European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Glyphosate

Volume 3 – B.5 (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.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Analysis of the plant protection product

Methods for the determination of the active substance and/or variant in the plant protection product

Data point	CP 5.1.1/001
Report author	Bates, C.
Report year	2001
Report title	Determination of glyphosate content in formulations MON 78043, MON 78044 and MON 2139 (glyphosate 360 g/L) SL by HPLC: validation of the analytical method
Report No	MSL – 17401
Document No	-
Guidelines followed in study	SANCO 3030/99 rev. 4
Deviations from current test guideline	None (SANCO/3030/99 rev. 5)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes
Category study in AIR 5 dossier (L docs)	Category 2a
Test facility	Agricultural Reaseach Centre, Phytopharmacy department, rue du Bordia, 11, B – 5030 – GEMBLOUX BELGIUM

Principle of method (AOAC-CIPAC method 284/SL/(M)/3):

An amount of the test item is accurately weighed in a 100 mL volumetric flask and dissolved with the mobile phase (0.84 g/L of KH_2PO_4 in 4 % methanol/water adjusted to pH 1.9 with o-phosphoric acid) using an ultrasonic bath during about 10 minutes. The volumetric flask is filled up to volume resulting in solutions containing approximately 4.0 mg/mL glyphosate. The glyphosate content is determined by HPLC using ion-exchange chromatography with UV detection and the external standardization procedure.

Details to the HPLC system and chromatographic parameters are summarised below.

HPLC system:	Liquid chromatograph (pump) : Hewlett-Packard HP 1050 series; Automatic sampler : Hewlett-Packard HP 1050 series; Detector : Hewlett-Packard G 1315 A (HP 1050 series);
Detection wavelength:	195 nm
Column:	Whatman PARTISIL 10 SAX, 250 x 4.6 mm i.d.
Column temperature:	Room temperature
Mobile phase:	Water - methanol (96 - 4 v/v) containing about 0.84 g/L of KH_2PO, and adjusted with phosphoric acid to pH 1.9.
Flow rate:	2.3 mL/min
Injection volume:	20 µL
Retention time:	Approx. 2.7 min

Validation

Specificity:
A confirmatory of identity is not required for methods used to determine the profile of batches/formulations. The UV-wavelength chosen is specific for the analyte glyphosate. The identification was based on the selected wavelength and the retention time. Furthermore, a comparison of the UV-spectra between 196 nm and 274 nm of glyphosate in a sample and a standard solution showed a match of 99.9°%.
Chromatograms of calibration solution of glyphosate, blank solution and sample solution for MON 78043, MON 78044 and MON 2139 have been provided. No significant interference in blank formulation was observed at the retention time of interest. Therefore, the method is specific for the determination of glyphosate in MON 78043, MON 78044 and MON 2139.

Linearity:
Linearity of detector response was tested using five calibration standard concentrations in the range of 2.05 mg/mL to 5.97 mg/mL covering concentration of the analyte in analytical solutions with an appropriate range of at least ± 20 %. Linear regression was performed with $r^2 > 0.99$. The calibration standards were prepared in mobile phase (0.84 g/L of KH_2PO_4 in 4 % methanol/water adjusted to pH 1.9 with o-phosphoric acid). Therefore, the method complies with EU guideline document SANCO/3030/99 rev. 5.

Table 5.1.1-1: Linearity data for glyphosate

Analyte	Calibration range (mg/mL)	Calibration curve	Correlation coefficient (r^2)
Glyphosate	2.05 to 5.97 (n=5)	y = 215.02x - 11.67	1.0000

Accuracy
The accuracy of the method has been determined by analysing four replicates of "synthetic" MON 78043, MON 78044 and MON 2139 samples (blank sample + technical material, at nominal range), respectively.
Acceptable mean recovery values at 30 % (w/w) between 99.7 % and 100 % for glyphosate were found for MON 78043, MON 78044 and MON 2139. For the accuracy, SANCO/3030/99 rev. 5 guideline requires recovery values in the range 97 to 103 % for active substance content higher than 10 °% w/w. Therefore the method complies with EU guideline document SANCO/3030/99 rev. 5.
Results of accuracy data are summarized in the table below.

Table 5.1.1-2 Accuracy data for glyphosate in MON 78043, MON 78044 and MON 2139

Formulation	Analyte	Fortification level (% w/w)	No of replicates	Mean recovery (%)	RSD (%)
MON 78043	Glyphosate	30.53	4	100.1	0.19
MON 78044	Glyphosate	30.50	4	99.7	0.10
MON 2139	Glyphosate	30.63	4	100.1	0.10

Repeatability (precision)
The precision of the analytical method was determined by analysing five replicates for the determination of glyphosate in MON 78043, MON 78044 and MON 2139 samples. The detailed results are given in the table below.

Table 5.1.1-3 Repeatability data for glyphosate in MON 78043, MON 78044 and MON 2139

Formulation	Analyte	Mean content (% w/w)	No of replicates	RSD (%)	RSD_r (%)	Horrat value (H_r)[1]
MON 78043	Glyphosate	29.78	5	0.73	1.61	0.45
MON 78044	Glyphosate	30.43	5	0.18	1.60	0.11
MON 2139	Glyphosate	30.92	5	0.12	1.60	0.08

[1] Horrat value (Hr) = %RSD/%RSD_r (Horwitz equation %$RSD_r = 0.67 * 2^{(1-0\ 5*\log(c))}$), it is calculated for the purposes of the SANCO 3030/99 rev. 5 requirements

The determined relative standard deviation (% RSD) and the expected repeatability obtained with modified Horwitz equation (% RSD_r) for the complete analytical procedure of glyphosate in formulations fulfils the criteria given in SANCO/3030/99 rev. 5. Furthermore, the Horwitz ratio value is acceptable with $H_r \leq 1$ for glyphosate in all tested formulations, namely. MON 78043, MON 78044 and MON 2139.

Conclusion

The analytical method was successfully validated for the determination of glyphosate in MON 78043, MON 78044 and MON 2139 with respect to specificity, linearity, accuracy and precision according to EU guideline requirements as outlined in SANCO/3030/99 rev. 5.

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate in MON 78043, MON 78044 and MON 2139 was previously evaluated and accepted at EU level. It was performed under GLP and according to the requirements applicable at the time of the study (EU guideline SANCO/3030/99 rev. 4) and it also matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations to the applied test guideline were reported.

Assessment and conclusion by RMS: A comparison table of MON 52276 (representative formulation) with the 3 similar formulations of the study has been included in the Vol 4 of Bayer. Considering the slight difference in composition between MON 52276 and MON 78043, the analytical method is considered validated for the determination of glyphosate in the plant protection product.

Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

Data point:	CP 5.1.1/002
Report author	[redacted]
Report year	2012
Report title	NNG and Formaldehyde method validations in MON 52276 and MON 77973
Report No	MSL0024115
Document No	PCH-2012-0207
Guidelines followed in study	SANCO 3030/99 rev. 4
Deviations from current test guideline	None (SANCO 3030/99 rev. 4)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes
Category study in AIR 5 dossier (L docs)	Category 2a
Test facility	Monsanto Compagny, 800 N. Lindbergh Blvd, St. Louis, Missouri 63167

Determination of formaldehyde:

Principle of method PC-ME-1137-02

Samples are diluted in water and an aliquot of the solution is injected into an ion exclusion column. Formaldehyde is determined by a post-column reaction (Hantzsch reaction). The column effluent is mixed with the post-column reagent (PCR) containing ammonium acetate and acetyl acetone. Formaldehyde reacts to produce 3:5-diacetyl-1: 4-dihydrolutidine which is then determined by visible detection at a wavelength of 420 nm. The amount of formaldehyde that is in a sample or a standard is directly proportional to the amount of lutidine formed in the reaction.

Typical equipment and chromatographic conditions:

HPLC system:	Autosampler: Varian ProStar Model 410 Isocratic HPLC pumps: Varian 9002 UV/Vis detector: Varian 9050 HPLC column heater: Eppendorf CH-30 column heater
Detector wavelength:	420 nm
Column:	BioRad Fast Acid Analysis, 100 mm x 7.8 mm ID, 9 µm particle size, p/n 125-0100
Column temperature:	50-55 °C
Mixing coil temperature:	50-55 °C
Mobile phase:	HPLC grade water
Flow rate:	1.0 mL/min
PCR flow rate:	0.8 mL/min
Injection volume:	10 µL
Retention time:	Approx. 4.2 min
Typical run time:	10 min

Validation

Specificity:
The identification was based on the selected wavelength and the retention time. The post-column reaction used in this method is very similar to the method referenced in the 2016 FAO specification on glyphosate (FAO, 2016) [1]that is based on the long established Hantzsch reaction chemistry and is selective for formaldehyde (Nash, 1953)[2]. Therefore, other formulation components are not expected to affect this determination and the method is specific for the determination of formaldehyde and no additional testing for specificity was performed.

While not typically found at significant levels in glyphosate technical materials, methanol are known possible interferences. While not a chromatographic interference, methanol at concentrations greater than 1 % interfere with the post-column reaction in a way that adversely affects peak shape. Therefore it is important to ensure that any samples analysed contain less than 1 % methanol.

Linearity:
Linearity of detector response was tested using 5 calibration standard concentrations in the range of 1.883 ppm to 173.6 ppm. Linear regression was performed with correlation coefficients of > 0.99. The calibration standards were prepared in water. Furthermore, samples are diluted in the range of the calibration standards used.

Accuracy:
Two dilution levels of a spiking solution (785 ppm) were used to generate accuracy data. Triplicate injections of two separate spike solution dilutions were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 5.1.1-4 Accuracy data for formaldehyde in MON 52276

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Formaldehyde	156	6	86.3	2.9
	311	6	90.2	0.6

[1] Food and Agricultural Organization of the United Nations (FAO). 2016. FAO Specifications and Evaluations for Agricultural Pesticides, Glyphosate.
[2] The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochemical Journal, 55(3), 416–421.

Acceptable mean recovery values between 86.3 % and 90.2 % for formaldehyde were found for MON 52276. For the accuracy, SANCO/3030/99 rev. 5 guideline requires recovery values in the range 75 to 125 % for impurity content below ≥**0.01** % **(w/w) - <0.1% (w/w)**.

Repeatability (precision):

Repeatability of this method was determined through the analysis of formaldehyde in solutions. Triplicate injections of each of five separate sample preparations (11 ppm) were used to generate precision data. The data for the repeatability of formaldehyde in solutions are summarised in the table below.

Table 5.1.1-5 Repeatability data for formaldehyde in MON 52276

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	11.327	15 (5*3)	2.88	0.8

[1]Horrat value (Hr) = %RSD/%RSD_r (Horwitz equation %RSD_r = 0.67 * $2^{(1\text{-}0\ 5*\log(c))}$)

Horwitz ratio value calculated using the relative standard deviation of 2.88 % is acceptable with $H_r \leq 1$.

Sensitivity (Limit of Quantitation)

The LOQ is defined as the lowest concentration tested, at which an acceptable recovery and an acceptable precision (repeatability), is obtained. . The LOQ is 156 mg/kg

Derivatisation

Concerning derivatisation step, as it is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Moreover, both post-column derivatisation steps are well described in FAO 2016 methods and are currently used for most glyphosate technical materials. Therefore, a full validation of the derivatization step is not considered necessary.

Conclusion

The analytical method was successfully validated for the determination of formaldehyde in formulated product MON 52276 with respect to specificity, linearity, accuracy and precision. However, the precision has been addressed only with the analysis of standard solutions. An analysis using a fortified sample should have been performed. This is considered as a data gap.

Determination of *N*-Nitrosoglyphosate (NNG):

Principle of method PC-ME-0766-02

The solution are injected into a HPLC system equipped with an cation exchange clean-up column, post column reactor, and UV detection at 550 nm. The analytical column separates the components of interest which are reacted post column with a solution of N-(1-naphthyl)ethylenediamine/HBr and sulfanilamide reagents. The azo dye formed is detected using a colorimeter set at 550 nm and quantitated by external standards using peak area.

Typical equipment and chromatographic conditions:

HPLC system:	Autosampler: Varian Marathon Pumps: Varian Model 2010, Spectra Physics Model SP8800, Technicon Pump m Colorimeter: Technicon Auto Analyzer II S.c. Colorimeter
Detector wavelength:	550 nm
Column:	7 micron, 4.6 mm ID x 25 cm: AX-300 Aquapore, Brownlee Part No. 0712-0040
Guard column:	7 micron, 3.2 mm ID x 15 mm, AX-300 anion: Brownlee New Guard Part No. 0711-0102
Mobile phase:	50 g of KH_2P0_4 in 3600 mL of HPLC grade water and 400 mL methanol stirred and adjusted to pH 2.3 with 85 °% H_3P0_4
Reactor temperature:	94 °C
Flow rate:	1.8 mL/min
Injection volume:	500 µL
Retention time:	Approx. 23 min

Validation

Specificity:
The identification was based on the selected wavelength and the retention time. Furthermore, no interferences were observed in MON 52276. The post-column reaction used in this method is very similar to the method referenced in the 2016 FAO specification on glyphosate (FAO, 2016) which is selective for nitroso containing compounds so that other formulation components are not expected to affect this determination. Therefore, the method is specific for the determination of NNG and no additional testing for specificity was performed.

Linearity
Linearity of detector response was tested using 6 calibration standard concentrations in the range of 0.00708 ppm to 0.1173 ppm. Linear regression was performed with correlation coefficients of > 0.99. The calibration standards were prepared in pH 7 0.1 M K_2HPO_4 buffer.

Accuracy
Two dilution levels of a spiking solution (5.008 ppm) were used to generate accuracy data. Triplicate injections of at least three separate sample preparations were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 5.1.1-6 Accuracy data for *N*-Nitrosoglyphosate (NNG) in MON 52276

Analyte	Fortification level (mg/kg)	No of replicates	Overall mean recovery (%)
N-Nitrosoglyphosate (NNG)	0.4	15 (3*5)	94.7
	0.6	9 (3*3)	

Acceptable mean recovery values at **< 0.01** °% **(w/w)** for *N*-Nitrosoglyphosate (NNG) were found for MON 52276. For the accuracy, SANCO/3030/99 rev. 5 guideline requires recovery values in the range 70 to 130 % for impurity content below **< 0.01** °% **(w/w)**. Therefore, the method complies with EU guideline document SANCO/3030/99 **rev. 5.**

Repeatability (precision)
Repeatability of this method was determined through the analysis of *N*-Nitrosoglyphosate (NNG) solutions. Triplicate injections of each of five separate sample preparations (low level spike; 0.4 ppm) were used to generate

precision data. The data for the repeatability of *N*-Nitrosoglyphosate (NNG) in MON 52276 are summarised in the table below.

Table 5.1.1-7: Repeatability data for *N*-Nitrosoglyphosate (NNG) in MON 52276

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
N-Nitrosoglyphosate (NNG)	0.427	15 (3*5)	1.54	0.25

[1]Horrat value (Hr) = %RSD/%RSD_r (Horwitz equation %RSD_r = 0.67 * $2^{(1-0\ 5*\log(c))}$)

Horwitz ratio value calculated using the relative standard deviation of 1.54 % is acceptable with $H_r \leq 1$.

Sensitivity (Limit of Quantitation)

The limit of quantitation was determined through the analysis of the low spike sample. Triplicate injections of each of five separate sample preparations support an LOQ of 0.4 ppm.

Derivatisation

Concerning derivatisation step, as it is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Moreover, both post-column derivatisation steps are well described in FAO 2016 methods and are currently used for most glyphosate technical materials. Therefore, a full validation of the derivatization step is not considered necessary.

Conclusion

The analytical method was successfully validated for the determination of *N*-Nitrosoglyphosate (NNG) in MON 52276 with respect to specificity, linearity, accuracy and precision. However, the precision has been addressed only with the analysis of standard solutions. An analysis using a fortified sample should have been performed. This is considered as a data gap.

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of formaldehyde and NNG in formulated product MON 52276 was previously evaluated at EU level. It was performed under GLP. The methods are fit-for-purpose to analyse formaldehyde and NNG in MON 52276.

Assessment and conclusion by RMS: Analytical methods for the determination of formaldehyde and NNG in MON 52276 are considered partially validated at LOQ of 11ppm and 4ppm respectively.

As the analytical methods for determination of two relevant impurities, formaldehyde and *N*-Nitrosoglyphosate (NNG) were not validated according to the current guidance SANCO/3030/99 rev. 5, new method validations have been performed in below studies to meet the validation criteria. Furthermore, with regard to maximum allowable limit for the relevant impurity *N*-Nitrosoglyphosate (NNG) in glyphosate technical material and glyphosate based formulated product, a position paper is submitted as CP 5.1.1-005. For more details, please refer to the respective report.

Data point	CP 5.1.1/003
Report author	[redacted]
Report year	2019
Report title	*N*-Nitrosoglyphosate method validation in MON 52276, MON 76610, MON 79351, MON 79545 and MON 79991
Report No	MSL0028536
Document No	PCH-2017-0062
Guidelines followed in study	SANCO 3030/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company, 800 N. Lindbergh Blvd. St. Louis, Missouri 63167

Principle of method ME-2070-01

The formulation MON 52276 is diluted in distilled water and passed through a 0.45 µm PTFE filter. The solution is injected into a HPLC system equipped with an ion exchange column, post column reactor, and UV detection at 550 nm. The analytical column separates the components of interest which are reacted post column with a solution of N-(1-naphthyl)ethylenediamine/HBr and sulfanilamide reagents. The azo dye formed is detected using a colorimeter set at 550 nm and quantitated by external standards using peak area.

Typical equipment and chromatographic conditions:

HPLC system:	Autosampler: Shimadzu SIL-20A Isocratic HPLC pumps: Shimadzu LC-20AT Post column reaction solution pump: LC-10AI HPLC controller: Shimadzu SCL-10AVP System Controller UV/Vis detector: Shimadzu SPD-20A with PEEK flow cell HPLC column heater: Brinkmann CH-430 column heater
Detector wavelength:	550 nm
Column:	7 micron, 4.6 mm ID x 25 cm: AX-300 Aquapore, Brownlee Part No. 0712-0040
Guard column:	7 micron, 3.2 mm ID x 15 mm, AX-300 anion: Brownlee New Guard Part No. 0711-0102
Reactor temperature:	97 °C
Mobile phase:	40.00 g potassium phosphate monobasic, 800 mL methanol, and 36.00 g of 85 °% phosphoric acid in 3 liters of distilled water
Flow rate:	1 mL/min
PCR flow rate:	0.5 mL/min
Injection volume:	900 µL
Retention time:	Approx. 24 min
Typical run time:	35 min

Validation

Specificity:
The identification was based on the selected wavelength and the retention time. Chromatograms of a NNG standard, a formulation sample and a spike sample were provided. No interferences were observed in MON 52276 with NNG peak. The post-column reaction used in this method is very similar to the method referenced in the 2016 FAO specification on glyphosate (FAO, 2016) which is selective for nitroso containing compounds so that other formulation components are not expected to affect this determination. Therefore, the method is specific for the determination of NNG and no additional testing for specificity was performed.

Linearity:
Linearity of detector response was tested using five calibration standard concentrations in the range of 0.0216 ppm to 0.2462 ppm. Linear regression was performed with correlation coefficients of > 0.99. The calibration standards were prepared in water.

Table 5.1.1-8: Linearity data for *N*-Nitrosoglyphosate (NNG)

Analyte	Calibration range (mg/kg)	Calibration curve	Correlation coefficient (r)
N-Nitrosoglyphosate (NNG)	0.0216 to 0.2462 (n=5)	y = 2736.1491x–13.3843	0.9990

Accuracy:
Standard additions at two concentration levels were used to generate accuracy data. Duplicate injections of at least three separate sample preparations were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 5.1.1-9: Accuracy data for *N*-Nitrosoglyphosate (NNG)

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery[1] (%)	RSD (%)
N-Nitrosoglyphosate (NNG)	0.36	10	98.5	6.5
	0.73	6	96.3	5.6

[1] Based on marginal recovery

Acceptable mean recovery values at **< 0.01** % **(w/w)** between 96.3 % and 98.5 % for *N*-Nitrosoglyphosate (NNG) were found for MON 52276. For the accuracy, SANCO/3030/99 rev. 5 guideline requires recovery values in the range 70 to 130 % for impurity content below **< 0.01** % **(w/w)**. Therefore, the method complies with EU guideline document SANCO/3030/99 **rev. 5.**

Repeatability (precision):
Repeatability of this method was determined through the analysis of MON 52276. Duplicate injections of each of five separate sample preparations (spiked sample at low level spike; 0.36 ppm) were used to generate precision data. The data for the repeatability of *N*-Nitrosoglyphosate (NNG) in MON 52276 are summarised in the table below.

Table 5.1.1-10: Repeatability data for *N*-Nitrosoglyphosate (NNG) in MON 52276

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
N-Nitrosoglyphosate (NNG)	0.52	10	6.46	0.55[2]

[1]Horrat value (Hr) = %RSD/%RSD$_r$ (Horwitz equation %RSD$_r$ = 0.67 * $2^{(1\text{-}0\ 5*\log(c))}$)
[2]Since NNG was present at low levels in the product, the precision data at low fortification level in accuracy tests are included

Horwitz ratio value calculated using the relative standard deviation of 6.46 % is acceptable with $H_r \leq 1$.

Sensitivity (Limit of Quantitation)

The limit of quantitation defined as the lowest level at which acceptable accuracy and precision is obtained was determined through the analysis of the low spike (0.36 ppm) sample. Duplicate injections of each of five separate sample preparations support an LOQ of 0.52 ppm.

Derivatisation

Concerning derivatisation step, as it is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Moreover, both post-column derivatisation steps are well described in FAO 2016 methods and are currently used for most glyphosate technical materials. Therefore, a full validation of the derivatization step is not considered necessary.

Conclusion

The analytical method was successfully validated for the determination of *N*-Nitrosoglyphosate (NNG) in MON 52276 with respect to sensitivity, specificity, linearity, accuracy and precision according to EU guideline requirements as outlined in SANCO/3030/99 **rev.5**.

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of N-Nitrosoglyphosate (NNG) in MON 52276 was not previously evaluated at EU level. It was performed under GLP and according to recent requirements (EU guideline SANCO/3030/99 **rev.5)**. No deviations to the applied test guideline were reported.

Assessment and conclusion by RMS:
The analytical method is considered validated according to SANCO/3030/99 rev.5 for the determination of NNG in MON 52276 with a LOQ of 0.52 mg/kg.

Data point:	CP 5.1.1/004
Report author	[redacted]
Report year	2020
Report title	Validation of the analytical method for the analysis of *N*-nitrosoglyphosate (NNG) in MON 52276
Report No	TRR0000189
Document No	PCH-2020-0230
Guidelines followed in study	SANCO 3030/99 rev. 5
Deviations from current test guideline	None, (SANCO/3030/99 **rev.5)**
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company Product and Process Analytical Chemistry 800 N. Lindbergh Blvd. St. Louis, Missouri 63167

Principle of method ME-2070

The sample is diluted in distilled water and passed through a 0.45µm PTFE filter. The solution is injected into a HPLC system equipped with an ion exchange column, post column reactor, and UV detection at 550 nm. The analytical column separates the components of interest which are reacted post column with a solution of N-(1-naphthyl)ethylenediamine/HBr and sulfanilamide reagents. The azo dye formed is detected using a colorimeter set at 550 nm and quantitated by external standards using peak area.

Typical equipment and chromatographic conditions:

HPLC system:	Autosampler: Shimadzu SIL-20A HPLC pumps: Shimadzu LC-20AT HPLC Pump UV/Vis detector: Shimadzu SPD-20A HPLC column heater: Brinkmann CH-430column heater
Detector wavelength:	550 nm
Column:	Brinkmann CH-430, 250 mm x 4.6 mm ID, 7 µm particle size, Guard column: 15 mm x 3.2 mm ID, 7 µm particle size,
Reactor temperature:	97 °C
Mobile phase:	Adding 40.00 g potassium phosphate monobasic, 800 mL methanol, and 36.00 g of 85 % phosphoric acid to 3 liters of distilled water. Additional distilled water was added to bring the total volume to 4 liters
Flow rate:	1.0 mL/min
PCR flow rate:	0.5 mL/min
Injection volume:	900 µL
Retention time:	Approx. 24 min
Typical run time:	35 min

Validation

Specificity:

The identification was based on the selected wavelength and the retention time. Chromatograms of a NNG standard, a formulation sample and a spike samples (low and high level) were provided. No interferences were observed in MON 52276 with NNG peak. The post-column reaction used in this method is very similar to the method referenced in the 2016 FAO specification on glyphosate (FAO, 2016) which is selective for nitroso containing compounds so that other formulation components are not expected to affect this determination. Therefore, the method is specific for the determination of NNG and no additional testing for specificity was performed.

Linearity:

Linearity of detector response was tested using 5 calibration standard concentrations in the range of 0.0206 ppm to 0.2431 ppm. Linear regression was performed with correlation coefficients of > 0.99. The calibration standards were prepared in water. Furthermore, samples are diluted in the range of the calibration standards used. Therefore, the method complies with EU guideline document SANCO/3030/99 rev. 5.

Table 5.1.1-11: Linearity data for *N*-Nitrosoglyphosate (NNG)

Analyte	Calibration range (mg/kg)	Calibration curve	Correlation coefficient (r)
N-Nitrosoglyphosate (NNG)	0.0206 to 0.2431 (n=5)	y = 3065.9967x -16.6967	0.9992

Accuracy:
Standard additions at two concentration levels were used to generate accuracy data. Duplicate injections of at least three separate sample preparations were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 5.1.1-12: Accuracy data for *N*-Nitrosoglyphosate (NNG) in MON 52276

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
N-Nitrosoglyphosate (NNG)	0.202	12	88.2	5.5
	1.217	6	84.7	6.0

Acceptable mean recovery values at **< 0.01** % **(w/w)** between 84.7 % and 88.2 % for *N*-Nitrosoglyphosate (NNG) were found for MON 52276. For the accuracy, SANCO/3030/99 rev. 5 guideline requires recovery values in the range 70 to 130 % for impurity content below **< 0.01** % **(w/w)**. Therefore, the method complies with EU guideline document SANCO/3030/99 **rev.5.**

Repeatability (precision):
Repeatability of this method was determined through the analysis of MON 52276. Duplicate injections of each of six separate sample preparations of MON 52276 (residue in unspiked sample) and spiked sample (low level spike; 0.2 ppm) were used to generate precision data. The data for the repeatability of *N*-Nitrosoglyphosate (NNG) in MON 52276 are summarised in the table below.

Table 5.1.1-13: Repeatability data for *N*-Nitrosoglyphosate (NNG) in MON 52276

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
N-Nitrosoglyphosate (NNG)	0.095	12	5.8	0.38
	0.202	12	5.5	0.40

[1]Horrat value (Hr) = %RSD/%RSD$_r$ (Horwitz equation %RSD$_r$ = 0.67 * $2^{(1\text{-}0\ 5*\log(c))}$)

Horwitz ratio value calculated using the relative standard deviation of 5.8 % and 5.5 % is acceptable with $H_r \leq 1$.

Sensitivity (Limit of Quantitation)

The limit of quantitation defined as the lowest level at which acceptable accuracy and precision is obtained was determined through the analysis of the low spike (70 ppm) sample. Duplicate injections of each of six separate sample preparations support an LOQ of 0.202 ppm.

Derivatisation

Concerning derivatisation step, as it is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Moreover, both post-column derivatisation steps are well described in FAO 2016 methods and are currently used for most glyphosate technical materials. Therefore, a full validation of the derivatization step is not considered necessary.

Conclusion

The analytical method was successfully validated for the determination of *N*-Nitrosoglyphosate (NNG) in MON 52276 with respect to specificity, linearity, accuracy and precision according to EU guideline requirements as outlined in SANCO/3030/99 **rev. 5**.

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of *N*-Nitrosoglyphosate (NNG) in MON 52276 was not previously evaluated at EU level. It was performed under GLP and according to recent requirements (EU guideline SANCO/3030/99 **rev.5)**. No deviations to the applied test guideline were reported.

Assessment and conclusion by RMS: The analytical method is considered partially validated according to SANCO/3030/99 rev.5 for the determination of NNG in MON 52276 with a LOQ of 0.202 mg/kg.

Data point	CP 5.1.1/005
Report author	[redacted]
Report year	2020
Report title	Position Paper supporting the Approval Renewal Dossier for an Active Substance: Glyphosate & the IPA-, K-, DMA and NH4-salts of Glyphosate (hereafter Glyphosate)
Report No	Not allocated
Document No	-
Guidelines followed in study	Not applicable (position paper)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (Position paper)
Acceptability/Reliability	Not relevant
Category study in AIR 5 dossier (L docs)	Category 1

Full summary of the study

For MON 52276, based on permissible limits for nitrites in water sources used in the production process (as well as other glyphosate-based SL products), *N*-Nitrosoglyphosate can form during the production process and result in levels greater than the RMS proposed (proportional) Maximum Allowable Limit of 0.324 mg/kg. The FAO concluded that an absolute maximum allowable limit of 1 mg/kg (ppm) *N*-Nitrosoglyphosate is sufficient to protect human health and the environment, the EU Commission made the same conclusion in its 2012 guidance document. The FAO also concluded the absolute 1 mg/kg Maximum Allowable Limit is appropriate based on the propensity for *N*-Nitrosoglyphosate to form during the formulation process and storage due to the presence of nitrites and nitrating agents in formulation water but also in air and co-formulants. As such, no scientific basis exists for establishing a more restrictive specification for *N*-Nitrosoglyphosate per the EU Council mandate in EU Regulation 1107/2009 for placing plant protection products on the market. Bayer production sites have established nitrosamine quality specifications and processes that ensure the level of NNG in finished product remain below the 1 mg/kg Maximum Allowable Limit.

It is requested that the FAO Specification maximum allowable limit of 1 mg/kg for *N*-Nitrosoglyphosate is adopted across all glyphosate formulated products.

Assessment and conclusion

Assessment and conclusion by applicant:
It is requested that the FAO Specification maximum allowable limit of 1 mg/kg for *N*-Nitrosoglyphosate is adopted across all glyphosate formulated products.

Assessment and conclusion by RMS:
The nitrites present in water used for the formulation of glyphosate products lead to the formation of the NNG during the formulation and the storage of glyphosate products. The quality of water used for the formulation can be managed by applicants in order to have a content of NNG relevant impurity in the final product as low as possible.
For the representative product of the renewal of glyphosate, RMS considers that the specification limit used to assess the storage stability (FAO or EU) is not an issue here as the contents of NNG before and after storage in the product are below the EU specification which is the most conservative.
Concerning other glyphosate products, RMS considers that the specification limit of NNG in products should be set on a case by case basis, taking into account the formulation process and the water quality used

An issue was raised during the assessment on the maximum content of impurities in the formulation: whether the content should be recalculated using the actual content of AS in the product, or if the maximum content of impurities should be set in line with the FAO specification.
This is a general point of discussion and is not specifically related to the evaluation of glyphosate. It was concluded that this point needs to be agreed on at EU level as a general matter between physchem experts. In the framework of the renewal of the approval of glyphosate, this does not lead to an open point for the representative formulation, as the content of NNG and formaldehyde were below both maximum levels.
Therefore, no a final decision was made for this issue.

Data point	CP 5.1.1/006
Report author	[redacted]
Report year	2020
Report title	Validation of the Analytical Method ME-1137 for Formaldehyde in MON52276
Report No	TRR0000082
Document No	PCH-2019-0599
Guidelines followed in study	SANCO 3030/99 rev. 5
Deviations from current test guideline	None, (SANCO/3030/99 **rev.5)**
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company, 800 N. Lindbergh Blvd. St. Louis, Missouri 63167

Principle of method ME-1137

The formulation MON 52276 is diluted in water and an aliquot of the solution is injected into an ion exclusion column. Formaldehyde is determined by a post-column reaction (Hantzsch reaction). The column effluent is mixed with the post-column reagent (PCR) containing ammonium acetate and acetyl acetone. Formaldehyde reacts to produce 3:5-diacetyl-1: 4-dihydrolutidine, which is then determined by visible detection at a wavelength of 420 nm. The amount of formaldehyde that is in a sample or a standard is directly proportional to the amount of lutidine formed in the reaction.

Typical equipment and chromatographic conditions:

HPLC system:	Autosampler: Shimadzu SIL-20A Isocratic HPLC pumps: Shimadzu LC-20AT UV/Vis detector: Shimadzu SPD-20A HPLC column heater: Eppendorf CH-30 column heater
Detector wavelength:	420 nm
Column:	BioRad Fast Acid Analysis, 100 mm x 7.8 mm ID, 9 µm particle size, p/n 125-0100
Column temperature:	50-55 °C
Mixing coil temperature:	50-55 °C
Mobile phase:	HPLC grade water
Flow rate:	1.0 mL/min
PCR flow rate:	0.8 mL/min
Injection volume:	10 µL
Retention time:	Approx. 5.4 min
Typical run time:	10 min

Validation

Specificity:

The identification was based on the selected wavelength and the retention time. Chromatograms of a formaldehyde standard, a formulation sample and spike samples were provided.

The post-column reaction used in this method is very similar to the method referenced in the 2016 FAO specification on glyphosate (FAO, 2016)[1] that is based on the long established Hantzsch reaction chemistry and is selective for formaldehyde (Nash, 1953)[2]. Therefore, other formulation components are not expected to affect this determination and the method is specific for the determination of formaldehyde and no additional testing for specificity was performed.

Linearity:

Linearity of detector response was tested using 7 calibration standard concentrations in the range of 0.6558 ppm to 122.8 ppm. Linear regression was performed with correlation coefficients of > 0.99. The calibration standards were prepared in water. Furthermore, samples are diluted in the range of the calibration standards used. Therefore, the method complies with EU guideline document SANCO/3030/99 rev. 5.

Table 5.1.1-14: Linearity data for formaldehyde

Analyte	Calibration range (mg/kg)	Calibration curve	Correlation coefficient (r)
Formaldehyde	0.6558 to 122.8 (n=7)	y = 366.2048x – 6.3005	1.0000

Accuracy:
Standard additions at three levels were used to generate accuracy data. Triplicate injections of at least three separate sample preparations were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 5.1.1-15: Accuracy data for formaldehyde in MON 52276

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Formaldehyde	70	18 (6*3)	101.38	0.091
	164	9 (3*3)	99.76	0.117
	400	9 (3*3)	99.20	0.068

Acceptable mean recovery values at > **0.01** % **(w/w)** between 99.2 % and 101 % for formaldehyde were found for MON 52276. For the accuracy, SANCO/3030/99 rev. 5 guideline requires recovery values in the range 75 to 125 % for impurity content below > **0.01** °% **(w/w)**. Therefore, the method complies with EU guideline document SANCO/3030/99 **rev.5.**

Repeatability (precision):
Repeatability of this method was determined through the analysis of MON 52276. Triplicate injections of each of six separate sample preparations (spike sample at low level spike; 70 ppm) were used to generate precision data. The data for the repeatability of formaldehyde in MON 52276 are summarised in the table below.

Table 5.1.1-16: Repeatability data for formaldehyde in MON 52276

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	70	18	0.091	0.016[2]

[1]Horrat value (Hr) = %RSD/%RSD_r (Horwitz equation %$RSD_r = 0.67 * 2^{(1-0\ 5*\log(c))}$)
[2]Since formaldehyde was not detected in the product, the precision data at low fortification level in accuracy tests are included

Horwitz ratio value calculated using the relative standard deviation of 0.091 % is acceptable with $H_r \leq 1$.

Sensitivity (Limit of Quantitation)

The limit of quantitation defined as the lowest level at which acceptable accuracy and precision is obtained was determined through the analysis of the low spike (70 ppm) sample. Triplicate injections of each of six separate sample preparations support an LOQ of 70 ppm.

Derivatisation

Concerning derivatisation step, as it is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Moreover, both post-column derivatisation steps are well described in FAO 2016 methods and are currently used for most glyphosate technical materials. Therefore, a full validation of the derivatization step is not considered necessary.

Conclusion

The analytical method was successfully validated for the determination of formaldehyde in MON 52276 with respect to sensitivity, specificity, linearity, accuracy and precision according to EU guideline requirements as outlined in SANCO/3030/99 **rev. 5**.

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of formaldehyde in MON 52276 was not previously evaluated at EU level. It was performed under GLP and according to recent requirements (EU guideline SANCO/3030/99 **rev. 5)**. No deviations to the applied test guideline were reported.

Assessment and conclusion by RMS: The analytical method is considered validated according to SANCO/3030/99 rev.5 for the determination of formaldehyde in MON 52276 with a LOQ of 70 mg/kg.

Two new relevant impurities (formic acid and trimethylamine) have been identified following the assessment of the renewal of active substance. The revised EU reference specifications for these impurities are:
Formic acid < 4 g/kg
Triethylamine < 2 g/kg

No methods for the determination of the 2 new impurities have been provided. This is considered as a data gap.

Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

MON 52276 does not contain any relevant co-formulants or components of co-formulants.

B.5.1.2. Methods for the determination of residues

Overview Table for Analytical Methods Used for Determination of Residues

Annex point Reference within Assessment Report	Author, date	Study title	Analytical method Author, date, No.	Technique, LOQ of the method, validated working range	Method meets analytical validation criteria	Remarks (in case validation criteria are not met)	Acceptability of the method
CP 5.1.2/002 (CP 10.2.1/001) (CP 5.1.2/001)	[redacted] 1992 Report No. J9108002b (TO-91-296)	MON 52276: Acute toxicity to rainbow trout, *Oncorhynchus mykiss*, under flow-through test conditions	N/A [redacted] [redacted] 1992 Report No. J9107004b (TO-91-320) [redacted], 1992 Report No. J9108002b (TO-91-296)	HPLC-UV LOQ 1.0 µg/mL 3.7 - 750 mg/L HPLC-UV LOQ 1.0 µg/mL 130 - 1000 mg/L	Yes	Method fit-for-purpose	Y

Overview Table for Analytical Methods Used for Determination of Residues

Annex point Reference within Assessment Report	Author, date	Study title	Analytical method Author, date, No.	Technique, LOQ of the method, validated working range	Method meets analytical validation criteria	Remarks (in case validation criteria are not met)	Acceptability of the method
CP 5.1.2/003 (CP 10.2.1/002) (CP 5.1.2/001)	[redacted] 1992 Report No. J9108002c (TO-91-298)	MON 52276: Acute toxicity to the common carp, *Cyprinus carpio*, under flow-through test conditions	N/A [redacted] [redacted] 1992 Report No. J9107004b (TO-91-320) [redacted] 1992 Report No. J9108002c (TO-91-298)	HPLC-UV LOQ 1.0 µg/mL 3.7-750 mg/L HPLC-UV LOQ 1.0 µg/mL 130 - 1000 mg/L	Yes	Method fit-for-purpose	Y
CP 5.1.2/004 (CP 10.2.1/003) (CP 5.1.2/001)	[redacted] 1992 Report No. J9108002a (TO-91-295)	MON 52276: Acute toxicity to the water flea, *Daphnia magna*, under flow-through test conditions	N/A [redacted] [redacted] 1992 Report No. J9107004b (TO-91-320) [redacted] 1992 Report No. J9108002a (TO-91-295)	HPLC-UV LOQ 1.0 µg/mL 3.7-750 mg/L HPLC-UV LOQ 1.0 µg/mL 130- 1000 mg/L	Yes	Method fit-for-purpose	Y
CP 5.1.2/005 (CP 10.2.1/005)	[redacted] 2002 Report No. 20021186/01-AALg	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed *Lemna gibba*	N/A [redacted] 2002 Report No. 20021186/01-AALg	HPLC-UV LOQ 0.0309 mg/L 0.0309-78.8 mg/L	Yes	Method validated	Y
CP 5.1.2/006 (CP 10.2.1/006)	[redacted] 2012 Report No. CHE-016/4-80/A	Effect of MON52276 (glyphosate formulation) on the growth of *Myriophyllum aquaticum* in the presence of sediment, with a subsequent recovery period	N/A [redacted] 2012 Report No. CHE-016/4-80/A	LC-MS/MS LOQ 0.25 mg/L 0.25-2.5 mg/L	Yes	Method validated	Y

Overview Table for Analytical Methods Used for Determination of Residues

Annex point Reference within Assessment Report	Author, date	Study title	Analytical method Author, date, No.	Technique, LOQ of the method, validated working range	Method meets analytical validation criteria	Remarks (in case validation criteria are not met)	Acceptability of the method
CP 5.1.2/007 (CP 10.3.1.5/ 001)	[redacted] 2011 Report No. V7YH1002	Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions	N/A [redacted] 2011 Report No. V7YH1002	LC-MS/MS LOQ 1 mg/kg between 1-200 mg/kg and 1-700 mg/kg, depending on the matrix	Yes	Method cannot be assessed as the analytical phase report is not available	N
CP 5.1.2/008 (CP 10.6.2/001)	[redacted] 2019 Report No. S19-03634	Effects on the seedling emergence and growth of ten non-target terrestrial plant species under greenhouse conditions	N/A [redacted] 2019 Report No. S19-03634	LC-MS/MS LOQ 0.01 g/L 0.01- 20.1 mg/ L	Yes	Method validated	Y
CP 5.1.2/009 (CP 10.6.2/002)	[redacted] 2013 Report No. 80477	MON 52276: Effects on the vegetative vigor of non-target terrestrial plants (Tier II)	N/A [redacted] 2013 Report No. 80477	LC-MS/MS LOQ 0.02 mg/mL 0.1 - 14.5 mg/L	Yes, with deficits	Method fit-for-purpose	Y

B.5.1.2.1. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Study previously submitted to the EU

Determination of glyphosate in test medium (reconstituted water)

1. Information on the study

Data point	CP 5.1.2/001
Report authors	[redacted]
Report year	1992
Report title	Validation of method to determine the concentration of MON 52276 in freshwater by liquid chromatography

Report No	J9107004b
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Toxikon Environmental Sciences, Florida

Data point	CP 5.1.2/002 (CP 10.2.1/001)
Report authors	[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	-
Guidelines followed in study	US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Data point	CP 5.1.2/003 (CP 10.2.1/002)
Report authors	[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	-
Guidelines followed in study	OECD guideline 203
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Data point	CP 5.1.2/004 (CP 10.2.1/003)
Report authors	[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	-
Guidelines followed in study	US EPA FIFRA 72-2 (1982), OECD 202 (1984), and EEC Method C.2 (1992).
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Toxikon Environmental Sciences, Florida

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate acid in freshwater by HPLC-UVD. The analysis of MON 52276 involved the derivatization of each water sample with NBD-C1 (4-chloro-7-nitrobenzeno-2-oxa-1, 3-diazole) reagent. The NBD-C1 reagent forms a chromophoric and fluorescent product with amines such as glyphosate. Quantitation of MON 52276 was performed by liquid chromatography using a UV/VIS detector and the external standard technique.

Chromatographic conditions:

HPLC system:	Shimadzu LC-600
HPLC detector:	Perkin-Elmer LC-75 UV/VIS detector (500 nm)
HPLC column:	Zorbax ODS, 150 mm x 4.6mm
Column oven temperature:	Room temperature
Mobile phase:	95% 10 mM phosphate, pH 3.60/5% ACN
Flow rate:	2.0 mL/min
Derivatisation agent (pre-column):	NBD-C1 (4-chloro-7-nitrobenzeno-2-oxa-1, 3-diazole)
Detection:	500 nm
Retention time:	Glyphosate-NBD: ~ 1.2 min

Findings
Recoveries
The method proved to be suitable to determine residues of glyphosate in freshwater. Samples were spiked with the analyte at two fortification levels of 3.7 mg/L and 750 mg/L. All average recovery values (mean of three replicates per fortification level) were between 70 % and 120 %. The detailed results are given in the table below.

Table 5.1.2-1: Results of method validation (spike recovery) for the determination of glyphosate in freshwater

Report No.	Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
J9107004b	Freshwater	Glyphosate	3.7	110 – 120	116	5.1	4.4	3
			750	95 – 97	96	1.0	1.0	3
			Overall	95 – 120	106	11	11	6

[1] Recovery values are not corrected for interference with matrix compounds/respective control samples. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Additionally duplicate samples of water samples with concentrations ranging from 130 mg/L to 1000 mg/L measured at zero-time were analysed using the analytical method. The results of these analyses are provided in the table below. These are not true validation recovery data; however, the results show the good performance of the method.

Table 5.1.2-2: Results of test water analysis

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
J9108002b	Water	Glyphosate	130	91.5 – 95.4	93.5	–	–	2
			216	93.5 – 113	103	–	–	2
			360	99.2 – 102	101	–	–	2
			600	97.3 – 99.8	98.6	–	–	2
			1000	99.4 – 103	101	–	–	2
			Overall	91.5 – 113	99.4	6.0	6.0	10
J9108002c	Water	Glyphosate	130	85.4 – 86.2	85.8	–	–	2
			216	79.2 – 109	94.0	–	–	2
			360	103 – 110	106	–	–	2
			600	95.0 – 103	99.1	–	–	2
			1000	102 – 105	103	–	–	2
			Overall	79.2 – 110	97.7	11	11	10

Table 5.1.2-2: Results of test water analysis

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
J9108002a	Water	Glyphosate	130	93.8 – 107	100	–	–	2
			216	100 – 106	103	–	–	2
			360	103 – 104	103	–	–	2
			600	98.8 – 102	100	–	–	2
			1000	96.1 – 96.9	97	–	–	2
			Overall	93.8 – 107	101	4.3	4.2	10

[1] Recovery values are not corrected for interference with matrix compounds/respective control samples. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity
The UV-wavelength chosen is specific for the analyte glyphosate. The identification was based on the selected wavelength and the retention time.

J9107004b: Chromatograms of standards solution, of blank and fortified sample are provided. No significant interferences were observed at the retention time of interest in the shown chromatograms of control samples.
J9108002b: No chromatograms were provided
J9108002c : No chromatograms were provided
J9108002a : No chromatograms were provided

Linearity
Linearity of detector response was tested using six calibration standard concentrations in the range of 2.5 µg/mL to 50 µg/mL with correlation coefficients of > 0.99. The calibration standards were prepared in mobile phase. Details to the calibration are provided below.

Table 5.1.2-3: Linearity parameters

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
Glyphosate	Linear	2.5 – 50	6	y = 429.16 x –357.68	0.99902

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level (where applicable) were < 20 %. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantitation (LOQ) in the study was determined at 1.0 µg/mL for glyphosate based on measurement of noise level. The limit of detection (LOD) was 0.3 µg/mL.

Matrix effects
Not assessed.

Stability of analyte in samples
Not assessed. However it was shown that glyphosate was stable over the whole test period of up to 96 hours in the studies J9108002b, J9108002c and J9108002a.

Conclusion
The analytical method was successfully validated for the determination of glyphosate in freshwater. The analytical method fulfils the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level (with exception of the analytical method validation report). It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (matrix effect not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is suitable to support the ecotoxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4 in several points: no chromatogram for several studies
The whole recovery data are in acceptable range. The linearity is acceptable. Concerning the derivatisation step, it can be considered as demonstrated as the calibration has been performed with standard solution prepared in the same manner as the test item
The method can be considered as fit for purpose for the determination of glyphosate in the fresh water.

Study previously submitted to the EU

Determination of glyphosate acid in test medium (reconstituted water)

1. Information on the study

Data point	CP 5.1.2/005 (CP 10.2.1/005)
Report authors	[redacted]
Report year	2002
Report title	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed *Lemna gibba*
Report No	20021186/01-AALg
Document No	-
Guidelines followed in study	SANCO/825/00 rev.6 (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effect not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Urnweltanalytik GmbH

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate acid in test medium (reconstituted water) by HPLC-fluorescence. Aqueous samples (2 mL) were derivatised by adding 0.2 mL borate buffer (pH 9) and 0.5 mL FMOC-CL in acetonitrile (10 g/L), vials closed with PTFE screw caps, mixed and incubated for 2 h at ambient temperature. Finally, 1 mL of toluene was added, the vials were closed again with PTFE screw caps and mixed. After phase separation, the samples were injected from the lower aqueous phase into HPLC system.

Chromatographic conditions:

HPLC system:	HPLC (Waters 5 10 solvent delivery system) equipped with fluorescence detector (Shimadzu RF-535)
HPLC column:	Spherisorb SAX, 220 × 4.0 mm id, 5 µm particvle size
Column oven temperature:	40 °C
Mobile phase:	Acetonitrile/deionised water/acetic acid/phosphoric acid (200/800/3/0.75, v/v/v/v)
Flow rate:	2.0 mL/min
Injection volume:	4 µL – 20 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Detection:	Excitation wavelength: 254 nm Emission wavelength: 310 nm
Retention time:	AMPA-FMOC: ~ 8 min Glyphosate-FMOC: ~ 13 min

Findings
Recoveries
For method validation, samples of test water were spiked with the analyte at four fortification levels, i.e. at 0.1, 1 10 and 255 mg test item/L, corresponding to 0.0309, 0.309, 3.09 and 78.8 mg glyphosate acid/L. The fortified samples were then submitted to derivatisation as described above. The recovery values at each fortification level and overall were between 70 % and 110 %, with relative standard deviations of below 20 %. The detailed results are summarised in the table below. Blank samples were also analysed (n = 3), where no signal above 30 % of the response at the lowest concentration were observed at the same retention time window as used for integration of glyphosate acid response.

Table 5.1.2-4: Results of method validation (spike recovery) for the determination of glyphosate in test medium (reconstituted water)

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate acid	0.0309	91.9 – 98.7	94.0	2.7	2.9	5
		0.309	94.8 – 97.7	96.3	1.2	1.2	5
		3.09	92.6 – 94.2	93.5	0.6	0.6	5
		78.8	96.3 – 97.8	97.1	0.6	0.6	5
		Overall	91.9 – 98.7	95.2	2.1	2.2	20

[1] Recovery values are not corrected for interference with matrix compounds/respective control samples.

Specificity
Chromatograms of spiked samples, of test samples, of blank are provided. No interfering peaks were observed at the retention time of the analyte. The method consists of a derivatisation step which is considered to be specific to the target compound.

Linearity
The linearity of the detector response was tested using calibration standard concentrations of glyphosate-FMOC prepared in water/acetonitrile (1/1, v/v). The analytical system gave a linear response between 0.04 ng/injection and 2.0 ng/injection (low range; 4 – 20 µL injection volume), between 2 ng/injection and 20.0 ng/injection (medium range; 4 – 20 µL injection volume) and between 20.0 ng/injection and 200 ng/injection (high range; 4 – 20 µL injection volume) of glyphosate acid. The calibration curves and the parameters of the curves were calculated by linear regression. Details on the calibrations are provided below.

Table 5.1.2-5: Linearity parameters

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
Glyphosate acid	Linear	0.01 – 0.1	20 (10 levels)	y = 173468 x + 2992.74	0.997482
Glyphosate acid	Linear	0.1 – 1.0	12 (6 levels)	y = 186074 x – 21959.0	0.999045
Glyphosate acid	Linear	1.0 – 10	11 (6 levels)	y = 185849 x – 116743	0.999414

Repeatability (Precision)
The relative standard deviations (RSDs) at each concentration level and overall were below 20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.
Moreover, for precision testing, six replicate determinations were made of glyphosate-FMOC standard solutions at four concentrations. Relative standard deviations (RSDs) of 6.4 %, 1.9 %, 0.9 % and 0.5 % were found for the 0.01, 0.1, 1.0 and 10 µg/mL concentration level, respectively.

Limit of Quantification and Detection
The limit of quantitation (LOQ) in the study was 0.0309 mg/L for glyphosate acid. The limit of detection (LOD) was not reported.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed. However it was shown that glyphosate acid was stable over the whole test period of seven days.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. The method showed good performance and is considered as fit-for-purpose for the determination of glyphosate acid in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev. 4) in most points with minor deficits (matrix effect not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the ecotoxicological study concerned.

Assessment and conclusion by RMS: The analytical method fulfil the European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4.

Concerning the derivatisation step, it can be considered as demonstrated as the calibration has been performed with standard solution prepared in the same manner as the test item

The recovery data are in acceptable range. The specificity and the linearity are acceptable.

The method can be considered validated for the determination of glyphosate in Test medium (reconstituted water) with an LOQ of 0.0309mg/L.

Study previously submitted to the EU

Determination of glyphosate acid in test medium (reconstituted water)

2. Information on the study

Data point	CP 5.1.2/006 (CP 10.2.1/006)
Report authors	[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	SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4 (analytical phase)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME) 57377 Schmallenberg, Germany

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed and validated for the determination of glyphosate acid in aqueous growth medium (reconstituted water) by LC-MS/MS with a limit of quantification (LOQ) of 0.25 mg/L. Sample aliquots of 1000 µL of the aqueous test medium, 100 µL methanol and 50 µL of the IS-solution were pipetted successively into 1.8 mL HPLC vials. Where necessary, sample aliquots less than 1000 µL were filled up to 1000 µL with purified water in a pre-dilution step. After tightly closing and vigorous manual shaking 10 µL of the mixture were analysed directly by LC-MS/MS.

Chromatographic conditions:

LC-MS/MS system:	Waters 2695 HPLC coupled with Waters/Micromass LC/MS/MS Quattro Micro (triple quadrupole system)
HPLC column:	Phenomenex Gemini C18, 150 mm × 3.0 mm, 5 µm particle size
Guard column:	Phenomenex Gemini C18, 4.0 mm × 3.0 mm, 5 µm particle size
Column temperature:	30 °C
Mobile phase:	A: Methanol containing 2 mmol ammonium acetate B: Purified water/methanol containing 2 mmol ammonium acetate, 90/10 (v/v)
Gradient:	Time (min) / % A / % B / Flow rate (mL/min) 0.0 / 0 / 100 / 0.5 2.0 / 0 / 100 / 0.5 2.1 / 100 / 0 / 0.5 3.5 / 100 / 0 / 0.5 3.6 / 0 / 100 / 0.5 7.0 / 0 / 100 / 0.5
Injection volume:	10 µL
Retention time:	Glyphosate acid: ~ 1.8 - 2.25 min Glufosinate ammonium (IS): ~ 1.7 min
Detection mode:	MS/MS
Scan type:	MRM
Ionisation mode:	ES negative
Mass transition for evaluation:	Glyphosate acid: *m/z* 168.0→150.0 Glufosinate ammonium (IS): *m/z* 180.1→136.1

Findings

Recoveries

For method validation, aliquots of test medium (reconstituted water) were spiked with the analyte at two fortification levels at 0.25 and 2.5 mg/L. The mean recovery values at each fortification level and overall were between 70 % and 110 %. The detailed results are summarised in the table below.

Table 5.1.2-6: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Fortification level (mg/L)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate acid	0.25	94.8 – 104.8	99.0	4.1	4.2	5
		2.5	91.5 – 97.9	95.9	2.7	2.8	5
		Overall	91.5 – 104.8	97.4	3.7	3.8	10

Specificity
The method allows the determination of glyphosate acid using HPLC-MS/MS, which is a highly selective detection technique. The specificity of the method is shown by LC-MS/MS chromatograms of untreated fortification samples (blanks) and control samples of the investigated matrix. No interference is observed at the retention time of glyphosate.

Linearity
The linearity of the detector response was tested using seven calibration standard concentrations in the range of 0.20 to 25.0 mg/L, which were prepared by diluting an intermediate analyte solution with blank test medium. A linear correlation was found with a correlation coefficient (r) of 0.9990.

Repeatability (Precision)
The relative standard deviations (RSDs) at each fortification level and overall were < 20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The validated limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % and a relative standard deviation (RSD) of ≤ 20 %. These criteria were fulfilled for the 0.25 mg/L fortification level for aqueous growth medium. The determination of LOD was not addressed.

Interference
No significant interferences were observed at the retention time of the analyte in example chromatograms.

Matrix effects
Matrix effects were eliminated by using matrix matching solvent for calibration solutions.

Stability of glyphosate acid in sample extracts
Not assessed. However the analyte was proved to be stable in test solution for the duration of the test (14 days).

Conclusion
The analytical method was validated for the determination of glyphosate acid in aqueous test medium at a limit of quantification (LOQ) of 0.25 mg/L and fully meeting criteria set in SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4). The method is considered as fully validated to support the ecotoxicological study concerned.

Assessment and conclusion by RMS: The analytical method fulfils the European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4. with an LOQ of 0.25mg/L.

Study previously submitted to the EU

Determination of glyphosate in larvae, pollen and nectar

1. Information on the study

Data point	CP 5.1.2/007 (CP 10.3.1.5/001)
Report authors	[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	SANCO/3029/99 rev. 4 (with relevance to analytical method)
Deviations from current test guideline	None (SANCO/3029/99 rev. 4)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	No the report is not available
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Eurofins AgroScience D-75223 Nieferm Germany

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was validated for the determination of glyphosate in nectar, pollen and larvae by LC-MS/MS. The samples were extracted with acetonitrile/water (1/4, v/v), cleaned up by solid-phase extraction (SPE) over C18 phase and derivatised with FMOC-Cl. A second clean-up was done on Oasis HLB; elution was performed with methanol. After changing the solvent to 5 % acetonitrile solution, final analysis was performed by HPLC-MS/MS.

Chromatographic conditions:

HPLC system:	HPLC system (Shimadzu-LC-10AD) with MS/MS detector (API 4000 triple stage quadrupole mass spectrometer)
HPLC column:	Phenomenex Synergi Max-RP (20 mm × 2.0 mm, 2.5 µm)
Guard column:	4 mm guard column
Column temperature:	40 °C
Mobile phase:	A: 0.1 % acetic acid in water B: 0.1 % acetic acid in methanol C: 100 mM ammonium acetate solution in methanol
Gradient:	Time (min) / Eluent A (%) / Eluent B (%) / Eluent C (%) / Flow rate (µL/min) 0.00 / 80 / 15 / 5 / 500 5.00 / 0 / 95 / 5 / 500 10.00 / 0 / 95 / 5 / 500 10.01 / 80 / 15 / 5 / 500 12.00 / 80 / 15 / 5 / 500
Injection volume:	50 µL
Derivatisation agent:	FMOC-Cl
Retention time:	Glyphosate: approx. 2.8 min
Ionization mode (polarity):	ESI (-)
Ion transitions:	390.0 → 149.8 (quantifier) 390.0 → 167.8 (qualifier)

Findings
Recoveries
For method validation, samples of nectar, pollen and larvae were spiked with the analyte at two or three fortification levels, i.e. at the LOQ of 1 mg/kg and one or two higher levels, with mean recoveries found as 87-108 %. The recovery values were between 70 % and 110 %. The detailed results are summarised in the table below.

Table 5.1.2-7: Results of method validation (spike recovery) for the determination of glyphosate in nectar, pollen and larvae

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Nectar	Glyphosate	1	80 – 102	92	10	11	6
		500	85 – 97	92	4.6	5.0	5
		Overall	80 – 102	92	7.8	8.4	11
Pollen	Glyphosate	1	71 – 97	87	9.7	11	5
		500	107 – 108	108	N/A	N/A	2
		700	98 – 107	103	3.5	3.4	6
		Overall	71 – 108	98	11	11	13
Larvae	Glyphosate	1	83 – 109	96	11	11	6
		200	96 – 109	103	5.3	5.1	5
		Overall	83 – 109	99	9.1	9.1	11

1 Recovery values are not corrected for interference with matrix compounds/respective control samples. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity
No interfering peaks were observed at the retention time of the analyte. Determination by LC-MS/MS is considered to be highly specific. A second ion transition was measured.

Linearity
The linearity of the detector response was tested using six to seven calibration standard concentrations in the range of 1.0 to 5000 µg/L (nectar and larvae) or to 3500 µg/L (pollen) prepared in acetonitrile/water (1/4, v/v). A linear function was found (1/x weighting, nectar: $y = 0.00831 x + 0.00279$, pollen: $y = 0.0105 x + 0.0182$, larvae: $y = 0.00911 x + 0.0162$) with coefficients of determination (r) of > 0.999.

Repeatability (Precision)
The relative standard deviations (RSD) of all recovery values (n = 5 - 6) were < 20 %, i.e. in compliance with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantitation (LOQ) in the study was 1.0 mg/kg. The limit of detection (LOD) was 0.3 mg/kg.

Interference
No interfering peaks (< 30 % LOQ) were observed at the retention time of the analyte.

Matrix effects
Matrix effects were not checked because of the use of an internal standard, which obviate possible ion enhancement or suppression effects in HPLC-MS/MS analysis. To prevent such effects, matrix-matched standards were used.

Stability of glyphosate in sample extracts
Sample extracts were stored 5 - 8 days prior the clean-up 1 deep-frozen in the dark (below -18°C). The stability of glyphosate in the extracts was verified by analysing the recovery samples which were extracted with the stored samples. The results indicate that glyphosate was stable in the extract over these storage periods.

Conclusion
The analytical method was validated for the determination of glyphosate in nectar, pollen and larvae samples. The method validation fully meets criteria set in SANCO/3029/99 rev. 4.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4). The method is considered suitable to support the ecotoxicological study concerned.

Assessment and conclusion by RMS: The analytical phase report is not available in the study report. In consequence, the method cannot be assessed to be in accordance with SANCO/3029/99 rev. 4.

Study submitted to the EU for the first time

Determination of glyphosate in water

1. Information on the study

Data point	CP 5.1.2/008 (CP 10.6.2/001)
Report authors	[redacted]
Report year	2019
Report title	Effects on the seedling emergence and growth of ten non-target terrestrial plant species under greenhouse conditions
Report No	S19-03634 (Analytical Phase: S19-03634-L2)
Document No	-
Guidelines followed in study	SANCO/3029/99 rev. 4 (with relevance to analytical phase)
Deviations from current test guideline	None (SANCO/3029/99 rev.4)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	TRIALCAMP S.L.U Polígono Industrial L'Alter Avda. Antic Regne de València, 25 46290 Alcàsser (València) Spain

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was validated for the determination of glyphosate in tap water by LC-MS/MS. Samples were diluted as required and directly analysed by LC-MS/MS with external calibration.

Chromatographic conditions:

HPLC system:	1290 Infinity HPLC system (Agilent) equipped with a SCIEX API 6500 triple quad mass spectrometric detector
HPLC Column:	Bio-Rad Aminex Fast Acid (100 × 7.8 mm; 25 µm particle size)
Column temperature	30 °C

Mobile phase:	Water containing 0.1 % formic acid
Flow rate:	1500 µL/min
Injection volume:	80 µL
Evaporation solvent (post column):	Methanol at 0.7 mL/min combined to the aqueous eluent from analyical column and used for better vaporisation. Slit ratio to MS source: 1/1.15
Retention time:	Glyphosate: approx. 2.7 min
Ionization mode (polarity):	ESI (negative ion mode)
Scan type:	Multiple reaction monitoring (MRM)
Ion transition:	168 → 63 (quantifier) 168 → 79 (qualifier)

Findings

Recoveries

For method validation, tap water was fortified spiked with the analyte at two fortification levels, i.e. at the LOQ of 0.01 mg glyphosate/L and one higher level (20.1 mg glyphosate/L). Two control samples were also analysed without detecting glyphosate above the LOD. The recovery values were between 70 % and 110 %. The detailed results are summarised in the table below.

Table 5.1.2-8: Results of method validation (spike recovery) for the determination of glyphosate in tap water (*m/z* 168 → 63)

Matrix	Analyte	Fortification level (g/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Tap water	Glyphosate	0.01	88 – 99	92.8	5.4	5.8	5
		20.1	84 – 94	89.2	4.2	4.7	5
		Overall	84 – 99	91.0	4.9	5.4	10

[1] Recovery values are not corrected for interference with matrix compounds/respective control samples. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity

For both transition, chromatograms of standards solution, of control sample, treated and fortified samples are provided. No interfering peaks were observed at the retention time of the analyte. Determination by LC-MS/MS is considered to be highly specific. Two ion transitions were measured.

Linearity

The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five concentration levels ranging from 10 to 150 ng/mL. This range covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any diluted sample. The calibration curve was linear (1/x weighting) with coefficient of correlation (r) of ≥ 0.995. Details to the calibration function are provided below.

Table 5.1.2-9: Calibration parameters

Analyte	Calibration function	Calibration concentrations (ng/mL)	Number of deter-minations	Equation	Coefficient of correlation (r)
Glyphosate (*m/z* 168 → 63)	Linear (1/x weighting)	10 – 150	7 (7 levels)	y = 733 x – 3040	0.9982

Repeatability (Precision)
The relative standard deviations (RSD) of all recovery values were < 20 %, i.e. in compliance with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantitation (LOQ) of the analytical method was 0.0324 g test item/L (MON 52276), which corresponds to 0.01 g glyphosate/L. The limit of detection (LOD) of the analytical method was set at 0.003 g glyphosate/L (30 % LOQ).

Interference
No interfering peaks (<30 % LOQ) were observed in control samples at the retention time of the analyte.

Matrix effects
Matrix effects were < ± 20 % and deemed to be insignificant. Nevertheless matrix-matched standards were used to account for potential matrix effects.

Stability of glyphosate in sample extracts
The maximum storage period from sampling to analysis was 97 days within the study. Freezer storage stability was proven by analysis of the fortified solution.

Conclusion
The analytical method was fully validated for the determination of glyphosate in tap water. The method validation meets criteria set in SANCO/3029/99 rev. 4.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4). The method is considered suitable to support the ecotoxicological study concerned.

Assessment and conclusion by RMS: The analytical method fulfils the European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4. with an LOQ of 0.01g/L.

Study previously submitted to the EU

Determination of glyphosate in test water

1. Information on the study

Data point:	CP 5.1.2/009 (CP 10.6.2/002)
Report authors	[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	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited validation data from spike recoveries • No chromatograms provided • Limited details to calibration provided • Stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202 USA

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate in test water by HPLC with tandem mass spectrometry (MS/MS) detection. Samples were diluted as necessary with deionized water to provide final sample concentrations within the analytical standard concentration range and directly injected to the HPLC with external calibration.

Chromatographic conditions:

HPLC system:	ABSciex API-5000
HPLC Column:	Bio-Rad Guard Cation-H (30 × 4.6 mm)
Column temperature:	Not provided
Mobile phase:	A: Water + 0.1 % formic acid B: Methanol + 0.1 % formic acid
Gradient:	Time (min) / % A / % B / Flow rate (mL/min) 0.00 / 90 / 10 / 1 2.00 / 90 / 10 / 1
Injection volume:	20 µL
Derivatisation agent (pre-column):	Not applicable, not derivatised
Retention time:	Glyphosate: not provided (no chromatograms available)
Ionisation mode/polarity:	Turbo spray/negative
Scan mode:	MRM
Ion transitions:	Glyphosate: 168.0 → 63.0 (quantifier)

Findings
Recoveries (accuracy)
Blank samples of test water were fortified with reference item at relevant concentrations of 0.1 and 14.5 mg/L and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 0.02 mg/mL). The average recovery values at each fortification level and overall were between 70 % and 110 %, with an overall relative standard deviation of 3.0 %. The detailed results are summarised in the table below.

Table 5.1.2-10: Results of method validation (spike recovery) for the determination of glyphosate in test water

Matrix	Analyte	Fortification level (mg/L)	Recovery[(a)]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test water	Glyphosate	0.1	105 – 109	107	2.0	1.9	3
		14.5	101 – 106	103	2.9	2.8	3
		Overall	101 – 109	105	3.1	3.0	6

(a): Recovery values are not corrected for interference with matrix compounds/respective control samples. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity
No chromatograms are provided. Determination by LC-MS/MS is considered to be highly specific.

Linearity
Details to the linearity of detector response (tested range, number of determinations) were not reported. The lowest standard concentration was 0.05 µg/mL. Linear calibration functions were found, one calibration is reported as y = 341.9507 x + 3064.767, without providing a coefficient of correlation.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level and overall were <20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantitation (LOQ) was defined on the basis of the lowest calibration level and set at 0.02 mg/mL. The determination of LOD was not addressed.

Matrix effects
Not assessed and not required, matrix is distilled water.

Stability of glyphosate in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate in test water. The method validation meets criteria set in SANCO/3029/99 rev. 4 with deficits but is considered as fit-for-purpose for the determination of glyphosate in test water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level and considered acceptable. It was performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation data, no chromatograms provided, limited details to calibration provided, stability not assessed). Nevertheless the method is considered as fit-for-purpose to support the ecotoxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4. Indeed, some data are missing: chromatograms, details data for linearity. Regarding data on the accuracy, the specificity of the method could be considered acceptable.

The method can be considered as fit for purpose for the determination of glyphosate in test water.

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	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	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Test facility	TRIALCAMP S.L.U Polígon Industrial L'Alter Avda. Antic Regne de València, 25 46290 Alcàsser (València) Spai

The study objective was to determine the effects of MON52276 on early growth of non-targer plant species under greenhouse conditions. Dose verification was performed by analysis of the application solution samples for glyphosate.

Principle of the method
An analytical method was developed for the determination of glyphosate in test water by HPLC with tandem mass spectrometry (MS/MS) detection. Samples were diluted and directly injected to the HPLC with external calibration.

Findings
Recoveries (accuracy)
Data on recoveries are reported below

Matrix	Analyte	Fortification level (g/L)	Recovery			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Tap water	Glyphosate	0.1g/L (0.0333g glyphosate /L)	95 – 100	97	2	5
		21g/L (6.49g glyphosate/L)	95 – 98	97	1	5

Specificity
Chromatograms of of the lowest calibration level, a sample fortified at the LOQ and a treated sample are provided. No blank chromatograms is provided. No interference is expected at the retention time of glyphosate.

Linearity
Data on linearity is reported below:
-Y=ax+b (n>5, r>099)
-range: 20.2 -303ng/mL

Limit of Quantification and Detection
The limit of quantitation (LOQ) is 0.1g test item/L (0.0333g glyphosate /L)

Conclusion
The analytical method was validated for the determination of glyphosate in tap water. The method validation meets criteria set in SANCO/3029/99 rev. 4 with deficits.

3. Assessment and conclusion

Assessment and conclusion by RMS: The analytical method fulfils the European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4 with an LOQ of 0.1g test item/L (0.0333g glyphosate /L)

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

Analytical methods for post-authorisation control and monitoring purposes are active substance data; please refer to Volume 3 CA B.5.

Methods for the determination of residues in body fluids and tissues

Analytical methods for post-authorisation control and monitoring purposes are active substance data; please refer to Volume 3 CA B.5.

Methods for the determination of residues in soil

Analytical methods for post-authorisation control and monitoring purposes are active substance data; please refer to Volume 3 CA B.5.

Methods for the determination of residues in water

Analytical methods for post-authorisation control and monitoring purposes are active substance data; please refer to Volume 3 CA B.5.

Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Analytical methods for post-authorisation control and monitoring purposes are active substance data; please refer to Volume 3 CA B.5.

B.5.3. 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] Y/N If yes, for which data point?
KCP 5.1.1-001	[redacted]	2001	Determination of glyphosate content in formulations MON 78013, MON 78011 & MON 2139 (glyphosate 360g/l) SL by HPLC: validation of the analytical method. Report No.: MSL-17401 Document No.: Monsanto Company GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: IIIA 5
KCP 5.1.1-002	[redacted]	2012	NNG and Formaldehyde method validations in MON 52276 and MON 77973 Report No.: MSL0024115 Document No.: PCH-2012-0207 Monsanto Company GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: IIIA 5.1.2; IIIA 5.2.4
KCP 5.1.1-003	[redacted]	2019	N-Nitrosoglyphosate method validation in MON 52276, MON 76610, MON 79351, MON 79545 and MON 79991 Report No.: MSL0028536 Document No.: PCH-2017-0062 Monsanto Company GLP/GEP: Y Published: N	N	N	-	BCS	N
KCP 5.1.1-004	[redacted]	2020	Validation of the analytical method for the analysis of N-nitrosoglyphosate (NNG) in MON 52276	N	Y	First submission in EU	BCS	N

			Report No.: TRR0000189 Document No.: PCH-2020-0230 Monsanto GLP/GEP: Y Published: N					
KCP 5.1.1-005	[redacted]	2020	Position Paper supporting the Approval Renewal Dossier for an Active Substance: Glyphosate & the IPA-, K-, DMA and NH4-salts of Glyphosate (hereafter Glyphosate) Report No.: - Document No.: - Bayer Agriculture BVBA GLP/GEP: N Published: N	N	Y	First submission in EU	BCS	N
KCP 5.1.1-006	[redacted]	2020	Validation of the Analytical Method ME-1137 for Formaldehyde in MON 52276 Report No.: TRR0000082 Document No.: PCH-2019-0599 Monsanto Company GLP/GEP: Y Published: N	N	Y	First submission in EU	BCS	N
KCP 5.1.2-001	[redacted]	1992	Validation of method to determine the concentration of MON 52276 in freshwater by liquid chromatography Report No.: J9107004b Document No.: TO-91-320 Toxikon Environmental Sciences GLP/GEP: Y Published: N	N	N	-	BCS	N
KCP 5.1.2-002	[redacted]	1992	MON 52276: Acute toxicity to rainbow trout, Oncorhynchus mykiss, under	Y	N	-	BCS	N

			flow-through test conditions Report No.: J9108002b Document No.: Toxikon Environmental Sciences GLP/GEP: Y Published: N					
KCP 5.1.2-003	[redacted]	1992	MON 52276: Acute toxicity to the common carp, Cyprinus carpio, under flow-through test conditions Report No.: J9108002c Document No.: - Toxikon Environmental Sciences GLP/GEP: Y Published: N	N	N	-	BCS	N
KCP 5.1.2-004	[redacted]	1992	MON 52276: Acute toxicity to the water flea, Daphnia magna, under flow- through test conditions Report No.: J9108002a Document No.: - Toxikon Environmental Sciences GLP/GEP: Y Published: N	N	N	-	BCS	N
KCP 5.1.2-005	[redacted]	2002	Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed Lemna gibba Report No.: 20021186/01-AALg Document No.: - Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Urnweltanalytik GmbH GLP/GEP: Y Published: N	N	N	-	GTF	N

KCP 5.1.2-006	[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	Y	-	GTF	N
KCP 5.1.2-007	[redacted]	2011	Glyphosate: Study to determine potential exposure of honeybee colonies to residues under semi-field conditions Report No.: V7YH1002 Document No.: - Environmental Risk Team Food and Environmental Safety Programme The Food and Environment Research Agency GLP/GEP: Y Published: N	N	Y	-	BCS	N
KCP 5.1.2-008	[redacted]	2019	Effects on the seedling emergence and growth of ten non-target terrestrial plant species under greenhouse conditions Report No.: S19-03634 (Analytical Phase: S19-03634-L2) Document No.: - TRIALCAMP S.L.U GLP/GEP: Y Published: N	N	Y	-	BCS	N
KCP 5.1.2-009	[redacted]	2014	MON 52276: Effects on the vegetative vigor of non-target terrestrial plants (Tier II) Report No.: 80477 Document No.: -	N	N	-	BCS	N

			ABC Laboratories GLP/GEP: Y Published: N					
CP 10.6.2/005	[redacted]	2021	MON 52276: Effects on the Vegetative Vigour of Ten Non-Target Terrestrial Plant species under Greenhouse conditions	N	Y	-	BCS	N

[1] In order to facilitate the compilation of the final list of the tests and studies relied upon and the corresponding data protection, indicate whether the study was used in the previous DAR/RAR or, when the information is available, whether the study was already submitted in the framework of national authorisations.
[2] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[3] The RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).