed. Throughout the development of the EFSA (2009) bird and mammal guidance document, it was raised that indirect effects through trophic interactions should eventually be addressed, and it was decided when the guidance on how this could be achieved was finalized, that this topic would need to be addressed by revised guidance. However, many experts in the Member States who reviewed the guidance document commented that this is area that requires further research and that it may be preferable to manage indirect effects to birds through mechanisms other than that pesticide approvals (e.g., farmland management and/or conservation policies).

Furthermore, concerning specifically potential impacts on biodiversity, there currently is no EU wide guidance on how this should be assessed at the taxa group level within the context of a single active substance renewal risk assessment.

The following assessment approach considers both direct effects and the potential for indirect effects via trophic interactions, based on the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs based on direct effects assessment considering representative sensitive populations across the tested trophic levels. The biodiversity assessment, aimed to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals, that includes considering indirect effects via trophic interaction. For example, reduced application rates relative to previous Annex I renewals, a reduced overall application volume of product on the land, and inclusion of no-spray buffer zones as a standard mitigation measure to protect edge of field surface waters. When defining SPGs for mammals, it is the responsibility of the risk assessors in the Member States to acknowledge existing protection goals and regulatory data requirements, to propose possible SPG options, and describe the possible environmental consequences of each option. The risk assessors within the Member States will need to propose realistic SPGs and exposure assessment goals and the interrelationships between them in a clear and transparent manner.

From [redacted] 2020: crop residues left over on the soil surface from practicing conservation tillage increases cover and benefits to wildlife. The general rule is that the greater the amount of crop residue a tillage practice leaves on the surface, particularly standing residue, the better the practice is for birds and small mammals. The studies on the benefits of conservation tillage have shown that fields using conservation tillage have a greater diversity and a higher density of birds and nests than reduced till or conventionally tilled fields. Increases in crop residue tend to increase the diversity of small

mammals in crop fields. In addition, crop residues also harbor insects and other arthropods that are an important food source for birds and mammals.

Assessment and conclusion by the RMS

The RMS notes that the applicant refers to the evaluation of potential for secondary poisoning (e.g., consumption of earthworms and fish) as an example of indirect effects. The RMS does not agree with this statement, since effects from exposure to a chemical via contaminated food are, by definition, regarded as direct effects.

Further, [redacted] (2020) refer to benefits for wildlife as a consequence of conservation tillage in combination with glyphosate. See RMS remark on this in the Introduction to the Ecotoxicology section in this document (Vol. 3CP, Section 9).

- **Scientific literature that informs the avian and mammal indirect effects assessment**

Assessment and conclusion by the applicant

Farmland is the most important habitat for bird conservation in Europe, harbouring more than 50% of bird species in the European Union (EU) and 55% of European bird species listed in the IUCN Red List (Burfield, 2005; Donald et al., 2006).

In Europe, trend data are available from the Pan-European Common Bird Monitoring Scheme, which is currently implemented in 18 countries (Gregory et al. 2003; Traba and Morales, 2019). The data show trends in farmland and woodland birds since 1980. On average, populations of woodland birds in Europe have remained stable. In contrast, populations of farmland birds in Europe declined particularly in the 1980s and the downward trend over the next two decades continued, but at a slower rate (trend 1980–2002, 29%). This rapid decrease in farmland birds is believed to reflect deterioration in the quality of farmland habitats in Europe (Traba and Morales, 2019).

Several reviews and studies on indirect effects through trophic interactions to populations of farmland bird species are available. These studies and reviews mainly focus on arable landscapes in the UK (Campbell et al. 1997; Marshall et al., 2001; Boatman et al. 2004; DEFRA 2005; Bright et al. 2008; Jahn et al. 2013; Traba and Morales, 2019).

After forestry applications, changes in bird community composition, and reductions in abundance, densities and species richness of bird populations often occurred in the first few years after glyphosate application (Guiseppe et al. 1986, Easton and Martin, 1998, Santillo et al. 1989b), and in Santillo et al. (1989b) the decline in bird densities was correlated with the decline in habitat complexity. These changes were assessed against untreated control sites to differentiate the effects of glyphosate from other background environmental factors such as the recovery trajectory following tree harvest and showed similar declines in bird densities where habitats removed following the use of other herbicides commonly used in managed forests (Guvnn et al., 2004).

For mammals, studies on indirect effects through trophic interactions at the population level are generally lacking. However, a number of studies have investigated the potential for indirect effects on birds and mammals in managed forest systems.

Studies on small mammals (i.e., rodents, shrews, voles, chipmunks) have shown that some short-term changes after forestry applications of glyphosate were observed at the species (Anthony and Morrison, 2985; D'Anieri *et al*. 1987; Gagné *et al*. 1999) and functional feeding group levels (Santillo

et al., 1989a), which the authors attributed to the reduction in invertebrates, plant cover, and food. At the population level, glyphosate did not appear to have significant or long-lasting effects in the first few years after application (D'Anieri *et al*. 1987; Santillo *et al*., 1989a; Sullivan *et al*. 1987). Similar to small mammals, changes in bird community composition, and reductions in abundance, densities and species richness of bird populations often occurred in the first few years after glyphosate application (Guiseppe *et al*. 1986, Easton and Martin, 1998, Santillo *et al*. 1989b), and in Santillo *et al*. (1989b) the decline in bird densities was correlated with the decline in habitat complexity. These changes were assessed against untreated control sites to differentiate the effects of glyphosate from other background environmental factors such as the recovery trajectory following tree harvest and showed similar responses to other herbicides commonly used in managed forests (Guynn *et al*., 2004).

Additionally, [redacted] 2020 referred to a comprehensive review by Lautenschlager and Sullivan (2003), on the use of glyphosate-based products for forestry applications concluded that abundance of songbirds that prefer early successional deciduous cover generally decreases one to two years after conifer release treatments, whereas densities of species that avoid that cover tend to increase likely in a compensatory manner. Abundance of species that decrease soon after treatment often recovers during the following three years. Species-specific responses are linked to treatment-related habitat changes and not to herbicide treatments *per se*. Densities of some species have been reduced as much or more by mechanical cutting as by herbicide treatments. However, population increases or decreases associated with herbicide treatments seem to last longer than those associated with mechanical cutting.

Sullivan and Sullivan (2003) published a comprehensive glyphosate assessment addressing vegetation management and ecosystem disturbance focusing on plant and animal biodiversity that considered both direct effects at the individual level, but also indirect effects on habitats / refuges and resource. Their analysis was based on 60 published studies of terrestrial plants and animals in temperate forests and agroecosystems. Species richness of plants was either unaffected or increased in the case of herbaceous species in those receiving glyphosate treatments. Species richness and diversity of songbirds, in open habitats representative of agricultural lands, did not appear to be negatively impacted in glyphosate use areas. In fact, conservation tillage, which is enabled by glyphosate, promoted greater abundance of songbirds and other fowl compared with ploughed fields (McLaughlin and Mineau, 1995; Cunningham et al., 2005).

Overall, the magnitude of changes in species richness and diversity of plants, birds, and small mammals in the studies reviewed by Sullivan and Sullivan (2003) were within the mean range of natural fluctuations and considered direct and indirect effects.

Assessment and conclusion by the RMS

The RMS has reviewed the published literature studies referred to by the GRG. It is noted that for some of the references, there are typos in [redacted] text: Sullivan *et al*. 1987 should be Sullivan *et al*. 2003, Guiseppe *et al*. 1986 should be Guiseppe *et al*. 2006, and Lautenschlager and Sullivan (2003) appears to be Sullivan and Sullivan, 2003. The correct references are given in the RMS' WoE table.

Some of the studies referred to by the applicant are dealing with decreasing biodiversity in farmland and forestry in a general context, or due to use of pesticides (or limited herbicides) without specific focus on glyphosate. There are also some studies comparing effects on biodiversity from non-inversion tillage practice compared to conventional tillage in agricultural fields.

Studies conducted in forestry can be considered as partly relevant for the glyphosate use against invasive species, and partly for the use along railway tracks, while those conducted in general farmland are relevant for field uses of glyphosate. However, there is no clear link between the

exposure situation in the referred studies and the representative uses of glyphosate for the EU evaluation.

[redacted] (2020) referred to Appendix 1a to The EFSA Journal (2008) 734, 1-181, regarding a statement from one MS (response to a questionnaire related to the EFSA guidance) that indirect effects of pesticides have a lower impact on bird numbers than direct effects. However, it should be noted that this statement was broadly coupled to 'national research', while no specific source was given.

- **Biodiversity assessment**

Assessment and conclusion by the applicant

The assessment approach – as previously defined aims to assess the potential indirect effects via trophic interactions and the impact on biodiversity, by developing a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals. In the following table, the specific protection goals relevant to mammals are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relates directly to the effects study endpoints.

A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence).

Based on the measurement endpoints from the study types, and the direct effects assessment presented above in this section, direct effects from glyphosate on aquatic organisms are not anticipated.
The impact on mammalian species will be additionally supported by the required in-field no spray buffer area for the NTTPs, which will protect mammals occurring in field margins.

The following table assessment illustrates that ecological function of wild mammals in off-target areas/ edge of field, will be sufficiently maintained to achieve the SPG for wild mammals according to the protection goals as defined in the EFSA guidance that sustains habitat and food resources for other organisms.

Table […] Protection Goals and Associated Assessment and Measurement Endpoints for Birds and Wild Mammals.

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
No visible mortality and long-term impacts on abundance and diversity	No reduction in survival, growth, development, reproduction of avian populations.	Survival, growth, development and reproduction	Acute oral avian and rat Avian reproduction Rabbit teratology Rat 2-generation
Avian and Wild Mammal Biodiversity Assessment Based on the current direct effects risk assessment for glyphosate, there is acceptable acute and long-term risk assessments based on current guidance and the intended use patterns for glyphosate. However, if additional risk mitigation measures are determined to be required based on local conditions, to mitigate indirect effects resulting from in-crop weed control on mammalian populations, options to be considered by risk assessors and risk managers within Member States are presented in the following section.			

[1] When protection goals are defined more precisely by risk managers or legislators to address indirect effect, then the protection goals and assessment procedures should be revised.

Assessment and conclusion by the RMS

It is agreed that no guidance is available for systematic assessment of indirect effects on biodiversity. However, for the time being, the assessment can be broadly presented following a weight of evidence approach, as detailed below.

Among the 21 studies on the effects of pesticides on the biodiversity of birds and mammals, only 11 address glyphosate specifically. From these, a large fraction (45%, i.e., 5 studies) show *negative* effects that are indirectly linked to glyphosate application. Three studies show both a decrease in some biodiversity metrics, as well as an increase in others, while one study shows first an increase, followed by a decrease in biodiversity metrics. Based on this limited dataset, it appears that evidence for the negative effects prevails, and thus the RMS preliminary concludes that glyphosate is potentially posing a risk for indirect effects on biodiversity.

However, it should be noted that any method used for removal of weeds may have a similar impact on abundance and biodiversity of birds and mammals. From the available data, only two studies included treatments consisting of mechanical removal of vegetation in the control. Further, to reflect a comparable situation, the removed weed should be left on the ground. Without adequate controls, the actual impact of glyphosate must be regarded as uncertain.

- **Conclusion**

Assessment and conclusion by the applicant

Based on the current direct effects risk assessment for glyphosate, there is acceptable acute and long-term risk based on current guidance and the intended use patterns for glyphosate. Currently, the EFSA birds and mammals guidance does not include assessment methodology for indirect effects through trophic interactions. Addressing potential indirect effects to birds and mammals by limiting in-crop weed control or compensating for its effects may be better handled though policies and programs outside the Plant Protection framework. However, if additional risk mitigation measures are concluded to be required, to mitigate indirect effects resulting from in-crop weed control on avian populations, options to be considered by risk assessors and risk managers within Member States. These mitigation options will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field.

Assessment and conclusion by the RMS

Further information would be needed to confirm whether there is a link between the observed effects and expected exposure levels following the representative uses of glyphosate at the EU level.

It is noted that the indirect effects on birds and mammals would ideally have to be considered in the other guidances (aquatic, NTA, NTTP, soil orgs, etc.). This should be taken into consideration for updating SPGs for these groups and in the context of revision of the guidance document.

B.9.14.1.2. Aquatic organisms - Risk to biodiversity via Indirect Effects and Trophic Interactions

- **Indirect Effects via Trophic Interactions**

Assessment and conclusion by applicant:

The available regulatory ecotoxicology data for glyphosate and its main metabolite AMPA includes a battery of acute and chronic aquatic guideline studies, across multiple trophic levels, that have been designed to assess the potential for direct and indirect effects through trophic interactions. Consideration of indirect effects through trophic interactions has been used to derive a SPG that is consistent with the current EFSA aquatic guidance (2013) and the Regulation ((EC) No 1107/2009). The SPG used for the biodiversity assessment states: "Negligible acute and long-term effects to aquatic plant and animal populations from direct and indirect effects through trophic interactions" ([…]). Negligible in the context of this assessment, and the EFSA aquatic guidance, means that there is a sufficient margin of safety to conclude there will be no unacceptable effects to aquatic ecosystems for the intended uses.

As previously discussed, glyphosate is an important tool to realize the benefits that conservation tillage has on biodiversity in agroecosystems. Low soil disturbance leaves the surface with adequate crop residue and organic matter that resists soil aggregate breakdown and soil crusting that contribute to runoff and erosion and consequently soil / particulate matter reaching aquatic systems resulting in sedimentation. The primary nutrient forms carried in runoff are ammonium, nitrate, and phosphate that contribute to degradation and eutrophication of aquatic ecosystems. Therefore, using glyphosate within conservation agriculture schemes can minimize impact to aquatic biodiversity.

The groups of aquatic organisms that were tested are well suited for direct and indirect effects assessment through trophic interactions because it contains the key components of the aquatic food chain as well as macrophytes that are an important structural component of aquatic waterbodies. Indeed, the test battery includes numerous representative species of primary producers (i.e., chronic studies with algae, diatoms, aquatic macrophytes), representative primary consumers (i.e., acute and chronic studies with pelagic invertebrates and sediment dwelling invertebrates) and acute and chronic studies with secondary consumers (i.e., fish development and reproduction and larval amphibian development) ([…]).

The following assessment approach considers both direct effects and the potential for indirect effects via trophic interactions, based on the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs based on direct effects assessment considering representative sensitive populations across the tested trophic levels. The biodiversity assessment, aimed to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals, that includes considering indirect effects via trophic interaction. For example, reduced application rates relative to previous Annex I renewals, a reduced overall application volume of product on the land, and inclusion of no-spray buffer zones as a standard mitigation measure to protect edge of field surface waters. When defining SPGs for aquatic plants and animals, it is the responsibility of the risk assessors in the Member States to acknowledge existing protection goals and regulatory data requirements, to propose possible SPG options, and describe the possible environmental consequences of each option. The risk assessors within the Member States will need to propose realistic SPGs and exposure assessment goals and the interrelationships between them in a clear and transparent manner

The direct effects assessment covering a broad range of aquatic taxa groups, informs on the biodiversity assessment by highlighting an acceptable risk across multiple trophic layers of the aquatic food chain. Therefore, where an acceptable direct effects risk assessment is concluded upon after incorporation of standard mitigation measures to reduce off-target movement to surface waters (anyway required to

support the NTTP assessment) coupled with the other standard mitigation measures that are applied, they are considered protective of indirect effects occurring outside of the target area.

However, for the purpose of this biodiversity assessment, the SPGs developed for aquatic systems is considered consistent with current EFSA guidance and what will likely be adopted in future EFSA guidance. The SPG is aimed at achieving negligible acute and long term direct and indirect effects on aquatic plant and animal populations.

Available study results and the risk assessment for direct effects presented in […]show negligible risk from direct effects on the representative species for the various trophic levels. Moreover, glyphosate and its main metabolite AMPA, do not bioaccumulate (Log Pow less than 3 and a BCF = 1.1). Additionally, the basic principles that underlie an aquatic mixture assessment for glyphosate have been provided in [40]Appendix 1 of the biodiversity assessment document. In addition, based on predicted environmental concentrations, either from FOCUS surface water modelling or from surface water monitoring studies, the risk of additive effects of glyphosate in the presence of other plant protection products in surface waters is low to negligible.

Assessment and conclusion by RMS

The EFSA Aquatic Guidance (2013) states that, in principle, protecting against direct effects should not lead to unacceptable indirect effects. The risk assessment of direct effects on aquatic organisms is expected to be acceptable with comparison of tier 1 RAC with FOCUS step 1-2 PECsw.
The RAC proposed by RMS is of 100 µg/L glyphosate.
The risk assessment resulted in acceptable for all intended uses without necessity of mitigation measures.
RMS considers that comparison between PECsw and RAC (and subsequently the margin of safety) may be considered to cover most of the indirect effects on aquatic ecosystems. This approach is based on the assumption that the current assessment factors used for assessing direct effects are protective enough to cover indirect effects. Given the data provided by the applicant for glyphosate, it could not be considered that all indirect effects and food web interactions are addressed given that not all food sources are considered. Please see next RMS commenting box.

- **Biodiversity Assessment**

Assessment and conclusion by the applicant

The assessment approach – as previously defined aims to assess the potential indirect effects via trophic interactions and the impact on biodiversity, by developing a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals. In the following table, the specific protection goals relevant to aquatic organisms are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relates directly to the effects study endpoints.

A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence).

[40] [redacted] (2020) Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment (TRR0000305).

Based on the measurement endpoints from the study types, and the direct effects assessment presented above in this section, indirect effects from glyphosate on aquatic organisms are not anticipated.

The following table assessment illustrates that ecological function of aquatic organisms in off-field / off-target areas / edge of field surface water, will be sufficiently maintained to achieve the SPG for the aquatic organisms according to the protection goals as defined in the EFSA guidance (2016), that sustains habitat and food resources for other organisms whilst achieving negligible acute and chronic effects on aquatic plants and animals.

Table […]: The relationship between the Specific Protection Goal, assessment endpoints and measurement endpoints for aquatic systems (wetlands, rivers and lakes) exposed by runoff and/or spray drift.

Specific Protection Goal[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types[2]
Negligible acute and long-term effects to aquatic plant and animal populations from direct and indirect effects through trophic interactions.	Survival, growth and reproduction of aquatic populations	Acute and chronic toxicity to aquatic plants and animals and bioaccumulation	Algal Vascular plants Acute Daphnia Daphnia life-cycle* Chironomid emergence* Acute fish Fish ELS* Fish repro screening * Fish Full Life-cycle* Amphibian metamorphosis * Fish bioconcentration
Biodiversity Assessment for Aquatic Ecosystems Based on the specific protection goal, inclusion of a 1 m buffer between the application area and the adjacent surface water body, for applications of MON 52276 made according to the proposed GAP, is considered protective of both direct and indirect effects on biodiversity in aquatic ecosystems through trophic interactions.			

[1] By accepting no population-level effects on representative sensitive populations in edge-of-field surface waters, these populations will be protected and propagation of effects to the community-, ecosystem- and landscape-level will not occur (Option 1: EFSA aquatic guidance, 2013).

[2] Acute and chronic aquatic studies for aquatic plants and animals are presented in the ecotoxicology section. Endpoints for AMPA are similar to endpoints for the same studies with glyphosate.

* Note these studies were performed to assess the potential for impacts to the endocrine pathways. No effects to the four endocrine pathways can be concluded based on the results of these studies and a weight of evidence evaluation (USEPA, 2015, EFSA, 2017, KCA 5.8.3/010, 2020)

As a conservative approach for finalizing the aquatic biodiversity assessment, the lower tier assessment option known as the Ecological Threshold Option (ETO) from the EFSA's tiered guidance for aquatic risk assessments (EFSA (2013). This option aims at ensuring that negligible effects only, may occur in aquatic populations (transient effects followed by recovery are not accepted with this option). Both direct and indirect effects on the food chain are covered within this option. When applied to the representative sensitive populations in edge-of-field surface water, this option allows to conclude that aquatic populations will be protected, and that propagation of effects to the community-, ecosystem-, and landscape level will not occur.

The current direct effects aquatic risk assessment in […] shows that inclusion of a one-meter buffer between the applied area and the edge-of-field surface water for glyphosate applications is considered protective of both direct effects and indirect effects through trophic interactions on aquatic biodiversity for the intended uses.

Assessment and conclusion by the RMS

The approach followed in the EFSA guidance for aquatic organisms (2013) for the definition of specific protection goals is in line with EFSA guidance on specific protection goals (2016). As such it is the most suitable guidance document that allow to consider biodiversity and ecosystem services for aquatic organisms. The tiered approach developed aimed to protect populations of aquatic organisms by defining Regulatory Acceptable Concentration (RAC) based on two options: "(1) The ecological threshold option (ETO), accepting negligible population effects only, and (2) the ecological recovery option (ERO), accepting some population-level effects if ecological recovery takes place within an acceptable time period".
The assessment of glyphosate is performed using RAC values based on the ecological threshold option (ETO). An overview of the SPG as defined in the aquatic guidance is presented here.

Table 13: Overview of proposed specific protection goals for the ecological threshold option

Organism group	Ecological entity	Attribute	Magnitude	Time
Algae	Population	Abundance/biomass	Negligible effect	Not applicable
Aquatic plants	Population	Survival/growth Abundance/biomass		
Aquatic invertebrates	Population	Abundance/biomass		
Vertebrates	Individual	Survival		
	Population	Abundance/biomass		
Aquatic microbes	Functional group	Processes (e.g. litter break down)	RA will not be developed since tier 1 data requirements are not defined	

From this approach, when considering the magnitude of effects as negligible for each ecological entity of each of the aquatic organisms, the risk assessment should be protective of both direct effects as well as indirect effects including trophic interaction among the aquatic food chain. This assumes that the current assessment factors used for assessing direct effects are protective enough to cover indirect effects.
The aquatic risk assessment of glyphosate is based on ecological threshold option. As such, the approach used could be considered appropriate to protect both direct effects as well as indirect effects including trophic interaction among the aquatic food chain in the sense of the EFSA aquatic guidance document (2013). However, given the data provided by the applicant and their assessment by RMS for glyphosate, it could not be considered that all indirect effects and food web interactions are addressed given that not all food sources are considered. For example, valid studies to assess the effects on sediment-dwelling organisms or rooted macrophytes of glyphosate that has a potential to partition in sediment are missing. Additionally, information on impact on decomposition processes in aquatic systems, or effects on the biofilm (algae, fungi and bacteria-matrix) would need to be considered. Further information on the effect to the aquatic community could also contribute to assess risk to biodiversity via indirect effects and trophic interactions.

- **Ecotoxicological relevance of the glyphosate surface water monitoring data**

Assessment and conclusion by the applicant

In addition to the predicted environmental concentrations from FOCUS modeling used for the standard aquatic assessment, there is an extensive amount of surface water monitoring data that can be used to further evaluate potential effects of glyphosate on biodiversity in aquatic ecosystems.

Horth (2012) provided a review that covers glyphosate and AMPA monitoring results for surface waters from all 27 Member States. The maximum concentrations of glyphosate found in surface water ranged from 1.3 to 370 µg acid equivalents (a.e.)/L and the maximum concentrations of AMPA ranged from 0.22 to > 200 µg/L. Glyphosate and AMPA concentrations in the monitoring data exceeded the predicted environmental concentration (PECsw), using the FOCUS (2000) surface water model for glyphosate and AMPA at an exceedingly small frequency. When calculating TER values with the concentrations monitored in the study by Horth, the outcome of the assessment demonstrates that the risk for direct and indirect effects to aquatic organisms from the intended uses of glyphosate is acceptable.

Based on a more recent analysis of the European Environment Agency water monitoring ([redacted] 2020) database, it can be concluded that 99.99% of the measured glyphosate surface water concentrations are below a regulatory acceptable concentration (RAC). For surface water, there were > 250,000 analyses and exceedance rates of the RAC were 0.01% for glyphosate and 0.001% for AMPA ([redacted] 2020). The original RAC value (100 µg/L) concluded in the [redacted] report is considered highly conservative, as the underlying fish toxicity study on which the RAC had been based ([redacted] 2000; MCA 8.2.2.1/002) is not acceptable for use in risk assessment (KCA 8.2.2.1/002 and KCA 8.2.2.1/003). Based on the now proposed lowest RAC value (400 µg/L) from the available reliable ecotoxicology aquatic endpoints, evaluated against current validity criteria for the study types, a further 4-fold margin of safety may be applied to the evaluation of the surface water detects in the monitoring report.

Assessment and conclusion by RMS

RMS does not agree with the new RAC value proposed by the applicant (400 µg/L).
The RAC proposed by RMS is of 100 µg/L.

As highlighted by RMS in Volume 3 B8 7.5, very few justification on some values (considered outliers by the applicant) was provided in the report. Some maximum values over the RAC (up to 558 µg/L) should be further justified. This is identified as a data gap for the applicant. However, the environmental fate RMS concluded that, considering that only 58 samples considered "outliers" by applicant are above 57 µg/L (applicant indicated the maximum concentration is 57 µg/L when excluding outliers), the overall compliance with any lower RAC would not be significantly different than the one presented by applicant.

Compliance of the concentration with the AMPA RAC of 1200 µg/L was very high (99.999% of samples; 99.976% of sites) with infrequent exceedances (0.001% of samples from 0.024% of sites) occurring on 3 separate non-consecutive occasions. A small number of high maximum concentrations were confirmed to be outliers and once excluded indicated a maximum concentration of 224.4 µg/L, which is below the RAC. Assessment of the spatial distribution of locations of AMPA exceedance of the RAC did not indicate any specific pattern or bias.

- **Glyphosate aquatic risk assessment under the PPP regulation in the context of the Water Framework Directive (WFD)**

Assessment and conclusion by the applicant

The protection goal underlying the WFD refers to human and ecosystem health. Within the context of ecosystem health and setting Environmental Quality Standards (EQS) it is assumed that (1) ecosystem sensitivity depends on the most sensitive species population, and (2) protecting ecosystem structure protects community functioning. Aquatic risk assessments for the WFD focus on larger water bodies (e.g., river basins) and EQSs should be linked to an annual average concentration or the maximum of the measured concentrations (MAC-EQS). In contrast, the aquatic risk assessment for PPP Regulation focuses on concentrations that can be achieved in edge-of-field surface waters in agricultural landscapes and the exposure assessment uses harmonized exposure scenarios (FOCUS surface water scenarios). These scenarios, in combination with models that estimate the emissions and the fate and behavior of PPPs in surface waters, predict realistic worst-case exposure concentrations in edge-of-field surface waters.
In terms of effects endpoints, EQSs are derived on the basis of predicted no effect concentrations (PNECs) for all relevant populations of water organisms and is generally comparable to the ETO approach used for a PPP aquatic assessment. Overall, the general protection goal of the WFD and PPP Regulation do not differ substantially. EQS setting within the context of the WFD in principle is based on the Ecological Threshold Option approach (ETO, EFSA, aquatic guidance 2013), and glyphosate satisfies the ETO option as discussed above. Glyphosate was identified as "low risk" to the water compartment in the 2011 evaluation of candidate EU priority substances using a PNEC in water of 24 µg a.e./L. To put this value into perspective with the new surface water monitoring data, and including values identified as outliers, less than 0.042% of samples exceed 24 µg a.e./L ([redacted] 2020). Moreover, considering the large margin of safety (>350-fold) between the endpoint driving the standard aquatic risk assessment, and measured levels of glyphosate from monitoring studies, risk of direct effects and indirect effects through trophic interactions on aquatic communities is negligible.

Assessment and conclusion by RMS

Regarding Environmental Quality Standard (EQS), no EU-wide EQS values, annual average (AA) or maximum allowable concentration (MAC), were available for assessment as broader ecosystem endpoints. Consideration of the MS GLY surface water data against MS EQS values indicates that the presence of GLY is not expected to have any adverse impacts on ecosystems with a near total compliance (99.987%) across the large EQS-MAC dataset (~228 000 samples from ~9 000 sites) with very few exceedances (0.013% of samples; 0.22% of sites) identified. Similarly, 100% compliance for the large EQS-AA dataset (~11 000 years from ~1 600 sites) is indicated with no exceedances identified.

The results indicate that for both glyphosate and AMPA, there were no sites showing average annual concentration >EQS-AA in the MS where such trigger is defined. RMS however underlines that the details on the calculation of this average annual concentration (AA) from monitoring results for comparison against the MS EQS-AA is not provided and could not be checked. It is indicated that only sampling sites with 12 sampling event within a year were selected for calculating this AA. However, there is no indication whether the selected sites fulfilling these criteria are located in areas where glyphosate is used. No further analysis of spatial distribution of these sites and their potential relation to glyphosate containing product use is proposed in the study of [redacted] (2020). See volume _3CA_B-8 section B8.5.4 for further details.

- **Relevance of the Drinking Water Threshold to Biodiversity Assessment**

Assessment and conclusion by the applicant

The Drinking Water Directive (DWD) sets the compliance limits at the tap of the consumer as 0.1 µg/L for individual pesticides and 0.5 µg/L for total pesticides. Only those pesticides which are likely to be present in a given supply need be monitored. From the environmental monitoring report ([redacted], 2020), the analysis of the dataset available for drinking water for glyphosate and AMPA indicates that compliance is to these requirements very high. Indeed, detections above 0.1 µg/L are very rare. When they do sporadically occur, they occur at low concentrations that are well below human health thresholds. The measured environmental concentrations available show that neither glyphosate nor AMPA pose a risk to human health via drinking water where the point of compliance is at the tap of the consumer. The drinking water threshold is not therefore considered relevant to the ecotoxicological risk assessment.

Assessment and conclusion by RMS

Regarding the threshold of 0.1 µg/L, detection for glyphosate above the threshold of 0.1 µg/L was ~23% of samples (~54.0% of sites), ranging from 3.4% in AT to 57.5% in BE. These results compare well with the previous data collection (Horth, 2012; 2016) where ~21% of samples were found to exceed 0.1 µg/L.

RMS notes that this comparison with the 0.1 µg/L threshold is reported for information. The proportion of monitoring location potentially intended to supply drinking water is unknown. No indication on this was further given in the report of [redacted] 2020 and it is not known whether exceedance of the 0.1 µg/L trigger is observed in particular conditions. It could be assumed that the level of exceedance will be lower for larger water bodies from which drinking water is abstracted, and it may be expected that the exceedance rates given are a worst-case for drinking water abstraction locations, but this cannot be asserted unless the analysis is actually done.

- **Scientific Literature that informs the aquatic biodiversity assessment**

Assessment and conclusion by the applicant

Baker et al. (2016) investigated the potential for indirect effects on natural communities of phytoplankton and zooplankton with a glyphosate-based formulation at concentrations up to 2.88 mg a.e./L, which represents a concentration resulting from an overspray application to a shallow waterbody (approximately 4.3 kg a.e./ha over-sprayed in to 15 cm water). Their co-application of herbicide and nutrients resulted in a transient decline in dietary quality of phytoplankton and zooplankton community similarity. However, direct and indirect effects were not evident in wetlands treated only with the formulation.

Rolando et al. (2017) conducted an extensive review of the available scientific literature for glyphosate-based herbicides used in forest management, at applications up to a rate of 4 kg a.e./ha and concluded that glyphosate use does not pose a significant long-term risk of direct and indirect effects in aquatic environments. Indirect effects of glyphosate to aquatic fauna were observed when high concentrations of the product were applied as overspray to the waterbodies. Effects on the aquatic fauna were associated with changes in aquatic plant community composition and habitat structure, cover, and food sources as a consequence of glyphosate's phytotoxic effects, rather than resulting from the toxicity of glyphosate on the aquatic fauna. To help put this observation of indirect effects into perspective, Edge et al. (2020) investigated the potential for indirect effects on aquatic animals from using a glyphosate-based

formulation to control emergent aquatic vegetation. Results showed that control of the aquatic vegetation indirectly increased the abundance of benthic invertebrates and wood frog larvae. This study shows how glyphosate can be safely used to control aquatic vegetation and has benefits to aquatic biodiversity.

Edge et al. (2011, 212, 2013, 2014) conducted field studies to assess effects of a glyphosate-based formulation, commonly used in Canadian forestry, on larval tadpoles at concentrations representative of a direct overspray into shallow water (2.88 mg a.e./L). The results from these studies showed no impact on growth, development and survival and it was concluded that there was no unacceptable risk to larval amphibians. The absence of chronic effects was concluded to result from rapid dissipation of glyphosate and its adjuvant in the water column and showed the importance of testing under environmentally realistic conditions.

Assessment and conclusion by RMS

In a replicated split-wetland experiment Baker et al.,2016 (see Appendix to Volume 3 (PPP) on literature related to biodiversity, part 3 study 1), investigated the effects of Roundup WeatherMax, alone or in combination with nutrient additions, on the changes in the phytoplankton and zooplankton communities.
A worst case contamination of wetlands with the herbicide Roundup WeatherMax in combination with fertilizer nutrients resulted in transient and relatively minor disruptions of plankton community structure. These effects were not evident in wetlands treated only with the herbicide.
Unlike after the first application, there were no significant changes observed in phytoplankton or zooplankton endpoints in the herbicide and nutrient treated wetlands. For the herbicide only treated wetlands, phytoplankton abundance and quality appeared to decline, but not significantly. However, the richness of zooplankton in the herbicide alone treated wetlands was reduced by an average of 2.7 ± 0.6 taxa compared to controls. Zooplankton abundance and community similarity were not significantly different between treatment and control halves as a result of the second herbicide application for either treatment.
Indirect effects of the herbicide-nutrient mixture were evident in mid-summer, when glyphosate residues were no longer detectable in surface water. Zooplankton abundance tripled, and zooplankton taxa richness increased by an average of four taxa in the herbicide and nutrient treated wetlands.
Increased abundance of plankton was unlikely to have been a direct stimulatory effect of the herbicide, but it may have been a result of the combination of indirect (from the first application of herbicide) and direct (from the second application of herbicide) effects. The loss of some zooplankton taxa during the initial herbicide application might have released the remaining zooplankton and phytoplankton assemblages from competition and predation. It is also hypothesized that this observation represents an indirect effect of the reduction of zooplankton grazing pressure on some phytoplankton taxa in the community, resulting from the significant loss of zooplankton abundance after the first herbicide application. The timing of the increasing abundance and richness of zooplankton occurred approximately in parallel to the reduction of emergent vegetation; when the treated sides of all wetlands had visibly reduced macrophyte cover (average of 19 % reduction).
RMS however notes that significant reduction in plant cover on treated sides of wetlands relative to their control sides following glyphosate herbicide application is not surprising, as reducing plant cover is the intended purpose of glyphosate herbicides (information obtained from Mudge, 2019, also assessed by RMS). The purpose of the additional glyphosate application directly targeting the macrophyte community was to maximize the possibility of indirect impacts of glyphosate herbicides on the invertebrate or amphibian communities through direct effects to the plant community. This consistent amount of herbicide applied directly to the plant community on the treated sides of all wetlands was much higher than the dose received through the different treatment concentrations applied directly to the water's surface.

The study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift and may have resulted in indirect effect on phytoplankton and zooplankton communities.
RMS also notes that measured concentrations of glyphosate was far below the nominal targeted (2.88 mg acid equivalents/L) and fastly decreased in the wetlands. Besides, despite having the same target glyphosate concentrations, glyphosate residues appeared (not statistically significant) to decline more slowly in the herbicide with nutrients-treated wetlands than the herbicide alone-treated wetlands, where the herbicide and nutrient wetlands had higher glyphosate concentrations of 1173.1 ± 1256.5 µg a.e./L on Day 1 and 195.1 ± 205.1 µg a.e./L on Day 3 than was observed in the herbicide alone-treated wetlands had glyphosate concentrations of 424.5 ± 343 µg a.e./L on Day 1 and 22.3 ± 18.3 µg a.e./L on Day 3. This high variability could not be explained by any difference of water volume or microbial respiration rates. RMS considers the study reliable with restrictions. The study authors also suggest that ecotoxicological risk assessments should also consider scenarios in which other contaminants or stressors may co occur in the receiving system, as the possibility exists for joint activity.

Baker et al.,2014 (see Appendix to Volume 3 (AS) B.9 on general literature data[41]), is an other paper related to Baker et al, 2016 as these studies were conducted concomitantly. Baker et al.,2014 focussed on the emergence of Chironomidae (Diptera) before and after herbicide-induced damage to macrophytes.
There were no direct effects of treatment on the structure of the Chironomidae community or on the overall emergence rates. However, after macrophyte cover declined as a result of herbicide application, there were statistically significant increases in emergence in all but the highest herbicide treatment, which had also received no nutrients. There was a negative relationship between chironomid abundance and macrophyte cover on the treated sides of wetlands.
Although direct toxicity of Roundup WeatherMax was not apparent, the authors observed longer-term impacts, suggesting that the indirect effects of this herbicide deserve more consideration when assessing the ecological risk of using herbicides in proximity to wetlands. The authors hypothesized that (based on the negative relationship between chironomid emergence rates and plant cover across all treated sides of wetlands) the loss of macrophytes from herbicide treatments led to increased chironomid abundance, possibly through some intermediary mechanism such as a loss of predators or increased food amounts.
As above, RMS considers this study relevant only for aquatic uses where emergent macrophytes are directly exposed.

From the same experiments as above, Mudge J. F. et al., 2019 (see Appendix to Volume 3 (AS) B.9 on general literature data [42]), assessed how different concentrations of glyphosate-based herbicides affect wetland plant communities over two years of herbicide application (alone and in combination with agricultural fertilizers) and two subsequent years without herbicide (or fertilizer) application. Lingering effects in the years after herbicides were applied (i.e. recovery) were also investigated.
The application of glyphosate-based herbicides was found to result in a decrease in macrophyte species richness, an increase in macrophyte species evenness, a decrease in macrophyte cover and a reduction in community similarity. These effects were evident in the first year of herbicide application (in 2009), and became more pronounced in the second year of herbicide application (in 2010). However, when herbicides were not applied in 2011, recovery was observed in most endpoints, with the exception being species evenness, for which partial recovery was not observed until 2012.
As already noted above by RMS, the significant reduction in plant cover on treated sides of wetlands relative to their control sides following glyphosate herbicide application is not surprising, as reducing

[41] Baker L. F. et al. (2014) The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands. Environmental toxicology and chemistry (2014), Vol. 33, No. 9, pp. 2076-85

[42] Mudge J. F. et al. (2019) Wetland macrophyte community response to and recovery from direct application of glyphosate based herbicides. Ecotoxicology and Environmental Safety, (2019) Vol. 183, Art. No. 109475

plant cover is the intended purpose of glyphosate herbicides. The lack of a concentration dependent effect in this study likely results from the additional spray at the maximum recommended label rate targeted specifically at emergent macrophytes that all wetlands received immediately following the application of the target concentration to the water. The purpose of the additional glyphosate application directly targeting the macrophyte community was to maximize the possibility of indirect impacts of glyphosate herbicides on the invertebrate or amphibian communities through direct effects to the plant community. However, this consistent amount of herbicide applied directly to the plant community on the treated sides of all wetlands was also much higher than the dose received through the different treatment concentrations applied directly to the water's surface.
The study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift.

RMS notes that Edge et al. (2020) (see Appendix to Volume 3 (PPP) on literature related to biodiversity) included an investigation of indirect effect on abundance of benthic invertebrates. From the same experiments as above, (Baker et al, 2014, 2016 and Mudge J. F. et al., 2019, (see Appendix to Volume 3 (AS) B.9 on general literature data), indirect effects on the relative abundance of predatory benthic invertebrates (and the abundance of Wood Frog larvae) arose from the direct effects of the herbicide on macrophyte cover.
These indirect effects were in the opposite direction to the direct effects of the herbicide, resulting in a compensatory effect and no overall change. The study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift.

Three of the five studies on amphibians (i.e., Edge et al 2011, 2012, 2013) investigated direct effects of glyphosate application, and are thus discussed in more detail in the standard risk assessment. The remaining two studies (Edge et al. 2014 and Edge et al. 2020) are based on a common dataset that shows both an increase and a decrease in biodiversity metrics (e.g., increased green frog larval abundance at most treatments with glyphosate, and decreased wood frog larval survival at high glyphosate concentrations combined with nutrient enrichment during the first year of the study). The increase in green frog abundance was suggested to be due do dead plant material (from glyphosate treatment adjacent to the wetland) which provided an improved habitat for oviposition. The authors pointed out that the increased abundance of green frog is of concern, since this species is larger and capable of completely removing other frog species from wetlands by predation of egg masses. This is an illustrative example of an indirect effect on one species due to change in habitat, resulting in a subsequent effect on another species via trophic interactions.

Rolando et al. 2017 (see Appendix to Volume 3 (PPP) on literature related to biodiversity), presents an international overview of the current use of glyphosate-based herbicides in planted forests and the associated risks. It concludes that glyphosate-based herbicides, as typically employed in planted forest management, do not pose a significant risk to humans and the terrestrial and aquatic environments. Sediment sorption and degradation of glyphosate have been identified as a primary removal mechanism for glyphosate from the water column in forested freshwater environments, a potential source of risk, particularly to sediment dwelling organisms. However, these risks are tempered by the strong ionic sorption mechanisms which are considered to limit leaching or diffusion into the water column and bioavailability of sediment-bound residues. This paper also states that subtle, sub-lethal, long-term, indirect effects, or potential interactions of glyphosate-based herbicides with other environmentally relevant stressors (e.g., herbicide mixtures, low dissolved oxygen, pH, excess nutrient inputs, other chemical pollutants) are less well understood as compared to simple direct acute or chronic effects.

- **Conclusion**

Assessment and conclusion by the applicant

The current aquatic risk assessment for glyphosate, its environmental metabolites, and the representative formulation demonstrate that a 1 m no-spray buffer zone from edge-of-field is protective of aquatic biodiversity from direct effects and indirect effects through trophic interactions. By demonstrating negligible risk of population-level effects on representative sensitive populations in edge-of-field surface waters, aquatic populations will be protected and propagation of indirect effects to the community, ecosystem, and landscape levels will not occur. When performing our assessment using the measured levels of glyphosate and AMPA from aquatic monitoring programs, we come to the same conclusion that no direct or indirect effects to aquatic biodiversity are likely to occur.

Assessment and conclusion by RMS

The approach followed in the EFSA guidance for aquatic organisms (2013)[43] for the definition of specific protection goals is in line with EFSA guidance on specific protection goals (2016). As such it is the most suitable guidance document that allow to consider biodiversity and ecosystem services for aquatic organisms.

The aquatic risk assessment of glyphosate is based on ecological threshold option. As such, the approach used could be considered appropriate to protect both direct effects as well as indirect effects including trophic interaction among the aquatic food chain in the sense of the EFSA aquatic guidance document (2013). However, given the data provided by the applicant and their assessment by RMS for glyphosate, it could not be considered that all indirect effects and food web interactions are addressed given that not all food sources are considered. For example, valid studies to assess the effects on sediment-dwelling organisms or rooted macrophytes of glyphosate that has a potential to partition in sediment are missing. Additionally, information on impact on decomposition processes in aquatic systems, or effects on the biofilm (algae, fungi and bacteria-matrix) would need to be considered. Further information on the effect to the aquatic community could also contribute to assess risk to biodiversity via indirect effects and trophic interactions.

Monitoring data confirmed that glyphosate and AMPA are frequently detected (Horth, 2012, [redacted] 2020), with detection above the limit of quantification (>LOQ) occurring in ~40% of samples for glyphosate and ~64% for AMPA. Exceedances of regulatory acceptable concentration (RAC) and Environmental Quality Standard (EQS) are limited, with over 99% compliance reported for these different triggers for both glyphosate and AMPA

Information from the literature data show that effects on wetland plant communities, changes in the phytoplankton, zooplankton and aquatic plant communities in wetland (Baker et al.,2016, Baker et al.,2014 and Mudge J. F. et al., 2019). However the studies are of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift and may have resulted in indirect effect on phytoplankton and zooplankton communities. The information are relevant for for aquatic uses where emergent macrophytes are directly sprayed with glyphosate-based products.

[43] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

Edge et al. (2020) included an investigation of indirect effect on abundance of benthic invertebrates. From the same experiments as above, (Baker et al, 2014, 2016 and Mudge J. F. et al., 2019), indirect effects on the relative abundance of predatory benthic invertebrates (and the abundance of Wood Frog larvae) arose from the direct effects of the herbicide on macrophyte cover. These indirect effects were in the opposite direction to the direct effects of the herbicide, resulting in a compensatory effect and no overall change.

Edge et al. (2014) and Edge et al. (2020) are based on a common dataset that shows both an increase and a decrease in biodiversity metrics (e.g., increased green frog larval abundance at most treatments with glyphosate, and decreased wood frog larval survival at high glyphosate concentrations combined with nutrient enrichment during the first year of the study). This is an illustrative example of an indirect effect on one species due to change in habitat, resulting in a subsequent effect on another species via trophic interactions.

The proposals for possible risk mitigation options as proposed by the applicant is presented under B.9.14.1.6. The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments. Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review in order to establish the basis for harmonised set of measures to be implemented on MS level at product authorisation.

B.9.14.1.3. Bees - Risk to biodiversity via Indirect Effects and Trophic Interactions

The guidance documents related to regulatory ecotoxicological risk assessment are focussed on direct effect of an exposure to pesticides. The basic risk assessment for bees assumes that bees are able to find enough food resources. Glyphosate being a total herbicide, a diminution of availability of flowering plants in-field can be expected. This indirect effect through trophic interaction is not taken into account in the risk assessment. Given the context of glyphosate and its broad area of use, it was recommended to have a broader consideration of indirect effects (via the destruction of the weeds). An analysis of the public literature on effects of glyphosate on bees (including non-apis species) was advised.

- **Indirect Effects on bees via Trophic Interactions**

Assessment and conclusion by the applicant

The ecotoxicology regulatory studies database for glyphosate includes a battery of acute and chronic guideline studies, designed to assess the potential for direct effects to bees, covering a range of life stages and different bee species.

The following approach has been taken to assess potential indirect effects via trophic interactions considers the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs based on direct effects assessment considering representative sensitive populations across the tested trophic levels.

Currently, specific protection goals (SPGs) for bees have not been adopted. However, for the purpose of this biodiversity assessment, three SPGs have been developed (Table […]).

Concerning specifically potential impacts on biodiversity, there currently is no EU wide guidance on how this should be assessed at the taxa group level within the context of a single active substance renewal risk assessment.

The first SPG is derived from the Plant Protection Product (PPP) regulations to achieve no significant effect on honeybee colony survival and development. The second SPG is aimed at protection of pollination services and production of hive products. The third SPG is aimed at protecting bee biodiversity.

The submitted risk assessment for direct effects considering the proposed GAP, is based on the existing EPPO and EFSA approaches (section 10.3.1). This has concluded low to negligible acute and chronic risk to larval and adult bees from direct effects and no risk mitigation measures are considered necessary.

Indirect effects assessment for Bees

Indirect effects to bees, resulting from reduction of off-crop pollen and nectar sources, may be mitigated through required no-spray buffer zones implemented to protect non-target terrestrial plant (NTTP) communities ([…]).

Indirect effects to bees may potentially result from reducing pollen and nectar sources by control of in-crop flowering weeds. However, a recent analysis of the likelihood of indirect effects by reduction of in-crop flowering weeds shows that indirect effects are unlikely to occur because of the relatively low amount of flowering weeds in-crop (Last *et al*., 2019). This data was derived from herbicide efficacy trial control data from a range of arable crops (sunflower, maize, oilseed rape, cereals, sugar beet, potatoes, peas and beans) as well as some permanent crops (orchards, citrus and grapes) and from a large data set on the presence of weed species within trial plots. Relevant information was extracted from the efficacy data with the intention of demonstrating that, for some crops, the occurrence of attractive flowering weeds in treated fields is relatively rare and constitutes < 10% of the area of use, thereby highlighting that the presence of bee weeds in the treated field scenario, is not applicable for many commercially grown crops.

Assessment and conclusion by RMS

RMS reminds that the current guidances (EPPO and EFSA) assess the potential direct toxic effect. They do not consider the removal of weeds and the reduction of floral resources that could follow application of herbicides such as glyphosate.

Reference to Last et al, 2019[44] was made in an attempt to demonstrate the low abundance of weeds in a variety of crops. This would indicate a negligible impact of weed removal on the availability of the nectar and pollen collected by bees. The presence of weeds was investigated in control plots of herbicide efficacy trials from different crops. RMS considers this may provide relevant data.

In their analysis, Last et al, 2019, intended to demonstrate that, for some crops, the occurrence of attractive flowering weeds in treated fields is relatively rare and constitutes < 10% of the area of use. This was done because, in the EFSA Guidance Document for bees (EFSA, 2013), exposure through pollen and nectar from flowering weeds in the treated field was identified as one of the exposure scenarios that need to be considered in the risk assessment. However, this scenario could further consider as indicated in Section 2.3 of Appendix N of EFSA (2013), the following is stated: "*If the first step results in an unacceptable risk, it may be checked whether it is likely that a significant fraction of the surface area of the treated fields is covered by weeds at the application time. If this is likely in less than 10% of the area of use of the substance, no weeds will occur in a 90th percentile case and thus their exposure can be ignored. For example, weeds are usually not abundant in annual crops - abundant weed growth is more likely to occur in, for example, orchards. However, at this moment no guidance for the assessment of the abundance of weeds is available for most crops*".

[44] Last, G. et al., 2019. Regulatory report on the occurrence of flowering weeds in agricultural fields.

RMS highlights that this concept was designed to address the potential exposure of bees to pesticides via the weeds (i.e. direct toxic effect via foraging on flowering weeds). Nevertheless the same reasoning may be adapted for the indirect effects assessment subsequent to weed removal. In short, if presence of weeds (in the absence of weeding via chemicals) is likely in less than 10% of the area of use of the substance, no weeds will occur in a 90th percentile case and thus the indirect effect of weed removal could be ignored.

Using this concept, the underlying assumptions made in the analysis by Last et al 2019 should be reconsidered (see below).

Assessing whether weeds in the treated field is a relevant source of food comprises 2 separate steps:

1. Determine what fraction of the surface area of a single treated field has to be covered by weeds in order for this fraction to be considered as 'significant'.

In the EFSA, 2013, it is not further specified what that 'significant fraction' of the surface area of a field covered by weeds should be, or how it should be determined. Last et 2019 made assumptions that are not agreed by RMS (see details in the Appendix for Literature on Biodiversity).
There are doubts whether the distinction dicotyl – monocotyl is acceptable as criterion to distinguish between flowering weeds that are attractive to bees or not (acknowledged by the authors). In absence of another clear criterion, RMS considers that all weeds may be considered relevant.
Besides, focussing only on weeds from BBCH stage 60-69 might be too limited. Although it is stated in the study report that BBCH stage ≥30, ≥40, ≥40 and ≥70 were also included in the assessment, the outcome of this assessment is not reported. RMS believes this study may be relevant to address indirect effect issues via the reduction of food availability subsequent to herbicide use. In such purpose, even if it is likely that only a small proportion of weeds in the field will be flowering at the time of application (and flowering weeds that are sprayed will rapidly wilt and their flowers will no longer be attractive to bees), flowering weeds only represent a portion of all weeds (including those not yet flowering). In agricultural landscapes, weeds may be the only permanent source of food. Removing the weeds at their early development stage may deprive bees of the only source of food normally available later on. RMS then considers that (to address the relevance of weeds as food source), all weeds at BBCH 0 to 69 should be considered.
The authors set a threshold of 10% weed ground cover within a single field (as "significant fraction", referring to Appendix N of the EFSA, 2013). Specific data or an argumentation to underpin the assumption that a weed ground cover within a field of below 10% is not significant for bees has not been provided. It is therefore assumed that this threshold of 10% originates from a misinterpretation of the text in Appendix N of the EFSA, 2013.

2. Consider all fields in the area of use of the substance, and determine in what percentage of these fields the weed coverage is higher than that 'significant fraction of the surface area'. If this is the case in less than 10% of all fields, no weeds will occur in the 90th percentile case, and thus weed removal can be considered not relevant.

RMS notes that in the dataset used by Last et al, 2019, although data from trials where conservation tillage was applied is also available, intensive tillage operations were performed prior to sowing in the majority of cases. The complete analysis of Last et al., 2019 could be found in the appendix to Volume 3 (PPP) B.9 on literature review related to biodiversity.

Overall, RMS notes that the percentages of weed occurrences reported in this analysis (not considering only considering those that are flowering), already breached the "threshold" of 10% (occurrence) in several crops or were around this value (in a lesser extent for sugar beet and pea). Besides, the drawbacks identified in this analysis may underestimate the real relevance of weeds.

The results from these efficacy trials may actually indicate the weed are present and relevant in more than 10% of cases.
RMS nevertheless believes that weed relevance (in term of food supply) may depend on crops, tillage practices and timing of applications. The dataset available in Last et al, 2019 may be of use to define these specific conditions/crops in order to establish exposure scenario to assess both direct and indirect effects related to the availability of the food sources.

The risk resulting from losing food by the use of glyphosate should be minimized, and should be compensated if unavoidable. Nevertheless, obligatory compensation areas and extra mitigation measures are outside the current regulatory framework. Further discussions involving also risk managers would be necessary. In this purpose it should be bear in mind that the current GAP does not cover all currently authorized uses in Europe (e.g. non-agricultural, non-professional uses, etc…).

- **Ecotoxicological relevance of monitoring data for glyphosate residues in honey and pollen**

Assessment and conclusion by the applicant

The duration of exposure of honey bees to glyphosate in the environment will be transient and of limited duration. The reason for this is that only a small proportion of weeds in the field will be flowering at the time of application (Last et al., 2019) and flowering weeds that are sprayed – for example in crop inter-row applications, in recently emerged crops, will rapidly wilt and their flowers will no longer be attractive to bees (Thompson et al., 2014). In addition, levels of glyphosate in nectar and honey will rapidly decline with 50% of initial levels after only 1 to 2 days (Thompson et al., 2014).

Laberge et al., (1997) measured glyphosate levels in nectar and pollen in a field study conducted in an agro-forestry environment. For this study, hives were placed within or at various distances from treated sites. Detectable residues of glyphosate were observed in approximately 50% of the pollen samples and 3 of 9 honey samples, with maximal residues of 8.2 mg a.e./kg in pollen sampled 3 days post-treatment from a hive situated directly within the treated area. Based on their risk assessment, Laberge et al., (1997) concluded that risks associated with glyphosate were negligible.

Data, on the frequency of detection and the level of glyphosate in honey, are summarized within the EFSA residue database. These data show a 10% frequency of detection (42 out of 406 samples), with a maximum level detected of 0.61 ppm and an average of 0.09 ppm (minimum LOQ of 0.01 ppm and max LOQ of 0.14 ppm).

Another representative honey residue study was conducted by the US FDA with an LC-MS/MS assay (Chamkasem and Vargo, 2017). Their validated assay had an LOQ = 16 µg/kg, and 9 of 16 samples bought from a local market had glyphosate > LOQ. Of these, the median concentration of glyphosate was 0.026 ppm with a range of 0.017 to 0.121 ppm. Low levels of glyphosate in honey were likely as the outcome of processing of the nectar by the bee's, limited exposure to glyphosate in the environment, and/or dilution with untreated nectar in the hive.

Additional studies in the literature report similar residues in honey and have been summarized in Vicini et al., (2020). The results of these monitoring studies demonstrate low environmental exposures to glyphosate and the conservative nature of the exposure values used for glyphosate exposure assessment for bees.

Assessment and conclusion by RMS

Last et al., 2019 was provided and analysed by RMS. This study may be relevant to address indirect effect issues via the reduction of food availability subsequent to herbicide use (see above).
However even if it is likely that only a small proportion of weeds in the field will be flowering at the time of application and flowering weeds that are sprayed will rapidly wilt and their flowers will no longer be attractive to bees, flowering weeds only represent a portion of all weeds (including those not yet flowering). In agricultural landscapes, weeds may be the only permanent source of food. Removing the weeds at early development stage may deprive bees of the only source of food normally available later on. All weeds at BBCH 0 to 69 should be considered.

Laberge et al., (1997), Chamkasem and Vargo, 2017[45] and Vicini et al., (2020)[46] reports data on food contamination. These reports were requested by RMS.
The applicant indicated that Laberge et al. (2017) was not available and no summary was provided. It was then not assessed by RMS.
Chamkasem and Vargo (2017) and Vicini et al., (2020) were checked by RMS. Both papers contained data related to glyphosate contamination in honey. It is RMS opinion that it is not relevant for indirect effects/biodiversity issues. Such data may potentially be used in a risk assessment (via honey consumption) even if honey is not currently considered a "more" relevant matrice. The maximum level of glyphosate in honey that was retrieved in these paper is 163 µg/kg from literature (source cited: Rubio et al, 2014) and 610 µg/kg in honey from market survey (source cited in the report: EFSA). The complete analysis of Chamkasem and Vargo (2017) and Vicini et al., (2020)could be found in the appendix to Volume 3 CP B.9 on literature review related to biodiversity.
RMS however highlights that pollen and nectar are currently considered as the most relevant matrices for bee risk assessment (for the calculation of exposure estimates). The exposure estimates (in both EPPO and EFSA guidances) are calculated on the basis of the sugar content in nectar.
Honey, although being consumed by bees, is currently not a directly relevant matrice. RMS highlights that consumption depends on sugar content in nectar/honey. Considering a higher sugar content in honey (than in nectar) it is assumed that honey consumption is relatively lesser than for nectar. RMS also assumes that sugar content in honey is higher than the contaminated syrup (a 50% w/v sucrose solution) that was fed to bees in [redacted] 2012.
In [redacted] 2012, colonies were exposed to contaminated syrup at concentrations up to 266 mg glyphosate acid equivalent/kg syrup, (measured). No effect was noted for brood development of honey bees at this concentration.
Therefore, considering the residue concentration in honey available from these 2 articles, (that are below the residue concentration in the syrup used in Thompson et al, 2014), it is unlikely that such exposure levels result in adverse effect on bees.

- **Scientific Literature that informs the bee assessment**

Assessment and conclusion by the applicant

The potential for adverse effects of glyphosate and Roundup to honey bees have been extensively tested in colony level feeding studies (Ferguson, 1987, 1988; Burgett and Fisher, 1990; Thompson et al, 2014). The first colony feeding study was performed in Australia and found no significant effects to larval and adult honey bees after six consecutive days of whole-hive exposure to 5 mg a.e./kg sucrose solution

[45] Chamkasem, N. & Vargo, J.D., 2017. Development and independent laboratory validation of an analytical method for the direct determination of glyphosate, glufosinate, and aminomethylphosphonic acid in honey by liquid chromatography/tandem mass spectrometry.
[46] Vicini, J. L. et al., 2019. Glyphosate in livestock: feed residues and animal health. Journal of animal science (2019), Vol. 97, No. 11, pp. 4509. DOI: 10.1093/jas/skz295

(Ferguson, 1987; Ferguson, 1988). Ferguson concluded from her study that glyphosate could be safely used around honey bee hives. Further, Ferguson reported that levels for a range of pesticides rapidly decline in nectar and pollen, with > 90% dissipation in 3 to 4 days after spraying. Similar results, showing a rapid decline of glyphosate residues in nectar and pollen, were also reported by Thompson et al. (2014). This rapid decline of glyphosate residues in nectar and pollen greatly limits exposure of honey bee colonies to glyphosate.

These original findings by Ferguson were supported by colony feeding trials conducted by two well-established apicultural experts, Burgett and Fisher, from Oregon State University (Burgett and Fisher, 1990). In their first honey bee colony feeding study, colonies were fed Roundup in sucrose solution at a concentration that was 100 to 1000 times above worst-case glyphosate exposure levels reported by Thompson et al. (2014). No significant effects were observed to honey bee adults or brood production after 42 days of observation, which is an indicator of no effects to egg production, egg laying and brood maintenance. In their second whole-hive study, blooming bee-attractive vegetation adjacent to the hives were treated at 6.8 kg a.e./ha. As with the colony feeding study, there were no effects to adult honey bee or brood production over the 42-day post-application period. These earlier findings are supported by a more recently published colony feeding study followed international guidance for honey bee testing (OECD guidance document 75) and this study was found to be acceptable for risk assessment in the recent glyphosate Annex 1 renewal (Thompson et al, 2014). Thompson et al. demonstrated no effect to larval development, growth and survival and adult survival at glyphosate concentrations of 75, 150 and 300 mg a.e./L.

All of the other bee effect studies reviewed in the literature did not measure effects on survival, growth, development, or reproduction with the exception of one study that evaluated effects on survival after an extreme challenge with the opportunistic pathogen *Serratia marcescens* (Motta et al. 2018). The relevance of the laboratory study conducted by Motta et al. is questionable because of the relatively high exposure levels (10 mg a.e./L) and artificial nature of the study.

Assessment and conclusion by RMS

The study of Ferguson, 1987, 1988[47] is not relevant to address indirect effects/biodiversity issues (this feeding study only aims to investigate direct toxic effects). RMS does not consider the data available sufficient for a proper assessment of the study and its results.
Study poorly described (study design, environmental conditions, etc…), test item not identified, no results presented (only a statement that glyphosate did not significantly affect the brood and bees). For more details, please refer to the appendix of Volume 3 CP B.9 related to literature data on biodiversity.

Burgett and Fisher, 1990[48] conducted two types of field evaluations: (1) feeding trials whereby glyphosate was fed directly to honey bee colonies using a 40% sugar solution as the toxicant vehicle and (2) a spray trial where ca. 1.5 acres of blooming vegetation containing 5 colonies were aerially sprayed with a 5% RoundupR plus 0.25% Nalcotrol II. Again, RMS considers these trials poorly described (study design, results) and not relevant/reliable to address indirect effects/biodiversity issues. For more details, please refer to the appendix of Volume 3 CP B.9 related to literature data on biodiversity.

Thompson et al, 2014 was not assessed by RMS as the first stage (on exposure) of this publication actually corresponds to the study summarized and assessed by RMS (CP 10.3.1.5/001, [redacted] 2011, Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions. Ref V7YH1002).

[47] Ferguson, F., 1988.Long term effects of systemic pesticides on honey bees. Bee keeping in the year 2000: Second Australian and International Beekeeping Congress, Surfers Paradise, Gold Coast, Queensland, Australia, July 21-26, 1988

[48] Burgett, M. & Fisher, G., 1990 A review of the Belizean honey bee industry: Final report prepared at the request of The Belize Honey Producers Federation.

The second part (for effect) of this publication actually corresponds to the study summarized in Volume 3CA and already assessed by RMS (CA 8.3.1.4/001, [redacted] 2012, Glyphosate: Evaluating potential effects on honeybee brood (*Apis mellifera*) development, V7YH1001).
In this study colonies were exposed to contaminated syrup at concentrations up to 266 mg glyphosate acid equivalent/kg syrup, (measured). No effect was noted for brood development of honey bees at this concentration.

Motta et al., 2018[49] suggests that glyphosate had some effect on honeybee microbiota. These conclusions were also supported by Blot N, et al 2019. (also assessed by RMS). RMS notes the absence of clear conceptual link between effects on the honeybee microbiota.and the specific protection goals for bees (SPG). It is agreed that it may play a role in the colony/population health, but such link is not immediate in conceptual terms and not quantifiable. These studies do not provide any data that could be used for the risk assessment. RMS provided an assessment on the reliablility of these two studies. These studies were considered not reliable enough to confirm the effect on microbiota or potential adversity on bee health. For more details, please refer to the appendix of Volume 3 CA B.9 related to literature data on ecotoxicology for assessment of Motta et al. 2018 and to the table B.9.11.1.4-2 of Volume 3 CA B.9 under point B.9.11 for Blot N. 2019.

Balbuena M. S. et al., 2015 states that exposure to non-lethal concentrations of glyphosate causes sub-lethal effects, which modify the bees' foraging behavior. In this study homeward trajectories of honeybees were tracked using harmonic radar technology. Honeybees that had been fed with solution containing 10 $mg.l^{-1}$ glyphosate spent more time performing homeward flights than control bees or bees treated with lower concentrations. Overall these results were considered not reliable by RMS. Information on indirect effects, bee diversity or trophic interactions can not be concluded from this study. For more details, please refer to the table B.9.11.1.4-2 in Volume 3 CA B.9.

- **Assessment**

Assessment and conclusion by the applicant

After a through literature review and considering all recent guidance, the approach taken, aimed to assess potential indirect effects via trophic interactions and the impact on biodiversity for bees including *Apis* and non-*Apis* bee species, using a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals.

In the following table, the specific protection goals relevant to bees / pollinators are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relates directly to the effects study endpoints. A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence) and if necessary through the application of standard mitigation measures as recognised at the EU level.

Based on the measurement endpoints from the study types, and the direct effects assessment presented above in this section, it is anticipated that for the proposed uses on the GAP table, that there will be no indirect effects on bee populations in terms of loss of foraging habitat that is not protected by the required in-field buffer distance required to support the non-target terrestrial plant – direct effects risk

[49] Motta, E. V. S. et al., 2018. Glyphosate perturbs the gut microbiota of honey bees. Proceedings of the National Academy of Sciences of the United States of America (2018), Vol. 115, No. 41, pp. 10305 DOI: 10.1073/pnas.1803880115

assessment, required to meet the specific protection goal for NTTPs which will also support bees, given the limited relevance to bees of weed species found in-field.

Table [...]: The relationship between Specific Protection Goals, assessment and measurement endpoints for bees from contact and dietary exposure.

Specific Protection Goals	Assessment Endpoints	Measurement Endpoints	Study Types
No significant effect on honeybee colony survival and development.	Population size and stability of managed bees	Adult and larval survival and larval emergence	Adult honeybee acute Adult Bumble bee acute Adult solitary bee acute Adult honeybee chronic Larval honeybee emergence Honeybee semi-field brood study
Pollination services and production of hive products	Population size and stability of native and commercially managed bees and quantity and quality of honeybee hive products.	Adult and larval survival and larval emergence	
Bee Biodiversity	Species richness and abundance	Adult and larval survival and larval emergence	

Bee Biodiversity Assessment
The direct effects assessment demonstrates negligible acute and chronic risk to adult and larval bees and is protective of effects at the population level. Indirect effects to bee populations from in-crop weed control is unlikely because in-crop flowering weeds are not a significant resource for nectar and honey and the off-crop NTTP community will be protected by in-crop no spray zones. Taken together, impacts on bee biodiversity from the intended uses of glyphosate and following the required risk mitigation measures, impacts to bee biodiversity are unlikely.

Assessment and conclusion by RMS

RMS considers that the standard risk assessment addresses the direct effect only. The indirect effect, due to the loss of food resources, is not addressed. No study investigating biodiversity issues or indirect effects was found in the literature review.
RMS considers that the appropriateness of applicant's proposal concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control should be discussed during the EU peer-review.

- **Conclusion (Applicant's proposal)**

Assessment and conclusion by the applicant

Glyphosate is a critical tool to enable conservation tillage systems, which can greatly improve water quality in agroecosystems by reducing sediment and nutrient run-off. Negligible risk of direct effects to bee biodiversity is supported by measures of glyphosate residues in honey from monitoring programs. Indirect effects from in-crop weed control is unlikely to impact bee populations because in-crop flowering weeds are not a significant resource for nectar, pollen and honey. In addition, the off-crop NTTP community will be protected by in-crop no-spray zones as a required mitigation. Taken together, impacts on bee biodiversity from the intended uses of glyphosate and following the required risk mitigation measures, impacts to bee biodiversity are unlikely.

Assessment and conclusion by RMS

Based on standard risk assessment, no risk mitigation measure is necessary to protect bees (from direct effect). The applicant states that the no spray buffer areas in-field and the drift reducing technologies set to protect non-target plants in off-target areas will in turn support non-target arthropod communities including beneficial insects such as the pollinators in off-field areas. RMS notes that, in the absence of a reliable vegetative vigor study (for MON 52276), the risk assessment cannot be conducted and the importance of the no-spray zones (or other standard mitigation measures) is not stated yet.
Some references informed on the abundance of weeds in agricultural landscape (e.g. Last et al, 2019). The results from these efficacy trials may actually indicate the weed are present and relevant in more than 10% of cases. This concept was designed to address the potential exposure of bees to pesticides via the weeds (i.e. direct toxic effect via foraging on flowering weeds). Nevertheless the same reasoning may be adapted for the indirect effects assessment subsequent to weed removal. RMS believes that weed relevance (in term of food supply) may depend on crops, tillage practices and timing of applications. The dataset available in Last et al, 2019 may be of use to define these specific conditions/crops. In short, if presence of weeds (in the absence of weeding via chemicals) is likely in less than 10% of the area of use of the substance, no weeds will occur in a 90th percentile case and thus the indirect effect of weed removal may be assumed to be low.
However in agricultural landscapes, weeds may be the only permanent source of food. Removing the weeds at early development stage may deprive bees of the only source of food normally available later on.
The applicant considered that indirect effects from in-crop weed control is unlikely to impact bee populations because in-crop flowering weeds are not a significant resource for nectar and pollen. In addition, the applicant considered that risk mitigation to protect off-field non-target terrestrial plants will benefit to bees and therefore no impact on bee biodiversity is concluded.
However indirect effects following reduction of floral resources that could follow application of herbicides such as glyphosate are not taken into account. RMS considered that reduction of floral resources and its impact on bees is difficult to handle in a risk assessment approach based on local scale (field). It requires the development of tools that allow assessment at landscape level.

The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments. Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review in order to establish the basis for harmonised set of measures to be implemented on MS level at product authorisation. Indeed, given that flowering weeds can be considered as important food source for bees and other pollinators, the reduction of blossoming weeds should be prevented in-field and off-field too by applying risk mitigation measures - both non-standard and standard - as proposed by the applicant:

i) treated area restriction,

ii) reduction of spray drift and establishment of buffer zones (as required for off-crop NTTP).

Besides this, MSs might consider the need of compensation potential of landscape to be implemented at different scale (local, regional, Euopean).

B.9.14.1.4. Non-target arthropods other than bees - Risk to biodiversity via Indirect Effects and Trophic Interactions

The guidance documents related to regulatory ecotoxicological risk assessment are focus on direct effects. As stated in regulation (EU) 2017/2324, the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions should be considered. Thus, the potential impact of the loss of habitats for foliage dwelling arthropods has to be considered. Indeed, a loss of plant biodiversity due to application of plant protection products may affect the entire food web, including

arthropods. The presence of arthropods as food sources is vital to the survival of many bird and mammal species. RMS therefore recommended to the applicant to have a broader consideration of potential effects on NTAs by taking into consideration the most recent EFSA opinion (Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996) together with findings from the literature search.

- **Indirect Effects via trophic Interactions**

Assessment and conclusion by the applicant

The ecotoxicology regulatory study database for the representative formulation (MON 52276) includes guideline studies and risk assessment methodology that was designed to assess potential direct and indirect effects on beneficial insect communities (ESCORT 2, 2000). For the Tier 1 NTA assessment, studies were conducted using ecologically important and highly sensitive indicator species of adverse effects. Then at Tier II (extended studies) additional levels of realism were introduced into the exposure scenario, by introducing exposure on leaf-based substrates. Specific protection goals (SPGs) for non-target arthropods (NTAs) were developed at the ESCORT 2 and 3, (2000 and 2010) workshops, with separate SPGs developed for arthropods occurring in the crop / in-field and off the crop / off-field. ESCORT 3 saw further distinction between in-field and off-field scenarios. It was considered practical by the experts during the ESCORT 3 workshop to make distinctions and recognize trade-offs between in-crop and off-crop and in-field and off-field area, given the differences in the socio-economic and ecological functions of these two distinct areas. This is consistent with the recommendation of the EFSA problem formulation workshop that was convened to prepare guidance that would inform the development of SPGs (EFSA, 2010).
In [redacted] 2020 (KCA 8.7/001), the applicant also indicated the following: "However, the ESCORT3 guidance and in-field SPG did not consider indirect effects of in-crop weed control on NTA communities and cascading effects on birds and mammals from a reduction in food resources in their scheme."

The first SPG from the ESCORT workshop addresses in-crop applications, where the goal is to maintain pest control (i.e., activity of parasitoids and predators) and to also provide a food source for wildlife - minimizing indirect effects through trophic interactions. In turn the aim here is to enable an in-crop NTA community to recover.

The in-crop measurement endpoint and risk assessment procedures developed to achieve this SPG, allow for a maximum of a 50% direct effect on individuals in-crop from a Tier 1 - 2 assessment approach. At the 1st tier lethality effects are considered, whilst at the second tier, impacts on reproduction are considered. The rationale for 50% effect threshold for direct effects, is based on the principle that this level of effect would allow for in-field recovery via immigration of beneficial insects from the off-field areas to the in-field areas, or from in-field / off-crop areas, where for example, a no spray buffer in-field / off-crop buffer is included, thereby enhancing recovery.

The second SPG was derived to protect the off-crop NTA community, with the goal to maintain NTA biodiversity off-crop to facilitate in-field recovery of non-target arthropod species.

Assessment and conclusion by RMS

The applicant refers to a tier 1 assessment, however no tier 1 assessment with NTA could be performed as the glassplate tests with *T. pyri* and *A. rhopalosiphi* were not valid.
The terms beneficial arthropods and non-target arthropods appears to be used incorrectly in the applicant's proposal. The ESCORT 2 and 3 guidances cover the risk assessment for non-target arthropods i.e arthropods that are not considered as pest. The risk for beneficials arthropods that are introduced for use in IPM are still relevant under Regulation EC No 1107/2009 but are not in the scope of the regulatory

ecotoxicological risk assessment that focus on endogenous arthropods. For the ecotoxicology section, RMS therefore focus on endogenous non-target arthropods.

There is currently no specific guidance or harmonized assessment procedures at the EU level for conducting a comprehensive biodiversity assessment. Highlighting the need for Specific Protection Goals (SPGs) used for the biodiversity assessment, the proposals made by the applicant are based on ESCORT 3 workshop (2010).

The EFSA guidance on specific protection goals (2016) aims to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services. As such, RMS considered that it is the most suitable approach available to assess biodiversity and indirect effects in the context of regulatory risk assessment.
Reference to the most recent EFSA opinion[50] (Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996) together with findings from the literature search was recommended by RMS.

Specific protection goals were defined in the EFSA Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods (2015):

Ecosystem service	Specific protection goal options
Biodiversity and genetic resources	In-field habitats: small effects on abundance and occupancy of NTA populations Off-field habitats: negligible effects on individual densities of all NTA species occurring in the off-crop and on spatial abundance and occupancy of NTA species
Cultural services (aesthetic value)	Same as 'Biodiversity and genetic resources'
Pest control	In-field habitats: medium effects on abundance and occupancy of key driver functional groups (e.g. parasitoids, predators) Off-field habitats: negligible effects on abundance and occupancy of key driver populations
Food web support	In-field habitats: small effects on abundance and occupancy of key driver functional groups (e.g. soil or leaf-dwelling NTAs). Generally, no shortfall below the limits given by chick food indices Off-field habitats: negligible effects on abundance and occupancy of key driver populations
Pollination	In-field habitats: small effects on abundance and occupancy of key driver functional groups (NTA pollinators) during flowering of the crop Off-field habitats: negligible effects on abundance and occupancy of key driver populations

The EFSA Scientific Opinion (2015) on non-target arthropods provided also information on further data that may be requested for assessing effects on non-target arthropods (including new test endpoints). No assessment scheme is available yet. RMS nevertheless requested to address this via information from the open literature.

The EFSA Opinion (2015) on non-target arthropods follows the principles of the EFSA 2016 method for defining SPGs as ecosystem services and SPGs were already identified. Therefore, the methodology and the process implemented in the EFSA Opinion on non-target arthropods (2015) can be considered in line with the EFSA method for defining SPGs. The EFSA opinion on non-target arthropods (2015)

[50] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996, 212 pp. doi:10.2903/j.efsa.2015.3996

suggested a specification of five interrelated dimensions of the SPG (i.e. ecological entities, attribute, magnitude, temporal and spatial scale) in line with the third step of the EFSA method but these were not discussed with risk managers of DG SANTE and the European Union (EU) Member States.

- **Scientific Literature that informs the NTA assessment**

Assessment and conclusion by the applicant

The scientific literature review conducted for the last Annex I renewal (submitted in 2012) that appears in the RAR (2015) contains an extensive review of ecotoxicological papers considered relevant but supplementary to the Annex I renewal.

These papers presented information that could not be relatable to an EU level ecotoxicological risk assessment, but that were considered in the previous dossier, where they were also evaluated by the previous RMS (UBA). A further evaluation of these reviewed literature has not been conducted. The previous literature review has been submitted as part of the Literature review requirements and is presented in Annex M-CA 8-01 of the document M-CA Section 8.

Literature review for non-target arthropods from the previous Annex I (2012) submission.
In the area of arthropods other than bees, a total of 31 peer reviewed papers were submitted, with no paper considered relevant for use in risk assessment. The RMS (UBA) re-evaluated the submitted papers with 11 papers recognised as information having a low weight and a further 7 publications being considered as supportive information.

In the evaluation of the literature from the previous Annex I submission, the RMS (UBA) indicated that indirect effects on beneficial arthropod communities take place within treated areas and are principally due to vegetation changes subsequent to herbicide application. These vegetation changes, mainly decomposition / loss of plant cover, might result in a drastic reduction of the habitats of beneficial and other non-target arthropod communities and a loss of their refuges from predators. This would anyway be the case if a non-chemical means of weed control was applied.

The RMS (UBA) reviewed a multiyear study using pitfall trapping to collect mobile arthropod species on the soil surface, the combination of conservation tillage and herbicide treatment had less impact on biodiversity than conventional ploughing (Schier, 2006). The RMS (UBA) concluded that conservation tillage without the use of glyphosate is not practiced, due to the upcoming weed pressure on culture crops. Stating that it was not possible to identify the effects of glyphosate applications in the performed studies.

Arthropods in their natural environment can be exposed directly to pesticides after the application due to residues on food or due to contact with contaminated surfaces (such as plants, soil, surrounding substrate).
The RMS (UBA) also stated that risk analysis is currently based on beneficial arthropods important for biological control of agronomic pests, through predation or parasitism, including beetles, mites, wasps and spiders. They indicated that test species were selected for practical reasons because of their utility in agricultural production and feasibility in experimental setups than on the basis of their ecological relevance.
The Notifiers indicate that the species selected for these tests are the representative species selected for testing according to Annex I data requirements. These same species are considered in the current 'arthropods other than bees' risk assessment, that includes impacts on survival and reproduction, that

is considered relevant to assess the recovery potential of such populations in transient habitats such a field row crops.

The RMS (UBA) highlighted that effects on different life stages and on other species, not considered in the traditional risk assessment, together with the indirect effects of herbicide treatment on the vegetation of their habitat, receive less attention even though they might have implications for the success of survival and reproduction.

In the current risk assessment – including the assessment of indirect effects via trophic interaction, the implications of the observed effects at the habitat and population level are considered. Notable from the previous RMS (UBA) review is the fact that there are few studies available on the indirect effects of glyphosate and also on conventional tillage weed control practices on terrestrial arthropod populations. This is still the case, but the assessment based on the direct effects' assessment is considered within the following indirect effects via trophic interaction assessment.

Glyphosate is considered a conservation tool that facilitates biodiversity. As stated by the previous RMS (UBA) following an assessment of a wide range of terrestrial invertebrate taxa showed variable responses in abundance and their diversity being largely a function of the degree of vegetation control (Guiseppe et al., 2006; Sullivan and Sullivan, 2002). It was also identified that populations of arthropods in areas where conservation tillage is practiced - of which glyphosate is typically used to enable, often have more beneficial insects and consequently diversity of other wildlife (Warburton and Klimstra, 1984).
Concerning the current literature review, there were no literature articles considered relevant to the ecotoxicological risk assessment for Annex I renewal.

A number of relevant but supplementary papers were identified, that discuss the selectivity of glyphosate based herbicides on a range of non-target arthropods such as Culicidae (Bara et al., 2014, Mohammed et al., 2016)), Chrysoperla externa (Castilhos et al., 2011, Pasini et al., 2018), Colorado potato beetle (Rainio et al., 2018), rose-grain aphids (Saska et al., (2016), Hymenopterans (Stecca et al., 2016), Bombyx mori (You et al., 2010 and Zhang et al., 2011) that all report impacts either directly in terms of mortality or indirectly via effects on reproduction or changes in life history traits. However, the observed findings are based on exposure of specific foliar predators following either an 'over the top application' under unrealistic exposure conditions, using glyphosate-based herbicides that are not the representative formulation for the Annex I renewal. The endpoints presented in these papers are not relatable to a test design that is used in EU level non-target arthropod risk assessment and were therefore considered supplementary to the assessment. Please refer to the literature review for further information on the supplementary nature of these articles.

Assessment and conclusion by RMS

The papers submitted for the previous Annex I submission were not reassessed by RMS. None of them impacted the outcome of the risk assessment (RAR 2015). The above statement provided by the applicant reflects the previous conclusions of the RAR 2015:

RMS nevertheless reminds that (as previously highlighted in RAR 2015) effects on various developmental stages of arthropods, physiology, and behavior or prey consumption are not given consideration in traditional risk assessment. Glyphosate containing products can be harmfull towards egg stages of *Trichogramma*, whereas at other parasitoid stages the same product was harmless. Sublethal effects of glyphosate were assessed in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of a web weaver spider (*Alpaida veniliae*) in Argentina (Benamu et al., 2010) and on wolf spiders in north America (Evans et al., 2010). The authors concluded that the exposure to glyphosate containing products affects the

behavior of the animals and their capacity to grow and persist in agroecosystems. In contrast, short term exposures (2h and one-day residues) of spiders and carabid beetles, respectively *Pardosa agricola* and *Poecilus cupreus*, did not affect mating or avoidance of the arthropods, but (only) slightly slower movement (Michalkova et al., 2009).

RAR 2015 further stated that these effects together with the indirect effects of herbicide treatment on the vegetation of their habitat might have implications for the success of survival and reproduction.
RMS further consider that there is a need to investigate the impact of loss of habitats fro non-target arthropods. For this purpose it is requested to the applicant to consider the results of Pleasants et al (2012)[51] on the effect of glyphosate on populations of the monarch butterfly due to habitat loss (data gap).

According to the applicant, the following 3 publications were used in the previous RAR (2015):
- Guiseppe KFL, Drummond FA, Stubbs C, Woods S. 2006. The Use of Glyphosate Herbicides in Managed Forest Ecosystems and their Effects on Non-Target Organisms with Particular Reference to Ants as Bioindicators; Maine Agricultural and Forest Experiment Station Technical Bulletin 192; Maine Agricultural and Forest Experiment Station, University of Maine: Orono, ME, USA, p. 51.
- Sullivan TP, Sullivan DS. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicides on plant and animal diversity in terrestrial systems. Env Rev 11:37-59.
- Warburton DB, Klimstra WD. 1984. Wildlife use of no-till and conventionally tilled corn fields. Journal of Soil and Water Conservation. 39:327-330.

RMS did not find any trace of the studies of Guiseppe KFL, et al. 2006. and Warburton DB, Klimstra WD. 1984 in RAR 2015. In the study Sullivan TP, Sullivan DS. 2003, only findings on birds were considered in RAR 2015. These articles have been further assessed by RMS, please refer to the appendix of Volume 3 CP B.9 related to literature on biodiversity.

Guiseppe KFL et al, 2006 reviewed articles related to ecological effects of the herbicide glyphosate used in forested landscapes. Among these papers, some stated that homopteran densities were lower in herbicide-treated plots compared with brush-saw-treated plots and non-treated control plots. It was hypothesized that indirect effects of herbicide treatment altered the nutritional quality of tree and shrub species (as homoptera feed on either phloem or xylem). Also indirect effects of herbicides on communities of herbivorous arthropods, in most cases, were hypothesized to be a result of reduced floral resources and the effect that this reduction would have on arthropods that require them during at least one phase of their life cycle. Studies are referenced that stated that herbicides have indirect effects on beneficial wasp and bees. These studies present correlative relationships that suggest that decreases in flowering plants in agricultural fields results in decreases in the abundance of wasps and bees and often concomitant increases in the density of insect pests.

In Warburton DB, Klimstra WD. 1984, invertebrate, avian, and small mammal populations in a no-till corn field and a conventionally tilled corn field were compared.
This study states (with data) that no-till provides habitat that supports more abundant and stable animal communities. The relative richness of the no-till field as wildlife habitat was investigated. Crop residue and other interrow cover increased habitat complexity in the no-till field (no quantitative habitat measures were made but weedy vegetation obviously provided greater niche variety in the no-till field). When compared with that in the conventional field, this cover resulted in greater diversity

[51] Pleasants J.N. and Oberhauser K.S., 2012. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity doi: 10.1111/j.1752-4598.2012.00196.x

within the invertebrate community and a more stable small mammal population. However, the study does not include results specific for glyphosate or herbicides in general.
It is hypothesized that reliance of no-till agriculture on pesticides may have fewer off-farm environmental impacts than conventional tillage, but the sublethal and long-term effects of pesticides on animal populations using no-till fields are not well understood and must be considered.
The authors also hypothesized that maintaining uncultivated areas in the field and between narrow crop rows may establish an equilibrium between predator and prey populations as they noted the absence of serious pest related problems during the study. This is considered of importance for protection of biodiversity.

The review of Sullivan TP, Sullivan DS. 2003 concluded that the diversity of terrestrial invertebrates in glyphosate-treated areas is variable. Abundance and diversity of invertebrates in a given treated area is principally a function of the degree of vegetation control and changes in vegetation structure.

Regarding the studies related to the selectivity of glyphosate based herbicides on a range of non-target arthropods, such as Culicidae (Bara et al., 2014[52], Mohamed et al., 2016[53]), *Chrysoperla externa* (Castilhos et al., 2011[54], Pasini et al., 2018[55]), Colorado potato beetle (Rainio et al., 2019[56]), rose-grain aphids (Saska et al., (2016)[57], Hymenopterans (Stecca et al., 2016)[58], *Bombyx mori* (You et al., 2010[59] and Zhang et al., 2011[60]), these studies were categorised as "Relevant but supplementary after detailed assessment of full-text article" by the applicant. Tahir H. M. et al., 2019 investigated the effect of glyphosate on the mortality, avoidance behavior, foraging activity, and activity of acetylcholine esterase (AChE) and carboxylesterase (CarE) in *Neoscona theisi* (Araneae: Araneidae). RMS agreed with applicant that these studies are not relevant and/or reliable and was not considered further for weight of evidence (please refer to Table B.9.11.1.4-2 of Volume 3 CA B.9 for more details about the studies exclusion).

Garcia Ruiz E. et al., 2018[61] investigated the relationship between weed management and the beneficial predatory arthropods in a glyphosate-tolerant (GT) cotton crop. Glyphosate (applied post-emergence) in this three-year farm-scale study resulted in a shift in weed species composition, suggests a positive correlation between weed density and the diversity of carabids and interspecific competition may occur between predatory groups. This study is considered relevant for biodiversity and indirect effect issues. However its relevance is limited as it focusses on post-emergence glyphosate applications and results were compared to an other herbicide treatment only. So the differences observed in the study are very likely the consequence of the different timing of application.

[52] Bara J. J. et al. 2014. Sublethal effects of atrazine and glyphosate on life history traits of Aedes aegypti and Aedes albopictus (Diptera: Culicidae).
[53] Mohamed I. A-w. et al.,2016. Unique efficacy of certain novel herbicides against Culex pipiens (Diptera: Culicidae) mosquito under laboratory conditions
[54] Castilhos R. V. et al., 2011.Selectivity of pesticides used in peach orchard on adults of Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae). Original title: Seletividade de agrotoxicos utilizados em pomares de pessego a adultos do predador Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae).
[55] Pasini R. A. et al., 2018. Comparative selectivity of herbicides used in wheat crop on the predators Chrysoperla externa and Eriopis connexa. Planta Daninha (2018), Vol. 36,pp. E018179968
[56] Rainio M. J. et al., 2019. Effects of a glyphosate-based herbicide on survival and oxidative status of a non-target herbivore, the Colorado potato beetle (Leptinotarsa decemlineata). Comparative biochemistry and physiology. Toxicology & pharmacology (2019), Vol. 215, pp. 47
[57] Saska P. et al., 2016. Treatment by glyphosate-based herbicide alters life history parameters of the rose- grain aphid Metopolophium dirhodum. Scientific reports (2016), Vol. 6, pp. 27801
[58] Stecca C. S. et al., 2016. Side-Effects of Glyphosate to the Parasitoid Telenomus remus Nixon (Hymenoptera: Platygastridae). Neotropical entomology (2016), Vol. 45, No. 2, pp. 192
[59] You W-y. et al., 2010. Toxicity Evaluation of Sixteen Herbicides to Bombyx mori. Asian Journal of Ecotoxicology (2010), Vol. 5, No. 1, pp. 91
[60] Zhang Q. et al., 2011. An evaluation on acute toxicity of 29 pesticides to Bombyx mori. Canye Kexue (2011), Vol. 37, No. 2, pp. 343
[61] Garcia Ruiz E. et al., 2018. Weeds and ground dwelling predators' response to two different weed management systems in glyphosate tolerant cotton: a farm scale study. PloS one, (2018) Vol. 13, No. 1, pp. e0191408

Besides RMS noted that insecticides were applied (at sowing). Herbicides treatments (other than glyphosate) were also applied in conventional managed plots. Their direct (toxic) effect was not investigated. Drought may also have reduced herbicide effectiveness (by reducing absorption, translocation and metabolism of herbicides). RMS considers these results reliable with restrictions.

- **Assessment**

Assessment and conclusion by the applicant

The following approach has been taken to assess potential indirect effects via trophic interactions, considers the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs based on direct effects assessment considering representative sensitive populations across the tested trophic levels. The biodiversity assessment, aimed to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals, that includes considering indirect effects via trophic interaction.

For example, reduced application rates relative to previous Annex I renewals, a reduced overall application volume of product on the land, and inclusion of no-spray buffer zones - a standard mitigation measure to protect non-target plant communities in off-target areas, which indirectly supports non-target arthropod biodiversity, by maintaining habitat and refuges for arthropods to reside in the off-field areas.

Although for example, for crop inter row applications and for applications made to control actively growing weeds in perennial row crops, herbicide application will result in habitat losses and non-target arthropods will be displaced as the directs effects assessment, indicates that there would be a limited direct effect on arthropod populations. The would be the case if an alternate herbicide was applied or if the weeds were removed mechanically. Populations in off-target areas would not be impacted and movement of non-target arthropods onto the developing crop or to areas adjacent to the application areas would occur.

Therefore, where an acceptable direct effects risk assessment is concluded upon after incorporation of standard mitigation measures to reduce off-target movement via drift to off-target areas, coupled with the standard mitigation measures, is considered protective of indirect effects occurring outside of the target area. When defining SPGs for arthropods that reflects both direct and indirect effects, it is the responsibility of the risk assessors in the Member States to acknowledge existing protection goals and regulatory data requirements, to propose possible SPG options, and describe the possible environmental consequences of each option. The risk assessors within the Member States will need to propose realistic SPGs and exposure assessment goals and the interrelationships between them in a clear and transparent manner.

The approach to the biodiversity assessment is to assess potential indirect effects via trophic interactions and their impact on biodiversity, by developing a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals.

In the following table, the specific protection goals relevant to non-target arthropods are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relates directly to the effects study endpoints.

A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence).

Based on the measurement endpoints from the study types, and the direct effects assessment presented above in this section, for in-field exposure, direct effects from glyphosate on NTAs are not anticipated. Due to the mode of action of glyphosate, an indirect effect on habitat in the in-field areas cannot be avoided. It is important to remember that this would also be the case if a non-chemical means of weed removal was employed.

Where there is crop present in the field at the time of application such as inter-row applications for weed control – or for example, applications made in orchards and vineyards where the applications are made in strips around the base of trees, populations of non-target arthropods will still be maintained in the unsprayed areas between the tree rows. For in-crop inter-row weed control spray scenarios, NTAs will still be present on the crop.

The impact on NTA species in the off-crop / off-target areas will be supported by the required in-field no spray buffer area for the NTTPs, which will protect off-field populations of NTAs allowing for in-field recovery of populations either onto the developing crop or onto weed species developing from the seed bank.

The following table assessment illustrates that ecological function of beneficial NTAs both in-field / in crop and off-field / off-crop (off-target) will be sufficiently maintained to achieve the SPG for the non-target arthropods according to the protection goals as defined in the ESCORT 2 and 3, that sustains a food resource for other animals, primarily birds and mammals.

Table [...]: The relationship between Specific Protection Goals and associated assessment and measurement endpoints for non-target arthropods (NTAs).

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
In-field Maintenance of ecological function of beneficial NTAs (i.e., pest control by parasitoids and predators), food source for wildlife, and effects not exceed the ability to recover.	Tier 1, at the maximum use rate (MUR) achieve an assessment factor of ≥ 2 with mortality Tier 2, at the MUR no significant mortality and < 50% effect on reproduction.	Survival (LR_{50}) and if appropriate, assess reproduction effects	Primary: *Typhlodromus pyri* (predatory mite) and *Aphidius rhopalosiphi* (parasitic wasp) Secondary: *O. laevigatus, C. carnea, C. septempunctata A. bilineata*
Off-field [1] Maintenance of NTA biodiversity and the ability to support in-field recovery			

NTA Biodiversity Assessment

Following ESCORT3 risk assessment guidance there is low to negligible risk of unacceptable direct and indirect effects to NTA communities for the representative formulation. Risk mitigation measures required for protecting the off-crop NTTP community (e.g., in-field buffers) will be protective of off-crop NTA biodiversity. However, if additional risk mitigation measures are determined to be required, to mitigate indirect effects resulting from in-crop weed control on NTA communities, options to be considered by risk assessors and risk managers within Member States are presented in Table [...].

[1]The off-crop area is defined as the area in-field that is not the crop. For NTA RA, the off-crop area is a default 1 meter distance between the last sprayed row of the crop and the edge of the in-field area.

Assessment and conclusion by RMS

The applicant refers to a tier 1 assessment, however no tier 1 assessment with NTA could be performed as the glassplate tests with *T. pyri* and *A. rhopalosiphi* were not valid. Moreover in his table above, the applicant used incorrectly the terms beneficial arthropods and NTA. The ESCORT 2 and 3 guidances cover the risk assessment for non target arthropods i.e. arthropods that are not considered as pest. For beneficial arthropods that are used for IPM, the uniform principles are still relevant under Regulation EC No 1107/2009. For the beneficial arthropods, the 30% trigger should be considered. The risk for beneficials arthropods that are introduced for use in IPM are not in the scope of the regulatory ecotoxicological risk assessment that focus on endogenous arthropods. For the ecotoxicology section, RMS therefore focus on endogenous non-target arthropods.

RMS considers that the current standard risk assessment and protection goals addresse the direct effect only and have not been defined to address specifically indirect effects.. The indirect effect, due to the loss of habitat or food resources, is not considered to be addressed. No study investigating biodiversity issues or indirect effects was found in the literature review.
RMS considers that the appropriateness of applicant's proposal concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control should be discussed during the EU peer-review.

- **Conclusion**

Assessment and conclusion by the applicant

Following ESCORT3 risk assessment guidance there is negligible risk of unacceptable direct and indirect effects to NTA communities for the representative formulation. Risk mitigation measures required for protecting the off-crop NTTP community (e.g., in-field buffers) will be protective of off-crop NTA biodiversity. The existing SPG for the in-crop assessment has been designed to only allow for up to a transient 50% effect on the NTA community and it allows for in-crop recovery to minimize the likelihood of indirect effects to birds and mammals through trophic interactions. The SPG for the off-crop assessment is protective of biodiversity based on spray-drift mitigations developed to protect the NTTP community. However, if additional risk mitigation measures are determined to be required, to mitigate indirect effects resulting from in-crop weed control on NTA communities, options to be considered by risk assessors and risk managers within Member States are presented in the following table.
In [redacted] 2020 (KCA 8.7/001), the applicant also indicated the following: "These mitigation options will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field."

Assessment and conclusion by RMS

Based on standard risk assessment, no risk mitigation measure is necessary to protect non-target arthropods (from direct effect). The applicants states that the no spray buffer areas in-field and the drift reducing technologies set to protect non-target plants in off-target areas will in turn support non-target arthropod communities in off-field areas. The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments conducting with the EFSA guidance document for aquatic organisms (2013). Additional proposals for mitigation of

risk from indirect effects should be discussed further during the EU peer review in order to establish the basis for harmonised set of measures to be implemented on MS level at product authorisation .

The following was provided by the applicant. These are non-standard mitigation measures. Their relevance/efficiency is not addressed by the standard risk assessment. RMS considers that the appropriateness of applicant's proposal reported below concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control should be discussed during the peer review.
In the context of the renewal of glyphosate, the representative uses did not include all potential uses of glyphosate. Particularly it did not include uses on non agricultural areas, mainly on sports fields, amenity areas, industrial areas, even on roads and pavements. When considering protection on biodiversity, it may be necessary to include a wider range of uses than those intended for renewal of approval. It should be also balanced with the need to destroy the vegetation for safety reasons, as it could be the case in industrial areas.
The loss of habitats and food sources should be prevented in-field and off-field too by applying risk mitigation measures - both non-standard and standard - as proposed by the applicant:

i) treated area restriction,

ii) reduction of spray drift and establishment of buffer zones (as required for off-crop NTTP).

Moreover, as the protection of the off-field area is of importance to prevent indirect effects on non-target arthropods and subsequent effects on birds and mammals. There might be a need for a more conservative risk assessment for non-target plants in such situation.
Besides this, MSs might consider the need of compensation potential of landscape to be implemented at different scale (local, regional, Euopean).

B.9.14.1.5. Soil meso- and macroorganisms - Risk to biodiversity via Indirect Effects and Trophic Interactions

- **Indirect effects via Trophic Interactions**

Assessment and conclusion by the applicant

The ecotoxicology regulatory study dataset for glyphosate and AMPA includes a battery of OECD test guideline studies, designed to assess the potential long-term effects on the structure and function of soil organism communities. For the Tier 1 assessment, studies were conducted using ecologically important indicators of soil organism community structure and function. These studies include long-term reproduction studies using a representative earthworm, a representative collembolan, and a representative predatory mite. Earthworms are tested because they play an important role as detritivores in soil communities. Collembola, which are the most abundant soil macro-organism, are also tested because they play an important role as detritivores and nutrient cycling in soil organism communities. Predatory mites are important to the battery in that they provide information on potential impacts to food chain interactions and biological control within soil organism communities.

Soil organisms contribute to a wide range of essential ecosystem services important for the function of terrestrial ecosystems, acting as the primary driving agents of nutrient cycling and regulating the dynamics of soil organic matter formation and decomposition, soil carbon sequestration, and greenhouse gas emission.

Soil macro-organisms modify soil physical structure and hydraulic properties that influence root growth, root function, and nutrient acquisition. Soil biodiversity is responsive to the management of cultivated systems (Schreck et al., 2012; Trivino-Tarrades et al. 2019). Cultivation drastically affects the soil environment and hence the organisms present and their number (Trivino-Tarrades et al. 2019; Brussaard

et al. 2007). Conservation tillage or minimal tillage generally have positive impacts on soil organism densities, diversity, and microbial content. No-till fields typically have significantly more beneficial insects, earthworms and earthworm diversity, higher organic matter and microbial content (Chan, 2001).

The following approach has been taken to assess potential indirect effects via trophic interactions, considers the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs based on direct effects assessment considering representative sensitive populations across the tested trophic levels. The biodiversity assessment, aimed to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals, that includes considering indirect effects via trophic interaction.

For example, reduced application rates relative to previous Annex I renewals, a reduced overall application volume of product on the land, and inclusion of no-spray buffer zones - a standard mitigation measure to protect non-target plant communities in off-target areas, which indirectly supports soil macro-organisms biodiversity, by maintaining soil structure and function in both in-field and off-field areas.

When defining SPGs for soil macro-organisms that reflects both direct and indirect effects, it is the responsibility of the risk assessors in the Member States to acknowledge existing protection goals and regulatory data requirements, to propose possible SPG options, and describe the possible environmental consequences of each option. The risk assessors within the Member States will need to propose realistic SPGs and exposure assessment goals and the interrelationships between them in a clear and transparent manner

Specific protection goal (SPGs) for soil organisms still need to be adopted. However, for the purpose of this biodiversity assessment, two SPGs have been developed that overall, are considered consistent with current EFSA (2016) opinion on soil organisms and are likely be adopted in future EFSA guidance.

The first SPG is aimed at protecting the structure and function (e.g., detritivory) of soil macro-organism communities and the function of soil micro-organism communities.
The second SPG is related to the first and is aimed at the protection of soil services (e.g., decomposition and cycling of organic matter and nutrients).

In the Annex 1 renewal, glyphosate and the representative formulation were shown to have low toxicity and an acceptably low long-term risk on the structure and function of soil macro-organisms, the functioning of soil micro-organism communities (– see next section for soil micro-organisms), and risk mitigations were required (EFSA, 2015a). This is further supported by the direct effects assessment for soil meso-organisms as presented in this section above.

Assessment and conclusion by RMS

The regulatory laboratory studies provided in the context of renewal of glyphosate did not allow as such to assess indirect effects and trophic interactions. Please refer late the discussion on SPG.

RMS notes that the articles cited above by the applicant were not submitted. These were not required by RMS as none of them was related to glyphosate or herbicides with large spectrum of weeds control but to no or minimum tillage consideration. It is acknowledged that glyphosate could be used in such situation. However in order to perform a comparative assessment of the different practices in the context of glyphosate uses, these data without information of herbicide uses did not provide reliable information to explore the impact of biodiversity of the different combination.

There is currently no specific guidance or harmonized assessment procedures at the EU level for conducting a comprehensive biodiversity assessment.

Reference to the most recent EFSA opinion[62] (Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690) together with findings from the literature search was recommended by RMS.

The EFSA guidance on specific protection goals (2016) aims to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services. As such, RMS considered that it is the most suitable approach available to assess biodiversity and indirect effects in the context of regulatory risk assessment.

The EFSA Scientific Opinion (2017) for in-soil organisms provides information on further data that may be requested for assessing effects for in-soil organisms (including new test endpoints). No assessment scheme is available yet. RMS nevertheless requested to address this via information from the open literature.

The EFSA Opinion (2017) for in-soil organisms follows the principles of the EFSA 2016 method for defining SPGs as ecosystem services and SPGs were already identified. Therefore, the methodology and the process implemented in the EFSA Opinion for in-soil organisms (2017) can be considered in line with the EFSA method for defining SPGs. The EFSA opinion for in-soil organisms (2017) suggested a specification of five interrelated dimensions of the SPG (i.e. ecological entities, attribute, magnitude, temporal and spatial scale) in line with the third step of the EFSA method but these were not discussed with risk managers of DG SANTE and the European Union (EU) Member States.

RMS reported below the specific protection goals that were defined in the EFSA Opinion addressing the state of the science on risk assessment of plant protection for in-soil organisms (2017):
In order to support the long term performance of the functional role of in-soil organisms in several ecosystem services in agricultural soils, it is recommended to define the SPU as the abundance/biomass of the populations of species belonging to the different functional groups.
The in-field specific protection goals for in-soil animals proposed in this scientific opinion could be summarized as follows:

Organism group	**Ecological entity / attribute**	**Option: below the limit of operation** **Magnitude and Duration**	**Option: limit of operation** **Magnitude and Duration**	**Option: above the limit of operation Magnitude and Duration**
	In-field SPG			
Earthworms	Population / abundance – biomass	Negligible effects Small effect up to weeks	Small effect up to months	Medium effects for months

[62] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Ockleford C, Adriaanse P, Berny P, Brock T, Duquesne S, Grilli S, Hernandez-Jerez AF, Bennekou SH, Klein M, Kuhl T, Laskowski R, Machera K, Pelkonen O, Pieper S, Stemmer M, Sundh I, Teodorovic I, Tiktak A, Topping CJ, Wolterink G, Craig P, de Jong F, Manachini B, Sousa P, Swarowsky K, Auteri D, Arena M and Rob S, 2017. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690, 225 pp. doi:10.2903/j. efsa.2017.4690

Enchytraeids	Population / abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for months
Microarthropods	Population / abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Macroarthropods	Population /abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Gastropods	Population / abundance – biomass	Negligible effects Small effect up to weeks	Small effect up to months	Medium effects for month
Nematodes	Population / abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Consequences of option choice regarding the effects of intended PPP use on in-soil organisms See EFSA opinion 2017, Table 19 for more details		Protection goals are achieved.	Off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms in the middle and long term. The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009, the aims of Directive 2009/128 for achieving a sustainable use of pesticides, the aims of Council Directive 79/409/EEC on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are still implemented, as long as off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain	Protection goals not achieved.

		recovery and recolonisation of vulnerable soil organisms in the middle and long term	

According to the scientific opinion on soil organisms, for the off-field non-target areas, it is proposed that only negligible effects on the abundance/biomass of in-soil organisms' populations can be tolerated.

- **Scientific Literature that informs the Soil Organism Risk Assessment**

Assessment and conclusion by the applicant

Literature review for non-target soil organisms from the previous Annex I (2012) submission.
The scientific literature review conducted for the last Annex I renewal (submitted in 2012) contains an extensive review of ecotoxicological papers considered relevant but supplementary to the Annex I renewal.

These papers presented information that could not be relatable to an EU level ecotoxicological risk assessment, but that were considered in the previous dossier as being supportive following re-evaluation by the previous RMS. A further evaluation of these literature papers according to the EFSA literature review approach used in this dossier has not been conducted. The previous literature review has been submitted as part of the Literature review requirements and is presented in […].

The scientific literature review conducted for the last Annex I renewal (submitted in 2012) contains a review of ecotoxicological papers considered relevant to the area of soil macro-organisms and glyphosate. A total of 21 peer reviewed papers were submitted, with 5 citing studies focusing on earthworms and considered as supporting information for the risk assessment (Casabe et al., 2007; . Correia et al., 2012; Kaneda et al., 2009; Verrel et al., 2004 and Yasmin et al., 2003). […].

The previous RMS (UBA) concluded on the submitted references, several points on acute exposure effects which are not considered relevant to the risk assessment as acute effects on soil organisms is now not a data requirement under Regulation (EC) No 1107/2009.
There were effects on reproduction examined by Casabe et al., (2007) and Yasmin et al., (2003) that considered commercial formulations other than the representative formulation, but it was concluded that these effects were not relevant at the population level in nature.

In a reproduction test with *Eisenia fetida*, conducted with the active substance glyphosate (Correia et al., 2012), earthworms were maintained in treated soil and classified as alive after the evaluation period, but with bodyweight effects across all test concentrations. Moreover - morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg). Further behavioural abnormalities were described in terms of reduced casting production (Kaneda et al., 2009), reduced cocoon viability, a reduction in the feeding activity (Casabé et al., 2007) or reduced body weight (Yasmin et al., 2006). However, the test rates were similar or above the one tested in the officially submitted studies, so that the outcome of the risk assessment for earthworm did not change.

In the current direct effects assessment, the results of a recent earthworm reproduction study were presented with worms exposed to the representative formulation (MON 52276) where there were no sub-lethal effects up to the maximum rate (1000 mg a.e./kg soil dw) tested, in either bodyweight effects at 28 days nor juvenile production at 56 days.

Concerning the current literature review, there were no literature articles that were considered relevant and reliable on soil meso-organisms, for use in the ecotoxicological risk assessment for Annex I renewal. There were 9 peer reviewed papers considered relevant but supplementary to the risk assessment for soil meso-organisms (Correia et al., 2010, Dominguez et al., 2016, Gaupp-Berghausen et al., 2015, Jarmul-Pietraszczyk et al., 2015, Nathan et al., 2019, Pochron et al., 2019, Santos et al., 2012, Sihtmaee et al., 2013 and Stellin et al., 2017). An 11th paper was found relevant and reliable (Von Merey et al., 2016). These data reviewed in this paper, exist in the regulatory list of endpoints. They will not be considered further in this review as data from this paper is used in the presented risk assessment for soil meso-organisms in this dossier.

Correia et al., (2010), performed an earthworm reproduction study using a Brazilian soil at test concentrations between 1 and 1000 mg/kg soil dw. This study did not present any data that could be used in an EU level risk assessment for renewal purposes and was therefore considered to be supplementary. In studies by Dominguez et al., 2016, Santoz et al., 2012 and Santadino et al., 2014 despite being conducted according to recognised test guidelines, the validity of the studies could not be confirmed due to lack of critical information in the papers.

Concerning indirect effects that may inform on trophic interactions, the biological availability of glyphosate and AMPA in soil is considered relevant. In a comprehensive study of 317 European agricultural soils, glyphosate and AMPA were found in 21 and 42% of the samples, respectively (Silva et al. 2018). Concentrations of glyphosate or AMPA rarely exceeded 0.5 mg a.e./kg of soil, and the highest level detected was 2.05 mg a.e./kg. This maximum level of glyphosate detected is more than 2-times less than the predicted environmental soil concentration used for the standard glyphosate soil organism assessment, which considered a worst-case exposure scenario (i.e., the maximum use rate and maximum potential to build up in soil). See direct effects assessments for soil organisms above in this section.

Assessment and conclusion by RMS

The papers submitted for the previous Annex I submission were not reassessed by RMS. None of them impacted the outcome of the risk assessment (RAR 2015). The above statement provided by the applicant partially reflects the previous conclusions of the RAR 2015.

RMS nevertheless disagrees with the applicant to discard acute exposure effects because these are no longer a data requirement under Regulation (EC) No 1107/2009. RMS then reminds then the previous statement of RAR 2015: "*for acute effects on soil organisms, behaviour is not included as a sensitive endpoint. However, these responses might also have negative consequences, e.g. –when worms move to the surface of contaminated soil- exposure to predators or to detrimental light. It could be shown that the activity of worms was influenced by the exposure to environmentally relevant concentration of commercial formulation of glyphosate (Verrel and Buskirk, 2004). The worms emerged onto the surface within 2 h after exposure. Nevertheless, after 48 h animals were found to be buried in the soil again. Authors concluded that acute exposure to the glyphosate containing plant protection product may compromise the survival of earthworms even though its direct toxicity appears low (Verrel & Buskirk, 2004). Nevertheless it seems important to assess not only of the active ingredients, but also of the different formulations (Piola et al. 2013). Especially for aquatic organsims it was also demonstrated that commercial formulations can be more toxic than the active substance itself because of the adjuvants present in the formulations"*.
RMS considers such effects (sublethal) relevant even for a short-term exposure. Moreover the study of Piola et al (2013) includes earthworm toxicity data. According to the summary available in the RAR 2015, results of this study highlight the importance of ecotoxicological assessment not only of the active ingredients, but also of the different formulations. Median lethal concentration (LC50) showed that glyphosate-A was 4.5-fold more toxic than glyphosate-B. Sublethal concentrations caused a concentration-dependent weight loss, consistent with the reported effect of glyphosate as

uncoupler of oxidative phosphorylation. Glyphosate- A showed deleterious effects on DNA and lysosomal damage at concentrations close to the applied environmental concentrations (14.4 lg ae cm ^{2}). With glyphosate-B toxic effects were observed at higher doses, close to its LC50, suggesting that the higher toxicity of formulate A could be attributed to the effects of some of the so-called ''inert ingredients'', either due to a direct intrinsic toxicity, or to an enhancement in the bioavailability and/or bioaccumulation of the active ingredient. A data gap is set for the applicant to provide the full text of Piola et al 2013 together with a summary and assessment in light with both direct and indirect effects risk assessment related to glyphosate based products.

RAR 2015 further stated that it can not be excluded that with repeated applications of glyphosate containing plant protection products during the season or year by year will have negative effects on the biotic soil community. It was considered that herbicide application did not directly affect the mortality or reproduction but instead the biological activity of the animals (RAR 2015).

Concerning the current literature review on ecotoxicology, the RMS agrees with applicant justification (see Table Table B.9.11.1.4-2 in Volume 3 CA B.9) except for Correia et al., 2010 and Santos et al., 2012 (see appendix to Volume 3 CA B.9 related to literature data on ecotoxicology).
Correia et al (2010) and Santos et al., 2012 are summarised in the appendix to Volume 3 CA B.9 related to literature data on ecotoxicology. However the exposure in Correia et al (2010) exceed the PEC estimated for the intended uses. Santos et al., 2012 investigated the impact of glyphosate on the avoidance behaviour and reproduction of the earthworm Eisenia andrei and the collembolan Folsomia candida. Adverse effects seen only for Folsomia are considered to be product-dependant. Please refer to the study summaries for details (appendix to Volume 3 CA B.9 related to literature data on ecotoxicology) and synthesis in point B.9.8 of Volume 3 CP B.9.
Gaupp-Berghausen M. et al. (2015) showed that vertically burrowing earthworms (Lumbricus terrestris) almost ceased activity three weeks after herbicide application (no mortality was observed), while the activity of soil dwelling earthworms (*Aporrectodea caliginosa*) was not affected. RMS notes that the reduced surface casting activity after herbicide treatment might be that L. terrestris avoided plant residues contaminated with glyphosate on the surface. As a consequence these earthworms might have lived in deeper soil horizons and avoided surface foraging and casting (as hypothetized by the authors). So the relevance of this parameter for the risk assessment is questionable. The study was not considered reliable enough (see Table B.9.11.1.4-2 in Volume 3 CA B.9).
Regarding von Merey 2016 [63], this publication corresponds to the regulatory studies summarized and already assessed by RMS (for details see appendix to Volume 3 CA B.9 related to literature data on ecotoxicology).

Therefore, this publication was not assessed by RMS

Overall, RMS agrees with the applicant that these articles are not sufficiently relevant or reliable to be used in the risk assessment. However, taken together, several articles may be used in a weight of evidence approach to address biodiversity and indirect effects issues.

Regarding the study of Silva et al, 2018[64]; 300 samples have been collected as part of the LUCAS topsoil project between April and October of 2015 and 17 samples are from three independent vineyards in north-central Portugal taken in September 2015. Results from these data indicate GLY is quantified in ~21% of 317 soil samples, AMPA is quantified in ~42% of 317 soil samples, with the maximum concentration being 2.05 mg/kg for GLY and 1.92 for AMPA, measured in the Portugese

[63] von Merey G. et al. 2016. Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota. Environmental toxicology and chemistry (2016), Vol. 35, pp. 2742

[64] Silva, V. et al., 2018. Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union.

vineyard. RMS underlines that these maximum measured concentrations should be regarded with caution since the exact sampling depth is unknown (15/20cm), and in any case higher than the one that would be considered for risk assessment in permanent crops (5cm).
Also, this study concluded that maximum level of glyphosate detected is more than 2-times less than the predicted environmental soil concentration used for the standard glyphosate soil organism assessment, which considered a worst-case exposure scenario. However the direct comparison with expected PECsoil in vines is uncertain since this latter is calculated in the study on 5 cm depth while the sampling depth for the measured concentration is 15/20cm and cannot be related to a precise use pattern of the active substance (application rate, time past since last application….).

- **Biodiversity Assessment**

Assessment and conclusion by the applicant

After a though literature search and considering all relevant guidance, the following approach is taken to assess potential indirect effects via trophic interactions and the impact on biodiversity, was to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals. In the […], the specific protection goals relevant to soil meso-fauna are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relates directly to the effects study endpoints.

A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence).

Based on the measurement endpoints from the study types, and the direct effects assessment presented above in this section, it is anticipated that for the proposed uses on the GAP table, that there will be no impacts on soil meso-organisms population (e.g. earthworms, collembola and hypoaspis) survival growth and reproduction, which in turn meets the specific protection goal for soil meso-organisms.

The Table […] assessment illustrates that ecological diversity and function of soil meso-organisms within spray zones will be sufficiently maintained to achieve the SPG for this taxa group according to the protection goals as defined in the Terrestrial guidance document (SANCO/10329/2000) sustains a food resource for other animals, primarily birds and mammals within in -field areas, sustains soil structure and function that has a knock on effect of enabling soil function of soil microbial communities. This in turn helps to maintain the community structure within the soil.

Table […]. The relationship between Specific Protection Goals, assessment and measurement endpoints for soil macro- and micro-organisms from foliar applications.

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
Protection of structure (biodiversity) and function of soil macro-organism communities and function of soil micro-organism communities.	Structure and function of soil macro-organism communities Long-term effects on the function of soil micro-organism communities	Survival and reproduction N-transformation rate ≤25% difference from control at ≥28 days.	Earthworm chronic Collembola chronic Predatory mite chronic N-transformation rate

Protection of soil services (e.g., decomposition and cycling of organic matter and nutrients)	Long-term effects on the function of soil micro-organism communities (i.e., Nitrogen cycling).	Survival and reproduction N-transformation rate ≤ 25% difference from control at ≥28 days	

Soil Organism Biodiversity Assessment

Based on the direct effects' assessment, there is low to negligible risk to the structure and function of soil organism populations and communities (EFSA, 2015a) and the likelihood of indirect effects soil organism biodiversity is also considered to be negligible.

[1] EFSA still needs to receive input from risk managers on the definition of specific protection goals being led by DG SANTE. In the draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms1, negligible effects are considered to be ≤ 10% and small effects are considered to be ≤ 35%.

Assessment and conclusion by RMS

No study investigating biodiversity issues or indirect effects related to glyphosate uses was found in the literature review submitted. RMS considers that the appropriateness of applicant's proposal concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control should be discussed during the EU peer-review.

- **Conclusion**

Assessment and conclusion by the applicant

Glyphosate is a critical tool to enable conservation tillage systems, which can greatly improve the abundance and biodiversity of soil organisms. There is negligible risk of direct effects to soil community biodiversity and supporting/regulating services related to soil processes. This conclusion is not changed after reviewing reported levels of glyphosate from soil monitoring studies (Silva et al. 2018). In addition, based on a review of the relevant and supportive literature, the likelihood of indirect effects soil organism biodiversity is also considered to be negligible.

Assessment and conclusion by RMS

Based on standard risk assessment, no risk mitigation measure is necessary to protect soil macroorganisms (from direct effects). RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects. The applicants state that the no spray buffer areas in-field and the drift reducing technologies set to protect non-target plants in off-target areas will in turn support soil macroorganisms in off-field areas. The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments. Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review. The risk mitigation measures proposed for NTAs might be protective for soil organisms too; though, some more supporting information would be beneficial.

It should be noted that, in the context of the renewal of glyphosate, the representative uses did not include all potential uses of glyphosate. Particularly it did not include uses on non agricultural areas, mainly on sports fields, amenity areas, industrial areas, even on roads and pavements. When considering protection on biodiversity, it may be necessary to include a wider range of uses than those intended for renewal of approval. It should be also balanced with the need to destroy the vegetation

for safety reasons, as it could be the case in industrial areas.
Discussions among risk managers should be reinforced around the question of biodiversity in agricultural landscape in order to allow implementation on MS level based on a common and harmonised approach.

B.9.14.1.6. Soil microorganisms - Risk to biodiversity via Indirect Effects and Trophic Interactions

- **Indirect Effects via Trophic Interactions**

Assessment and conclusion by the applicant

As stated in the EFSA 2017 Scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, general protection goals are stated in the European legislation, but are not precisely defined. A precise definition is considered crucial for designing appropriate risk assessment schemes. Different groups of soil organisms have been identified as providers of important ecosystem services in the soil ecosystem. In the biodiversity position paper submitted with this submission, specific protection goals have been developed that consider the six dimensions, namely ecological entity, attribute, magnitude of effects, temporal scale of effect, spatial scale of effect and degree of certainty. SPGs are proposed for both in-field and off-field areas. Due to the specific traits and short generation times, it has been possible to study internal recovery of microbial populations or communities after PPP exposure. It has been demonstrated that microbial communities do recover quickly from effects at both the structural and functional levels of the microbial community (EFSA (2017).

The ecotoxicology dataset for glyphosate and AMPA includes a battery of OECD guideline studies, designed to assess potential long-term effects on the structure and function of soil organism communities. The presented direct effects assessment in this section of the dossier, demonstrates that ecological function and therefore regulation of essential nutrients within the soil microbial community is not lost following exposure to glyphosate at application rates that are considerably higher than those proposed on the GAP table. With max application per annum also being substantially reduced compared to the previous Annex I renewal (2017), the overall burden of product on the land is also reduced, for both the in-field and off-field areas.

For soil microbes Tier 1 direct effects assessments, studies were conducted using ecologically important indicators of soil organism community function (see Table […]). Soil microbes in combination with other soil organisms contribute to a wide range of essential services that are important for the function of terrestrial ecosystems by acting as the primary driving agents of nutrient cycling, decomposition, soil carbon sequestration, and greenhouse gas emission. As stated for the soil meso-organisms, conservation tillage or minimal tillage generally have positive impacts on soil organism densities, diversity, and also microbial content. No-till fields typically have significantly higher organic matter and microbial content (Chan, 2001).

The following approach has been taken to assess potential indirect effects via trophic interactions, considers the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs based on direct effects assessment considering representative sensitive populations across the tested trophic levels. The biodiversity assessment, aimed to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals, that includes considering indirect effects via trophic interaction. For example, reduced application rates relative to previous Annex I renewals, a reduced overall

application volume of product on the land, and inclusion of no-spray buffer zones as a standard mitigation measure to protect soil communities in off-target areas, which indirectly supports biodiversity by maintaining soil community function and structure, providing a substrate for habitat creation that provides refuge and food resource for other organisms in off-target areas. Therefore, where an acceptable direct effects risk assessment is concluded upon after incorporation of standard mitigation measures to reduce off-target movement via drift coupled with the other standard mitigation measures that are being applied, is considered protective of indirect effects occurring outside of the target area.

Specific protection goals (SPGs) for soil microbes still need to be adopted. However, for the purpose of this biodiversity assessment, two SPGs have been developed that overall are consistent with current EFSA guidance and what will likely be adopted in future EFSA guidance. The first SPG is aimed at protecting the function of soil micro-organism communities. The second SPG is related to the first and is aimed at the protection of soil services (e.g., decomposition and cycling of organic and nutrients) in which soil microbes play a critical role.

In the previous Annex 1 renewal, glyphosate and the representative formulation were shown to have low toxicity and negligible risk of long-term effects to the functioning of soil micro-organism communities, and no risk mitigations were required (EFSA, 2015a).

Assessment and conclusion by RMS

RMS notes that the article cited above by the applicant (Chan, 2001) was not submitted. It was not required by RMS as it is not related to glyphosate or herbicides with large spectrum of weeds control but to no or minimum tillage consideration. It is acknowledged that glyphosate could be used in such situation. However in order to perform a comparative assessment of the different practices in the context of glyphosate uses, these data without information of herbicide uses did not provide reliable information to explore the impact of biodiversity of the different combination.

There is currently no specific guidance or harmonized assessment procedures at the EU level for conducting a comprehensive biodiversity assessment.

Reference to the most recent EFSA opinion[65] (Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690) together with findings from the literature search was recommended by RMS.

The EFSA Scientific Opinion (2017) for in-soil organisms provides information on further data that may be requested for assessing effects for in-soil organisms (including new test endpoints). No assessment scheme is available yet. RMS nevertheless requested to address this via information from the open literature.

The EFSA guidance on specific protection goals (2016) aims to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services. As such, RMS considered that it is the most suitable approach available to assess biodiversity and indirect effects in the context of regulatory risk assessment.

[65] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Ockleford C, Adriaanse P, Berny P, Brock T, Duquesne S, Grilli S, Hernandez-Jerez AF, Bennekou SH, Klein M, Kuhl T, Laskowski R, Machera K, Pelkonen O, Pieper S, Stemmer M, Sundh I, Teodorovic I, Tiktak A, Topping CJ, Wolterink G, Craig P, de Jong F, Manachini B, Sousa P, Swarowsky K, Auteri D, Arena M and Rob S, 2017. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690, 225 pp. doi:10.2903/j. efsa.2017.4690

The EFSA Opinion (2017) for in-soil organisms follows the principles of the EFSA 2016 method for defining SPGs as ecosystem services and SPGs were already identified. Therefore, the methodology and the process implemented in the EFSA Opinion for in-soil organisms (2017) can be considered in line with the EFSA method for defining SPGs. The EFSA opinion for in-soil organisms (2017) suggested a specification of five interrelated dimensions of the SPG (i.e. ecological entities, attribute, magnitude, temporal and spatial scale) in line with the third step of the EFSA method but these were not discussed with risk managers of DG SANTE and the European Union (EU) Member States.

RMS reported below the specific protection goals that were defined in the EFSA Opinion addressing the state of the science on risk assessment of plant protection for microorganisms (2017).
In order to support the long term performance of the functional role of in-soil organisms in several ecosystem services in agricultural soils, it is recommended to define the SPU as the community structure of the different functional groups.

The in-field specific protection goals for in-soil animals proposed in this scientific opinion are:

Organism group	**Ecological entity / attribute**	**Option: below the limit of operation** **Magnitude and Duration**	**Option: limit of operation** **Magnitude and Duration**	**Option: above the limit of operation Magnitude and Duration**
	In-field SPG			
Mycorrhiza, other fungi and protozoa	Community / structure	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Soil bacteria and Archaea	Community / microbial community	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks Large effects up to days	Medium effects for months Large effects for weeks
Consequences of option choice regarding the effects of intended PPP use on in-soil organisms See EFSA opinion 2017, Table 19 for more details		Protection goals are achieved.	Off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms in the middle and long term. The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009, the aims of	Protection goals not achieved.

		Directive 2009/128 for achieving a sustainable use of pesticides, the aims of Council Directive 79/409/EEC on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are still implemented, as long as off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms in the middle and long term	

According to the scientific opinion on soil organisms, the off-field non-target areas, it is proposed that only negligible effects on the abundance/biomass of in-soil organisms' populations can be tolerated.

- **Scientific Literature that informs the soil organism assessment**

Assessment and conclusion by the applicant

Literature review for non-target soil organisms from the previous Annex I (2012) submission.
The scientific literature review conducted for the last Annex I renewal (submitted in 2012) contains a review of ecotoxicological papers considered relevant to the area of soil non-target micro-organisms. Out of 99 papers submitted, 21 papers were described in detail in the dossier. The RMS (UBA) re-evaluated the papers and mostly dealt with the rhizobia of glyphosate resistance crops and were therefore not relatable to an EU level ecotoxicological risk assessment. There were 28 papers considered to be informative with a low weight, with 18 papers considered to be supportive to the risk assessment and one publication considered critical with a high weight of evidence for use in risk assessment. The single study was conducted according to the recognised test guidelines (OECD 216 and 217) with glyphosate applied at the field rate of 4.5 mg/kg soil and also at a 5-fold factor higher (22.5 mg/kg soil). After 1, 7, 14 and 28 days incubation, soil respiration and nitrate formation rates did not significantly differ from the control soil.

The full evaluation of these papers by the previous RMS (UBA) may be found in […].

The conclusions of the previous RMS (UBA) literature review, included identifying effects on soil functional diversity (Liphadzi, et al. 2005).Where there were repeated applications, desiccation led to significant increases of microbial biomass (Ruzkova et al., 2011) but reduced nitrate transformation rates.

Some measured parameters were related as a function of time and site quality rather than pesticides application (Gomez et al.,2009), function of seasonality (Hart et al. ,2009), function of habitat and land use (Busse et al., 2001).

Glyphosate used as a source of P, C or N for soil bacteria (van Eerd et al., 2003), that correlated with increases in soil respiration (Accinelli et al., 2002), increased microbial biomass (Lupwayi, N.Z., et al., 2004), increased rates of C- and N- mineralizations (Lancaster et al., 2006; Haney et al., 2000a, 2002b), which led to a shift in community structure (Ratcliff et al., 2006) from fungal dominance to an equal ratio of fungal and bacteria communities. However, since no significant effects to the function of the fungal and bacterial communities have been observed, then no unacceptable indirect effects to the microorganisms' communities are anticipated.

The RMS (UBA) concluded in 2015, that the soil microorganisms play an important role in soil fertility, by assuming key ecological functions like matter decomposition and nutrient cycling. They indicated that plant biodiversity, productivity and variability are strongly dependant on the association with microorganisms and fungi in the soil. They also stated that the soil microbial diversity is extremely difficult to measure and therefore the risk assessment is restricted to the measurement of impact of pesticides on soil functional diversity. Currently, the data requirements for PPP registration in the EU require only studies on nitrogen transformation rates in artificial or field collected soils.

The RMS (UBA) indicated that there was a need to consider both microbial diversity and composition when considering the impact of plant protection products on soil non-target micro-organisms. However, the current test guidelines do not provide for such a study and based on the currently available test guideline considered relevant for risk assessment purposes, the direct effects assessment demonstrates an acceptable risk t considering the effects on soil function (nitrogen transformation).

Concerning the literature review for the current dossier:
There were no public domain literature papers in the field of soil microbes that were classified as being both relevant and reliable for use in the ecotoxicological risk assessment for soil micro-organisms. There were 17 papers considered to be relevant but supplementary, which are presented in the literature review submitted in […].

Further to the discussion on diversity, a number of papers were considered relevant to the biodiversity assessment. In a comprehensive study of 317 European agricultural soils glyphosate and AMPA were found in 21 and 42% of the samples, respectively (Silva et al. 2018). Concentrations of glyphosate or AMPA rarely exceeded 0.5 mg a.e./kg of soil, and the highest level detected was 2.05 mg a.e./kg of soil. This maximum level of glyphosate detected is more than 2-times less than the predicted environmental soil concentration used for the standard glyphosate soil organism risk assessment, which considered a worst-case exposure scenario (i.e., the maximum current use rate in the GAP and maximum potential to build up in soil).

Soil microbial populations and their associated biochemical processes are critical to maintain soil health and quality. Soil microbial communities are highly complex and are often characterized by high microbial diversity (Tiedje et al. 1999). The occurrence and abundance of soil microorganisms are affected by 1) soil characteristics like tilth, organic matter, nutrient content, and moisture capacity, 2) typical physico-chemical factors such as temperature, pH, and redox potential, and 3) soil management practices. Agricultural practices such as fertilization and cultivation may also have profound effects on soil microbial populations, species composition, colonization, and associated biochemical processes (Buckley and Schmidt, 2001, 2003). Consequently, significant variation in microbial populations is expected in agricultural fields. Minor changes in a single microbial species or group are difficult to measure in such a dynamic system and, moreover, the minor effects of such a change may be better assessed in more integrated measures such as soil fertility and carbon and nitrogen transformation.

The effects of glyphosate and glyphosate-based formulations on soil microorganisms have been extensively investigated (von Mérey et al., 2016; Cerdeira and Duke, 2010; Duke et al. 2012; Sullivan and Sullivan, 2000). Results of standardized tests with glyphosate formulations performed for submission to regulatory agencies indicate no long-term effects on two key functional endpoints, carbon (not a current data requirement) and nitrogen transformation, in soil even at rates that greatly exceed maximum use rates. In addition, independent researchers have reviewed numerous laboratory and field studies, investigating the effects of glyphosate on soil bacteria and fungi (Felsot, 2001; Giesy et al., 2000). Although some laboratory tests have shown effects on nitrogen-fixing bacteria and soil fungi, effects are typically observed only under laboratory conditions and at glyphosate concentrations well above normal field application rates. Several researchers have concluded that it is difficult to extrapolate results from some laboratory studies to the natural soil environment (Estok et al., 1989; Wan et al., 1998; Busse et al., 2001).

Arbuscular mycorrhizal fungi are obligate symbionts that transfer mineral nutrients to their plant hosts (Harrison, 2005; Hata et al. 2010). The potential impact of glyphosate effects on arbuscular mycorrhizal fungi (AMF) colonization on glyphosate tolerant cultivars of cotton, corn and soybean grown in soil under greenhouse conditions has been evaluated (Savin et al. 2009; Knox et al. 2008; Lu et al. 2018). AMF colonization of roots was not affected by glyphosate, and neither were acid nor alkaline phosphatase soil enzyme activities. Additional research has shown that symbiosis of mycorrhiza, rhizobium, and soybean, no adverse effects of glyphosate was observed (Powell et al. 2009). Collectively, these studies indicate that effects of glyphosate on plants through effects on AMF are unlikely.

Assessment and conclusion by RMS

The papers submitted for the previous Annex I submission were not reassessed by RMS. None of them impacted the outcome of the risk assessment (RAR 2015). The above statement provided by the applicant partially reflects the previous conclusions of the RAR 2015 but lacks relevant data.

For completeness, RMS reports further elements available from RAR 2015 that could inform on indirect effects.

This potential use of glyphosate as a source of P, C or N by soil non-target micro-organisms is likely to induce a shift in their community structures. Ratcliff et al. (2006) detected a community shift from fungal dominance to equal ratio with an enrichment of opportunistic copiotrophic bacteria. Community shifts from bacterial to fungal dominance were also recorded (Araujo et al., 2003). Lupwayi et al. (2004) observed herbicide-induced shifts in microbial composition even when diversity indices among treatments did not differ. This study points out the importance to assess both microbial diversity and composition when looking at the effects of pesticides on non-target micro-organisms. In microcosm experiments performed with sediment microbes, Widenfalk et al. (2008) focused their monitoring on various levels of microbial community organization. Community-level endpoints like bacterial activity, fungal and total microbial biomass were not affected by pesticide exposure, whereas endpoints recorded at the "sub-community level" (e.g. Phospholipid Fatty-acid Analysis, 16S rRNA genotyping, T-RFLP) demonstrated significant shifts in bacterial community composition even at environmentally relevant concentrations. The same authors concluded that "Any shifts in community structure will, however, only have consequences on ecosystem function if the tolerant microorganisms cannot compensate for biogeochemical functions normally carried out by inhibited or eliminated microbial groups". Such community shifts coupled with a loss of function are clearly illustrated in Lancaster et al. (2006). The authors looked at how the combinations of pesticides may affect soil microbial activity differently than pesticides applied alone. They found that after 30 days, soils treated with glyphosate alone (applied as Roundup WeatherMAX, Monsanto Co., St. Louis, MO) exhibited greater microbial biomass,

cumulative C and N mineralization than all other treatments. However, the addition of "Roundup WeatherMax" reduced C mineralization in soils treated with the pesticides fluometuron, aldicarb, or mefenoxam + pentachloronitrobenzene formulations. The authors concluded that glyphosate based herbicides might alter the soil microbial response to other pesticides.
Therefore, like stated in Lupwayi et al. (2004), community shifts might have long-term effects on soil biological processes and the relevance of microbial diversity and composition is of importance when assessing the ecosystem soil."

Concerning the literature review for the current dossier:
Regarding Von Mérey et al., 2016, this study does not bring any added value since it only reports the results of the regulatory studies assessed by RMS.

Cerdeira and Duke, 2010[66] provides an overview of GRC (Transgenic glyphosate-resistant crops) related studies and aim to contrast certain risks of GRCs with the risks that the GRCs displace.
It states that potential effects of glyphosate on soil and water are minimal, compared to the effects of the herbicides that are replaced when GRCs are adopted. It states that GRCs crops promote the adoption of reduced- or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination. Glyphosate and its degradation product, aminomethylphosphonate (AMPA), residues are not usually detected in high levels in ground or surface water in areas where glyphosate is used extensively. Furthermore, both glyphosate and AMPA are considered to be much more toxicologically and environmentally benign than most of the herbicides replaced by glyphosate.
RMS notes that some studies referenced in this paper concluded that there was still insufficient data to determine whether glyphosate application increases incidence of Fusarium spp. associated diseases in GR crops. Other stated that high doses of glyphosate in soil reduce colonization of pepper (Capsicum annuum) plant roots with mycorrhizae. Whether effects were due to a direct effect on the mycorrhizae or to effects on the plant is not known. The doses of glyphosate used also inhibited growth of pepper. However, plants with mycorrhizae were more resistant to the growth-inhibiting effects of glyphosate.

Duke et al. 2012[67] concludes that:
- although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR (glyphosate-resistant) crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate;
- most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease;
- yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops.

However, from this review, the finding that GR crops with only a change in their EPSPS are about 50-fold less sensitive to glyphosate than similar GS (glyphosate-sensitive) crops indicated that mineral nutrition is not involved in the mode of action of glyphosate.
RMS then considers that only data on GS crops are relevant for the purpose of risk assessment.
RMS notes that further statements are available in the review on GS crops (i.e. sensitive crops, relevant for EU). Mijangos et al. examined glyphosate effects on GS plants (triticale and peas) and their rhizosphere microbial communities. Ammonia concentrations increased in rhizosphere soil after glyphosate treatment compared to the control. Functional diversity of the rhizosphere microbial community was examined. Community diversity and richness were reduced at the highest rate of glyphosate application in rhizospheres of killed GS pea and GS triticale, but not in soil from triticale grown alone. (This study Mijangos et al was not assessed by RMS)

[66] Cerdeira, A. & Duke, S.O., 2010 Effects of glyphosate-resistant crop cultivation on soil and water quality.
[67] Duke, S.O. et al., 2012. Glyphosate Effects on Plant Mineral Nutrition, Crop RhizosphereMicrobiota, and Plant Disease in Glyphosate-Resistant Crops.

Also the review states that glyphosate can have effects on mineral nutrition of GS plants through its herbicidal effects on plant roots and other parts of the plant. It also states that treatment of GS plants with glyphosate can result in increased susceptibility to pathogens.

Sullivan and Sullivan, 2000[68] is a compendium of references and abstracts illustrating the available literature on the impact of glyphosate on non-target organisms. No specific examples are discussed.

Savin et al. 2009[69] aimed to determine if dynamics of the rhizosphere microbial community were altered by applications of glyphosate and P fertilizer to glyphosate-tolerant cotton, maize, and soybean growing in low-P soil in the greenhouse. The hypothesis tested was that glyphosate application to glyphosate tolerant crops changes the rhizosphere microbial community such that plant growth and crop productivity may be hindered under conditions of low phosphorus nutrition. Overall, the study concludes that when the indigenous soil community and potential inoculum was not altered by pasteurization, glyphosate was not inhibitory nor stimulatory to mycorrhizal infection rates after six weeks of plant growth. In contrast, soil pasteurization, while not reducing the total microbial biomass, did impose a stress on the microorganisms and likely inhibited particular microbes and biochemical functioning in the soil. The potential for glyphosate to alter arbuscular mycorrhizal fungal infection in glyphosate-tolerant plants may depend on whether soil microbial communities are compromised by other factors (e.g. fungicides,…).

Knox et al. 2008[70] states that field grown cotton, regardless as to whether it is conventional or genetically modified for either insecticidal or herbicide tolerance or both traits, is mycorrhizal.
It does not imply an application of glyphosate (only the genetically modified plant). The paper is not relevant for the assessment of glyphosate. Besides, in Europe, cropping systems are not carried out with glyphosate resistant crops (GMO).

Lu et al. 2018[71] analysed the soil rhizosphere microbial communities by 16S rRNA gene amplicons sequencing and shotgun metagenome sequencing analysis between the soybean line ZUTS31 foliar sprayed with diluted glyphosate solution and those sprayed with water only in seed-filling stage.
This study indicates that the formulation of glyphosate-isopropylamine salt did not significantly affect the alpha and beta diversity of the rhizobacterial community of the soybean line ZUTS31, whereas it significantly influenced some functional genes involved in PGPT (Plant Growth Promoting Traits) in the rhizosphere during the single growth season.

Powell et al. 2009[72] states that glyphosate applied at recommended field rates had no effect on *Glomus intraradices* or *Bradyrhizobium japonicum* colonization of soybean roots, or on soybean foliar tissue [P]. N_2-fixation was greater for glyphosate-treated soybean plants than for untreated plants in both experiments, but only when glyphosate was applied at the first trifoliate soybean growth stage. These data deviate from previous studies estimating the effect of glyphosate on the rhizobial symbiosis, some of which observed negative effects on rhizobial colonization and/or N_2-fixation. In this study, genetically modified soybean was used.

The following articles were assessed in RAR 2015:

[68] Sullivan DS, TP Sullivan. 2000. Non-target impacts of the herbicide glyphosate: A compendium of references and abstracts. 5th Edition. Applied Mammal Research Institute, Summerland, British Columbia, Canada.
[69] Savin, M.C. et al., 2009. Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize and cotton.
[70] Knox, O.C.G. et al., 2008. Genetically modified cotton has no effect on arbuscular mycorrhizal colonization of roots.
[71] Lu, G.H. et al., 2018. Impact of Glyphosate on the Rhizosphere Microbial Communities of An EPSPS-Transgenic Soybean Line ZUTS31 by Metagenome Sequencing. DOI:10.2174/1389202918666170705162405
[72] Powell, J.R. et al., 2009. Effect of glyphosate on the tripartite symbiosis formed by Glomus intraradices, Bradyrhizobium japonicum, and genetically modified soybean.

Estok et al., 1989 ***showed that Roundup*** *reduced the growth of the ectomycorrhizal fungi species at concentrations < 100 ppm.*
Busse et al. (2001) found that variation in microbial community size, activity and metabolic diversity was more a function of time of year and land-use that herbicide treatment.

Newman M. et al., 2016 (see summary in appendix to Volume 3 CA B.9 on general literature on ecotoxicology)[73], investigated the rhizosphere bacterial community composition response to the application of glyphosate in formulation Roundup PowerMax. The study is then of limited relevance for the risk assessment of glyphosate. Barcoded sequencing permitted detailed phylogenetic diversity analysis and was used to identify specific bacterial taxa shifts in the rhizosphere bacterial community in response to repeated glyphosate exposure on corn and soybeans.
The largest shifts in relative abundance were observed for Proteobacteria (specifically gammaproteobacteria) and Acidobacteria. The increase in γ-Proteobacteria relative abundance for both corn and soybean rhizosphere samples was driven by increases in bacteria from the family Xanthomonadaceae following glyphosate treatment, suggesting that Xanthomonadaceae are adapted to and/or enriched by environments containing glyphosate. Concomitantly, there were decreases in the relative abundance of Acidobacteria, particularly the Acidobacteria subgroup 6.
It is hypothesized by the study authors that long-term glyphosate application could affect rhizosphere nutrient status. RMS considers the parameters investigated (rhizosphere bacterial community) as relevant as the effects of glyphosate may be masked by "functional redundancy" where overall soil functions are unaffected while microbial community composition is altered and key functions mediated by specific microbial populations are affected. Alterations to soil microbial community composition and subsequent changes in microbial diversity could potentially have pronounced long-term effects on soil quality and plant health. This study does not provide any information on whether this shift affects functional capability of the soil. Due to the uncertainties around the test item, the exposure (no analytical verification), and low replication (2 rhizoboxes per treatment), this study is considered reliable with restrictions.

Thus, in view of the literature data submitted for the current reapproval dossier, and in view of the literature review of the previous RAR used in support to biodiversity assessment by the applicant, and the additional literature data available, RMS considers that a shift in their community structures of soil micro-organisms could not be excluded as glyphosate could be used as a source of P, C or N by soil micro-organisms.

- **Biodiversity Assessment**

Assessment and conclusion by the applicant

After the thorough literature search and considering the relevant guidance, the following approach was taken to assess potential indirect effects via trophic interactions and the impact on biodiversity. This was achieved by developing a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals.

In the Table […], the specific protection goals (as described above) relevant to soil microflora are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relate directly to the effects study endpoints.

[73] Newman M. et al. 2016. Glyphosate effects on soil rhizosphere associated bacterial communities. The Science of the Total Environment, (2016) Vol. 543, No. Pt A, pp. 155 60

A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence).

Based on the measurement endpoints from the study types, and the direct effects assessment presented in this section, it is anticipated that for the proposed uses on the GAP table, that there will be no impacts on soil microbial populations in terms of nitrogen transformation and impacts on soil function, which based on the data requirements, meets the specific protection goal for soil micro-organisms.

The Table […] assessment illustrates that ecological diversity and function of soil microbes within spray zones will be sufficiently maintained to achieve the SPG for this taxa group according to the protection goals as defined in the Terrestrial guidance document (SANCO/10329/2000).

Table […]. The relationship between Specific Protection Goals, assessment and measurement endpoints for soil micro-organisms from foliar applications.

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
Protection of function of soil micro-organism communities.	Long-term effects on the function of soil micro-organism communities	N-transformation rate ≤25% difference from control at ≥28 days.	N-transformation rate
Protection of soil services (e.g., cycling of organic matter and nutrients)	Long-term effects on the function of soil micro-organism communities (i.e., Nitrogen cycling).	N-transformation rate ≤ 25% difference from control at ≥28 days	

Soil micro-organism Biodiversity Assessment
Based on the direct effects assessment, there is low risk to functioning of soil microbial populations and communities (EFSA, 2015a) and the likelihood of indirect effects on soil function due to effects on microbial or bacterial biodiversity is considered low to negligible.

[1] EFSA still needs to receive input from risk managers on the definition of specific protection goals being led by DG SANTE. In the draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, low to negligible effects are considered to be ≤ 10% and small effects are considered to be ≤ 35%.

Assessment and conclusion by RMS

No study investigating biodiversity issues or indirect effects related to glyphosate uses was proposed by the applicant from the literature review. RMS considers that the appropriateness of applicant's proposal concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control should be discussed during the EU peer-review.
In the context of the renewal of glyphosate, the representative uses did not include all potential uses of glyphosate. Particularly it did not include uses on non agricultural areas, mainly on sports fields, amenity areas, industrial areas, even on roads and pavements. When considering protection on biodiversity, it may be necessary to include a wider range of uses than those intended for renewal of approval. It should be also balanced with the need to destroy the vegetation for safety reasons, as it

could be the case in industrial areas.
Discussions among risk managers should be reinforced around the question of biodiversity in agricultural landscape in order to allow implementation on MS level based on a common and harmonised approach.

- **Conclusion**

Assessment and conclusion by the applicant

Glyphosate is a critical tool to enable conservation tillage systems, which can greatly improve the abundance and biodiversity of soil organisms. There is low risk of direct effects to soil community biodiversity and supporting/regulating services related to soil processes. This conclusion is not changed after reviewing reported levels of glyphosate from soil monitoring studies. In addition, based on a review of the literature, the likelihood of indirect effects soil organism biodiversity is also considered to be low.

However, if additional risk mitigation measures are determined to be required, to mitigate indirect effects resulting from in-crop weed control on soil microbial populations, there are standard mitigation measure options that may be considered by risk assessors and risk managers within Member States.

Assessment and conclusion by RMS

Based on standard risk assessment considering direct effects, no risk mitigation measure is necessary to protect soil microorganisms. The applicants state that the no spray buffer areas in-field and the drift reducing technologies set to protect non-target plants in off-target areas will in turn support soil macroorganisms in off-field areas. RMS notes that, in the absence of a reliable vegetative vigor study (for MON 52276), the risk assessment based on direct effects cannot be conducted and the importance of the no-spray zones (or other standard mitigation measures) is not stated yet.

B.9.14.1.7. Non-target terrestrial plants - Risk to biodiversity via Indirect Effects and Trophic Interactions

- **Indirect effects via trophic interaction**

Assessment and conclusion by the applicant

The existing terrestrial ecotoxicology guidance for NTTP assessments provides risk assessment methods for evaluating potential effects to NTTP communities outside the cropped area. Historically, protection of in-crop non-target plants / weeds has not been considered in ecological assessments for PPPs.

Therefore, a general protection goal, based on existing guidance, was derived to protect 95% of the species 90% of the time off-crop (Table […]). Based on the current assessment for the representative formulation, implementation of standard risk mitigation measures (e.g., in-field buffers, drift

reduction technology nozzles, hooded / shielded sprayers) may be required to protect NTTP communities in off-target areas. ([…]).

In the revision of the PPP data requirements (Annex to Commission Regulation (EC) No 284/2013), the former phrase "*Non-target plants are non-crop plants located outside the treatment area*" was deleted. As an outcome of this revision, an EFSA Scientific Opinion addressing the state of the science on risk assessment for NTTPs was developed that defined SPGs for off-field and in-field and linking them to biodiversity. In the EFSA Scientific Opinion (2014), NTTPs were newly defined as "*all plants growing outside fields, and those growing within fields that are not the intended pesticide target*". The proposed general protection goal for NTTPs in the Scientific Opinion is to maintain the biodiversity of plant species in the agricultural area, including both the above- and belowground (seed bank) diversity, and is linked to ecosystem services. Further, three Specific Protection Goals (SPGs) were defined: (1) protection of off-field NTTPs because they are drivers for nutrient cycling, water regulation, food web support, aesthetic values and genetic resources (biodiversity); (2) protection of in-field NTTPs because they are key drivers for food web support (primary production, provision of habitat and food for other non-target organisms, e.g. arthropods, birds), aesthetic values and genetic resources; and (3) protection of endangered plant species including rare arable weeds. However, the EFSA Scientific Opinion (2014) does not have the status of an official guidance document. The definition and selection of SPGs and exposure assessment goals (i.e., exposure in-crop versus off-crop) for NTTPs requires further discussion and decision making between risk assessors and risk managers (e.g., those of SCoPAFF, the Standing Committee on Plants, Animals, Food and Feed, in which risk managers of EU Member states are represented). When defining SPGs for arable weeds and NTTPs, it is the responsibility of the risk assessors in the Member States to acknowledge existing protection goals and regulatory data requirements, to propose possible SPG options, and describe the possible environmental consequences of each option. The risk assessors within the Member States will need to propose realistic SPGs and exposure assessment goals and the interrelationships between them in a clear and transparent manner.

Assessment and conclusion by RMS

There is currently no specific guidance or harmonized assessment procedures at the EU level for conducting a comprehensive biodiversity assessment. Highlighting the need for Specific Protection Goals (SPGs) used for the biodiversity assessment, the proposals made by the applicant are based on the EFSA Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 2014;12(7):3800, 163 pp. doi:10.2903/j.efsa.2014.3800. Doing so, the applicant considers that the SPGs developed for the glyphosate biodiversity assessment are fit-for-purpose.

Specific protection goals were defined in the EFSA Opinion 2014 for:

(1) for off-field NTTPs as key drivers for nutrient cycling, water regulation, food web support, aesthetic values and genetic resources (biodiversity);

(2) for in-field NTTPs as key drivers for food web support (primary production, provision of habitat and food for other non-target organisms, e.g. arthropods, birds), aesthetic values and genetic resources;

(3) for endangered species including rare arable weeds.

A loss of plant biodiversity due to application of plant protection products may affect the entire food web, including birds and mammals. The presence of appropriate range of plants as food sources is vital to the survival of many bird and mammal species, as well as the presence of adequate habitat for a number of species, including leaf-dwelling arthropods that serve as food sources for birds and mammals, and small mammals.

The EFSA Scientific Opinion (2014) provided also information on further data that may be requested for assessing effects on non-target terrestrial plants (including new test endpoints such as effect on the whole life cycle i.e. germinating seeds, seedling, juvenile stages, flowering, seed production and germinability, other groups of species, …). No assessment scheme is available yet. RMS nevertheless requested to address this via information from the open literature. It was also advised to reinforce the protection of off-crop habitats. The risk mitigation measures to compensate from indirect effects may be also found from literature data.

The EFSA Opinion 2014 on NTTP follows the principles of the EFSA 2016 method for defining SPGs as ecosystem services and SPGs were already identified. Therefore, the methodology and the process implemented in the EFSA Opinion 2014 can be considered in line with the EFSA method for defining SPGs. The EFSA guidance on specific protection goals (2016) aims to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services. As such, RMS considered that it is the most suitable approach available to assess biodiversity and indirect effects in the context of regulatory risk assessment.

The EFSA opinion 2014 suggested a specification of five interrelated dimensions of the SPG (i.e. Ecological Entities, Attribute, Magnitude, Temporal and Spatial scale) in line with the third step of the EFSA method (see Table below). These were not discussed with risk managers of DG SANTE and the European Union (EU) Member States.

Overview of the SPGs as defined in the scientific opinion (EFSA PPR Panel, 2014) in light of the steps described in the EFSA framework for defining SPGs (EFSA Scientific Committee, 2016).

<table>
<tr><th>EFSA Scientific Committee (2016)</th><th>EFSA Opinion on NTTP 2014</th></tr>
<tr><td>Step 1
Definition of ecosystem services</td><td>Nutrient cycling, water cycling, primary production, aesthetic values and genetic resources, provision of habitat and food for other non-target organisms, e.g. arthropods, birds</td></tr>
<tr><td>Step2
SPU</td><td>Non-target terrestrial plants (NTTP)
Off-field NTTP
In-field NTTP for food web support
In-field NTTP for aesthetic values and genetic resources
Endangered species</td></tr>
<tr><td>Legal considerations</td><td>No unacceptable effects on the environment, having particular regard to impact on biodiversity of non-target species (Mandatory for risk assessment)</td></tr>
<tr><td>Step3
Specification of the level/parameters of protection of the SPUs based on five interrelated dimensions</td><td>
<table>
<tr><th>Dimensions</th><th>Choice</th></tr>
<tr><td>Ecological entity</td><td>Off-field NTTP: Population
The protection goal is to maintain the biodiversity in the agricultural area. It is possible to define a SPG that integrates structural as well as functional aspects of biodiversity. Owing to ecological redundancy, structural endpoints are generally more sensitive to PPP application than functional endpoints. Thus, effects at the population level of NTTP species should drive the risk assessment. It is assumed that biodiversity is maintained when the plant
populations will not be affected, even for a short period, by the use of PPPs

In-field NTTP for food web support: functional group food web support (e.g. leafy crops, grass, seeds,)
Since the function of non-target plants as a food source is more relevant in this context than structural endpoints (plant diversity), the SPG should be aimed at the conservation or restoration of those functions as food or habitat sources rather than at the protection of the populations of single species. The</td></tr>
</table>
</td></tr>
</table>

		functional group for food web support provides food (biomass of green material and seeds) and habitat (cover, host plant) provisioning for higher trophic levels. In-field NTTP for aesthetic values and genetic resources: population/meta population Endangered species: individuals/population
	Attribute	Off-field NTTP: survival / growth / reproduction, abundance / biomass In-field NTTP for food web support: biomass for food web support In-field NTTP for aesthetic values and genetic resources: survival/growth / reproduction, abundance / biomass Endangered species: survival/growth / reproduction, abundance / biomass
	Magnitude	Off-field NTTP: Negligible The SPG is thereafter defined as follows: • Negligible effects on reproduction at the edge of the field/field margin. • Negligible to small effects on biomass at the edge of the field/field margin (maintenance of plant species diversity may be hampered by direct impairment of reproduction (sexual and vegetative) as well as by indirect effects owing to competitive interactions in the field resulting from effects on growth, which is not covered by the reproductive endpoint). The SPG is further made operational with the following assumptions: • for exposure, the 90th percentile of expected concentrations at the downwind edges of the field is used; • of the available toxicity data (often six or more), the 5th percentile of the species sensitivity distribution will be used. When no effects are expected for either reproduction or biomass, it is assumed that also the biodiversity will be maintained. For reproduction, the ERrepro10 and for biomass the ERveg10 is proposed. They are the effect rates (ER) where 10% effect is seen. These values can be calculated from the dose–response relationship observed in the toxicity test and they are considered as a better representation for negligible effects than the no observed effect rate (NOER) values. The SPGs can therefore be described in the following way: 95% of the NTTP will not be exposed above their ER10 under consideration of realistic worst case off-field scenarios in 90% of the cases In-field NTTP for food web support: negligible (landscape) to medium effects (field) In-field NTTP for aesthetic values and genetic resources: medium (meta-population), large effects (population) (both in-field), negligible (landscape)

		Endangered species: no effects
	Temporal scale	Off-field NTTP: not applicable For reproduction (reproduction should not be hampered at all to maintain the biodiversity outside the field) In-field NTTP for food web support: weeks (no to few days during breeding/chick phase) In-field NTTP for aesthetic values and genetic resources: not applicable/day to weeks Endangered species: not applicable
	Spatial scale	Off-field NTTP: Edge of field/field margin One of the aims of the assessment is to maintain biodiversity in the off-field. However, it is not known where (at how many meters distance from the field) the assessment should be based to maintain biodiversity and therefore the edge of the field or field margin is chosen (thus assuming that when biodiversity is maintained just outside the field biodiversity is also maintained in the off-field) In-field NTTP for food web support: field/landscape In-field NTTP for aesthetic values and genetic resources: field/landscape Endangered species: field

The applicant states that definition and selection of SPGs and exposure assessment goals (i.e., exposure in-crop versus off-crop) for NTTPs requires further discussion and decision making between risk assessors and risk managers. The applicant reminded that when defining SPGs for arable weeds and NTTPs, it is the responsibility of the risk assessors in the Member States to acknowledge existing protection goals and regulatory data requirements, to propose possible SPG options, and describe the possible environmental consequences of each option. RMS agrees that discussions are needed between MSs on the way to tackle these issues.

RMS considers that given the efficacy spectrum of glyphosate (total herbicide), the in-crop protection goals for non-target plants should be considered depending on the crop groups and accounting for good agricultural practices. For example, in orchards and vineyards, application is generally made in the rows, below trees. The space between the rows should ideally be vegetated to allow the use of the part of the field by herbivorous vertebrates and arthropods and offer flowering resources for bees. The notifier was therefore encouraged to also address how the scope of use of glyphosate based on the GAPs in question, and also based on yearly use estimates, may impact non-target plants.

In a first step, and for transparency reasons, RMS reported below the applicant's proposals in grey boxes. RMS also provided some comments that may serve as basis for discussions throughout the text to facilitate the reading.

- **Scientific Literature that informs the NTTP assessment**

Assessment and conclusion by the applicant

The scientific literature review conducted for the last Annex I renewal contains an extensive review of ecotoxicological papers considered relevant but supplementary to the Annex I renewal. The papers presented information that could not be relatable to an EU level ecotoxicological risk assessment, but that were considered in the previous dossier, where they were evaluated by previous RMS (UBA). A further evaluation of these literature papers according to the EFSA literature review approach used in this dossier has not been conducted. The previous literature review has been submitted as part of the Literature review requirements and is presented in […].

Literature review for non-target terrestrial plants from the previous Annex I (2012) submission.
In the area of non-target terrestrial plants, a total of 87 peer reviewed papers were submitted, from which a single paper (Boutin et al., (2010) that measured variability in phytotoxicity testing using crop and wild plant species) was rated with the category 'Klimisch 2". All remaining papers were not considered relevant to risk assessment. The RMS (UBA) also evaluated the submitted papers, with 27 papers identified as being supportive. The RMS (UBA) identified that most of the cited studies used formulated products and not the active substance. An objective of the NTTP risk assessment by the UBA was to ensure that NTTPs will be harmed by unintended exposure via drift to the off-target / off-field area outside of the intended spray zones.
The full evaluations of all 87 non-target terrestrial plant papers submitted as part of the peer reviewed literature review for the previous Annex I renewal (2012) are presented in […].

Current public domain literature review of published literature since the last dossier submission
Recently, Koning et al. (2019) investigated the effects of mold-board plowing, chisel plowing, and glyphosate herbicide application on weed species density and diversity in agricultural fields. Their results showed that in-crop weed communities evolved over the years depending on the type and timing of treatment. However, overall biodiversity of the weed community, which is at the basis for any consideration of potential trophic interaction within the field boundaries, was not more negatively affected by one method compared to another. This is an important paper for the biodiversity assessment, because it demonstrates that conventional tillage weed control practices have a similar outcome as chemical weed control on in-crop plant biodiversity.

In [redacted] 2020 (KCA 8.7/001), the applicant indicated the following:
A recent and relevant study by Colbach et al., (2018) investigated the topic of land-sharing versus landsparing and how to reconcile crop production and biodiversity with a mechanistic model for simulating the effects of cultural practices on weeds and crops with a spatially-explicit representation of small landscapes. This made it possible to draw conclusions on how to associate different cropping systems and land-use types in order to reconcile contrasting objectives, in this case weed harmfulness control and weed-mediated biodiversity promotion. Land-sharing is where crop production and biodiversity are maximized within individual fields. Alternatively, land-sparing is where some fields or habitats are assigned for biodiversity conservation (e.g., field margins) while the remaining fields aim to maximize production.
They modeled these two strategies based on a case study with maize-based cropping systems including genetically modified glyphosate tolerant varieties that allow for post-emergence application of glyphosate to the crop. The virtual-field model FLORSYS simulated multi-species weed floras and their impact on crop production and biodiversity depending on cropping systems and pedoclimate. The land-sharing scenario combining fields aiming to maximize crop production with either fields aiming to maximize biodiversity (25% of landscape) or grass strips (10% of landscape) were best, resulting in high crop production and medium biodiversity at the landscape scale. Land-sharing scenarios always produced less biodiversity and less production. When more crops and cropping systems were grown each year in the landscape, the weed impact on production and biodiversity was higher and more stable

over the years. In order to move further towards practical application, the authors acknowledge that their conclusions need to be confirmed with further simulation studies and the performance of the solution option should be tested in field studies. The authors further acknowledge that convincing farmers to change their practices based on simulations with a model can be difficult when they have not participated in the design of the model. The authors also recommend that new mitigations aimed at enhancing biodiversity in agroecosystems should be co-designed in workshops with farmers and extension services. The take-away from this study is that land-sparing practices / mitigations such as field-margins are most effective at promoting biodiversity. Land-sparing would be most effective in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. However, it is anticipated that land-sparing will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field.

A follow up to the EFSA Scientific Opinion (2014) on NTTPs, Arts et al., (2017) developed a proposal for three possible SPGs for arable weeds: maximal weed reduction, moderate weed reduction, and beneficial weed protection. The "maximal weed reduction" option allows for trade-offs by allowing provisioning of the ecosystem service "crop production" as being of primary importance and considers all non-crop plants in the cropped area as weeds that are not protected. This option is consistent with the current NTTP guidance that only protects off-crop NTTP communities in line with the SANCO/10329/2002 rev 2 final 'Guidance document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC. Risk assessors and risk managers in the member states will need to consider the ecological consequences of this option in light of local properties of their agricultural landscapes. The "moderate weed reduction" option differs from the "maximal weed reduction option" in that it aims to support the presence of a moderate level of arable weeds in-crop to support ecosystem services provided by weeds in crop. These ecosystem services could provide supporting services such as provisioning habitat to invertebrates and food for farmland birds and cultural services such as protecting weeds of conservation concern. This option for "moderate weed control" would most practically be achieved by implementing non-spray crop areas along the field edges and/or at the corners of an agricultural field whilst the remaining in-crop area is maintained under 'maximal weed reduction'. The economic consequence of this option may be that the monetary value of the crop decreases due to competition of the crop with arable weed. In addition, where arable weeds are allowed to persist in-crop, it is important to consider potential seed returns, which may increase the seed burden in subsequent crops. Alternatively, the non-sprayed crop areas can be replaced by vegetation other than the crops. Finally, the "beneficial weed protection" option is challenging because it would be difficult to maintain effective in-crop control of problem weeds while sustaining beneficial species at economically acceptable levels. In addition, because of the broad-spectrum nature of glyphosate, this option would not be feasible without using advanced forms of precision agriculture.

The current NTTP assessment […] is highly protective of off-crop NTTP populations and communities based on the effects data used, the exposure assessment, and the risk assessment procedures. However, because of the broad spectrum of weed control that glyphosate offers, many uses (e.g., pre-planting uses, range-land restorations) will result in loss of the in-field weeds prior to tillage. Nonetheless, there are specific scenarios with orchards / vineyards, spot treatments, control of invasive species, and directed applications where only a portion of the weed biomass will be left untreated, minimizing the impact to birds adapted to farmlands from indirect effects through trophic interactions.

It is unclear the extent to which indirect effects of broad-spectrum herbicides impact farmland birds across the different geographies in the EU, in addition to the unknown magnitude of affect that habitat modification / destruction, also has on these populations at a local and EU wide scale. In cases, where indirect effects from in-field weed control may be considered to pose an unacceptable risk in individual Member States, risk mitigation measures may be applied to mitigate effects from in-field weed control. Risk mitigation options or Member States to address direct effects and indirect effects from in-crop weed control are outlined in Table […]after the conclusions below (insert cross reference) and are primarily derived from the risk mitigations discussed in the proceedings from the MAgPIE workshop (2013) and Arts et al. 2017.

In [redacted] 2020 (KCA 8.7/001), the applicant also indicated the following:
Rare arable weed species and endangered species are a special case. They are part of the historic segetal flora communities (i.e., weeds growing in crops) and are adapted to traditional and historical agricultural practices. There are several factors defining the habitat conditions of rare arable weed species within cropped areas. Their presence or absence is often linked to particular crop varieties (i.e., historical crop varieties, which have been progressively replaced by more competitive varieties), cultivation techniques (i.e. fertilization plays a large role since many species of the rare arable flora are competitive in soils with low fertility) and timing of crop management (i.e. sowing; harvest, which may determine which weeds are able to reseed and which not). Thus, the main driver for the change in the historic segetal flora is the general agricultural intensification and optimization that act as selection filter. The use of pesticides is just one element of agricultural practices, albeit one that gets more attention (Meyer et al. 2013, Richner et al. 2014, Pinke and Gunton, 2014). Conservation of the rare arable weeds requires to optimize the agroecosystem for their conservation at the expense of crop productivity and farmers agronomic needs (farming for conservation purpose vs. farming for agricultural production). As a result of the multiplicity of influencing factors, these species cannot be addressed in the SPG set in the context of pesticide evaluation and registration of today's crop management systems. Rather, specific conservation programs are needed within other legislations to actively create arable habitats for such species.

Assessment and conclusion by RMS

The papers submitted for the previous Annex I submission were not reassessed by RMS. None of them impacted the outcome of the risk assessment (RAR 2015), however as previously highlighted from these papers in the RAR 2015:

- due to the presence of surfactants in the formulations, it is essential for current regulatory risk assessment to take into account toxicity data on the possible synergistic effects of the products in the assessed formulation in order to avoid underestimation of the toxicity of glyphosate containing products.
- intrinsic variability in plant sensitivity to herbicides is not sufficiently addressed by current risk assessment
- reproductive endpoints in many cases were more sensitive that vegetative ones
- crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested under different environmental conditions
- sublethal effects of repeated drift events or exposure to mixtures of herbicides are unaddressed
- herbicides can influence plant communities in terms of species composition and diversity
- foliar applied glyphosate to target plants is released into the rhizosphere and might negatively affect non-target plants, disease problems and nutritional status
- disease development, including increasing soil pathogen populations immobilizing micronutrients involved in disease resistance
- even small effect of glyphosate itself on functionally important components of the agroecosystem can have an impact when bearing in mind the extensive usage of glyphosate in various indications and large area
- Ecological side effects might even be stronger in diverse and species rich forest ecosystems.
- glyphosate has several secondary or indirect effects on plant physiology which may also explain its herbicidal effects

Most of these concerns are also pointed out in the EFSA Opinion 2014. Therefore it is RMS opinion that the principles of this EFSA Opinion may serve as basis to investigate the biodiversity issues.

Within this new submission, the applicant suggested the following papers:

The study by Colbach et al., (2018) was submitted by the applicant and assessed by RMS (see Appendix to Volume 3 CP on literature data on biodiversity). This study is indeed deemed relevant as it aims to assess the pros and cons of different strategies i.e. land-sharing versus landsparing. This was made in an attempt to reconcile crop production and biodiversity with a mechanistic model for simulating the effects of cultural practices on weeds and crops with a spatially-explicit representation of small landscapes. RMS agrees with the applicant statement for the case studied in this publication. Indeed RMS considers that this study is only a case-study not to be generalised to other crop systems. The results of this publication are reliable with major restrictions.

The applicant mentions an other new study by Koning et al. 2019[74]. RMS agreed that an argumentation comparing different types of weed removal (does glyphosate have equal or greater impact on ecosystems and biodiversity?) could be interesting for risk managers and decision making in a context of comparative assessment. This paper investigates the effects of glyphosate applications versus tillage on the weed vegetation in a field experiment. Two different glyphosate doses were included in the experiment, 100% and 50% of the recommended dose on the product label, in order to assess the effect of both a normal frequent application as well as the effect of a frequently applied reduced dose. Two different tillage methods were investigated, chisel plow and mould board plow, to evaluate the influence of a minimal versus a fully soil turning approach to plowing.
Overall, any method employed influenced the weed composition in some way. Some species were favored over others depending on the weed management method, but the overall biodiversity of the weed community was not more negatively affected by one method compared to another.
Species which were distinctly more rare on plots treated with glyphosate than on tillaged plots belonged especially to two groups: root and rhizome propagating species (*Cirsium arvense, Equisetum arvense, Elymus repens, Rumex acetosella*) and annual agricultural weeds with no pronounced seasonality in their germination (*Stellaria media, Matricaria chamomilla, Capsella bursa-pastoris, Lamium purpureum*). The weed community in the glyphosate treatments with differing doses grew apart over time. Particularly *Chenopodium album* and *Epilobium tetragonum* were spared by the 50% glyphosate dose compared to the 100% dose.

The report of Arts et al., 2017[75] was not submitted but could be retrieved by RMS. This document explores and presents SPG options and related Exposure Assessment golas (EAG) options from a Dutch perspective, which might be used to develop guidance on environmental risk assessment procedures for PPPs and arable weeds in in-field areas and non-target terrestrial plants in off-field areas. The described options serve to facilitate discussions at the EU level.
Three options for in-field specific protection goals (SPGs) for arable weeds are proposed:
1/ Maximal weed reduction option. This is the current option in the EU risk assessment.
Characteristics of this options are:

- Maximal provision of the ecosystem service ‘crop production’,
- Lowere priority for other ecosystem services provided by non-crop plants,
- No protection of non-crop plants in-field.

2/Moderate weed reduction option. Characteristics of this options are :

- Support of a certain moderate level of arable weeds in in-field areas.
- Support of several ecosystems services provided by non-crop plants, such as regulating services (e.g. prevention of erosion), supporting services (e.g. provision of habitat to

[74] Koning, L.A. et al., 2019. Effects of management by glyphosate or tillage on the weed vegetation in a field experiment
[75] Arts G, T Brock, I Roessink. 2017. Arable weeds and nontarget plants in prospective risk assessment for non-target plant: specific protection goals and exposure assessment goal options. Wageningen University.

invertebrates and food for farmland birds) and cultural services (e.g. protecting weeds of conservation concern).
- Improvement of local biodiversity relative to the current status.
- Effects on the ecosystem service 'crop production' is limited and controllable if implemented via a pre-defined in-field fraction of non-sprayed areas or conservation headlands.

3/ Beneficial weed protection option. Characteristics of this option are:
- Protection of 'beneficial' and low-competitive non-target plants that could potentially be managed to maintain diverse ecosystem services.
- Control of weeds that hamper growth of crop plants and thus need to be controlled to secure crop production.
- Improvement of local biodiversity relative to the current status.
- Effects on the ecosystem service 'crop production' are less quantifiable because they are dependent on the availability of selective herbicides that control pernicious weeds but spare 'beneficial' ones.

For the off-field area, three options for SPGs are also described.

1/ Population recovery option for non-target terrestrial plants. Characteristics of this option are:
- Effects on the vegetative growth/biomass of non-target terrestrial plants in the operational edge-of-field strip are accepted if:
- a) recovery takes place within an acceptable time frame
- b) effects in the operational nearby off-field strip are negligible.
- Effects on reproductive endpoints might occur in the operational edge-of-field strip.
- Least restrictive for the provision of the in-field ecosystem service 'crop production'.
- Sustainable plant populations at the landscape level are likely not at stake under the condition that in the agricultural landscape enough ecological focus areas are available (7% is proposed in the reform of the Common Agricultural Policy.

2/ Threshold option for vegetative growth of non-target terrestrial plants (this option is similar to the current procedure in the EU risk assessment). Characteristics of this option are:
- Effects on the vegetative growth/biomass of non-target terrestrial plants in the operational edge-of-field are negligible.
- Effects on reproductive endpoints might occur at the local level.
- Sustainable plant populations at the landscape level are likely not at stake.

3/ Threshold option for vegetative growth and generative reproduction of non-target terrestrial plants. Characteristics of this option are:
- Effects on the vegetative growth/biomass and on generative endpoints (flower and seed production; viability of seeds) of non-target terrestrial plants in the operational edge-of-field strip are negligible;
- Improvement of sustainability of plant populations and biodiversity at local and landscape level.

Within all three possibilities for off-field SPGs, two options are proposed for the spatial unit of the exposure assessment goal (EAG). The two options are either a 10-cm or a 2-m width of off-field strip in the edge-of-field area (and for SPG option 1 in the nearby off-field area as well) for which these three possible SPGs are assessed. This 10 cm is considered a minimum width from a scientific point of view because a plant cannot grow on e.g. a 1-mm strip. The background for offering these options is that spray drift is the most importanty exposure route and that spray drift deposition decreases sharply with distance from the treated field. Thus protecting a 10-cm-wide strip leads to higher exposure estimates (e.g. a factor of two) than protecting a 2–m- wide strip.

Agronomic consequences of the in-field and off-field options for specific protection goals have not been studied so far and need further elaboration and research.

RMS notes that concerning the Integrated Weed Management, the applicant states that a "threshold approach" is often used by farmers. RMS highlights that even if such thresholds exist, they are only

indicative and they do not constitute a legal obligation. It is then likely that these thresholds are not implemented across all EU Member States.
These thresholds only take into account the direct damage to the crop, but the indirect effects of weeds such as changes in the seed stock are crucial. RMS considers that farmers should also balance the risk of rapid increase of weed seedbank and subsequent problems in following years.
Practical implementation in an IPM context should have to consider the farmers' concern about a build-up of high soil seed banks that may impact on the weed pressure in the following years.
Finally, no information was provided to demonstrate that these thresholds are sufficiently protective for the purpose of biodiversity conservation.

A variety of risk mitigation options for the in- and off-field risk are available in the EFSA Opinion 2014.

This latter states that:" *adequate protection of the plant communities in the off-field area can be achieved by exposure-reducing risk mitigation measures, this is mostly not an appropriate option for the management of the risk to non-target species in the in-crop area. (…). Therefore, risk mitigation measures for in-crop SPGs should aim to compensate for unavoidable effects rather than reduce exposure. Indeed, indirect effects both in field and off field owing to PPP use need to be compensated for by appropriate measures (MAGPie risk management workshop, 2013: mitigating the risks of PPPs in the environment), including describing compensation measures as an option for managing in-field effects of PPP.*
Risk mitigation measures implemented at the EU level in the authorisation procedure of PPPs focused on the reduction of exposure of the off-field area. The only measures currently accepted by all EU Member States are non-spray areas at the edge of the field by in-field buffer zones to adjacent off-field areas. The focus of a non-spray area in-field (buffer zone) is primarily on the reduction of drift and run-off entries from treated fields into adjacent off-field areas (see sections 2.3, 2.4.2 and 5).
Many EU Member States also apply drift-reducing application techniques such as low drift nozzles or directed applications in order to reduce the exposure via spray drift and dust drift outside the field of application (see sections 2.3 and 5). (…)
Additional measures exist in different Member States to mitigate or compensate risk owing to direct and indirect effects of PPPs both in field and off field (for details refer to DEFRA, 2004; Bright et al., 2008; Jahn et al., 2014). For example, some of these management measures are suggested by integrated pest management (IPM) (Prokopy, 2003; Ehler, 2006; Reichenbergeret et al., 2007; van Eerdt, 2014). IPM is mainly composed of exposure mitigation measures for PPPs. Several measures aim at reducing exposure such as using alternative PPP formulations, patch spraying, restriction of application of PPP in ecological hot spots (nesting sites, burrows, see Jahn et al., 2014), and alternative methods of cultivation or use such as low pesticide-input agriculture (e.g. mechanical weed control). Other measures have the primary aim of compensating for in-crop effects on higher trophic levels by providing alternative in-field areas with improved food availability that also serve as alternative habitats (e.g. conservation headlands; creation of areas with sparsely sown cereal crops and restriction of application of PPP; creation of flowering areas or strips; keeping over-wintered stubble with self-greening and as appropriate with maintenance measures, and whole-field set-aside). If designed as a buffer zone between in- and off-field areas, these compensation areas could additionally contribute to the exposure reduction for off-field areas.
There exists a large variety of options for the mitigation or compensation of inevitable effects of PPPs on arable plant species of high conservation value and biodiversity of the agroecosystem in general owing to indirect effects on higher trophic levels. Whereas most options mentioned above can be expected to improve the food provision to higher trophic levels, the appropriate options for the conservation of the arable flora are mostly those where cultivation of the area is still retained. The concrete risk management concept (including the choice of the adequate risk management measures and their combination) needs to be established on a national level, reflecting ecological and agricultural conditions such as the availability of drift-reducing application techniques or national

policies for implementation of management and mitigation options, e.g. obligatory vegetated buffer strips of a certain width."

RMS notes that risk mitigation and compensation measures are regarded as Member State issues (as strongly depending on ecological and agricultural conditions).

RMS would like to remind that the current risk assessment scheme is based on ER50 values as endpoints whereas ER10 values are proposed in the EFSA Opinion of 2014. Moreover SSD derived for NTTPs are currently compared to a safety factor of 1. In the RAR of 2015 already a number of arguments were mentioned and again presented in the biodiversity section of this RAR which show that the actual risk assessment of glyphosate is probably not protective enough. So RMS considered that the use by the applicant of the word "highly" to describe the level of protectivness is not appropriate. Following the recommandations of EFSA Opinion of 2014 on NTTPs, RMS considered that protection of plant biodiversity is not sufficiently addressed.

RMS wishes to remind the overall outcome of the extensive literature review conducted in the scope of the EFSA Opinion on NTTPs that are of interest when dealing with the question of impact on biodiversity and indirect effect through trophic interactions. The EFSA opinion stated that rare arable weeds are usually annual species that need regular soil disturbance and are preferably found in crop edges of conventional farming as well as in field centre and edges of organic fields. It was stated that many arable weeds have become rare owing to intensive management practices introduced in the last 50 years: extensive use of agrochemicals applied with ever increasing machinery size, increased field size and destruction of marginal habitats for the use of this machinery, better seed cleaning, use of high-density crop shading out weeds, and other modifications in crop types and management such as monoculture and timing of harvest. Studies pointed to the fact that uncropped cultivated (tilled) margins appear to be best for rare arable weeds. Herbicides are very detrimental to rare arable weeds. However, uncropped cultivated margins are not a preferred option by the farming community and, consequently, are not practised much among farmers. Although rare arable weeds need protection from pesticide use, their management should be considered in the light of the overall agricultural practices of crop margins (buffer zones or in-field non-treated strips, etc.). Management practices that favour rare arable weeds have been identified, e.g. uncropped tilled field edges with no herbicide spray.

- **Assessment**

Assessment and conclusion by the applicant

After a through literature review and considering all recent guidance, the following approach has been taken to assess potential indirect effects via trophic interactions considering the proposed Specific Protection Goals drawn from the existing EU guidance and working documents, and the 2016 EFSA Guidance on developing protection goals for ecological risk assessments (ERA) for pesticides. The SPGs were based on direct effects assessment considering representative sensitive populations across the tested trophic levels. The biodiversity assessment, aimed to develop a flexible framework that informs the development of risk mitigation options to achieve the specific protection goals, that includes mitigating against indirect effects via trophic interaction. For example, for NTTPs, the inclusion of no-spray buffer zones as a standard mitigation measure protects NTTP communities in off-target areas, which indirectly supports biodiversity by maintaining habitat as both a refuge and food source for other organisms in off-target areas. Therefore, where an acceptable direct effects risk assessment is concluded upon after incorporation of standard mitigation measures to reduce off-target movement via drift, this is considered protective of indirect effects occurring outside of the target area.

In the following table, the specific protection goals relevant to non-target terrestrial plants are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relate directly to the effects study endpoints.

A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence). For NTTPs an acceptable direct effects assessment by including a standard mitigation measure e.g., no-spray buffer zone.

The direct effects assessment requires a no-spray buffer zone to reduce the possible exposure risk to plants occurring in off-target areas. This is considered to meet the proposed SPG. *In [redacted] 2020 (KCA 8.7/001), the applicant also indicated the following:*
These measures are intended to address simplified landscapes intensified production areas, where the refuge areas for insects, birds and mammals are limited. However, it is anticipated that these mitigations will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field.
The relationship between study type, measured and assessed endpoints and the SPG are presented in Table […].

Table […]: The relationship between specific protection goals and associated assessment and measurement endpoints for non-target terrestrial (NTTP) plants from off-crop spray drift.

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
Negligible risk to off-field NTTP communities to support nutrient cycling, water regulation, food web, aesthetic values and genetic resources (biodiversity)	Protect 95% of the populations in 90% of the cases.	EC_{50} values for plant survival, height and weight.	Vegetative vigor Seedling emergence

NTTP Biodiversity Assessment
Based on the current direct effect assessment for the representative formulation, standard risk mitigation measures (e.g., in-field buffers, drift reduction technology nozzles, hooded sprayers) will be required on the label to protect NTTP communities outside the cropped area. However, if additional risk mitigation measures are considered to be required by risk managers at the Member States level, to mitigate indirect effects resulting from in-crop weed control, risk mitigation options that maybe considered are presented in Table […]of the [redacted] (2020) Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment. (TRR0000305) […].
[1] It is assumed that the biodiversity is maintained when most of the plant populations will not be affected using plant protection products. It is assumed that this goal can be reached when the plant populations are protected off-crop.

Assessment and conclusion by RMS

RMS considers that the applicant's proposal (concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control) should be discussed. The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments conducting with the EFSA guidance document for aquatic organisms (2013). Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review in order to establish

the basis for harmonised set of measures to be implemented on MS level at product authorisation.

- **Conclusion**

Assessment and conclusion by the applicant

The existing terrestrial ecotoxicology guidance for NTTP assessments provides risk assessment methods for evaluating potential direct effects to NTTP communities outside the cropped area. Historically, protection of in-crop non-target plants / weeds has not been considered in ecological assessments for PPPs. However, in the revision of the PPP data requirements, the former phrase "*Non-target plants are non-crop plants located outside the treatment area*" was deleted. As an outcome of this revision, an EFSA Scientific Opinion (2014) was developed that defined SPGs for off-crop and in-crop NTTPs and linking them to biodiversity. In the Scientific Opinion (2014), NTTPs were newly defined as "*all plants growing outside fields, and those growing within fields that are not the intended pesticide target*"; though the Scientific Opinion (2014) does not have the status of an official guidance document. The derivation of SPGs for NTTPs requires further discussion and decision making between risk assessors and risk managers as well as risk mitigation options to address indirect effects. Holistically addressing potential indirect effects to birds and mammals by limiting in-crop weed control may be better handled through policies and programs outside the PPP framework.

Based on the current direct effect assessment for the representative formulation, standard risk mitigation measures (e.g., in-field buffers, drift reduction technology nozzles, hooded sprayers) will be required on the label to protect NTTP communities outside the target area . However, if additional risk mitigation measures are required by risk managers at the Member State level, standard risk mitigation options are available at the EU level and are presented in the following table. Many of these have been considered in the current dossier submission.

Assessment and conclusion by RMS

The applicant seems to state that drift reduction measures like buffer zones, drift reduction nozzles, (…) will be favourable for biodiversity. These buffer zones or other risk mitigation measures are necessary according to the risk assessment in order to reduce the risk to the off-crop community of plants to an acceptable level. They are considered to contribute to protection of biodiversity but may not be sufficient alone. In intensive agricultural areas, the extent of the off-field areas may be limited and it appears essential to create sufficient compensation areas to protect the biodiversity within the landscape.

B.9.14.1.8. Risk mitigation options to protect biodiversity

Assessment and conclusion of the applicant

Risk mitigation options to address direct and indirect effects to ecological species

Environmental risk mitigation measures are a key component in defining the conditions of use of pesticides in crop protection in Europe ((EC) No 1107/2009) and (EU) No 547/2011). These risk mitigation measures are derived directly from the evaluation of pesticide products and the risk assessment conducted for each use and are specific of the type of risk they are intended to mitigate. They therefore range from the adjustment of the conditions of use, to minimizing transfers to surface and groundwater, to the setting of buffer zones at the edge of the crop, and to requiring compensatory measures (e.g., field margins).

Risk mitigation measures can be divided into "standard" mitigation measures where an impact can be calculated in the frame of environmental risk assessment and "non-standard" mitigation measures where the impact on biodiversity cannot be directly expressed in numerical values. It needs to be noted that biodiversity related mitigation measures need to be adapted to the local Member State level, to the local environmental circumstances (e.g. landscape), to the local biodiversity conservation status and to the desired protection and conservation goals.

It is therefore appropriate to consider the available mitigation tools available across the EU, that could be applied by risk managers. Currently, the most up-to-date compilation of plant protection mitigation tools available across Europe was compiled during a series of workshops in 2013 under the auspices of the Society of Environmental Toxicology and Chemistry (SETAC) and the European Commission. The goal of the MAgPIE workshops was to develop a toolbox of mitigation measures from across the EU. The outcome of these workshops was a proceedings published in 2017 "Mitigating the Risks of Plant Protection Products in the Environment MAgPIE".

The MAgPIE workshop proceedings and associated publications were inventories of the available risk mitigation options across the various Member States in the EU and included a toolbox of recommendations in view of future implementation. The types of standard mitigation measures are generally described in [table below]. The data were collated into Risk Mitigation Measure Technical Sheets (RMMTS) and reflect the risk mitigation tools available in most Member States.

Types of standard risk mitigation measures described in MAgPIE across the various Member States to mitigate effects on biodiversity and how they could be applied to glyphosate products.

Type of Mitigation Measure	Risk Mitigation Measure	Benefits	Glyphosate renewal dossier (2020)
Restrictions or modifications of products' conditions of application	Application rate, Application frequency, application timing, and interval between applications	Lower transfers to groundwater and surface water; Reduces exposure of organisms in-crop and off-crop.	**Significant reductions (50% in volume) in newly proposed application rates** compared with the representative use presented in the 2012 renewal dossier. See [76]Appendix 2 of the biodiversity document accompanying this submission. **Treated area restriction**

[76] [redacted] (2020) Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment (TRR0000305).

			1. for the representative use GAPs: applying to only 50% of the total area in orchard/vineyard area. 2. maximum of 50% of the total area for broad acre vegetable inter-row 3. Invasive species control e.g., couch grass – maximum of 20% of the cropland + extended application intervals. **Limited frequency and timing of application:** 28-day interval between applications and no pre-harvest applications
Application equipment with Spray Drift Reduction Technology (SDRT)	Spray drift reduction nozzles (SDRN), shields, Precision treatment, etc.	Reduces exposure of organisms in-crop (precision treatment) and off-crop	**Reduction of spray drift to the off-field:** 1. Use 75% drift reducing nozzles for pre-sowing/pre-planting in arable crops. 2. Use of ground directed, shielded spray for band application in orchards / vineyards and broad-acre vegetable inter-row application.
Buffer zones	Non-sprayed zone at the edge of a crop	Reduces exposure of organisms and off-crop	**Establishment of buffer zones:** Buffer zones of varying size (depending on the type of SDRT) are required as protection for off-crop NTTP communities from spray drift.

Furthermore, some biodiversity conservation measures are included in the MAgPIE toolbox of mitigation measures. These non-standard mitigation measures are introduced to maintain biodiversity in agroecosystems. […] presents examples of non-standard mitigation measures that could be considered at the EU level for glyphosate as recommendations for Member State consideration. However, these measures will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field.

Examples of non-standard mitigation described in MAgPIE across the various Member States and risk mitigation options, and Arts et al. 2017, to address potential indirect effects and how they could be applied to glyphosate products.

Type of Mitigation Measure	Risk Mitigation Measure	Benefits	Comments
Moderate weed reduction in-crop	A certain part of the in-crop area would not be sprayed.	Aims to support a moderate level of arable weeds incrop to support provisioning of habitat to invertebrates and food for farmland birds / mammals to overcome indirect effects from in-crop weed control. The option for "moderate weed control"	Mitigation option for broad acre row crops to protect against indirect effects through trophic indirections. This measure will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high

		could be achieved by implementing non-spray crop areas along the edge and/or at the corners and/or in subfield areas of lower productivity of an agricultural field while for the remaining in-crop area a maximal weed reduction can apply.	ecological benefit in complex landscapes where enough refuges are available off-field. The economic consequence of this option may be that the monetary value of the crop decreases due to competition of the crop with arable weed. The agronomic consequence of this measure is the progressive increase in the weed seed bank in-field, which may increase the weed pressure and thereby the need for weed control in subsequent years.
Multi-functional field margins	Supportive measures: Using seed mixtures: wild flower-sown mix, pollen and nectar flower mix, adapted wild bird cover mix, vegetated filter strips.	Reduces exposure of organisms in-crop and offcrop, provides habitat and food resources and mitigates indirect effects on biodiversity.	Field margins provide benefits for conservation in terms of biodiversity (species) and the provision of biotic and abiotic agro-ecosystem services. This measure will bring the greatest ecological benefit when their creation is spatially targeted in simplified landscapes and intensified crop production areas, where the refuge areas for insects, birds and mammals are limited. It has been shown that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field Sown flower areas bear the risk of also generating disservices to crop management such increased pest pressures and/or noxious or invasive alien plant species, if seed mix are not properly selected.
Compensation areas	Recovery areas (ecological focus areas, biodiversity refuge) => part of the agricultural area that is not cultivated anymore (land sparing) Supporting measures: hedges, trees, landscape features, biotopes, afforested area.	Provides habitat and food resource, reduces exposure of organisms in-crop, and depending on location in the farmland, may reduce exposure of non-target organisms.	Availability of habitats is key to support food webs and biodiversity. The creation of semi-natural habitats and corridors across the landscape is especially important in intensively cropped areas, to ensure sufficient connectivity between available habitat patches. Since different species have different habitat requirements noncrop/ semi-natural habitat creation measures should be adapted to the local situation. This measure will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high

			ecological benefit in complex landscapes where enough refuges are available off-field.

In addition, to the non-standard mitigation options in […], over 200 measures (including habitats, GAPs, input use reduction) have been recommended by various stakeholders in EU studies to enhance biodiversity conservation in field crops at landscape level (e.g., Dicks et al, 2013). Most of the habitats are targeted at the conservation of single or specific groups of species (birds, pollinators) and include the recommendation to create specific, often expensive habitats such as ponds or hedges. Other less costly habitat recommendations exist that can benefit multiple species and ecosystem services (e.g., water regulation, soil erosion regulation, pollination) at the same time. Most of these habitats are defined by numerous names (fallow land and ecological set aside meaning the same thing) and vary in application forms and duration (areas, margins, strips, annual, or longer etc.). Their farm economic aspects in terms of profitability and costs (labor, time) involved nor their benefits to agro-ecosystem services have not been researched. Thus, when identifying habitats as to their broader suitability for use in field crops (orchard, perennial crops are different) dual benefits for both crop production and biodiversity conservation should be aimed at to increase the motivation for their creation. When analyzing the many habitats recommended for use in field crops in more details their numbers can be reduced to a few key groups: fallow land (uncropped land, fallow areas, crop edges, headlands), managed margins (also bordering sensitive areas such forests, water bodies), managed flower subfield areas or areas/filed cropped at reduced intensity. These habitats could provide a low effort, cost-effective tool to support associated biodiversity to crop production and may benefit crop profitability if applied on less productive land. Creation of such habitat in addition to applying traditional GAPs, or the saving of natural resources such as land, water, energy would provide an additional tool fundamental to efforts that enhance agroecosystem services and cropland associated biodiversity (e.g., bees, birds), increase ecological resilience, sustainably intensify and improve crop production as required by Regulation 1107, Article, 6. Such in-field or beyond field habitats could especially contribute to better biodiversity outcomes in simplified landscape types where field crops are intensively cropped and should complement the application of broader landscape planning efforts.

Assessment and conclusion by RMS

The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments conducting with the current guidance documents.
The applicant seems to state that drift reduction measures like buffer zones, drift reduction nozzles, (…) will be favourable for biodiversity. These buffer zones or other risk mitigation measures are necessary according to the risk assessment in order to reduce the risk to the off-crop community of plants to an acceptable level. They are considered to contribute to protection of biodiversity but may not be sufficient alone. In intensive agricultural areas, the extent of the off-field areas may be limited and it appears essential to create sufficient compensation areas to protect the biodiversity within the landscape.

Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review in order to establish the basis for harmonised set of measures to be implemented on MS level at product authorisation.

B.9.14.2. Overall conclusion of RMS regarding risk to biodiversity assessment via indirect effects and trophic interaction

The regulation (EU) 2017/2324 related to the approval of glyphosate stated that "Member States shall pay particular attention (…) to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions". A loss of plant biodiversity following the application of plant protection products may affect the entire food web. It could affect the presence of adequate habitats for arthropods, as well as for birds and mammals. Moreover the presence of appropriate range of plants as food sources is vital to the survival of foliage eating arthropods, birds and mammals, as well as nectar and pollen sources for bees. However, there is currently no validated tools nor methodology for a European harmonized risk assessment of biodiversity and consideration of indirect effects via trophic interactions available. RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects. Moreover, even if indirect effects and trophic interactions are linked to biodiversity, there is much more to consider to protect biodiversity and the providing ecosystem services in Europe in adequacy with the various EU and national legislations.

For aquatic organisms and bees, EFSA guidance documents proposed specific protection goals that followed methodology reported in the EFSA guidance on specific protection goals (2016). As the aim of this guidance is to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services, RMS considered that it is the most suitable approach available to assess biodiversity in the context of regulatory risk assessment.
For aquatic organisms, according to the guidance document in force for aquatic organisms (EFSA, 2013), risk assessment based on ecological threshold option could be considered protective of both direct effects as well as indirect effects including trophic interaction among the aquatic food chain when the magnitude of effects is considered as negligible for each ecological entity of each of the aquatic organisms. However, for glyphosate, it could not be considered that all indirect effects and food web interactions are addressed given that not all food sources are considered. For example, study to assess the effects on sediment-dwelling organisms is missing. Additionally, information on impact on decomposition processes in aquatic systems, or effects on the biofilm (algae, fungi and bacteria-matrix) would need to be considered. Further information on the effect to the aquatic community could also contribute to assess risk to biodiversity via indirect effects and trophic interactions. Thus some uncertainties remain.
For bees, risk to bee biodiversity from direct effects can be considered covered by the risk assessment for glyphosate that is based on standard laboratory tests. However indirect effects that may result to the reduction of weeds availability could not be addressed via current risk assessment. One option could be to implement compensatory area but for the time being the effectiveness of such method is only qualitative.

For soil organisms, non-target arthropods and non-target terrestrial plants, the applicant attempted to define what could be specific protection goals for these organisms by considering recent EFSA Scientific Opinions. However, RMS noted that functional/organism groups used are limited to the regulatory species. However the options proposed to set SPG for these organisms should be agreed by risk managers and guidance documents should have to be revised accordingly.
Regarding the indirect effects through trophic interactions to farmland birds by reducing in-crop food resource as consequence of glyphosate application, one option could be to consider additional mitigation measures that allow birds to find food resources from adjacent non-treated area. Considering this, a reflection should be made on the desired option manageable at the European landscape for approval of active substance as well as at more local level (MS, field…). System-based approach exist that may help risk managers to choose the more appropriate approach (landsharing vs. landsparing) considering the biodiversity goal of the European legislation.

Regarding the indirect effects linked to the loss of habitats for non-target arthropods and cascading effects to birds and mammals, one option could be to compensate this loss. Same concept as for indirect effects related to non-target plants as food source could be considered.

Overall, there is a need of practical harmonised risk assessment tools for the assessment of active substance and plant protection products before their placement on the market. For that purpose, guidance documents used for risk assessment should be revised to take into account specific protection goals as defined according to the principles of EFSA guidance (2016).
In the meantime, given the importance of agroecosystems as habitats and food/ressources supply location, discussions among risk managers should be reinforce around the question of biodiversity in agricultural landscape. There is a balance to find between reducing indirect effects and impact on biodiversity and benefits to use plant protection products such as glyphosate to maintain agricultural food and livestock production sustainable.
Implementation of mitigation measures dedicated to biodiversity could be part of the environmental risk assessment in the context of plant protection products considering definition of SPG. Under Regulation (EC) No 1107/2009, the evaluation of effects on biodiversity via indirect effects and trophic interactions are limited to effects caused by the intrinsic properties of the active substance itself. The consideration of the extent of uses of a specific plant protection active substance should be considered by risk managers during the decision making process. Proposals for mitigation of risk from indirect effects/tophic interaction should be discussed further during the EU peer review and decided on MS level.

Monitoring programs and indicators such as farmland bird index, grassland butterfly index, (…) should be developed and harmonised. As reported Maes J. et al. (2020)[77] in a recent JRC report "Monitoring biodiversity is essential to be able to assess if policy targets have been met (e.g. halting biodiversity loss)." JRC also indicated that "While availability of information on species and habitats is improving, indicators on genetic diversity are still missing from the overall picture, and in particular organised information, at the EU level, on the number, amount and geographical distribution of traditional breeds, cultivars, landraces, wild crop relatives, traditional and ancient varieties." Harmonised approach to report results and assessment of monitoring programs and indicators will allow to have feedback on the effectiveness of mitigation measures taken. System-based approach could also be used for that purpose as they represent tools that could be used by both risk assessors and risk managers.

[77] Maes, J., et al., 2020. Mapping and Assessment of Ecosystems and their Services: An EU ecosystem assessment, EUR 30161 EN, Publications Office of the European Union, Ispra, 2020, ISBN 978-92-76-17833-0, doi:10.2760/757183, JRC120383.

B.9.15. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1]
KCA 8.7-001	[redacted]	2020	Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment Report No.: TRR0000305 Document No.: -- - GLP/GEP: N Published: N	N	N	-	GRG	N
KCP 10.1	Ebeling, M., Wang, M.	2018	Dissipation of Plant Protection Products from Foliage. Environmental Toxicology and Chemistry. Wiley Online Library.	N	N	N	-	-
KCP 10.2.1-001	[redacted]	1992	MON 52276: Acute toxicity to rainbow trout, *Oncorhynchus mykiss*, under Flowthrough test conditions Report No.: J9108002b Document No.: [redacted]-91-296 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Yes RAR 2017: IIIA 10.2.1
KCP 10.2.1-002	[redacted]	1992	MON 52276: Acute toxicity to common carp, *Cyprinus carpio*, under flow- through test conditions Report No.: J9108002c Document No.: [redacted]-91-298 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Yes RAR 2017: IIIA 10.2.1
KCP 10.2.1-003	[redacted]	1992	MON 52276: Acute toxicity to the water flea, *Daphnia magna*, under flow- through test conditions Report No.: J9108002a Document No.: TO-91-296 Toxikon Environmental Sciences GLP/GEP: Y Published: N	N	N	-	BCS	Yes RAR 2017: IIIA 10.2.1
KCP 10.2.1-005	[redacted]	2002	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed *Lemna gibba* Report No.: GA-2002-051 Document No.: 20021186/01-AALg GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Yes RAR 2017: IIIA 10.2

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KCP 10.2.1-006	[redacted]	2012	Effect of MON52276 (Glyphosate formulation) on the growth of *Myriophyllum aquaticum* in the presence of sediment, with a subsequent recovery period Report No.: CHE-016/4-80/A Document No.: Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME) GLP/GEP: Y Published: N	N	N	--	GTF	Yes RAR 2017: IIIA 10.2
KCP 10.3.1.1.1-001	[redacted]	2001	Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the honeybee, *Apis mellifera* Report No.: MON-00-2 version 2 Document No.: Mambo-Tox Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Yes RAR 2017: IIIA 10.4.1
KCP 10.3.1.1.2-001	[redacted]	2001	Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the honeybee, *Apis mellifera* Report No.: MON-00-2 version 2 Document No.: Mambo-Tox Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Yes RAR 2017: IIIA1 10.4.2
KCP 10.3.1.5-001	[redacted]	2011	Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions Report No.: V7YH1002 Document No.: The Food and Environment Research Agency (Fera) GLP/GEP: Y Published: N	N	N	--	GTF	Yes RAR 2017: IIA 8.7.3
KCP 10.3.1.5-002	Thompson, H.M., Levine, S.L. *et al.*	2014	Evaluating Exposure and Potential Effects on Honeybee Brood (*Apis mellifera*) Development Using Glyphosate as an Example Report No.: DOI: 10.1002/ieam.1529 E-ISSN: 1551-3793 - Document No.: - Integrated environmental assessment and management, (2014) Vol. 10, No. 3, pp. 463-70. GLP/GEP: N Published: Y	N	N	-	LIT	No

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1]
KCP 10.3.2.1 -001	[redacted]	1995	Testing toxicity to beneficial arthropods Cereal aphid parasitoid - *Aphidius rhopalosiphi* (Destefani-Perez) / Imagines according to IOBC Guideline (Mead-Briggs 1992). Roundup Ultra Report No.: 95 10 48 054 Document No.: - BioChem GmbH Karlruhe GLP/GEP: Y Published: N	N	N	-	BCS	Yes Monograph 1998: AIIIA-10.5.1
KCP 10.3.2.1 -002	[redacted]	1995	Testing toxicity to beneficial arthropods. Predacious mite - *Typhlodromus pyri* (Scheuten) according to IOBC Guideline (Overmeer 1988 and Louis 1994). Roundup Ultra Report No.: 95 10 48 056 Document No.: - BioChem GmbH Karlruhe GLP/GEP: Y Published: N	N	N	-	BCS	Yes Monograph 1998: AIIIA-10.5.1
KCP 10.3.2.1 -003	[redacted]	1995	Testing toxicity to beneficial arthropods - carabid beetle - *Poecilus cupreus* L. according to BBA Guideline VI, 23-2.1.8 (1991) Report No.: 95 10 48 055 Document No.: - BioChem GmbH Karlruhe GLP/GEP: Y Published: N	N	N	-	BCS	Yes Monograph 1998: AIIIA-10.5.1
KCP 10.3.2.1 -004	[redacted]	1995	Testing toxicity to beneficial arthropods - Spider - *Pardosa* spp. According to BBA Guideline (Proposal 1994) Report No.: 95 10 48 053 Document No.: - BioChem GmbH Karlruhe GLP/GEP: Y Published: N	N	N	-	BCS	Yes Monograph 1998: AIIIA-10.5.1
KCP 10.3.2.2 -001	[redacted]	2010	An extended laboratory bioassay of the effects of fresh residues of MON 52276 on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae) Report No.: MON-09-3 Document No.: MT-2009-404 Mambo-Tox Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Yes RAR 2017: IIA 8.8.2.2
KCP 10.3.2.2 -003	[redacted]	1998	Testing toxicity to beneficial arthropods - Predatory mite - *Typhlodromus pyri* (Scheuten) (extended laboratory test) according to IOBC Guideline	N	N	-	BCS	Yes RAR 2017: IIA 8.7.2

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1]
			(Oomen 1988) Report No.: 95 10 48 065 Document No.: - BioChem agrar GLP/GEP: Y Published: N					
KCP 10.3.2.2-004	[redacted]	2010	A rate-response extended laboratory test to determine the effects of MON 52276 on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) Report No.: MON-09-2 Document No.: MT-2009-405 Mambo-Tox Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Yes RAR 2017: IIA 8.8.2.1
KCP 10.3.2.2-005	[redacted]	1999	Testing toxicity to beneficial arthropods Cereal aphid parasitoid - *Aphidim rhopalosiphi* (Destefani-Perez) (extended labortory test) following the IOBC Guideline proposal (Mead-Briggs 1994) Report No.: 95 10 48 066 Document No.: - BioChem GmbH Karlsruhe GLP/GEP: Y Published: N	N	N	-	BCS	Yes Endpoint reported in RAR 2017
KCP 10.3.2.2-006	[redacted]	1999	Testing toxicity to beneficial arthropods Cereal aphid parasitoid - *Aphidim rhopalosiphi* (Destefani-Perez) (extended labortory test) following the IOBC Guideline proposal (Mead-Briggs 1994) - Amendment No.1 to Final Report Report No.: 95 10 48 066 Amend Document No.: - BioChem agrar GLP/GEP: Y Published: N	N	N	-	BCS	Yes Endpoint reported in RAR 2017
KCP 10.3.2.2-007	[redacted]	2010	An extended laboratory test to determine the effects of MON 52276 on the ground-active beetle, *Aleochara bilineata* (Coleoptera; Staphylinidae) Report No.: MON-09-4 Document No.: MT-2009-403 Mambo-Tox Ltd. GLP/GEP: Y Published: N	N	N	-	BCS	Yes RAR 2017: IIA 8.8.2.3
KCP 10.3.2.2-008	[redacted]	1999	A Laboratory Evaluation of the Effects of MON 52276 on the Green Lacewing, *Chrysoperla carnea* Report No.: MON-99-3	N	N	-	BCS	Yes RAR 2017:

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1]
			Document No.: US-99-093 Agrochemical Evaluation Unit (AEU) GLP/GEP: Y Published: N					
KCP 10.4.1.1-001	[redacted]	2020	MON 52276: Effects on survival, growth and reproduction of the earthworm *Eisenia andrei* tested in artificial soil Report No.: 20 48 TEC 0028 Document No.: BI-2019-0632 BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	BCS	No
KCP 10.4.2.1-001	[redacted]	2020	MON 52276: Effects on reproduction of the collembolan Folsomia candida Report No.: 20 48 TCC 0037 Document No.: BI-2020-0179/ TRR 0000493 BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	BCS	No
KCP 10.4.2.1-002	[redacted]	2020	MON 52276: Effects on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* tested in artificial soil Report No.: 20 48 THC 0031 Document No.: BI-2020-0183/TRR0000517 BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	BCS	No
KCP 10.5-001	[redacted]	2012	MON 52276: Effect on soil microbial activity, Carbon and Nitrogen transformations Report No.: CEMR-5259 Document No.: CE-2011-0537 CEM Analytical Services Limited (CEMAS) GLP/GEP: Y Published: N	N	N	-	GTF	Yes RAR 2017: IIIA 10.7.1
KCP 10.6.2-001	[redacted]	2019	MON52276: Effects on the Seedling Emergence and Growth of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report No.: S19-03634 Document No.: EUR-2019-0233 Trialcamp S.L.U.	N	Y	First submission in EU	BCS	No

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1]
			GLP/GEP: Y Published: N					
KCP 10.6.2-002	[redacted]	2014	MON 52276: Effects on the Vegetative Vigor of Non-Target Terrestrial Plants (Tier II) Report No.: 80477 Document No.: - ABC Laboratories, Inc. GLP/GEP: Y Published: N	N	N	--	BCS	Yes RAR 2017: KIIIA1 10.8(OECD)
KCP 10.6.2-004	[redacted]	2012	Comparative Post-Emergence Phytotoxicity of AMPA and Glyphosate to Crop and Annual Weed Species Report No.: MSL0024009 Document No.: - Monsanto company GLP/GEP: Y Published: N	N	N	-	BCS	Yes RAR 2017: IIA 8.16.1
KCP 10.6.2-005	[redacted]	2021	Vegetative vigour study with MON 52276 Report No.: S20-05300 Document No.: Trialcamp S.L.U. GLP/GEP: Y Published: N	N	Y	First submission in EU	BCS	No
CP 10.1.1 CP 10.1.2	Last, G. et al.	2019	Regulatory report on the occurrence of flowering weeds in agricultural fields. Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	No
CP 10.1.1 CP 10.1.2	Warburton, D.B. et al.	1984	Wildlife use of no-till and conventionally tilled corn fields. Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	
CP 10.1.1 CP 10.1.2	Cerdeira, A. & Duke, S.O.	2010	Effects of glyphosate-resistant crop cultivation on soil and water quality. Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	
CP 10.1.1 CP 10.1.2	Savin, M.C. et al.	2009	Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize and cotton. Report No.: Document No.: -	-	N	-	LIT	

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1]
			GLP/GEP: N Published: Y					
CP 10.1.1 CP 10.1.2	Knox, O.C.G. et al.	2008	Genetically modified cotton has no effect on arbuscular mycorrhizal colonization of roots. Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	
CP 10.1.1 CP 10.1.2	Lu, G.H. et al	2018	Impact of Glyphosate on the Rhizosphere Microbial Communities of An EPSPS-Transgenic Soybean Line ZUTS31 by Metagenome Sequencing Report No.: DOI:10.2174/1389202918666170705162405 Document No.: DOI:10.2174/1389202918666170705162405 - GLP/GEP: N Published: Y	-	N	-	LIT	
CP 10.1.1 CP 10.1.2	Silva, V. et al.	2018	Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	
CP 10.1.1 CP 10.1.2	Koning, L.A. et al.	2019	Effects of management by glyphosate or tillage on the weed vegetation in a field experiment Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	
CP 10.1.1 CP 10.1.2	Powell, J.R. et al.	2009	Effect of glyphosate on the tripartite symbiosis formed by *Glomus intraradices*, *Bradyrhizobium japonicum*, and genetically modified soybean. Report No.: Document No.: - GLP/GEP: N Published: Y	-	N	-	LIT	

[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