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 (AS)

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

Version History

When	What
2021/06	Initial RAR

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B.5. METHODS OF ANALYSIS

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

B.5.1.1.1 Methods for the analysis of the active substance as manufactured

Barclay

Analytical methods for the determination of glyphosate in the technical substance of different sources of the notifier Barclay are provided and reported below.

Source 1

Data point:	J-CA 4.1.1/001
Report author	[redacted]
Report year	2009
Report title	Determination of active content and impurity profile of glyphosate
Report No	OS-012
Document No	-
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

Principle of method

Samples and standards were dissolved in phosphate buffer. The solution was directly analysed by reverse phase high performance liquid chromatography (HPLC) using an UV detector at 196 nm. Quantitation was performed by external standards.

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

HPLC system:	LC-10ADVP Pump, SIL-10ADVP Autosampler, SPD-M10AVP, PDA Detector (Shimadzu)
Column:	ExsilTM Amino column, 4.6 × 250 mm
Column temperature:	30 °C
Mobile phase:	Phosphate buffer, KH_2PO_4 4.0 g/L H_3PO_4 3.0 g/L
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detector:	PDA
Wavelength:	196 nm
Retention time:	Approx. 6.1 min

Findings

Specificity:

Chromatograms of standard solution, of blank solvent, and sample are provided. No interference was observed. Specificity was confirmed by the use of a DAD detector: The examination of the response at different wavelengths, comparison of UV spectra for the glyphosate reference standard and in samples and determination of peak purity showed that the analyte chromatographic peak is not attributable to more than one component – glyphosate.

Linearity
Linearity was determined with at 5 standard levels containing glyphosate with correlation coefficient > 0.99.

Table 4.1.1-1: Linearity data for glyphosate

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r^2)
Glyphosate	1.3664 – 2.5559 g/L	y = 440.59 x + 33026	0.9991

Note: The linearity range in % or g/kg is not available. Data required

Accuracy
Accuracy is not required for active substance.

Repeatability (precision)
System precision was evaluated by injecting seven times of a glyphosate standard with concertation of 2015.2 mg/L. The respective %RSD is 0.25.

To evaluate sample repeatability a single batch of glyphosate (AFS08/1973, batch no. 080527-01) was analysed seven times and each of the remaining batches were analysed in duplicate.

Table 4.1.1-2: Repeatability data for glyphosate

Analyte	Mean content	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	96.8 % w/w	7	0.2	0.148

[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

Conclusion

The analytical method for determination of glyphosate in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS:
The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Source 2

Data point:	J-CA 4.1.1/002
Report author	[redacted]
Report year	2008
Report title	Qualitative and quantitative profile of the test substance glyphosate technical (five batch analysis)
Report No	3996.030.288.07
Document No	-
Guidelines followed in study	EPA OPPTS 830.1700 EPA OPPTS 830.1000 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

Principle of method

The methodology for the determination of glyphosate comprised the following stages, solubilization in water, separation using HPLC and detection by UV absorption. The responses were calibrated by external standard method. The quantification of Glyphosate was determined by a liquid chromatograph coupled to UV detector. The Glyphosate concentrations in samples were determined in triplicate for each batch.

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

HPLC system:	HPLC-UV
Column:	PRP – X 100, 4.1 × 250 mm, 10 µm
Column temperature:	Not reported
Mobile phase:	Water / methanol / KH_2PO_4 – 960/40/0.844 (v/v/w) pH 2.1 (with phosphoric acid)
Flow rate:	1.5 mL/min
Injection volume:	20 µL
Detector:	UV detection
Wavelength:	Not reported
Retention time:	Approx. 6.0 min

Findings

Specificity:
Chromatograms of sample, of standards solution and blank are provided. There were no interference peaks at the retention time of glyphosate, thus the method is selective for the active ingredients. ^{1}H-NMR, IR, UV and LC-MS/MS were used to confirm the identity of active substance glyphosate of both standard and technical material.

Linearity
Linearity was determined with at 5 standard levels containing glyphosate with correlation coefficient > 0.99.

Table 4.1.1-3: Linearity data for glyphosate

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r^2)
Glyphosate	997.50ng/L – 3990.0 ng/L	y = 50.3 x - 806	1.000

Note: The linearity range in % or g/kg is not available. Data required

Accuracy

Once the instrument had reached equilibrium, solutions with three levels of concentration were injected. Each one of the solutions was injected two times

Table 4.1.1-4: Accuracy

Analyte	Fortification level (g/L)	No of replicates	Mean recovery (%)	RSD (%)
Glyphosate	2.22	2	102.7	N/A
	2.35	2	103.0	N/A
	2.49	2	102.1	N/A
	2.35 (mean)	**6**	**102.63**	**0.416**

Repeatability (precision)

System precision was evaluated by injecting ten times of a glyphosate standard with concertation of 1.995 g/L. The respective %RSD is 0.13.

The test sample precision of the HPLC-UV determination of the concentrations of Glyphosate was assessed from the results obtained for identical samples of the test substance by two different analysts working independently.

Table 4.1.1-5: Repeatability

Analyte	Mean content	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	98.64 % w/w	20	0.30	0.223

[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

Conclusion

The analytical method for determination of glyphosate in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Method for determination of active substance glyphosate in **source 2 (QC data)**

Data point:	J-CA 4.1.1/003
Report author	[redacted]
Report year	2017

Report title	The summary 5-batch quantitative analysis of glyphosate 97% min. tech. for quality control
Report No	D20150128
Document No	-
Guidelines followed in study	EPA OPPTS 830.1700 EPA OPPTS 830.1000
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes
Category study in AIR 5 dossier (L docs)	Category 1

Principle of the method

The same validated analytical method from the 5-batch report by [redacted] (2008) was applied. The method was revalidated in [redacted] (2017). The summary of validation data is present in table below.

Table 4.1.1-6: Validation data for glyphosate in technical material (QC data)

Parameter	**Results**
Specificity	No interference
Linearity	R = 0.99991
Accuracy	Recovery = 99.54 %
Repeatability (precision)	System precision: %RSD = 0.31 Sample precision: %RSD = 0.59

Conclusion

The validation of the method for analysis of glyphosate in glyphosate technical material was not previously evaluated at EU level. It was performed according to the requirements applicable at the time of the study (EU guideline SANCO/3030/99 rev. 4) and it matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS:: The analytical [redacted] (2008) is revalidated in [redacted] (2017) for the determination of active substance is used for the quantification in QC data. As the method used was already fully validated, validation data are considered sufficient.
See conclusion of the [redacted] 2008 method

Data point:	J-CA 4.1.1/004
Report author	[redacted]
Report year	2020
Report title	The summary 5-batch quantitative analysis of 97% min. glyphosate technical for QA
Report No	F20200305
Document No	-

Guidelines followed in study	EPA OPPTS 830.1700 EPA OPPTS 830.1000
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes
Category study in AIR 5 dossier (L docs)	Category 1

Principle of the method

The same validated analytical method from the 5-batch report by [redacted]. (2008) was applied. The method was re-validated in [redacted] (2017). The summary of validation data is present in table below.

Table 4.1.1-7: Validation data for glyphosate in technical material (QC data)

Parameter	**Results**
Specificity	No interference
Linearity	R = 0.9987
Accuracy	Recovery = 100.54 %
Precision	System precision: %RSD = 0.48 Sample precision: %RSD = 0.29

Conclusion

The validation of the method for analysis of glyphosate in glyphosate technical material was not previously evaluated at EU level. It was performed according to the requirements applicable at the time of the study (EU guideline SANCO/3030/99 rev. 4) and it matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS:: The analytical [redacted] (2008) is revalidated in [redacted] (2020) for the determination of active substance is used for the quantification in QC data. As the method was already fully validated, validation data are considered sufficient.
See conclusion of the [redacted] 2008 method

Source 3

Data point:	J-CA 4.1.1/005
Report author	[redacted]
Report year	2017
Report title	Qualitative and quantitative profile of the test substance glyphosate technical HDF (five batch analysis)
Report No	15846.030.002.16
Document No	-
Guidelines followed in study	EPA OPPTS 830.1700 EPA OPPTS 830.1800 EPA OPPTS 830.1000 SANCO/3030/99 rev. 4

Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes
Category study in AIR 5 dossier (L docs)	Category 1

Principle of method

The content of the active ingredient was determined by external calibration using validated method of Liquid Chromatography coupled to UV detector with diode array (HPLC/UV). Solutions from the test substance were prepared by weighing of approximately 100 mg of sample in volumetric flask of 50.0 mL. The volumes were completed with ultra pure water and the solutions were stirred and sonicated until complete solubilisation, producing work solutions with approximately 2000.00 mg/L.

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

HPLC system:	Liquid chromatograph Agilent 1100 series
Column:	PRP – X 100, 4.1 × 250 mm, 10 µm
Column temperature:	40 °C
Mobile phase:	Solution of monobasic potassium phosphate acidified with phosphoric acid + Methanol (100%)
Flow rate:	1.5 mL/min
Injection volume:	20 µL
Detector:	UV detection
Wavelength:	195 nm
Retention time:	Approx. 4.3 min

Findings

Specificity:
Chromatograms of standards solution, of blank and sample are provided. No interfering peaks at the retention time of Glyphosate were detected, thus the method is selective to determine the active ingredient. The specificity for active ingredient was assured by analysis of the UV spectra of glyphosate (please refer to spectrum on pages 224 to 228), which shows that the peak is pure; therefore, there is no other compound co-eluting with it. The active ingredient in the test samples of glyphosate technical was identified as glyphosate by Ultraviolet Spectrophotometry (UV), Mass Spectrometry (MS), Infrared Spectroscopy (IR) and ^{1}H-NMR.

Linearity
Linearity was determined with five standard levels containing glyphosate with correlation coefficient > 0.99.

Table 4.1.1-8: Linearity data for glyphosate

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Glyphosate	1.012 – 3.642 g/L	y = 0.349745 + 19.31913	0.99997

Note: The linearity range in % or g/kg is not available. Data required

Accuracy
Recovery was determined with three standard levels (1530 mg/L (67.2% w/w), 2013 mg/L (88.3% w/w) and 2537 mg/L (111.3 % w/w)) containing glyphosate.

Table 4.1.1-9: Accuracy data

Analyte	Nominal concentration	No of replicates	Mean recovery (%)	RSD (%)
Glyphosate	1.53 g/L (67.2 % w/w)	1	100.82	N/A
	2.013 g/L (88.3 % w/w)	1	100.01	N/A
	2.537 g/L (111.3 % w/w)	1	99.32	N/A
	Overall	**3**	**100.05**	**0.75**

N/A: not applicable

Repeatability (precision)

The repeatability test is evaluated by seven replicates of sample determinations. The same sample (batch 20160301) is prepared and analyzed by the same analyst (Repeatability 1). A second analyst also prepares and analyzes the sample seven times (Repeatability 2).

Table 4.1.1-10: Repeatability data

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	96.54	7	0.11	0.081
	96.51	7	0.10	0.074

[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

Conclusion

The analytical method for determination of glyphosate in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5) No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Bayer

Analytical method for the determination of glyphosate in the technical substance of different sources of the notifier Bayer is provided and reported below. The same method ME 1847-02 is used for the determination of glyphosate in all Bayer sources.

The validation data of the method ME 1847-02 is not provided in the dossier. Data required.

Another method AG-ME-0765-05 has been provided and reported below:

Data point:	CA 4.1.1/007
Report author	[redacted]
Report year	2020

Report title	Amended from MSL0026166: Glyphosate, NNG, and Formaldehyde Method Validations in Glyphosate Technical (Wetcake) MON 77973
Report No	PCH-2013-0656
Document No	TRR0000235
Guidelines followed in study	SANCO 3030/99 rev. 5
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

Only method validation of active substance part is summarized below.

Analytical method for determination of active substance glyphosate in glyphosate technical MON 77973 (glyphosate wetcake)

Principle of method

The glyphosate-containing technical material is diluted in water and injected into an HPLC. Separation is achieved with an anion exchange column. The wetcake is dried for glyphosate assay. Quantification is performed using peak area responses from a UV or RI (Refractive Index) detector and external standards calibration.

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

HPLC system:	HPLC system equipped with an autosampler, isocratic pump, and UV or RI detector
Column:	Platinum SAX Rocket, 7.5 × 4.6 mm, 5µm particle size
Column temperature:	40 °C
Mobile phase:	3.39 g of KH_2PO_4 ;4000 mL of water pH adjusted to 2.1 with 85% H_3PO_4 ;540 mL of methanol
Flow rate:	3.0 mL/min
Injection volume:	20 µL
RI Detector:	Agilent G-1362A
Retention time:	Approx. 5.5 min

Findings

Specificity:
The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the samples (resolution ratio > 3.0; nearest peak). Chromatogram of standards solution and of samples are provided. However, the chromatogram of blank is missing. There were no impurities or interferences co-eluted with the analyte of interest.

Linearity
Linearity was determined with at 5 standard levels containing glyphosate with correlation coefficient >0.99.

Table 4.1.1-11: Linearity data for glyphosate

Analyte	Calibration ranges (% w/w)	Calibration curve	Correlation coefficient (r)
Glyphosate	0.0993 to 0.5892	y = 152313.0754 x – 18.3861	0.9999

Accuracy

The accuracy was determined by analysis of a MON 77973 sample followed by analysis of the same sample spiked with two levels of glyphosate. The samples were diluted to bring in the calibration range. Triplicate injections of three separate sample preparations at each of two spiking levels were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 4.1.1-12: Accuracy data

Analyte	Fortification level (ppm)	No of replicates	Mean recovery (%)
Glyphosate	53 % w/w	9	101.8
	105 % w/w	9	100.5

Repeatability (precision)

Repeatability of this method was determined through the analysis of MON 77973. Triplicate injections of each of five separate sample preparations were used to generate precision data. The data for the repeatability of active substance glyphosate in MON 77973 are summarised in the below table.

Table 4.1.1-13: Repeatability data (dry basis)

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	96.56	15	0.60	0.462

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

Conclusion

The analytical method for determination of glyphosate in glyphosate technical material MON 77973 (glyphosate wetcake) has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5.

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate in glyphosate technical material MON 77973 (glyphosate wetcake) was not previously evaluated at EU level. It was performed under GLP and according to current requirements (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material MON 77973 (glyphosate wetcake).

Assessment and conclusion by RMS: The analytical method is validated for the determination of glyphosate in technical substance MON 77973 (glyphosate wetcake).

Nufarm

Analytical method for the determination of glyphosate in the technical substance is provided and reported below.

Data point:	J-CA 4.1.1/019
Report author	[redacted]
Report year	2019
Report title	Validation of analytical methodology for the assay of active ingredient and impurities in glyphosate technical
Report No	ABC-2019-039
Document No	-
Guidelines followed in study	US EPA OCSPP 830.1700, 830.1800 Brazilian Normative – ABNT NBR 14029-2016 SANCO 3030/99 rev. 5
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

Analytical method for determination of active substance glyphosate in glyphosate technical material (Method ABCTM-2019-039-01).

Principle of method

The glyphosate-containing technical material is diluted in water and injected into an HPLC-DAD. Quantification is performed using peak area responses from a UV detector and external standards calibration. Details to the HPLC system and chromatographic parameters are summarised below.

HPLC system:	Agilent 1260 HPLC system with DAD
Column:	ZORBAX SAX 4.6 mm × 250 mm, 5.0 µm
Column temperature:	30 °C
Mobile phase:	Isocratic: Water (0.1 M KH_2PO_4 + 0.1 % H_3PO_4
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detector:	DAD detection
Wavelength:	195 nm
Retention time:	Approx. 8.8 min

Confirmatory method (ABCTM-2019-040-01)

HPLC system:	Agilent 1200/6130 Single Quadrupole LC/MS system
Column:	Agilent ZORBAX-SB-Aq, 4.6 × 250 mm, 5 µm
Column temperature:	30 °C
Mobile phase:	Isocratic: 90 % water (0.1 formic acid) + 10 % methanol
Flow rate:	0.3 mL/min
Injection volume:	2.0 µL
Detector:	MS detection with SIM mode
Ionization mode:	API-ES
Retention time:	Approx. 3.1 min

Findings

Specificity:
Chromatograms of blank solution, of standard solution of test item are provided. No interference was observed. The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the samples. There was no impurities or interferences co-eluted with the analyte of interest.

The identification of the glyphosate in glyphosate technical material was confirmed by MS spectrum using HPLC/MS analysis (method ABCTM-2019-054-02), UV/Vis spectrum, FT-IR spectrum and ^{1}H-NMR. The retention time and respective spectrum (MS, UV/Vis, FT-IR and ^{1}H-NMR) were compared to the ones of referent standard under same analysis conditions.

Linearity
Linearity was determined with 5 standard levels containing glyphosate with correlation coefficient > 0.99.

Table 4.1.1-14: Linearity data for glyphosate

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Glyphosate	1733.9833 - 3220.2547 mg/L (69.36 ~ 128.81%)	y = 0.8946 x – 30.0155	0.9999

Accuracy
The accuracy is not required for active substance.

Repeatability (precision)
To evaluate sample repeatability a single batch of glyphosate (batch no. GH0162) was analysed five times. Intermediate precision was further investigated by another analyst on another time.

Table 4.1.1-15: Repeatability data

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	97.50	5	0.36	0.27[2]
	97.62	10	0.36	0.27[3]

[1]Horrat value (Hr) = %RSD/%RSD$_r$ (Horwitz equation %RSD$_r$ = 0.67 * $2^{(1\text{-}0\ 5*\log(c))}$)
[2] Calculated based on precision data
[3] Calculated based on intermediate precision data.

Conclusion

The analytical method for determination of glyphosate in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate in glyphosate technical material as manufactured was not previously evaluated at EU level. It was performed under GLP and according to the requirements of EU guideline SANCO/3030/99 rev. 5. No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Industrias Afrasa

Analytical methods for the determination of glyphosate in the technical substance of different sources of the notifier Industria Afrasa are provided and reported below.

Source 1

Report:	J-KCA 1.11/16 [redacted] (2017)
Title:	5-Batch Analysis of Glyphosate TGAI in Accordance with Regulation (EC) No 1107/2009, Reference SANCO 3030/99 rev. 4
Document No:	EPP00297, AN16111117
Guidelines:	Regulation (EC) No. 1107/2009 SANCO/3030/99 rev. 4
GLP:	Yes
Acceptability	Yes

Determination of glyphosate: Method CIPAC 284/TC/M/3

The glyphosate content in the active substance technical material has been determined according to the established validated method, CIPAC 284/TC/M/3. Data requirement CA 4.1.1 of Regulation (EC) No 283/2013 states that *"In case of use of a CIPAC method, further validation data shall not be required, but example chromatograms shall be submitted, where available"*. Only the provision of example chromatograms generated using the method are therefore strictly necessary in accordance with the data requirements. However, additional validation data generated as part of the 5-batch study to demonstrate the acceptability of the method are presented below in the interests of completeness and transparency.

Principle of method
The glyphosate content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection, based upon the validated method CIPAC 284/TC/M/3.

Preparation of standard solutions
Approximately 400 mg of a glyphosate analytical standard is weighed in a 100 mL volumetric flask and made up to volume with the mobile phase (see analysis of test samples) and dissolved by sonication. This gives a glyphosate standard solution of concentration 4 mg/mL.

Preparation of test samples
400 mg of glyphosate technical material (to the nearest 0.1 mg) is weighed into a 100 mL volumetric flask and made up to volume with the mobile phase and dissolved by sonication. This gives a test sample solution of the technical material of concentration 4 mg/mL.

Analysis of test samples
50 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1100 series HPLC
Column:	Hichrom Partisil 5 SAX, 250 x 4.6, 5 µm

Mobile Phase:	96:4 (v/v) HPLC grade water with potassium dihydrogen phosphate (0.8437 g/L) / methanol, adjusted to pH 1.9 using orthophosphoric acid.
Injection Volume:	50 µL
Flow Rate:	2.3 mL/min
Temperature:	Ambient
Detection:	195 nm (UV)
Run Time:	13 minutes
Retention Time:	Glyphosate at ~2.8 minutes

All standard and test sample solutions are injected in duplicate.

Findings

The following method validation data for quantification of glyphosate content using CIPAC 284/TC/M/3 were reported:

Specificity
Representative chromatograms are provided of a solvent blank, an analytical standard (3.997 mg/mL) and the glyphosate active substance technical material (~4.0 mg/mL). The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no interfering peaks with the active substance peak. The data are therefore sufficient to demonstrate the specificity of the method.

Linearity
No linearity data for the active substance is presented. As the analytical method being used is a validated CIPAC method, further validation data to demonstrate the method's linearity of response is not necessary in accordance with the requirements of Regulation (EC) No 283/2013. No further consideration is needed.

Accuracy
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material as manufactured. However, in the interests of completeness and transparency, the assessment of the method accuracy which was performed as part of the 5-batch analysis is presented here.

A solution of glyphosate analytical standard was prepared in the chromatographic mobile phase at a concentration of ~4 mg/mL. Five determinations of the active substance content were performed according to CIPAC/284/TC/M/3. The recoveries were in the range of 99.83-100.24% w/w (998.3-10024 g/kg) with a mean value 99.98 % w/w (999.8 g/kg), supporting the acceptability of the method accuracy.

Precision
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material.

Five determinations of the active substance content were made using a sample of the active substance technical material. The mean content of the active substance material was determined to be 99.98% (999.8 g/kg) with a %RSD of 0.19. The modified Horwitz equation for a sample concentration based on the mean recovery (999.8 g/kg) gives a value of 1.34. The experimentally derived %RSD and the modified Horwitz value ($\%RSD_r$) give a Howitz ratio (H_r) = 0.19/1.34 = 0.14. As $H_r < 1$, the method precision is concluded to be acceptable.

Confirmation of analyte identity
It is not a requirement to confirm the identity of the active substance according to the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements laid down in Regulation (EC) No. 283/2013.

However, in the interests of transparency and completeness it is highlighted that spectral characterisation of the glyphosate active substance technical material using Infrared (IR) Spectroscopy was reported as part of the 5-batch analysis report. Spectra of samples of the each batch of technical material used in the 5-batch analysis were obtained as potassium bromide (KBr) dispersions (~2.5% w/w substance in KBr) over a wavelength range of 400-4000 cm^{-1}

using an FTIR spectrometer operated in diffuse reflectance mode. An IR spectrum was also acquired for an analytical standard of glyphosate. Assignment of the FTIR bands observed in the spectra from each batch of technical material, and comparison with the spectrum obtained from the analytical standard confirmed the identity of the active substance.

Table 4.1.1-1 Summary of validation data for method CIPAC 284/TC/M/3 for quantification of glyphosate content in glyphosate active substance technical material as manufactured

LOQ (g/kg)	Recovery Fortification Level (g/kg)	Recoveries % range (mean)*	Repeatability % RSD (n)	Linearity	Specificity
SANCO/3030/99 rev. 5 states that according to Regulation (EU) No 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for the technical active substance.	SANCO/3030/99 rev. 5 states the determination of recovery for the active substance in the technical material is not required. In the interests of completeness and transparency, the following recoveries data were reported for a sample of the glyphosate active substance technical material as manufactured: % Range = 99.96 – 100.24 Mean = 99.98 n = 5		Mean content = 999.8 g/kg (99.98 % w/w) % RSD = 0.19 (5) Modified Horwitz value ($\%RSD_r$): 1.34 Horwitz ratio (H_r = $\%RSD/\%RSD_r$): 0.14 $H_r < 1$	No additional linearity data is presented. As the method is a validated CIPAC method, it is not a requirement according to Regulation (EC) No 283/2013 to provide further linearity of response data. No further consideration is therefore needed.	Representative chromatograms are provided of a solvent blank, an analytical standard (3.997 mg/mL) and the glyphosate active substance technical material (~4.0 mg/mL). The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no interfering peaks with the active substance peak. The data demonstrate the specificity of the method.

Conclusions

The analytical method used to determine the active substance content in the active substance technical material as manufactured is a validated CIPAC method. In accordance with the data requirements of Regulation (EU) No 283/2013, only the provision of chromatograms, where available, to demonstrate the method specificity is required when a CIPAC method is used. Representative chromatograms are available which confirm the method specificity. Though not a formal requirement, in the interests of completeness and transparency, supplementary data to demonstrate the accuracy and precision of measurements are also presented. Overall, sufficient information is provided to confirm the acceptability of CIPAC 284/TC/M/3 for quantification of glyphosate content in the glyphosate active substance technical material as manufactured. The method is considered fully validated in accordance with SANCO/3030/99 rev. 5 and no further consideration is needed.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Source 2

Report:	**KCA Section 1/017 [redacted] (2016)**
Title:	Qualitative and Quantitative Profile of the test substance Glyphosate (Five Batch Analysis)
Document No:	15425.030.027.15
Guidelines:	Regulation (EC) No. 1107/2009 SANCO/3030/99 rev. 5
GLP:	Yes
Acceptability	Yes

Determination of glyphosate

The glyphosate content in the active substance technical material has been determined using an in-house liquid chromatography coupled UV detection method. Validation data generated as part of the 5-batch study to demonstrate the acceptability of the use of the method are presented below in the interests of completeness and transparency.

Principle of method

The glyphosate content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-Vis detection at 195 nm

Preparation of standard solutions
A stock solution of the active ingredient was prepared by weighing 40.53 mg of glyphosate analytical standard into a 10 mL volumetric flask. The flask was made up to the mark with ultra-pure water and the solution sonicated with stirring until complete dissolution, giving a concentration of 4032.74 mg/L of glyphosate analytical standard. The solution was further diluted to provide five calibration concentration with a working range of 1532.44-4032.74 mg/L.

Preparation of test samples
Duplicates of approximately 130 mg of technical material from each of the five batch samples was transferred to a separate 50 mL volumetric flask. Samples were then dissolved and made up to the 50 mL mark in ultra-pure water to provide the test solutions with concentrations of approximately 2600 mg/L glyphosate.

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1100 series HPLC
Column:	PRP-X 100: 10 µm internal diameter, 250 x 4.6 mm
Mobile Phase:	Solution of monobasic potassium phosphate acidified with phosphoric acid and methanol
Injection Volume:	20 µL
Flow Rate:	1.5 mL/min
Temperature:	40 ºC
Detection:	195 nm (UV)
Run Time:	10 minutes
Retention Time:	Glyphosate at ~5.4 minutes

All standard and test sample solutions are injected in duplicate.

Findings

The following method validation data for quantification of glyphosate content were reported:

Specificity
Representative chromatograms are provided of a solvent blank, an analytical standard and the glyphosate active substance technical material. The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no

interfering peaks with the active substance peak. The data are therefore sufficient to demonstrate the specificity of the method.

Linearity
The analytical calibration was performed using five analytical standards of glyphosate, ranging in concentration from 1532.44-4032.74 mg/L (~59-155% of the nominal concentration of test material). Overall, the calibration data provided is considered sufficient to demonstrate the method shows good linearity, where the correlation coefficient (r) is >0.99

Accuracy
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material. However, in the interests of completeness and transparency, the assessment of the method accuracy which was performed as part of the 5-batch analysis is presented here.

The accuracy of the method was evaluated by the determination of the analyte content in spiked samples (spiking concentrations of 1760.83, 2321.10, and 2881.36 mg/L of glyphosate). The accuracy / recovery was then calculated based on: Accuracy = 100 x amount found / amount expected.

The results obtained show a good accuracy within the acceptability limits as outlined by SANCO/3030/99/rev.5. Recovery rates for spiked samples ranged from 99.10-99.85 %. Overall, the method is concluded to be sufficiently accurate.

Precision

The repeatability (precision) of the method was assessed through the sevenfold determination of the active ingredient in one batch of test item (Batch N° HB20150214) in two separate experiments. The results obtained show a relative standard deviation of 0.10 % (mean value: 982.4 g/kg number of values: 14) which indicates a good consistency of the data. (relative standard deviation acceptable limit: 1.34 %, according to SANCO 3030/99/rev 5).

Confirmation of analyte identity
It is not a requirement to confirm the identity of the active substance according to the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements laid down in Regulation (EC) No. 283/2013.

However, additional analysis on the active substance was performed as part of the 5-batch study and is referenced here in the interests of completeness and transparency. The identity of glyphosate technical material within the five batches was confirmed by comparison with a reference item of glyphosate by each of HPLC/MS, UV-Vis, and ^{1}H-NMR.

Table 4.1.1-1 (b) Summary of validation data for method CIPAC 284/TC/M/3 for quantification of glyphosate content in glyphosate active substance technical material as manufactured

LOQ (g/kg)	**Recovery Fortification Level (g/kg)**	**Recoveries % range (mean)***	**Repeatability % RSD (n)**	**Linearity**	**Specificity**

SANCO/3030/99 rev. 5 states that according to Regulation (EU) No 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for the technical active substance.	SANCO/3030/99 rev. 5 states the determination of recovery for the active substance in the technical material is not required. In the interests of completeness and transparency, the following recoveries data were reported for a sample of the glyphosate active substance technical material as manufactured: % Range = 99.10 – 99.85 Mean = 99.36 n = 3	Mean content = 982.5 g/kg (98.25 % w/w) % RSD = 0.10 (5) Modified Horwitz value ($\%RSD_r$): 2.01 Horwitz ratio (H_r = $\%RSD/\%RSD_r$): 0.05 $H_r < 1$	Calibration plot: y = 0.499758x + 17.30592 Coefficient of determination: R^2 = 0.9994 Range: 1532.44 – 4032.74 mg/L (~59 – 155%) Number of determinations: 5 Standards	Representative chromatograms are provided of a solvent blank, an analytical standard (3.997 mg/mL) and the glyphosate active substance technical material (~4.0 mg/mL). The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no interfering peaks with the active substance peak. The data demonstrate the specificity of the method.

Conclusions

The analytical method used to determine the active substance content in the active substance technical material as manufactured was an in-house HPLC-UV coupled detection method. Data has been provided to address method specificity, linearity, accuracy, and precision. The method is considered fully validated in accordance with SANCO/3030/99 rev. 5 and no further consideration is needed.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Source 3

Report:	**KCA 1.11/001 Study No.OS-012, [redacted] (2009)**
Title:	Determination of Active Content and Impurity Profile of Glyphosate
Document No:	OS-012
Guidelines:	CIPAC
GLP:	Yes
Acceptability	Yes

Determination of glyphosate

The glyphosate content in the active substance technical material has been determined using an in-house liquid chromatography coupled UV detection method. Validation data generated as part of the 5-batch study to demonstrate the acceptability of the use of the method are presented below in the interests of completeness and transparency.

Principle of method

The glyphosate content in the glyphosate active substance technical material as manufactured was quantified by reverse phase HPLC with UV-vis detection at 196 nm

Preparation of standard solutions
Standard solutions were prepared by weighing various amounts of glyphosate analytical standard (approximately 13.5 mg, 16 mg, 20 mg, 22.5 mg, and 25.5 mg) into a 10 mL volumetric flask. The samples were dissolved, and the flasks made to the mark using the mobile phase buffer (4 g/L KH_2PO_4, 3 g/L H_3PO_4). This provided a working range of 1366.4 – 2555.9 mg/L of glyphosate (68.32- 127.80 % w/w of test sample concentration).

Preparation of test samples
Approximately 50 mg of test item from each of the give batches was weighed into 25 mL volumetric flasks. The samples were dissolved, and the flasks filled to the mark using the phosphate buffer mobile phase (4 g/L KH_2PO_4, 3 g/L H_3PO_4).

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	LC-10ADVP pimp, SIL-10ADVP Autosampler, SPD-M10AVP PDA Detector
Column:	Exsil Amino column: 250 x 4.6 mm
Mobile Phase:	Phosphate buffer (4 g/L KH_2PO_4, 3 g/L H_3PO_4).
Injection Volume:	20 µL
Flow Rate:	1.0 mL/min
Temperature:	30 ºC
Detection:	196 nm (UV)
Run Time:	11 minutes
Retention Time:	Glyphosate at ~6.1 minutes

All standard and test sample solutions are injected in duplicate.

Findings
The following method validation data for quantification of glyphosate content were reported:

Specificity
The selectivity of the HPLC method was assessed by examination of peak homogeneity and purity using a diode array detector. The examination of response at different wavelengths for blank samples, the glyphosate reference standard and the test item were compared. Examination of the UV spectra and determination of peak purity demonstrated that chromatographic peaks were not attributed to more than one component.
Linearity
The analytical calibration was performed using five analytical standards of glyphosate, ranging in concentration from 1366.4 – 2555.9 mg/L of glyphosate (68.32- 127.80 % w/w of test sample concentration). Overall, the calibration data provided is considered sufficient to demonstrate the method shows good linearity, where the correlation coefficient (r) is >0.99

Accuracy
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material.

Precision

According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material.

The repeatability (precision) of the method was assessed through the sevenfold determination of the active ingredient in a single batch of glyphosate technical material (AFS08/1973). The results obtained show a relative standard deviation of 0.2 % (mean value: 968 g/kg, number of values: 7) which indicates a good consistency of the data. (relative standard deviation acceptable limit: 1.34 %, according to SANCO 3030/99/rev 5).

Confirmation of analyte identity
It is not a requirement to confirm the identity of the active substance according to the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements laid down in Regulation (EC) No. 283/2013.

However, additional analysis on the active substance was performed as part of the 5-batch study and is referenced here in the interests of completeness and transparency. The identity of Glyphosate technical material within the five batches was confirmed by comparison with a reference item of Glyphosate by FTIR.

Table 4.1.1-1 (c)Summary of validation data for method CIPAC 284/TC/M/3 for quantification of glyphosate content in glyphosate active substance technical material as manufactured ([redacted] source)

LOQ (g/kg)	**Recovery Fortification Level (g/kg)**	**Recoveries % range (mean)***	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
SANCO/3030/99 rev. 5 states that according to Regulation (EU) No 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for the technical active substance.	SANCO/3030/99 rev. 5 states the determination of recovery for the active substance in the technical material is not required.		Mean content = 968 g/kg (96.8 % w/w) % RSD = 0.20 (7) Modified Horwitz value (%RSD_r): 2.010 Horwitz ratio (H_r = %RSD/%RSD_r): 0.1 $H_r < 1$	Calibration plot: y = 440.59x + 33026 Coefficient of determination: R^2 = 0.9991 Range: 1366.4 – 2555.9 mg/L (68.32-127.80 % w/w) Number of determinations: 5 Standards	Representative chromatograms are provided of a solvent blank, an analytical standard and the glyphosate active substance technical material. The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material (~6.1 mins). Comparison of the chromatograms show no interfering peaks with the active substance peak. The data demonstrate the specificity of the method.

Conclusions
The analytical method used to determine the active substance content in the active substance technical material as manufactured was an in-house HPLC-UV coupled detection method. Data has been provided to address method specificity, linearity, accuracy, and precision. The method is considered fully validated in accordance with SANCO/3030/99 rev. 5 and no further consideration is needed.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Source 4

Report:	**KCA section 1/021 - SSL04409: [redacted] (2010)**
Title:	Glyphosate TC Analytical Profile of 5 Batches
Document No:	SSL04409
GLP:	Yes
Acceptability	Yes

Determination of glyphosate

The glyphosate content in the active substance technical material has been determined according to the established validated method, CIPAC 284, handbook C, pp. 2132. Data requirement CA 4.1.1 of Regulation (EC) No 283/2013 states that *"In case of use of a CIPAC method, further validation data shall not be required, but example chromatograms shall be submitted, where available"*. Only the provision of example chromatograms generated using the method are therefore strictly necessary in accordance with the data requirements. However, additional validation data generated as part of the 5-batch study to demonstrate the acceptability of the method are presented below in the interests of completeness and transparency.

Principle of method

The glyphosate content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection, based upon the validated method CIPAC 284/TC/M/3.

Preparation of the mobile phase
The mobile phase was prepared by dissolving 3.37g KH_2PO_4 in 3.84 L of HPLC grade water. 160 mL of methanol was added to the solution and the pH adjusted to 9 using concentrated phosphoric acid

Preparation of standard solutions
The glyphosate standard was ground into a fine powder and dried overnight at 105 ºC. 392.32 mg of the dried material was weighed into a 100 mL volumetric flask and the flask filled to the mark with the previously described mobile phase.

Preparation of test samples
Approximately 400 mg of glyphosate technical material was weighed into a 100 mL volumetric flask and made up to volume with the mobile phase. The technical material was dissolved with magnetic stirring giving a test sample solution of the technical material of concentration 4 mg/mL.

Analysis of test samples
25 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Column:	Reprosil 80 SAX, 10 µm, 250 x 4.6 mm
Mobile Phase:	KH_2PO_4 0.084% / MeOH 4% / H_2O, pH 1.9
Injection Volume:	25 µL
Flow Rate:	2.3 mL/min
Temperature:	35 ºC
Detection:	195 nm (UV)
Run Time:	20 minutes
Retention Time:	Glyphosate at ~2.3 minutes

All standard and test sample solutions are injected in duplicate.

Findings

The following method validation data for quantification of glyphosate content using CIPAC 284 were reported:

Specificity
Representative chromatograms are provided of a solvent blank, an analytical standard (~3.92 mg/mL) and the glyphosate active substance technical material (~4.0 mg/mL). The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no interfering peaks with the active substance peak. The data are therefore sufficient to demonstrate the specificity of the method.

Linearity
No linearity data for the active substance is presented. As the analytical method being used is a validated CIPAC method, further validation data to demonstrate the method's linearity of response is not necessary in accordance with the requirements of Regulation (EC) No 283/2013. No further consideration is needed.

Accuracy
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material as manufactured. However, in the interests of completeness and transparency, the assessment of the method accuracy which was performed as part of the 5-batch analysis is presented here.

A solution of glyphosate analytical standard was prepared in the chromatographic mobile phase at a concentration of ~3.92 mg/mL. Fifteen determinations of the active substance content were performed according to CIPAC 284. The recoveries were in the range of 98.3-100.5% w/w (983-1005 g/kg) with a mean value 99.3 % w/w (993 g/kg), supporting the acceptability of the method accuracy.

Precision
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material.

Five determinations of the active substance content were made using a sample of the active substance technical material. The mean content of the active substance material was determined to be 97.6% (976 g/kg) with a %RSD of 0.43. The modified Horwitz equation for a sample concentration based on the mean recovery (976 g/kg) gives a value of 1.34. The experimentally derived %RSD and the modified Horwitz value ($\%RSD_r$) give a Howitz ratio (H_r) = 0.43/1.34 = 0.32. As $H_r < 1$, the method precision is concluded to be acceptable.

Confirmation of analyte identity
It is not a requirement to confirm the identity of the active substance according to the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements laid down in Regulation (EC) No. 283/2013.

However, in the interests of transparency and completeness it is highlighted that spectra were obtained through ^{1}H-NMR and ^{31}P-NMR for each of the five batches and glyphosate commercial reference.

Table 4.1.1-1 (d)Summary of validation data for method CIPAC 284 for quantification of glyphosate content in glyphosate active substance technical material as manufactured

LOQ (g/kg)	**Recovery Fortification Level (g/kg)**	**Recoveries % range (mean)***	**Repeatability % RSD (n)**	**Linearity**	**Specificity**

SANCO/3030/99 rev. 5 states that according to Regulation (EU) No 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for the technical active substance.	SANCO/3030/99 rev. 5 states the determination of recovery for the active substance in the technical material is not required. In the interests of completeness and transparency, the following recoveries data were reported for a sample of the glyphosate active substance technical material as manufactured: % Range = 98.3 – 100.5 Mean = 99.3 n = 15	Mean content = 976 g/kg (97.6 % w/w) % RSD = 0.43 (5) Modified Horwitz value (%RSD_r): 1.34 Horwitz ratio (H_r = %RSD/%RSD_r): 0.32 $H_r < 1$	No additional linearity data is presented. As the method is a validated CIPAC method, it is not a requirement according to Regulation (EC) No 283/2013 to provide further linearity of response data. No further consideration is therefore needed.	Representative chromatograms are provided of a solvent blank, an analytical standard (3.92 mg/mL) and the glyphosate active substance technical material (~4.0 mg/mL). The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material (~2.3 minutes). Comparison of the chromatograms show no interfering peaks with the active substance peak. The data demonstrate the specificity of the method.

Conclusions

The analytical method used to determine the active substance content in the active substance technical material as manufactured is a validated CIPAC method. In accordance with the data requirements of Regulation (EU) No 283/2013, only the provision of chromatograms, where available, to demonstrate the method specificity is required when a CIPAC method is used. Representative chromatograms are available which confirm the method specificity. Though not a formal requirement, in the interests of completeness and transparency, supplementary data to demonstrate the accuracy and precision of measurements are also presented. Overall, sufficient information is provided to confirm the acceptability of CIPAC 284 for quantification of glyphosate content in the glyphosate active substance technical material as manufactured. The method is considered fully validated in accordance with SANCO/3030/99 rev. 5 and no further consideration is needed.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Source 5

Report:	**KCA section 1/028 -038: [redacted] (2015)**
Title:	Qualitative and Quantitative Profile of the test substance Glyphosate Technical JiangNan (Five Batch Analysis)
Document No:	RF.14613.030.067.14
Guidelines:	Commission Regulation (EU) No 283/2013 of 1 March 2013 SOP-M 1573 – Estudo de Cinco Bateladas (Five Batch Analysis) – Revision 05
GLP:	Yes
Acceptability	Yes

Determination of glyphosate

Principle of method

The glyphosate content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection.

Preparation of standard solutions
A stock solution of the active ingredient was prepared by weighing of 41.92 mg of the glyphosate analytical standard in volumetric flask of 10 mL. The volume was completed with ultra pure water and the solution was stirred and sonicated until complete solubilization, producing the stock solution with 4171.04 $mg.L^{-1}$ of glyphosate analytical standard.

Preparation of test samples
Solutions of the test substance were prepared in duplicate by weighing of approximately 130 mg of sample in volumetric flask of 50 mL. The volumes were completed with ultra pure water and the solutions were stirred and sonicated until complete solubilization, producing work solutions with approximately 2600 $mg.L^{-1}$.

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1260 series HPLC
Column:	PRP-X 100, 250mm x 4.6mm, 10 µm
Mobile Phase:	Solution of monobasic potassium phosphate acidified with phosphoric acid + Methanol (100%)
Injection Volume:	20 µL
Flow Rate:	1.5 mL/min
Oven Temperature:	40ºC
Detection:	195 nm (UV)
Run Time:	10 minutes
Retention Time:	Glyphosate at ~4.0 minutes

Findings

The following method validation data for quantification of glyphosate content were reported:

Specificity
Representative chromatograms are provided of a solvent blank, 5 analytical standards (ranging between 834.21-4171.04 mg/L) and the glyphosate active substance technical material (~2550 mg/L). The peak position of the glyphosate analytical standard coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no interfering peaks with the active substance peak. The data are therefore sufficient to demonstrate the specificity of the method.

Linearity
The linearity of response was demonstrated by preparing 5 analytical standards at concentrations ranging from 834.21-4171.04 mg/L (corresponding to 32.1-160.4 % w/w, *i.e.* 321.0-1604 g/kg content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 0.362119x - 7.00785$

and a correlation coefficient (r) of 0.99997. The data meets the criteria of acceptability according to SANCO/3030/99 rev. 5. The method linearity therefore considered to have been adequately addressed.

Accuracy
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material as manufactured. However, in the interests of completeness and transparency, the assessment of the method accuracy which was performed as part of the 5-batch analysis is presented here.

A solution of glyphosate analytical standard was prepared in ultra-pure water at a concentration of ~4000 mg/L and further diluted to give recovery solutions of 3162.61 mg/L, 2530.09 mg/L and 1897.56 mg/L. Three determinations of the active substance content were performed according to the method described above for determination of glyphosate content. The recoveries were in the range of 100.35-101.67% w/w (1003.5-1016.5 g/kg) with a mean value 100.96 % w/w (1009.6 g/kg), supporting the acceptability of the method accuracy.

Precision
According to the criteria of SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013, it is not required to report recoveries (accuracy) data to support validation of methods for quantification of the active substance in the active substance technical material. However, in the interests of completeness and transparency, the assessment of precision of the measurements was performed as part of the 5-batch analysis is presented here.

Determinations of the active substance content were made using a sample of the active substance technical material and evaluated by seven replicates of sample determinations in duplicate. The mean content of the active substance material was determined to be 96.58% (965.8 g/kg) with a %RSD of 0.35. The modified Horwitz equation for a sample concentration based on the mean recovery (965.8 g/kg) gives a value of 1.35. The experimentally derived %RSD and the modified Horwitz value ($\%RSD_r$) give a Howitz ratio (H_r) = 0.35/1.35 = 0.26. As $H_r < 1$, the method precision is concluded to be acceptable.

Confirmation of analyte identity
It is not a requirement to confirm the identity of the active substance according to the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements laid down in Regulation (EC) No. 283/2013, however details of the confirmation of analyte identity are presented here for transparency and completeness. The active ingredient was identified through comparison of the test samples to that of the analytical standard for glyphosate, by mass spectrometry (HPLC-MS) and UV-Vis spectrophotometry (HPLC-UV) with peak purity obtained using a DAD detector. Further confirmation of identity was provided by Nuclear Magnetic Resonance (^{1}H-NMR) and Infrared spectroscopy (IR).

Table 4.1.1-1 (e)Summary of validation data for the method for quantification of glyphosate content in glyphosate active substance technical material as manufactured

LOQ (g/kg)	**Recovery Fortification Level (g/kg)**	**Recoveries % range (mean)***	**Repeatability % RSD (n)**	**Linearity**	**Specificity**

SANCO/3030/99 rev. 5 states that according to Regulation (EU) No 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for the technical active substance.	SANCO/3030/99 rev. 5 states the determination of recovery for the active substance in the technical material is not required. In the interests of completeness and transparency, the following recoveries data were reported for a sample of the glyphosate active substance technical material as manufactured: % Range = 100.35-101.67 Mean = 100.96 n = 3	Mean content = 965.8 g/kg (96.58 % w/w) % RSD = 0.35 (7 for each analyst)* Modified Horwitz value (%RSD_r): 1.35 Horwitz ratio (H_r = %RSD/%RSD_r): 0.26 $H_r < 1$	834.21 – 4171.04 mg/L 32.1 – 160.4 % of nominal content R=0.99997 (n=5)	Representative chromatograms are provided of a solvent blank, 5 analytical standards (ranging between 834.21-4171.04 mg/L) and the glyphosate active substance technical material (~2550 mg/L). The peak position of the glyphosate analytical standards coincides with the retention time of the peak from the glyphosate active substance technical material. Comparison of the chromatograms show no interfering peaks with the active substance peak. The data demonstrate the specificity of the method.

*Repeatability was evaluated by seven replicates of sample determinations made by 2 separate analysts. Therefore, the stated %RSD corresponds to each analyst overall.

Conclusions

Representative chromatograms are available which confirm the method specificity and sufficient linearity of response was demonstrated through analysis of 5 analytical standards. Though not a formal requirement, in the interests of completeness and transparency, supplementary data to demonstrate the accuracy and precision of measurements are also presented. Overall, sufficient information is provided to confirm the acceptability of the method for quantification of glyphosate content in the glyphosate active substance technical material as manufactured. The method is considered fully validated in accordance with SANCO/3030/99 rev. 5 and no further consideration is needed.

Assessment and conclusion by applicant:

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Assessment and conclusion by RMS: The analytical method used for the determination of glyphosate in technical substance is validated according to SANCO 3030/99 rev 5.

Albaugh

Analytical method for the determination of glyphosate in the technical material for the notifier Albaugh is provided and reported below.

Data point:	J-CA 1.11/017
Report author	[redacted]
Report year	2020
Report title	Analysis of five batches of Gloyphosate wet cake to determine the content of active ingredient and specified impurities, with associated method validation, in compliance with good laboratory practice
Report No	DNA5494
Document No	-
Guidelines followed in study	Not stated
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

Principle of method

The assay of Glyphosate was performed using approximately 0.05g of each batch of Technical Material (dried). The mass of the Technical Material was accurately recorded, transferred to a 50mL volumetric flask and made to partial volume with Deionised Water (5 samples in duplicate). These solutions were sonicated for 5 minutes and made to final volume once cooled. These solutions were then used for assay by injecting each solution once into the HPLC-DAD under the following conditions:

HPLC-DAD Conditions :

Instrument:	Agilent 1200 Series HPLC-DAD
Mode:	Isocratic Reverse Phase (Anion Exchange)
Column:	Phenomenex SAX, 250mm x 4.6mm
Packing:	SAX, 5µm
Eluent:	4.2185g Monopotassium Phosphate in 4800mL Deionised Water and 200mL Methanol adjusted to pH2 with Phosphoric Acid
Wavelength:	195nm
Injection Volume:	100µl
Flow Rate:	0.9mL/minute
Column Temperature:	25ºC
Data Collection:	LabSolutions
Retention Time:	Approximately 5.0 to 5.1 minutes

Validation

Specificity:
In the Specificity chromatograms, the Glyphosate eluted at 5.1 minutes. Other significant peaks were accounted for by assaying a solvent blank and reference standards for Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5, Impurity 6, Impurity 7, Impurity 8, Impurity 9 and Impurity 10. There were no significant peaks present in these chromatograms at the same elution time as the Glyphosate (Impurity 5 eluated at 6 minutes). This demonstrates that there were no analyte interferences.
The UV, MS, FTIR and NMR Spectra for Glyphosate confirm the species identification.

Linearity:
The linearity was determined from twenty-two injections of eleven concentrations of standard ranging from 0.0025mg/mL to 2.0mg/mL (corresponding to 0.25% to 200%). The samples were prepared for analysis at a sample concentration of 1.0mg/mL. From the sample assay it is known that the samples contain approximately 100% Glyphosate. This equates to a concentration of 1.0mg/mL, which falls within the limits of the linearity range. The mean area of each duplicate injection for each standard concentration has been plotted on a graph displayed below. The plot possesses a correlation coefficient of 1.0000, based on individual values.

Sample Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2):

To show the Sample Precision (Repetitiveness Assay 1), six samples of approximately 0.05g of Technical Material were weighed into a 50mL volumetric flask and made to partial volume with Deionised Water. The samples were sonicated for 5 minutes, made to volume with Deionised Water once cooled and injected into the HPLC-DAD. The results are indicated in the table below.

Intermediate Precision:

The results obtained from the Repetitiveness Assay 1 and Repetitiveness Assay 2 were combined to show the Sample Precision of the method and Percentage Relative Standard Deviation (%RSD) and Grubbs Test Criteria for the 12 Measurements. The results are indicated in the table below.

80% Recovery
80% Recovery samples were prepared for analysis at 0.80mg/mL using the certified reference standard material. This was achieved by weighing 0.02g Glyphosate reference standard into a 25mL volumetric flask and making to partial volume with Deionised Water. The samples were sonicated for 5 minutes and made to volume with Deionised Water once cooled. Six separate solutions were prepared in this way and then injected into the HPLC-DAD. The results indicate a percentage recovery range of 99.58% to 99.94%, a standard deviation of 0.147 and a relative standard deviation of 0.147. The results are summarized in the table below.

100% Recovery
100% Recovery samples were prepared for analysis at 1.0mg/mL using the certified reference standard material. This was achieved by weighing 0.025g Glyphosate reference standard into a 25mL volumetric flask and making to partial volume with Deionised Water. The samples were sonicated for 5 minutes and made to volume with Deionised Water once cooled. Six separate solutions were prepared in this way and then injected into the HPLC-DAD. The results indicate a percentage recovery range of 99.51% to 100.8%, a standard deviation of 0.472 and a relative standard deviation of 0.472. The results are summarized in the table below.

120% Recovery
120% Recovery samples were prepared for analysis at 1.20mg/mL using the certified reference standard material. This was achieved by weighing 0.03g Glyphosate reference standard into a 25mL volumetric flask and making to partial volume with Deionised Water. The samples were sonicated for 5 minutes and made to volume with Deionised Water once cooled. Six separate solutions were prepared in this way and then injected into the HPLC-DAD. The results indicate a percentage recovery range of 99.80% to 100.2%, a standard deviation of 0.152 and a relative standard deviation of 0.152. The results are summarized in the table below.

LOQ Recovery
The LOQ is defined as the lowest point on the linearity, which for Glyphosate is 0.0025mg/mL. This equates to 0.25% as the samples were prepared at 1.0mg/mL concentration.

LOQ Recovery samples were prepared for analysis at 0.0025mg/mL using the certified reference standard material. This was achieved by diluting the 100% Recoveries 1:400 by taking 125µL of this solution into a 50mL volumetric flask and making to volume with Deionised Water. Five separate solutions were prepared in this way and then injected into the HPLC-DAD. The results obtained indicate a percentage recovery range of 91.68% to 96.71% with a mean of 94.42%, a standard deviation of 2.112 and a percentage relative standard deviation of 2.237.

Validation summary Table – Active ingredient Glyphosate

Validation Parameter	Results Obtained	Acceptance Criteria under SANCO/3030/99 rev.5
Linearity	R^2 = 1.0000 n=11*2 0.25% to 200%)	R^2 = >0.99
Sample Precision (Repetitiveness Assay 1)	%RSD = 0.539 Hr = 0.401 n = 6	Horwitz %RSD less than 1.34 Horrat (Hr) ≤ 1 at 97.84% (w/w)
Sample Precision	%RSD = 0.357	Horwitz %RSD less than 1.34

(Repetitiveness Assay 2)	Hr = 0.265 n = 6	Horrat (Hr) ≤ 1 at 97.74% (w/w)
Sample Precision (Intermediate Precision)	%RSD = 0.418 Hr = 0.311 n = 12	Horwitz %RSD less than 1.34 Horrat (Hr) ≤ 1 at 97.79% (w/w)
Grubbs Test (maximum) G_n	G_n = 1.788	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
Grubbs Test (minimum) G_1	G_1 = 1.354	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
80% Recovery at 0.80mg/mL	Mean Recovery = 99.82% %RSD = 0.147 Hr = 0.106 n = 6	Between 97%-103% Horwitz %RSD less than 1.39 Horrat (Hr) ≤ 1 at 79.85% (w/w)
100% Recovery at 1.0mg/mL	Mean Recovery = 100.0% %RSD = 0.472 Hr = 0.352 n = 6	Between 97%-103% Horwitz %RSD less than 1.34 Horrat (Hr) ≤ 1 at 100.0% (w/w)
120% Recovery at 1.20mg/mL	Mean Recovery = 100.1% %RSD = 0.152 Hr = 0.116 n = 6	Between 97%-103% Horwitz %RSD less than 1.34 Horrat (Hr) ≤ 1 at 120.1% (w/w)
LOQ Recovery at 0.25%	Mean Recovery = 94.42% %RSD = 2.237 Hr = 0.672 n = 5	Between 80%-120% Horwitz %RSD less than 3.33 Horrat (Hr) ≤ 1 at 0.236% (w/w)
Theoretical Limit of Quantification (LOQ)	LQ = 0.216%	Based on 10 times (S/R) (Signal-to-Noise ratio)

Assessment and conclusion

Assessment and conclusion by applicant:

Assessment and conclusion by RMS: The analytical method for the determination of glyphosate in glyphosate technical material is validated in accordance with SANCO 3030/99 rev 5.

Sinon ([redacted] Source and [redacted] source)

[redacted] source:

Analytical method for the determination of glyphosate in the technical material (Method No. LCG01) for the [redacted] source is provided and reported below.

Data point:	J-CA 4.1.1/023
Report author	[redacted]
Report year	2020
Report title	Five Batches Analysis of Technical Grade Active Ingredient (TGAI) Glyphosate
Report No	SNCL Report No. SB03
Document No	-
Guidelines followed in study	Not stated
Deviations from current test guideline	Not applicable

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

Principle of method

The glyphosate-containing technical material is diluted in buffer solution (potassium dihydrogen phosphate, pH 1.9 – 2.2) and injected into an HPLC-DAD. Quantification is performed using peak area responses from a UV detector and external standards calibration.

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

HPLC system:	Agilent 1100 HPLC system with DAD detector
Column:	5 SB (100A), 4.6 mm x 250 mm or 10 SB (100A) 4.6 mm x 250 mm or equivalent
Column temperature:	30 °C
Mobile phase:	Isocratic: buffer solution (potassium dihydrogen phosphate in deionized water and methanol, adjusted to pH 1.90 – 2.20 with 85 % phosphoric acid)
Flow rate:	1.5 mL/min
Injection volume:	50 µL
Detector:	DAD detection
Wavelength:	195 nm
Retention time:	Approx. 6.9 min (for 5SB) 3.3 min (for 10SB)

Validation

Specificity:
No interference was observed. The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the samples. There was no impurities or interferences co-eluted with the analyte of interest.

The glyphosate analytical standards and technical glyphosate batches were confirmed by IR spectrum, UV-Vis spectrum, LC-MS, ^{1}H and ^{13}C-NMR spectrum. Glyphosate in technical material was further confirmed by retention time with comparison to analytical standard.
The DAD spectrum and chromatograms of blank, standard and a glyphosate technical sample and spiked samples have been provided.

Linearity
Linearity was determined with at 5 standard levels containing glyphosate with correlation coefficient > 0.99.

Linearity data for glyphosate

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Glyphosate	193.33 – 966.67 mg/L (equivalent to 32% - 162%)	y = 0.7009 x – 4.3767	1.0000

Accuracy:

The recovery test for glyphosate was performed by standard addition method. One of one batch of the test substances (batch no. SNGA1910010) was prepared and spiked with three different levels of standard solutions.

Accuracy data for glyphosate

Analyte	Fortification level (mg/L)	No of replicates	Mean recovery (%)	RSD (%)
Glyphosate	386.67 (65 % w/w)	2	99.70	N/A
	580.00 (97 % w/w)	2	100.00	N/A
	773.34 (130 % w/w)	2	100.06	N/A
	Total sum	6	99.92	0.514

N/A: not applicable

Repeatability (precision):
The system precision (%RSD) was found to be 0.13 by analysing standard solution with concentration of 580.00 mg/L. To evaluate sample repeatability a single batch of glyphosate (batch no. SNGA1910010) was analysed six times.

Repeatability data for glyphosate

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	97.28	6	0.13	0.096

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

Assessment and conclusion

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate in glyphosate technical material ([redacted] source) as manufactured was not previously evaluated at EU level. It was performed under GLP and meets the criteria stated in the current guidance document SANCO/3030/99 rev. 5. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS:

The analytical method for the determination of glyphosate in glyphosate technical material is validated in accordance with SANCO 3030/99 rev 5.

[redacted] source:

Analytical method for the determination of glyphosate in the technical material (CIPAC Method 284/TC/M/3) for the [redacted] source is provided and reported below.

Data point:	J-CA 4.1.1/024
Report author	[redacted]
Report year	2017
Report title	5-Batch analysis of glyphosate TGAI in accordance with regulation (EC) No. 1107/2009, referencing SANCO 3030/99 rev. 4
Report No	AN16111117
Document No	-
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

Principle of method

The glyphosate-containing technical material is diluted in mobile phase solution (96:4 (v/v) HPLC Grade water with potassium dihydrogen phosphate (0.8437 g/L) / methanol, adjusted to pH 1.9 using orthophosphoric acid) and injected into an HPLC-UV. Quantification is performed using peak area responses from a UV detector.

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

HPLC system:	Agilent 1100 series HPLC
Column:	Hichrom Partisil 5 SAX, 250 × 4.6 mm, 5 µm
Column temperature:	Ambient
Mobile phase:	Isocratic: 96:4 (v/v) HPLC Grade water with potassium dihydrogen phosphate (0.8437 g/L) / methanol, adjusted to pH 1.9 using orthophosphoric acid
Flow rate:	2.3 mL/min
Injection volume:	50 µL
Detector:	UV detection
Wavelength:	195 nm
Retention time:	Approx. 2.8 min

Validation

Specificity:
No interference was observed. The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the samples. There was no impurities or interferences co-eluted with the analyte of interest.

Each of 5 production batches of glyphosate technical materials and glyphosate reference standard employed during the course of the study were characterised using Infrared Spectroscopy (IR).

Chromatograms of a glyphosate analytical reference standard, of each glyphosate TGAI batch and of a blank have been provided.

Linearity:
The linearity of active substance is not established since a calibration bracketing technique was employed (Standard, sample, sample, sample, QC, Standard, etc). Content of active substance is quantified based on response factors calculated from peak area between analytical standard and sample.

Accuracy:
A concentration of 3.999 mg/mL (ca 100 % w/w equivalent) glyphosate sample solution (batch no. 5884100) was analysed five times to determine the assay accuracy.

Accuracy data for glyphosate

Analyte	Nominal concentration (% w/w)	No of replicates	Mean recovery (%)	RSD (%)
Glyphosate	100	5	99.98	0.19

Repeatability (precision):
A concentration of 3.999 mg/mL (ca 100 % w/w equivalent) glyphosate sample solution (batch no. 5884100) was analysed five times to determine the assay precision.

Repeatability data for glyphosate

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Glyphosate	100	5	0.19	0.142

[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

Assessment and conclusion

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate in glyphosate technical material ([redacted] source) was not previously evaluated 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 with the applied test guidelines were reported. The method is suitable for the determination of glyphosate impurities in glyphosate technical material.

Assessment and conclusion by RMS:

The analytical method for the determination of glyphosate in glyphosate technical material (CIPAC Method 284/TC/M/3) is validated in accordance with SANCO 3030/99 rev 5 in term of specificity, and accuracy. For linearity, the calibration bracketing technique was employed. RMS considered that it is not sufficient to demonstrate the linearity. Nevertheless, the method used is a CIPAC method, a full validation of the linearity is not necessary. In consequence, no futher data is necessary.

B.5.1.1.2 Method for the analysis of relevant impurities in the active substance as manufactured

Barclay

Source 1

Data point:	J-CA 4.1.1/001
Report author	[redacted]
Report year	2009f

Report title	Determination of active content and impurity profile of glyphosate
Report No	OS-012
Document No	-
Guidelines followed in study	OPPTS 830.1700; OPPTS 830.1800 SANCO 3030/99 rev. 5
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes (further data is required)
Category study in AIR 5 dossier (L docs)	Category 1

Principle of the method

A method for determination of the content of formaldehyde in technical grade material by HPLC-UV has been adapted from an FAO recommended method.
The Hantzsch reagent reacted with formaldehyde present in aqueous glyphosate solutions. The resulting derivative, diacetyldihydrolutidine or DDL, was determined by reversed phase HPLC with UV detection.
Quantitation was based on the area of the DDL peak. This response was compared to the response of external standards prepared in the same manner as the samples. Samples and standards were dissolved in Milli-Q water and the solutions were analysed by reverse phase HPLC using an UV detector at 412 nm.
Confirmation of identity of the analyte is performed by comparison of retention time of standards solution and spiked samples.

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

HPLC system:	LC-10ADVP Pump, SIL-10ADVP Autosampler, SPD-M10AVP, PDA Detector (Shimadzu)
Column:	Alltima C 18, 4.6 × 250 mm
Column temperature:	30 °C
Mobile phase:	Water/Acetonitrile = 70/30
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detector:	PDA
Wavelength:	412 nm
Retention time:	Approx. 5.9 min

Findings

Specificity:
The combination of the HPLC column, mobile phase composition and the column temperature was designed to ensure separation of the active substance from any impurities that possibly could interfere with the determination of the active substance. The selectivity of the HPLC method was assessed by examination of peak homogeneity and peak purity using diode array detector. Chromatograms of standards solution of solvent blank, of samples and fortified samples are provided. No interference is observed at the retention time of the analyte.

Linearity

Linearity was determined with at six standard levels containing impurities with correlation coefficient > 0.99. The standard solutions were prepared in the same manner than the test item solution wuth derivatisation step.

Table 4.1.1-16: Linearity data for formaldehyde

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r^2)
Formaldehyde	1.280 – 12.80 mg/L (equivalent to 0.35 g/kg - 3.5 g/kg)	y = 137670 x - 30105	0.9999

Accuracy

The standard addition method was utilised. Sample AFS08/1973 (batch no. 080527-05) was spiked with a known amount of analyte standards at three levels.

Table 4.1.1-17: Accuracy data

Analyte	Fortification level (g/kg)	No of replicates	Recovery (%)	RSD (%)
Formaldehyde	1.15	1	106	N/A
	1.19	1	109	N/A
	1.55	1	103	N/A

N/A: not applicable

Repeatability (precision)

System precision was evaluated by injecting seven times of impurity standards with concertation of approximate concentration 10.24 mg/L (2.8g/kg) with %RSD of 0.41.

Analyte	Mean Area (mAUsec)	No of replicates	RSD (%)
formaldehyde	1380290	7	0.41

To evaluate sample repeatability a single batch of glyphosate technical (AFS08/1973, batch no. 080527-01) was analysed seven times and each of the remaining batches were analysed in duplicate.

Table 4.1.1-18: Repeatability data

Analyte	Mean content (g/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	<LOQ	7	-	-

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

As formaldehyde was not detected in the test samples, Horwitz value could not be calculated. The repeatability using fortified samples should have been performed to confirm the precision sample. Nevertheless, base on the recovery data at 1.15, 1.19 and 1.55 g/kg the precision can be calculated. .

Recoveries%	Mean recovery%	RSD%	RSDr%	Horrat
106, 109, 103	106	2.83%	3.64%	0.77

Horrat value (Hr) = %RSD/%RSDr (Horwitz equation %RSDr = 0.67 * 2(1-0.5*log(c))), calculated from values given in the report for the purposes of the SANCO 3030/99 rev. 5 requirements. The concentration c is the mean value of the three fortification level, which is 1.30g/kg.

Limit of Quantification

For the LOQ, a standards solution of approximately 1.280mg/L each was injected seven times and mean area, standards deviation and coefficient of variation were determined. Results are reported below:

Analyte	Fortification at 1.280mg/L	No of replicates	RSD (%)	Horrat value (Hr)
formaldehyde	0.35g/kg	7	1.07	0.24

The LOQ level for formaldehyde is 0.35 g/kg.

Note: No recovery data are determined at the the LOQ. Data required.

Conclusion
The analytical method for determination of relevant impurity formaldehyde in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:
The validation of the method for analysis of formaldehyde in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of formaldehyde in glyphosate technical material

Assessment and conclusion by RMS: The analytical method for determination of relevant impurity formaldehyde in glyphosate technical material has been partially validated in accordance with SANCO/3030/99 rev. 5.
The LOQ corresponds to the level for which the recovery and repeatabily are validated. The LOQ proposed by applicant show a validated repeatability but no recovery has been performed at this level. Therefore the LOQ cannot be validated without further data. Recovery data at this level 0.35 g/kg should be provided.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Analytical method for determination of relevant impurity ***N*-Nitroso-glyphosate (NNG)**

Principle of the method

The method was based on a FAO recommended method and adjusted to the test facility equipment. NNG was derivatised by reaction with hydrobromic acid to form nitrosyl cation. Nitrosyl cation then reacted with *N*-(1-naphthyl)ethylenediamine and sulphanilamide to form a purple azo dye which was detected with HPLC using an UV detector at 480 nm. Confirmation of identity of the analyte is performed by comparison of retention time of standards solution and spiked samples.

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

HPLC system:	LC-10ADVP Pump, SIL-10ADVP Autosampler, SPD-M10AVP, PDA Detector (Shimadzu)
Column:	Alltima C 18, 4.6 × 250 mm
Column temperature:	40 °C
Mobile phase:	Mobile phase A: Acetonitrile Mobile phase B: 50 mM CH_3COONH_4 pH 6.4 Time (min) %A %B 0 25 75 12 30 70 15 40 60 20 25 75
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detector:	PDA
Wavelength:	480 nm
Retention time:	Approx. 18.3 min

Specificity:
Chromatograms of standards solution of solvent blank, of samples and fortified samples are provided. No interference is observed at the retention time of the analyte.

Linearity
Linearity was determined with at six standard levels containing impurities with correlation coefficient > 0.99. The standard solutions were prepared in the same manner than the test item solution with derivatisation step.

Table 4.1.1-19: Linearity data for *N*-Nitroso-glyphosate (NNG)

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r^2)
NNG	0.047 – 0.748 mg/L (equivalent to 0.54 – 8.59 mg/kg)	y = 141939 x – 821.88	0.9999

Accuracy
The standard addition method was utilised. Sample AFS08/1973 (batch no. 080527-05) was spiked with a known amount of analyte standards at three levels.

Table 4.1.1-20: Accuracy data

Analyte	Fortification level (mg/L)	No of replicates	Recovery (%)	RSD (%)
NNG	0.751mg/kg	1	94	N/A
	0.854 mg/kg	1	97	N/A
	1.004 mg/kg	1	94	N/A

N/A: not applicable

Repeatability (precision)
System precision was evaluated by injecting seven times of impurity standards with concertation of approximate concentration 0.561 mg/L (6.44mg/kg) with %RSD of 0.44.

Analyte	Mean Area (mAUsec)	No of replicates	RSD (%)	RSDr
NNG	78742	7	0.44	1.46

To evaluate sample repeatability a single batch of glyphosate technical (AFS08/1973, batch no. 080527-01) was analysed seven times and each of the remaining batches were analysed in duplicate.

Table 4.1.1-21: Repeatability data

Analyte	Mean content (g/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
NNG	<LOQ	3	-	-

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

As NNG was not detected in the test samples, Horwitz value could not be calculated.

The repeatability using fortified samples should have been performed to confirm the precision sample. Nevertheless, base on the recovery data at 0.751, 0.854, 1.004 the precision can be calculated by compilation.

Recoveries%	Mean recovery%	RSD%	RSDr%	Horrat
94, 97, 94	95	1.82%	7.74%	0.23

Horrat value (Hr) = %RSD/%RSDr (Horwitz equation %RSDr = 0.67 * 2(1-0.5*log(c))), calculated from values given in the report for the purposes of the SANCO 3030/99 rev. 5 requirements. The concentration c is the mean value of the three fortification level, which is 0.000869g/kg.

Limit of Quantification

For the LOQ, a standards solution of approximately 0.047mg/L (0.54mg/kg) each was injected seven times and mean area, standards deviation and coefficient of variation were determined. Results are reported below:

Analyte	Fortification at 0.047mg/L	No of replicates	RSD (%)
NNG	0.54mg/kg	7	2.27

The LOQ level for NNG is 0.54 mg/kg.

Note: No recovery data are determined at the the LOQ. Data required

Conclusion

The analytical method for determination of relevant impurity NNG in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:

The validation of the method for analysis of NNG in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of NNG in glyphosate technical material

Assessment and conclusion by RMS:

The analytical method for determination of relevant impurity NNG in glyphosate technical material has been partially validated in accordance with SANCO/3030/99 rev. 5.

The LOQ corresponds to the level for which the recovery and repeatabily are validated. The LOQ proposed by applicant show a validated repeatability but no recovery has been perfpormed at this level. Therefore the LOQ cannot be validated without further data. Recovery data at this level 0.54mg/kg should be provided

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Source 2

Data point:	J-CA 4.1.1/002
Report author	[redacted]
Report year	2008
Report title	Qualitative and quantitative profile of the test substance glyphosate technical (five batch analysis)
Report No	3996.030.288.07
Document No	-
Guidelines followed in study	EPA OPPTS 830.1700 EPA OPPTS 830.1000
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

Analytical method for the determination of the relevant impurity **formaldehyde**

Principle of the method

The methodology for the determination of formaldehyde comprised the following stages: solubilization in water, derivatization with Hantzch reagent, and separation using HPLC and detection by UV. The responses were obtained by external calibration. This method describes a liquid chromatographic procedure for the selective determination of formaldehyde in glyphosate samples. The Hantzch reagent was used to react with formaldehyde present in aqueous glyphosate solutions. The standard solutions and samples solutions were prepared freshly for every analysis.

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

HPLC system:	HPLC-UV
Column:	ACE C18, 4.0 × 250 mm, 5 µm
Column temperature:	45 °C
Mobile phase:	Isocratic Mode – A : B (20/80 v/v) Solvent A: acetonitrile Solvent B: water
Flow rate:	0.4 mL/min
Injection volume:	5.0 µL
Detector:	UV detection
Wavelength:	412 nm
Retention time:	Approx. 4.9 min

Specificity:

Chromatograms of standards solution, of samples of fortified samples and blank are provided. No interference is observed at the retention time of formaldehyde. The identity of formaldehyde standard was confirmed by UV, MS (LC-MS/MS), ^{1}H-NMR and IR spectroscopy.

Linearity
Linearity was determined with at five standard levels containing formaldehyde derivatized with Hantzch reagent combining equal volumes of standard solution and Hantzch reagent. Correlation coefficient is > 0.99.

Table 4.1.1-22: Linearity data for formaldehyde

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r^2)
Formaldehyde	0.31 – 12.91 mg/L (0.05 to 1.0g/kg)	y = 94997 x – 787.845	0.9999569

Accuracy
The recovery test was evaluated by spiking method. A sample was assayed, a known amount of analytical standard was added, and the sample was again assayed. The recovery was determined two times at three concentration levels.

Table 4.1.1-23: Accuracy data

Analyte	Fortification level	No of replicates	Mean recovery (%)	RSD (%)
Formaldehyde	0.00031 g/L (0.05 g/kg)	2	104.08	-
	0.00129 g/L (0.2 g/kg)	2	99.92	-
	0.0646 g/L (1.0 g/kg)	2	103.18	-

N/A: not applicable

Repeatability (precision)
The system precision of the HPLC-UV determination of the concentrations of impurities in a solution was assessed by making repeated injections of a single solution (0.00129 g/L) (n=10) prepared from analytical standard of formaldehyde. %RSD was found to be 0.56.

The Intermediate precision of the HPLC-UV determination of the concentrations of formaldehyde in the test substance technical grade was assessed from the results obtained for identical samples. These solutions were prepared by two different analysts working independently. Both analysts followed the same procedure.

Table 4.1.1-24: Repeatability data

Analyte	Mean content (g/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	0.11	20	5.46	1.033

[1] Horrat value (Hr) = %RSD/%RSD$_r$ (Horwitz equation %RSD$_r$ = 0.67 * $2^{(1\text{-}0\ 5*\log(c))}$), calculated from values given in the report for the purposes of the SANCO 3030/99 rev. 5 requirements

Note: The precision of the method on fortified samples should be demonstrated. **Data gap**

Limit of Quantification (Limit of Detection)
The LOQ corresponds to the level for which the recovery and repeatabily are validated. According to the guidance document SANCO/3030/99 re.5, the repeatability is validated at the level of 0.11g/kg. No recovery has been measured at this level. However, the recoveries have been performed at 0.05 g/kg and at 0.2g/kg and are validated. Therefore, the LOQ level for formaldehyde can be set at 0.11g/kg

Conclusion
The analytical method for determination of relevant impurity formaldehyde in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:
The validation of the method for analysis of formaldehyde in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline

SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of formaldehyde in glyphosate technical material

Assessment and conclusion by RMS: The analytical method for determination of relevant impurity formaldehyde in glyphosate technical material is validated with an LOQ of 0.11g/kg according to the guidance document SANCO/3030/99 rev.5. The precision of the method on fortified samples should be demonstrated. Data gap

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Analytical method for the determination of the relevant impurity ***N*-Nitroso-glyphosate (NNG)**

Principle of the method

The methodology for the determination of impurity glyphosate-*N*-nitroso comprised the following stages: solubilization of glyphosate in water, acidification of the medium with 6N HCl and polarographic measure with a dropping mercury electrode (DME) of -0.50V to 0.98V. The responses were calibrated by an external standard.

Details to polarographic parameters are summarised below.

Polarograph:	757VA Computrace-Metrohm
Potential	- 0.50 a- 0.98 V
Voltage step time	0.005 V
Pulse amplitude:	0.1 V
Pulse time	0.04 s
Mode	Differential pulse (DP)
Eletrolite	6N HCl

Findings

Specificity:
The selectivity of the method was tested by evaluation of the polarographic curve (samples and standards solution) of an acid solution prepared with water and 6N HCl in the same manner used for samples and at the same voltage of the NNG response. The polarogram was recorded under the same conditions as for the calibration solutions, and inspected carefully for any evidence of interference at the half wave voltage of NNG. The specificity of NNG is demonstrated by the comparison of NNG response in the polarogram of the standards solution and the sample at the same voltage. There were no interference peaks at the half wave of NNG. Thus the method is selective for this substance. The identity of NNG standard was confirmed by UV, MS (LC-MS/MS), ^{1}H-NMR and IR spectroscopy.

Linearity
Linearity was determined with at six standard levels containing impurities with correlation coefficient > 0.99.

Table 4.1.1-25: Linearity data for *N*-Nitroso-glyphosate (NNG)

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r^2)
NNG	0.2 – 8.6 mg/L	y = 97.776 x – 4.6621	0.9986

Note: the calibration range in % or g/kg is missing and required.

Accuracy

For this test, three solutions of technical Glyphosate (2.5 g in 10 mL of HCl 6N) were spiked with NNG resulting solutions with concentrations of 0.70 ng/µL. These solutions were analysed. The procedure of preparation of this solution was repeated nine times.

Table 4.1.1-26: Accuracy data

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
NNG	2.8 (0.700ng/µL)	9	93.11	2.22

Repeatability (precision)

System precision was evaluated by analyzing nine times of a single solution prepared from analytical standard of NNG of 0.7 ng/µL. The %RSD was found to be 1.12.

The sample precision of the polarographic determination of the concentrations of NNG in the reference technical grade test substance was assessed from the results obtained for three samples of NNG of nominal concentration 0.700 ng/µL of the test substance in two different days. In each day each the determinations of NNG concentration were evaluated in triplicate.

Table 4.1.1-27: Repeatability data

Analyte	Mean content (mg/kg)	No of replicates[1]	Mean %	RSD (%)	Horrat value (H_r)[2]
NNG	2.8 (0.700ng/µL)	10x2	90.34	4.06	0.446

[1] 10 measurements in two different days,

[2] Horrat value (Hr) = %RSD/%RSD$_r$ (Horwitz equation %RSD$_r$ = 0.67 * $2^{(1\text{-}0\ 5*\log(c))}$), calculated from values given in the report for the purposes of the SANCO 3030/99 rev. 5 requirements

Limit of Quantification

The limit of quantification of the method (LOQ) was evaluated from the practical limit of detection. The practical limit of detection was determined by successive dilutions of the standard solution of Glyphosate-N-nitroso arriving to the lowest concentration that gives a signal at the polarographic of voltage corresponding to the reduction at –0.50 V to 0.98V. This limit was determined to be 0.2 mg L-1 that corresponded to a signal that can be distinguished from the base line. The LOQ level for NNG is 0.8 mg/kg. However, according to the guidance document SANCO/3030/99 re.5, the LOQ corresponds to the level for which the recovery and repeatabily are validated. Therefore, base on available data, the LOQ should be set at 2.8 mg/kg.

Conclusion

The analytical method for determination of relevant impurity NNG in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:

The validation of the method for analysis of NNG in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of NNG in glyphosate technical material

Assessment and conclusion by RMS: The analytical method for determination of relevant impurity NNG in glyphosate technical material has been provided. The method is considered validated for the determination of the impurity. Base on available data, the LOQ should be set at 2.8 mg/kg that higher than the specification limit. Therefore, additional data should be provided to validate the method with an LOQ un agreement with the specification level 1mg/kg

Note that the method is not a common method and a new method is required in case of monitoring purpose.

Source 3

Data point:	J-CA 4.1.1/005
Report author	[redacted]
Report year	2017
Report title	Qualitative and quantitative profile of the test substance glyphosate technical HDF (five batch analysis)
Report No	15846.030.002.16
Document No	-
Guidelines followed in study	EPA OPPTS 830.1700 EPA OPPTS 830.1800 EPA OPPTS 830.1000 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

Analytical method for the determination of relevant impurity **formaldehyde**

Principle of the method

The relevant impurity formaldehyde was quantified in glyphosate technical by use of liquid chromatography coupled to UV-visible detector. The quantities of Formaldehyde in sample solutions were determined by external calibration method. The methodology for the determination of formaldehyde comprised the following stages: solubilization in water, derivatization with reagent Hantzsch, separation using HPLC and detection by UV-visible

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

HPLC system:	Liquid Chromatograph Agilent 1100 series
Column:	Phenomenex Luna C18, 2.0 × 250 mm, 5 µm
Column temperature:	40 °C
Mobile phase:	Ultra pure water / Acetonitrile (80:20 % v/v)
Flow rate:	0.4 mL/min
Injection volume:	20 µL
Detector:	UV detection
Wavelength:	412 nm
Retention time:	Approx. 5.0 min

Specificity

The selectivity of the analytical method is demonstrated by injection of the same solvent used in the samples preparation. Chromatograms of blank, of standards solution, of sample and fortified samples are provided. There was no interference peak at the retention time of formaldehyde. Thus the method is selective to determine this impurity. The specificity for impurity was assured by analysis of the UV spectrum of formaldehyde, which shows that the peak is pure; therefore, there is no other compound co-eluting with it. The relevant impurity formaldehyde was not detected in the samples, therefore, the identification was based only on the ultraviolet and infrared spectra of its analytical standard.

Linearity
Linearity was determined with at seven standard levels containing impurities with correlation coefficient > 0.99. The standard solutions were prepared in the same manner than the test item solution wuth derivatisation step.

Table 4.1.1-28: Linearity data for formaldehyde

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Formaldehyde	1.01 – 10.12 mg/L (equivalent to 0.033 – 0.33% w/w)	y = 768.23444 x + 22.19384	0.99998

Accuracy
The recovery test for the impurity formaldehyde was performed by standard addition method. Three solutions of a batch of the test substance (20160301) were prepared and spiked with three different levels.
All these solutions were derivatized with the Hantzsch reagent by combining equal volumes of sample solution and reagent Hantzsch, the solutions reposed for two hours before analysis.

Table 4.1.1-29: Accuracy data

Analyte	Fortification level (% w/w)	No of replicates	Mean recovery (%)	RSD (%)
Formaldehyde	0.1	1	99.09	N/A
	0.16	1	98.67	N/A
	0.27	1	98.99	N/A

N/A: not applicable

Repeatability (precision)
The repeatability test is evaluated by seven replicates of sample determinations. The same sample (batch 20160301) is prepared and analyzed by the same analyst (Repeatability 1). A second analyst also prepares and analyzes the sample seven times (Repeatability 2). The intermediate precision test is evaluated by combination of the data obtained in the Repeatability test (Repeatability 1 – Analyst 1 and Repeatability 2 – Analyst 2). The impurity formaldehyde was not detected in the test substance; therefore, the repeatability test for this impurity was performed by standard addition method. All these solutions were derivatized with the Hantzsch reagent by combining equal volumes of sample solution and reagent Hantzsch, the solutions reposed for two hours before analysis.

Table 4.1.1-30: Repeatability data

Analyte	Mean content (g/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	1.5350	7	3.50	0.986
	1.5407	7	2.75	0.775
	1.5379	**14**	**3.02**	**0.850**

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

Note: The precision of the method on fortified samples should be demonstrated. **Data gap**

Limit of Quantification (Limit of Detection)
The limit of quantification of the method was evaluated by injecting of six replicates of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. The limit of quantification was defined as the lowest concentration used in the calibration range.

Analyte	content (g/kg)	No of replicates	Mean recoveries	RSD (%)
Formaldehyde	0.3367	6	100.84	0.45

The LOQ of Formaldehyde is 0.3367 g/kg (LOD of 0.0087 g/kg)

Conclusion
The analytical method for determination of impurity formaldehyde in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:
The validation of the method for analysis of formaldehyde in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of formaldehyde in glyphosate technical material

Assessment and conclusion by RMS: The analytical method for determination of impurity formaldehyde in glyphosate technical material has been validated with an LOQ of 0.33g/kg in accordance with SANCO/3030/99 rev. 5. The precision of the method on fortified samples should be demonstrated. Data gap

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Analytical method for the determination of relevant impurity ***N*-nitrosoglyphosate (NNG)**

Principle of the method

The relevant impurity *N*-nitrosoglyphosate (NNG) in Glyphosate technical was quantified by use of liquid chromatography coupled to mass spectrometer (HPLC/MS) at selected ion monitoring (SIM) mode. The concentrations of impurity in the sample solutions were determined by external calibration method after derivatization with NED/HBr and sulphanilamide.

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

HPLC system:	Liquid chromatograph Agilent 1100 series
Column:	Phenomenex Luna C18, 2.0 × 250 mm, 5 µm
Column temperature:	40 °C
Mobile phase:	Mobile phase A: acetonitrile Mobile phase B: solution of ammonium acetate (10 mM) acidified with 0.1% (v/v) of acetic acid Time (min) %A %B 0 15 85 7 20 80 12 50 50 25 50 50
Flow rate:	0.3 mL/min
Injection volume:	10 µL
Detector:	MS detection with SIM mode
Ionization mode:	ESI
Monitored ion:	368 m/z
Retention time:	Approx. 15.2 min

Specificity

The selectivity of the analytical method is demonstrated by injection of reagent blank used in the samples preparation. Chromatograms of blank, of standards solution, of sample and fortified samples are provided. There was no interference peak at the retention time of NNG. Thus the method is selective to determine this impurity. The specificity for this impurity was assured by the technique employed in the analysis (HPLC/MS), which monitors a specific ion; therefore, there is no other compound with different mass-to-charge ratio being analyzed by this method. The identification of relevant impurity NNG was based on retention time of the chromatographic peak attributed to this impurity. Furthermore, the identity was confirmed by the recovery test in the validation method, the mass spectrum (HPLC/MS) at Scan mode and Infrared spectrum of the analytical standard.

Linearity

Linearity was determined witht seven standard levels containing impurities with correlation coefficient > 0.99. The standard solutions were prepared in the same manner than the test item solution wuth derivatisation step.

Table 4.1.1-31: Linearity data for NNG

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
NNG	56.52 – 314.00 µg/L (equivalent to 0.000037 – 0.00037 %w/w)	y = 280.18634 x + 2082.04366	0.99551

Accuracy

The recovery test for the impurity NNG was performed by standard addition method. Three solutions of a batch of the test substance (20160218) were prepared and spiked with three different levels of standard solution derivatized with NED/HBr and sulphanilamide solution by heating to 95 °C for 15 minutes using the dry block.

Table 4.1.1-32: Accuracy data

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
NNG	0.4	1	89.28	N/A
	1.0	1	86.08	N/A
	1.3	1	81.72	N/A

N/A: not applicable

Repeatability (precision)

The repeatability test is evaluated by seven replicates of sample determinations. The same sample (batch 20160218) is prepared and analyzed by the same analyst (Repeatability 1). A second analyst also prepares and analyzes the sample seven times (Repeatability 2). The intermediate precision test is evaluated by combination of the data obtained in the Repeatability test (Repeatability 1 – Analyst 1 and Repeatability 2 – Analyst 2)

Table 4.1.1-33: Repeatability data

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
NNG	0.788	7	9.39	0.845
	0.821	7	6.21	0.563
	0.804	**14**	**7.84**	**0.708**

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

Limit of Quantification (Limit of Detection)

The limit of quantification of the method was evaluated by injecting of six replicates of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. The limit of quantification was defined as the lowest concentration used in the calibration range.

Analyte	content (mg/kg)	No of replicates	Mean recoveries%	RSD (%)
NNG	0.377	6	104.44	0.92

NNG: 0.377 mg/kg (LOD of 0.067 mg/kg)

Conclusion

The analytical method for determination of impurity NNG in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Assessment and conclusion by applicant:

The validation of the method for analysis of NNG in glyphosate technical material was not previously evaluated 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 matches the requirements of the current guideline (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of NNG in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method for determination of impurity NNG in glyphosate technical material has been validated with an LOQ of 0.377mg/kg in accordance with SANCO/3030/99 rev. 5.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Bayer

Data point:	CA 4.1.1/016
Report author	[redacted]
Report year	2020
Report title	N-nitrosoglyphosate and formaldehyde method validations in MON 77973
Report No	PCH-2019-0095
Document No	MSL0030752
Guidelines followed in study	SANCO 3030/99 rev. 5
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

Analytical method ME-1848-02 for formaldehyde

Determination of formaldehyde (relevant impurity) in glyphosate technical MON 77973 (Glyphosate wetcake).

Principle of method

Analytes are separated on an ion exclusion column. Formaldehyde is determined by a post-column 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 is directly proportional to the amount of lutidine. Results are reported on a wet-cake basis.
The identity of the formaldehyde is confirmed by the comparison of the retention time of the standard solution of the formaldehyde and the test item.

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

HPLC system	HPLC system equipped with an autosampler, 2 isocratic pumps, a column heater, and UV detector
Column	BioRad Fast Acid Analysis, 100 mm × 7.8 mm ID, 9 µm particle size
Mobile phase:	HPLC grade water or 0.05% sulphuric acid
Column temperature:	50 – 55 °C
Mixing coil temperature:	50 – 55 °C
Flow rate:	Mobile phase: 1.0 mL/min Post-column reagent (PCR): 0.8 mL/min
Injection volume:	10 µL
Detector:	UV detection
Wavelength:	420 nm
Run time:	10 min
Retention time:	Approx. 4 min (formaldehyde)

Findings

Specificity:

Chromatograms of standards solution, of fortified samples and unspiked samples are provided. No interference is observed at the retention time of the analyte.

Linearity

Linearity was determined with 6 standard levels containing formaldehyde with correlation coefficient > 0.99. The standard solutions were prepared in the same manner than the test item solution wuth derivatisation step.

Table 4.1.1-34: Linearity Data for formaldehyde

Analyte	Calibration ranges (ppm)	Calibration curve	Correlation coefficient (r)
Formaldehyde	0.4047 to 80.1554	y = 102.9538x – 2.3724	0.9999

All samples and spiked samples were diluted 1:25 to fit within the standard curve.

Accuracy

The accuracy was determined by analysis of a MON 77973 sample followed by analysis of the same sample spiked with two levels each of formaldehyde. Triplicate injections of five separate sample preparations at each of two spiking levels were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 4.1.1-35: Accuracy data for formaldehyde in MON 77973

Analyte	Fortification level (ppm)	No of replicates	Mean recovery (%)	Standard deviation (%)	RSD (%)
Formaldehyde	202.3	15	102.26	0.355	0.347
	1109.5	15	103.21	0.203	0.197

Note: samples and spiked samples for the determination of formaldehyde were diluted 1:25 to fit within the calibration curve

Repeatability (precision)

Repeatability of this method was determined through the analysis of MON 77973. Triplicate injections of each of five separate sample preparations were used to generate precision data. The data for the repeatability of impurity formaldehyde in MON 77973 are summarised in the below table.

Table 4.1.1-36: Repeatability data for formaldehyde in MON 77973

Analyte	Mean content (ppm)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	202.3	15	0.355	0.072[2]
	1109.5	15	0.197	0.053[3]

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

[2]Since formaldehyde was present at low levels in technical material, the precision data at low fortification level in accuracy tests are included;

[3]Since formaldehyde was present at low levels in technical material, the precision data at high fortification level in accuracy tests are included.

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. Therefore, a full validation of the derivatization step is not considered necessary.

Limit of Quantification

The limit of quantification (LOQ) of the method is defined as the lowest spike level where both precision and accuracy criteria are met according to SANCO 3030/99 rev. 5.

The defined LOQ levels for formaldehyde: 0.2023 g/kg (202.3 ppm)

Conclusion
The analytical method for determination of formaldehyde in glyphosate technical material MON 77973 (Glyphosate wetcake) has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5.

Assessment and conclusion by applicant:
The validation of the methods for analysis of formaldehyde in glyphosate technical material MON 77973 (glyphosate wetcake) was not previously evaluated at EU level. It was performed under GLP and according to current requirements (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of formaldehyde in glyphosate technical material MON 77973 (glyphosate wetcake).

Assessment and conclusion by RMS: The analytical method for determination of formaldehyde in glyphosate technical material MON 77973 (Glyphosate wetcake) has been validated with an LOQ of 0.20g/kg in accordance with SANCO/3030/99 rev. 5.
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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Data point:	CA 4.1.1/016
Report author	[redacted]
Report year	2020
Report title	N-nitrosoglyphosate and formaldehyde method validations in MON 77973
Report No	PCH-2019-0095
Document No	MSL0030752
Guidelines followed in study	SANCO 3030/99 rev. 5
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

Analytical method ME-2070-01 for *N*-nitrosoglyphosate (NNG)
Determination of NNG (relevant impurity) in glyphosate technical MON 77973 (Glyphosate wetcake).

Principle of method

A cation exchange column is used to separate N-nitrosoglyphosate (NNG) from some other components present in the sample. Before NNG elutes from the clean-up column, an electric valve switches and a cut containing NNG is eluted onto the anion exchange analytical column. After NNG elutes, the valve then switches back and the clean-up column effluent goes to waste. The analytical column separates NNG from other components and the effluent is air segmented prior to addition of N-(1-naphthyl)ethylenediamine/ HBr and sulphanilamide reagents. The azo dye formed is detected using a colorimeter set at 550 nm and quantified by external standards. Results are reported on a wet-cake basis.
The identity of NNG is confirmed by the comparison of the retention time of the standard solution of the NNG and the test item

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

HPLC system	HPLC system equipped with an autosampler, 2 isocratic pumps, a post-column reaction heating apparatus, and UV detector
Column	Analytical column: AX-300 Aquapore, 250 mm × 4.6 mm ID, 7 µm particle size Guard column: AX-300 anion: 15 mm × 3.2 mm ID, 7 µm particle size
Mobile phase:	Analytical mobile phase: Methanol / water / phosphate buffer Post-column reagent (RCR): 2.175 g N-(1-naphthyl)ethylenediaminedihydrochloride (NED), sulphanilamide, hydrobromic acid solution and hydrochloric acid solved in distilled water
Column temperature:	Not reported
Flow rate:	Mobile phase: 1.0 mL/min Post-column reagent (PCR): 0.5 mL/min
Injection volume:	900 µL
Detector:	UV detection
Wavelength:	550 nm
Run time:	35 min
Retention time:	Approx. 26.5 min (NNG)

Findings

Specificity:
Chromatograms of standards solution, of fortified samples and unspiked samples are provided. No interference is observed at the retention time of the analyte.

Linearity
Linearity was determined with 6 standard levels containing NNG with correlation coefficient > 0.99.

Table 4.1.1-37: Linearity Data for NNG

Analyte	Calibration ranges (ppm)	Calibration curve	Correlation coefficient (r)
NNG	0.0202 to 0.2393 (n>5)	y = 2988.5422x – 16.5904	0.9999

Accuracy
The accuracy was determined by analysis of a MON 77973 sample followed by analysis of the same sample spiked with two levels each of NNG. Duplicate injections of five separate sample preparations at each of two spiking levels were used to generate accuracy data. Results of accuracy data are summarized in the below table.

Table 4.1.1-38: Accuracy data for NNG in MON 77973

Analyte	Fortification level (ppm)	No of replicates	Mean recovery (%)	Standard deviation (%)	RSD (%)
NNG	0.4271	10	102.6	3.993	3.891
	1.2718	10	103.1	2.267	2.198

All samples and spiked samples were diluted 1:13.3 to fit within the standard curve.
Note: samples and spiked samples for the determination of N-nitrosoglyphosate were diluted 1:13.3 to fit within the calibration curve.

Repeatability (precision)
Repeatability of this method was determined through the analysis of MON 77973. Duplicate injections of each of five separate sample preparations were used to generate precision data. The data for the repeatability of impurity NNG in MON 77973 are summarised in the below table.

Table 4.1.1-39: Repeatability data for NNG in MON 77973

Analyte	Mean content (ppm)	No of replicates	RSD (%)	Horrat value (H_r)[1]
NNG	0.4271	10	3.8917	0.32[2]
	1.2718	10	2.198	0.21[3]

[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 technical material, the precision data at low fortification level in accuracy tests are included;
[3]Since NNG was present at low levels in technical material, the precision data at high fortification level in accuracy tests are included

Limit of Quantification
The limit of quantification (LOQ) of the method is defined as the lowest spike level where both precision and accuracy criteria are met according to SANCO 3030/99 rev. 5.
The defined LOQ levels for NNG: 0.4271 mg/kg (0.4271 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. Therefore, a full validation of the derivatization step is not considered necessary.

Conclusion
The analytical method for determination of NNG in glyphosate technical material MON 77973 (glyphosate wetcake) has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5.

Assessment and conclusion by applicant:
The validation of the methods for analysis of formaldehyde in glyphosate technical material MON 77973 (glyphosate wetcake) was not previously evaluated at EU level. It was performed under GLP and according to current requirements (EU guideline SANCO/3030/99 rev. 5). No deviations with the applied test guidelines were reported. The method is suitable for the determination of formaldehyde in glyphosate technical material MON 77973 (glyphosate wetcake).

RMS Conclusion
The analytical method for determination of NNG in glyphosate technical material MON 77973 (Glyphosate wetcake) has been validated with an LOQ of 0.42mg/kg in accordance with SANCO/3030/99 rev. 5.

Nufarm

Data point:	J-CA 4.1.1/019
Report author	[redacted]
Report year	2019
Report title	Validation of analytical methodology for the assay of active ingredient and impurities in glyphosate technical
Report No	ABC-2019-039
Document No	-
Guidelines followed in study	SANCO 3030/99 rev. 5
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

Analytical method for the determination of relevant impurity **formaldehyde** in technical material **(method ABCTM-2019-039-03)**

Principle of method
Formaldehyde in the technical substance was derivatized with Hantzsch reagent and quantified by use of HPLC-DAD. Quantification is performed using peak area responses from a UV detector and external standards calibration.

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

HPLC system:	Agilent 1260 HPLC system with DAD
Column:	Agilent Eclipse Plus, 4.6 × 100 mm, 3.5 µm
Column temperature:	30 °C
Mobile phase:	Isocratic: 75 % water + 25 % acetonitrile
Flow rate:	0.5 mL/min
Injection volume:	5.0 µL
Detector:	DAD
Signal	412 nm
Retention time:	Approx. 5.5 min

Confirmatory method (ABCTM-2019-040-03)

GC system:	Agilent 6890N/5975B GC/MS system
Column:	HP-5, 30 m × 0.32 mm, 0.25 µm
Column temperature:	60 °C, hold 3.0 min, 60 °C to 300 °C, 30 °C/min, 300 °C, hold 10 min
Carrier gas:	Helium
Flow rate:	Ramped flow Initial flow: 2.0 mL/min Initial time: 3.0 min Rate: 10.0 mL/min Final flow: 4.0 mL/min
Injection temperature:	250 °C
Injection volume:	1 µL
Detector:	MS detection
Acquisition mode:	Scan mode
Retention time:	Approx. 27.3 min

Derivative yield for formaldehyde

The derivative yield was determined to be 104.29 % (derivative standard 1005mg/L and formaldehyde standard 1005mg/L).

Specificity
The specificity of the analytical method is demonstrated by injection of the blank (10% NaOH in acetone), reference item and test item solution to HPLC/MS. There was no interference peak at the retention time of formaldehyde. Thus the method is specific to determine this impurity.

The identification of the formaldehyde in glyphosate technical material was confirmed by MS using GC/MS analysis (method ABCTM-2019-040-04). The retention time and spectrum were compared to the ones of referent standard under same analysis conditions.

Linearity

Linearity was determined with five standard levels containing impurities with correlation coefficient > 0.99.

Table 4.1.1-40: Linearity data for formaldehyde

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Formaldehyde	0.3015-5.025g/kg	y = 53.3297 x + 2.4259	0.9999

Accuracy

The recovery test for the impurity formaldehyde was performed by standard addition method. Five solutions of one batch of the test substance (batch no. GH0162) were prepared and spiked with two different levels.

Table 4.1.1-41: Accuracy data

Analyte	Fortification level (g/kg)	No of replicates	Mean recovery (%)	RSD (%)	%RSDr
Formaldehyde	0.5025	5	106.83	0.24	4.20
	4.0200	5	104.28	0.31	3.07

Repeatability (precision)

To evaluate sample repeatability a single batch of glyphosate (batch no. GH0162) was analysed five times. As formadehyde is present as lower than limit of quantification (LOQ) in test batch, standard addition in test item solution was applied for precision test.

Intermediate precision was further investigated by another analyst on another time.

Table 4.1.1-42: Repeatability data

Analyte	Mean content (g/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	0.54	5	1.85	0.44[2]
	0.54	10	1.85	0.44[3]

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

[2] Calculated based on precision data

[3] Calculated based on intermediate precision data.

Limit of Quantification (Limit of Detection)

The LOQ is defined as the lowest spiking concentration of toluene in accuracy test, at which an acceptable mean recovery with an acceptable RSD according to SANCO/3030/99 rev. 5. The LOQ of formaldehyde is 0.5025 g/kg.

Assessment and conclusion by applicant:

The validation of the method for analysis of formaldehyde in glyphosate technical material as manufactured was not previously evaluated at EU level. It was performed under GLP and according to the requirements of EU guideline SANCO/3030/99 rev. 5. No deviations with the applied test guidelines were reported. The method is suitable for the determination of formaldehyde in glyphosate technical material

Assessment and conclusion by RMS:

The method ABCTM-2019-039-03 is validated with an LOQ of 0.50g/kg for the determination of formaldehyde in the technical substance according to the SANCO 3030/99 rev 5.

Concerning the derivatisation step, it can be considered as demonstrated as the derivatisation yield is at 104.29% and thecalibration has been performed with standard solution prepared in the same manner as the test item. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Analytical method for the determination of relevant impurity **NNG** in technical material **(method ABCTM-2019-039-02)**

Principle of method
NNG in the technical substance was quantified by use of liquid chromatography coupled to mass spectrometer (HPLC/MS) at selected ion monitoring (SIM) mode. The concentrations of NNG were determined by external calibration.

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

HPLC system:	Agilent 1200/6130 Single Quadrupole LC/MS system
Column:	Agilent ZORBAX-SB-Aq, 4.6 × 250 mm, 5 µm
Column temperature:	30 °C
Mobile phase:	Isocratic: water (0.1 formic acid)
Flow rate:	0.5 mL/min
Injection volume:	5.0 µL
Singnal:	242
Detector:	MS detection with SIM mode
Ionization mode:	API-ES
Retention time:	Approx. 6.2 min

Confirmatory method (ABCTM-2019-040-02)

HPLC system:	Agilent 1200/6130 Single Quadrupole LC/MS system
Column:	Agilent ZORBAX-SB-Aq, 4.6 × 250 mm, 5 µm
Column temperature:	30 °C
Mobile phase:	Isocratic: water (0.1 formic acid)
Flow rate:	0.5 mL/min
Injection volume:	5.0 µL
Detector:	MS detection with SIM mode
Ionization mode:	API-ES
Retention time:	Approx. 6.0 min

Specificity
The specificity of the analytical method is demonstrated by injection of the blank (water), reference item and test item solution to HPLC/MS. There was no interference peak at the retention time of NNG. Thus the method is specific to determine this impurity.

The identification of the NNG in glyphosate technical material was confirmed by MS using HPLC/MS analysis (method ABCTM-2019-040-02). The retention time and spectrum were compared to the ones of referent standard under same analysis conditions.

Linearity
Linearity was determined with at five standard levels containing impurities with correlation coefficient > 0.99.

Table 4.1.1-43: Linearity data for NNG

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
NNG	0.298-5.094mg/kg	y = 3882383.43 x – 6229.14	0.9996

Accuracy
The recovery test for the impurity NNG was performed by standard addition method. Five solutions of one batch of the test substance (batch no. GH0162) were prepared and spiked with two different levels.

Table 4.1.1-44: Accuracy data

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)	%RSDr
NNG	0.497	5	99.45	9.03	11.91
	4.09	5	127.15	1.24	8.67

Repeatability (precision)
To evaluate sample repeatability a single batch of glyphosate (batch no. GH0162) was analysed five times. As NNG was not detected in test batch, standard addition in test item solution was applied for precision test.
Intermediate precision was further investigated by another analyst on another time.

Table 4.1.1-45: Repeatability data

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
NNG	0.49	5	9.18	0.77[2]
	0.53	10	11.51	0.98[3]

[1]Horrat value (Hr) = %RSD/%RSD$_r$ (Horwitz equation %RSD$_r$ = 0.67 * $2^{(1-0\ 5*\log(c))}$)
[2] Calculated based on precision data
[3] Calculated based on intermediate precision data.

Limit of Quantification (Limit of Detection)
The LOQ is defined as the lowest spiking concentration of toluene in accuracy test, at which an acceptable mean recovery with an acceptable RSD according to SANCO/3030/99 rev. 5. The LOQ of NNG is 0.49725 mg/kg. LOD is calculated to be 0.085 mg/kg.

Assessment and conclusion by applicant: The validation of the method for analysis of NNG in glyphosate technical material as manufactured was not previously evaluated at EU level. It was performed under GLP and according to the requirements of EU guideline SANCO/3030/99 rev. 5. No deviations with the applied test guidelines were reported. The method is suitable for the determination of NNG in glyphosate technical material

Assessment and conclusion by RMS: The method ABCTM-2019-039-02 is validated with an LOQ of 0.49mg/kg for the determination of NNG in the technical substance according to the SANCO 3030/99 rev 5.

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Source 1

Report:	J-KCA 1.11/01; J-KCA 4.1.1/01 : [redacted] (2017)
Title:	5-Batch Analysis of Glyphosate TGAI in Accordance with Regulation (EC) No 1107/2009, Reference SANCO 3030/99 rev. 4
Document No:	EPP00297, AN16111117
Guidelines:	Regulation (EC) No. 1107/2009 SANCO/3030/99 rev. 4
GLP:	Yes
Acceptability	Yes

Determination of formaldehyde: In-house method AN161111117-A

Principle of method
The formaldehyde content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection following derivatisation of the analyte with 2,4-dinitrophenylhydrazine. Acetaldehyde is employed as an internal standard as part of the method design.
The identity of the formaldehyde is confirmed by the comparison of the retention time of the standard solution of the formaldehyde and the test item

Preparation of standard stock solutions
Approximately 1 g of a formaldehyde analytical standard (~36-38 % w/w) is weighed into a 100 mL volumetric flask containing some water, dissolved and then made up to volume with water giving a stock standard of concentration ~3.66 mg/mL.

Preparation of internal standard solutions
Acetaldehyde (*ca* 200 mg) is accurately weighed into a volumetric flask (100 mL) dissolved in water, and made up to volume with water to give an internal standard stock solution of *ca* 2.0 mg/mL.

Preparation of calibration standards
To each of eight 25 mL vials, 10 mL of 2,4-dinitrophenylhydrazine (DNPH) reagent (3 g DNPH dissolve in 1.5 L of 2 M HCL solution), and then spiked with 0, 50, 150, 200, 250, 300 and 350 µL of the stock standard solution, respectively, along with 250 µL of internal standard. This gives eight calibration solutions containing formaldehyde in quantities of 0, 183, 366, 549, 732, 915, 1098 and 1282 µg, respectively. These solutions represent a range of 0-0.26% w/w content in glyphosate technical material (assuming a target weight of 500 mg glyphosate active substance technical material).

Preparation of test samples
500 mg of glyphosate technical material is weighed into a vial and 10 mL of DNPH reagent added, followed by spiking with 250 µL of internal standard.

Further treatment and standards and test samples
Samples are placed in a shaker at 25 ^{o}C for ~ 2 hours, shaking at 77 rpm, followed by manual shaking and addition of ~5 g NaCl and 5 mL methylisobutylketone (MIBK). The bottle is shaken vigorously for ~1 minute and the layer allowed to settle. 100 µL of the MIBK layer is diluted to 10 mL volume using acetonitrile / water (60:40 v/v).

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1100 series HPLC
Column:	Hichrom Partisil 5 SAX, 250 x 4.6, 5 µm
Mobile Phase:	50:50 (v/v) water / acetonitrile
Injection Volume:	20 µL
Flow Rate:	1.2 mL/min
Temperature:	Ambient
Detection:	240 nm (UV)
Run Time:	30 minutes
Retention Time:	Formaldehyde at ~3.8 minutes

Findings
Specificity
Representative chromatograms of a blank (internal standard only), formaldehyde analytical standard (0.43 µg/mL) and glyphosate active substance technical material. The blank chromatogram of the internal standard only shows a peak at the same retention time as formaldehyde. No interference below 3% is observed at the retention time of formaldehyde.

Linearity
The linearity of response was demonstrated by preparing 7 analytical standards at concentrations ranging from 0.43-3.03 µg/mL (corresponding to 0.04-0.30 % w/w, *i.e.* 0.4-3.0 g/kg content in the active substance technical material).

A representative linearity of response plot gives a calibration of plot equation of $y = 1.49486x - 0.00145$ and a correlation coefficient (r) of 0.9988.

Accuracy
Recoveries data were generated on samples of the glyphosate active substance technical material spiked with an analytical standard of formaldehyde at levels of 0.84 µg/mL (0.8 g/kg), 1.68 µg/mL (1.7 g/kg) and 2.1 µg/mL (2.1 g/kg). 5 determinations were made at each spiking level. 3 determinations of unspiked samples of the glyphosate active substance technical material were also analysed to determine the background level of formaldehyde inherent to the samples; the level of formaldehyde inherent to the technical material was determined to be <LOQ. The mean recovery for the 0.8 g/kg spiking level is 86.0 % and for the 1.7 g/kg and 2.1 g/kg spiking levels are 85.5 % and 85.7 %.

Precision
The method precision could not be calculated in a meaningful way using samples of the glyphosate active substance technical material as the formaldehyde content in the technical material was <LOQ in all cases.

However, an assessment of the method precision was performed on samples of the technical material spiked with an analytical standard of formaldehyde at three different spiking levels representing: 0.84 µg/mL (0. 8 g/kg), 1.68 µg/mL (1.7 g/kg) and 2.10 µg/mL (2.1 g/kg). The mean concentrations determined for each set of these spiked samples were 0.72 µg/mL (0.7 g/kg), 1.44 µg/mL (1.4 g/kg) and 1.8 µg/mL (1.8 g/kg), respectively, with associated %RSDs of 4.7, 0.9, and 1.5. The modified Horwitz values ($\%RSD_r$) for each spiked level tested are 3.92, 3.50, and 3.39, respectively, corresponding to Horwitz ratios (H_r) of 1.2, 0.26, and 0.44. Whilst the Horwitz ratio is > 1 in the case of samples spiked at 0.8 g/kg, it is only slightly above 1. Furthermore, the method linearity and accuracy have all been shown to be acceptable. This slight exceedance of the acceptable Horwitz ratio value of 1 is therefore not expected to adversely impact on the overall performance and the method and the reliability of the data generated. It is therefore proposed that the method precision at this level is adequate to supporting determination of formaldehyde content down to 0.8 g/kg.

LOQ
The LOQ is defined as the lowest spiking concentration in accuracy test, at which an acceptable mean recovery with an acceptable RSD according to SANCO/3030/99 rev. 5. The LOQ is 0.8g/kg.

Conclusions
Supporting validation data have been provided for the use of analytical method AN161111117-A to quantify the formaldehyde content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. An assessment of the method precision at the lowest spiking level of 0.8 g/kg gave a Horwitz ratio slightly above 1 (1.2). However, given the value was only slightly in exceedance of the level of acceptability and the method linearity and accuracy were all found to be acceptable, the method precision is concluded to be adequate, not adversely impacting on the overall method performance. The method has been shown to be fit-for-purpose for quantifying formaldehyde content in the active substance technical material with adequate accuracy and precision down to levels of 0.8 g/kg. The validation data is therefore sufficient to support the determination of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fit-for-purpose and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method linearity, accuracy and precision with an LOQ of 0.8g/kg in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013.
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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-2 (a) Summary of validation data for determination of the impurity formaldehyde in the glyphosate active substance technical material as manufactured

Analyte	LOQ (g/kg)	Recovery Fortification Level	Recoveries % range (mean)*	Repeatability % RSD (n)	Linearity	Specificity

		(g/kg)				
Formaldehyde	0.8	0.8	81.0 – 91.7 (86.0) n = 5	Mean content = 0.7 g/kg %RSD: 4.7 (5) Modified Horwitz value: 3.92 Horwitz ratio (Hr = %RSD/%RSDr): 1.2 Hr > 1.	**Calibration plot:** $y = 1.49486x - 0.00145$ **Correlation coefficient:** R = 0.9988 **Range:** 0.43 – 3.03 µg/mL (corresponding to 0.04 – 0.30 % w/w content in the active substance technical material **Number of determinations:** 7 Standards	Representative chromatograms of a blank (internal standard only), formaldehyde analytical standard (0.43 µg/mL), and glyphosate active substance technical material. The data demonstrates the specificity of the method to be acceptable.
		1.7	84.5 – 86.3 (85.5) n = 5	Mean content = 1.4 g/kg %RSD: 0.9 (5) Modified Horwitz value: 3.50 Horwitz ratio (Hr = %RSD/%RSDr): 0.26 Hr < 1.		
		2.1	84.3 – 87.1 (85.7) n = 5	Mean content = 1.8 g/kg %RSD: 1.5 (5) Modified Horwitz value: 3.39 Horwitz ratio (Hr = %RSD/%RSDr): 0.44 Hr < 1.		

Determination of N-nitrosoglyphosate: In-house method AN161111117-B

Principle of method
The N-nitrosoglyphosate content in the glyphosate active substance technical material as manufactured was quantified by ion chromatography with UV-vis detection.
The identity of the NNG is confirmed by the comparison of the retention time of the standard solution of the NNG and the test item

Preparation of standard stock solutions
~29.5 mg of N-nitrosoglyphosate, anilinium salt is weighed into a 100 mL volumetric flask, and made to volume with 0.1 M H_2SO_4, giving a stock solution of concentration 200 µg/mL of N-nitrosoglyphosate free acid.

2.5 mL of the standard stock solution is pipetted into a 100 mL volumetric flask and made up to volume with 0.1 M H_2SO_4 to give an intermediate standard solution of concentration 5 µg/mL N-nitrosoglyphosate free acid.

Preparation of calibration standards
To each of eight 100 mL volumetric flasks, 0, 1, 2, 3, 4, 6, 8, and 10 mL of the intermediate stock solution are added and made up to volume with 0.1 M NaCl, giving standard solutions of 0, 0.05, 0.10, 0.15, 0.20, 0.30, 0.40 and 0.50 µg/mL of N-nitrosoglyphosate. These solutions equate to a content range covering 0-2.0 mg/kg of the impurity in the active substance technical material.

Preparation of test samples
25 mL vials are first rinsed with salfamic acid solution, then rinsed thoroughly with water and dried. 2 g of the glyphosate active substance technical material is added to the vial, and 8 mL of 0.1 M H_2SO_4 added and then sonicated for ~1 hour. Samples are then filtered using 0.45 µm filters.

Analysis of test samples
100 µL of the prepared sample solution is injected into the ion chromatography system and analysed under the following chromatographic conditions:

Instrument:	Agilent 1100
Column:	Dionex IonPac AS 11, 250 x 4.0 mm with Dionex IonPac AG 11-HC guard column
Mobile Phase:	50 mM sodium carbonate in water
Injection Volume:	100 µL
Flow Rate:	1.75 mL/min
Temperature:	25 ºC
Detection:	244 nm (UV)
Run Time:	25 minutes
Retention Time:	N-nitrosoglyphosate at ~8.0 minutes

Findings
Specificity
Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard (0.20 µg/mL; 0.8 mg/kg), and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to quantification of N-nitrosoglyphosate content.

Linearity
The linearity of response was demonstrated by preparing 7 analytical standards at concentrations ranging from 0.05-0.50 µg/mL (corresponding to 0.00002-0.0002 % w/w, *i.e.* 0.2-2.0 mg/kg content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 1.73077x - 0.00310$ and a correlation coefficient (r) of 1.000.

Accuracy
Recoveries data were generated on samples of the glyphosate active substance technical material spiked with an analytical standard of N-nitrosoglyphosate at levels of 0.15 µg/mL (0.6 mg/kg), 0.26 µg/mL (1.04 mg/kg) and 0.31 µg/mL (1.24 mg/kg). 5 determinations were made at each spiking level. 3 determinations of unspiked samples of the glyphosate active substance technical material were also were also analysed to determine the background level of N-

nitrosoglyphosate inherent to the sample. The mean recoveries for the 0.6 mg/kg, 1.04 mg/kg and 1.24 mg/kg spiking levels are 96.0 %, 99.2 % and 105.2 %

Precision
The method precision could not be calculated in a meaningful way using samples of the glyphosate active substance technical material as the N-nitrosoglyphosate content in the technical material was <LOQ (0.6 mg/kg) in all cases.

However, an assessment of the method precision was performed on samples of the technical material spiked with an analytical standard of N-nitrosoglyphosate at three different spiking levels representing: 0.15 µg/mL (0.6 mg/kg), 0.26 µg/mL (1.04 mg/kg) and 0.31 µg/mL (1.24 mg/kg).

The mean spiked concentrations determined for each set of these samples were 0.14 µg/mL (0.56 mg/kg), 0.26 µg/mL (1.04 mg/kg) and 0.33 µg/mL (1.30 mg/kg), respectively, with associated %RSDs of 6.5, 3.4 and 3.2. The modified Horwitz values ($\%RSD_r$) for each spiked level tested are 11.6, 10.7 and 10.4, respectively, corresponding to Horwitz ratios (H_r) of 0.56, 0.32, and 0.31. As the Horwitz ratios are not greater than 1 in any instance, the method precision at all spiking levels investigated is considered acceptable.

LOQ
The LOQ is defined as the lowest spiking concentration in accuracy test, at which an acceptable mean recovery with an acceptable RSD according to SANCO/3030/99 rev. 5. The LOQ is 0.6mg/kg.

Conclusions
Supporting validation data have been provided for the use of analytical method AN161111117-B to quantify the N-nitrosoglyphosate content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The method has been shown to be validated for determination of N-nitrosoglyphosate content in the active substance technical material with acceptable accuracy and precision down to levels of 0.6 mg/kg. The validation data is therefore sufficient to support quantification of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS:. The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision with an LOQ of 0.6mg/kg in accordance with the criteria laid down in SANCO/3030/99 rev. 5.

Table 4.1.1-3 (a) Summary of validation data for determination of the impurity N-nitrosoglyphosate in the glyphosate active substance technical material as manufactured

Analyte	**LOQ (mg/kg)**	**Recovery Fortification Level (mg/kg)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
N-nitroso-glyphosate	0.6	0.6	86.7 – 113.3 (96.0) n = 5	Mean content = 0.56 mg/kg %RSD: 6.5 (5) Modified Horwitz value: 11.6 Horwitz ratio (Hr = %RSD/%RSDr): 0.56 Hr = 1.	**Calibration plot:** $y = 1.73077x + 0.00310$ **Correlation coefficient:** R: 1.0000 **Range:** 0.05-0.50 µg/mL (corresponding to 0.00002-0.0002 % w/w, i.e. 0.2-2.0 mg/kg **Number of determinations:** 7 Standards	Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard (0.20 µg/mL), and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. The data demonstrates the specificity of the method to be acceptable.
		1.04	92.3 – 107.7 (99.2) n = 5	Mean content = 1.04 mg/kg %RSD: 3.4 (5) Modified Horwitz value: 10.7 Horwitz ratio (Hr = %RSD/%RSDr): 0.32 Hr < 1.		
		1.24	100.0 – 109.7 (105.2) n = 5	Mean content = 1.30 mg/kg %RSD: 3.2 (5) Modified Horwitz value: 10.4 Horwitz ratio (Hr = %RSD/%RSDr): 0.31 Hr < 1.		

Source 2

Report:	[redacted] **(2016)**
Title:	KCA section 1/017 - 020 - Qualitative and Quantitative Profile of the test substanceGlyphosate(Five Batch Analysis)
Document No:	15425.030.027.17
Guidelines:	Regulation (EC) No. 1107/2009 SANCO/3030/99 rev. 5
GLP:	Yes
Acceptability	Yes

Determination of formaldehyde

Principle of method

The formaldehyde content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection following derivatisation of the analyte with Hantzch reagent.
The identity of formaldehyde is confirmed by the comparison of the retention time of the standard solution of formaldehyde and the test item.

Specificity
Preparation of blank solution
The blank solution was prepared by the addition of 180 µL of 11% sodium hydroxide solution to a 10 mL volumetric flask which was then filled to the mark with ultra-pure water. 500 µL of this solution was then combined with 500 µL Hantzsch reagent. The solution then reposed for two hours providing the blank solution.

Linearity
Preparation of calibration standards
A stock solution of formaldehyde was prepared by weighing 5.35 mg of the formaldehyde analytical standard into a 5 mL volumetric flask. The volume was completed with ultra-pure water and the solution was stirred until complete dissolution producing a concentration of 394.83 mg/L of formaldehyde analytical standard. An aliquot of 260 µL of this stock solution was diluted to a final volume of 5 mL with ultra-pure water producing the solution Standard A (20.53 mg/L). Standard solutions were prepared from dilutions of the Standard A. These solutions were then derivatised using Hantzsch reagent as shown in 2.6.2 of the 5-batch report, producing a working concentration of 10.27-1.03 mg/L (representing a range of 0.3423-0.0343 % w/w).

Accuracy
Preparation of spiked solutions
Three solutions of test substance (batch HB2015214) were prepared by weighing approximately 60 mg of sample into a 10 mL volumetric flask. Standard A (20.53 mg/L formaldehyde) was used as the spiking solution at different concentrations. 180 µL of 11% sodium hydroxide solution was added and the flasks made to the mark with ultra-pure water. 500 µL of each solution was combined with 500 µL for derivatisation. Samples were analysed after 2 hours with spiking concentration of 3.16 mg/L (0.1047% w/w), 5.14 mg/L (0.1612% w/w), and 7.11 mg/L (0.2396% w/w) formaldehyde.

Precision
Preparation of spiked solutions
60 mg of test material (batch HB20150214) was weighed into seven 10 mL flask and spiked with 160 µL of standard A (20.53 mg/L formaldehyde). 180 µL of 11% sodium hydroxide solution was added and the flask filled to the mark with ultra-pure water. Samples were derivatised by combining equal volumes (500 µL) of sample solution and Hantzsch reagent. Samples were reposed for two hours prior to analysis, providing a spiking concentration of 3.16 mg/L (0.1044% w/w) of formaldehyde.

Test item
Preparation of test samples
Solutions of the test item were prepared by weighing approximately 60 mg of sample into 10 mL volumetric flasks. To each flask, 180 µL of 11% sodium hydroxide solution was added and made up to the mark with ultra-pure water. Samples were stirred and sonicated until complete solubilization. These samples were then derivatized with the Hantzsch reagent (outlined in point 2.2.1 of the give batch analysis report) by the combining of equal parts Hantzsch

reagent and test item solution. The samples reposed for two hours before analysis, producing a final concentration of 3000 mg/L of technical

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1260 series HPLC
Column:	Phenomenex Luna C18: 5 µm internal diameter, 250 x 2.0 mm
Mobile Phase:	80:20 (v/v) water / acetonitrile
Injection Volume:	20 µL
Flow Rate:	0.4 mL/min
Temperature:	40 ºC
Detection:	412 (UV)
Run Time:	10 minutes
Retention Time:	Formaldehyde at ~3.8 minutes

Specificity
Representative chromatograms of a blank (solvent only), formaldehyde analytical standard, and glyphosate active substance technical material have been provided (pages 180-185 of the 5-batch report). The retention time of the peak of the formaldehyde analytical standard matches that of the impurity peak from the active substance technical material (~5.6 minutes). Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the formaldehyde impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to determination of formaldehyde content.

Linearity
The linearity response was demonstrated by preparing five analytical standards of formaldehyde, ranging in concentration from 1.03-10.27 mg/L (corresponding to 0. 0.0343- 0.3423% w/w content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 671.34942x + 6.98456$ and a correlation coefficient (r) of 0.99972.

Accuracy
Recovery data was generated on samples of glyphosate technical material spiked with an analytical standard of formaldehyde. Formaldehyde was spiked at concentrations of 3.16 mg/L (0.1047% w/w), 5.14 mg/L (0.1612% w/w) and 7.11 mg/L (0.2396% w/w). The accuracy of the method was evaluated by the determination of the analyte content in the spiked samples The recovery rates fell within this range with 101.41% for the 3.16 mg/L (0.1047% w/w) spiking solution, 101.28% for the 5.14 mg/L (0.1612% w/w) spiking solution, and 99.84% for the 7.11 mg/L (0.2396% w/w) spiking solution.

Precision
The method precision could not be calculated in a meaningful way using samples of the glyphosate active substance technical material as the formaldehyde content in the technical material was <LOQ in all cases.

However, an assessment of the method precision was performed on samples of the technical material spiked with an analytical standard of formaldehyde at a spiking concentration of ~1 g/kg (0.1 % w/w). The sample repeatability (precision) was assessed through the seven-fold determination of the analyte in the test item in two separate experiments. The results obtained indicate a good precision of the data (acceptability according to SANCO/3030/99/rev.5, RSD < RSD max given by modified Horwitz equation based on the amount in spiked samples, 3.16 mg/mL).The associated %RSDs was 3.6 with a modified Horwitz value (%RSD_r) of 3.77. This corresponded to a Horwitz ratios (H_r) of 0.95.

LOQ
The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 1.03%) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. The LOQ of the method is 0.034% .

Analyte	content (g/kg)	No of replicates	Mean recoveries%	RSD (%)
formaldehyde	0.3433	6	88.9	1.03

Conclusions
Supporting validation data have been provided for the analytical method used to quantify the formaldehyde content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The validation data is therefore sufficient to support the quantification of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method linearity, accuracy and precision with an LOQ of 0.034% in accordance with the criteria laid down in SANCO/3030/99 rev. 5.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-2 (b) Summary of validation data for determination of the impurity formaldehyde in the glyphosate active substance technical material as manufactured

Analyte	**LOQ (g/kg)**	**Accuracy**		**Repeatability % RSD (n)**	**Linearity**	**Specificity**
		Recovery Fortification Level (g/kg)	**Recoveries % range (mean)***			
Formaldehyde	The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 1.03%; Mean recoveris:88.9) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. The LOQ of the method is 0.034%	1.047 (0.1047 % w/w)	101.41 n=1	**Analyst 1:** Mean content: 1.04 g/kg %RSD = 3.6 (7) Modified Horwitz value ($\%RSD_r$): 3.77 Horwitz ratio (H_r = $\%RSD/\%RSD_r$) : 0.95 As H_r < 1, precision is acceptable.	**Calibration plot:** y = 671.34932x + 6.98456 **Coefficient of determination :** R^2 = 0.99972 **Range:** 10.27 – 1.03 mg/L (0.3423 - 0.0343% w/w content in the active substance) **Number of determinations:** 5 Standards	Example chromatograms of a solvent blank, analytical standards of each impurity, and the active substance technical material are available. Comparison of the solvent blank and analytical standards of the impurities show no significant peak interferences with formaldehyde. The retention time of the analytical standard matches that of the analyte peak from the active substance technical material (~5.6 min). The data is considered sufficient to demonstrate the specificity of the method with respect to formaldehyde
		1.612 (0.1612 % w/w)	101.28 n=1	**Analyst 2:** Mean content = 1.05 g/kg %RSD: 3.37 (5) Modified Horwitz value: 3.76 Horwitz ratio (Hr = %RSD/%RSDr): 0.87 Hr < 1.		
		2.396 (0.2396 % w/w)	99.84 n=1			

Determination of N-nitrosoglyphosate

Principle of method

The N-nitrosoglyphosate content in the glyphosate active substance technical material as manufactured was quantified by an in-house method using HPLC coupled mass spectrometer- based detection.

Specificity
Preparation of blank solution
The blank solution was prepared by a mixture of 500 µL ultra-pure water, 250 µL of NED/HBr solution, and 250 µL of sulphanilamide solution. The solution was heated to 95ºC on a heat block for 15 minutes and then analysed.

Linearity
Preparation of calibration standards
Calibration solutions were prepared by weighing approximately 2.22 mg of the analytical standard of impurity 2 into a 5 mL volumetric flask. The standard solution was dissolved in ultra-pure water and the volumetric flask filled to the mark, providing a standard solution with 393.61 mg/L analytical standard in acid form. An aliquot of 85 µL of this solution was diluted in 50 mL ultra-pure water producing solution A with a concentration of 669.14 µg/L of analytical standard. Differing volumes of standard A solution were used to prepare standard solutions by addition of 250 µL NED/HBr and 250 µL sulphanilamide to provide a working range of 56.88-334.57 µg/L (3.792E-5 to 2.23E-4 % w/w of the test item) of N-nitrosoglyphosate.

Accuracy
Preparation of spiked solutions
Three solutions of test substance (batch HB201541230) were prepared by weighing approximately 1500 mg of sample into a 5 mL volumetric flask. Standard A (3936.10 µg/L N-nitrosoglyphosate) was used as the spiking solution at different volumes (140, 390, and 510 µL respectively) and the flasks filled to the mark with ultra-pure water. 500 µL of each sample was combined with 250 µL of NED/HBr and 250 µL of sulphanilamide solution. The solutions were heated to 95 ºC for 15 minutes using a drying block to produce test items with approximately 150000000 µg/L of test substance with spiking concentrations of 55.11 µg/L (3.51E-5% w/w), 153.51 µg/L (1.00E-4% w/w), and 200.74 µg/L (1.30E-4% w/w) N-nitrosoglyphosate respectively.

Precision
Preparation of solutions
Approximately 1500 mg of test item (batch HB20151230) was weighed into a 5 mL volumetric flask. The samples were dissolved, and the flasks filled to the mark with ultra-pure water. 500 µL of each sample solution was combined with 250 µL of NED/HBr solution and 250 µL of sulphonamide solution. The solutions were incubated at 95ºC for 15 minutes to provide the test sample.

Test item
Preparation of test samples
Approximately 1500 mg of sample was weighed and dissolved in ultra-pure water by sonication for 5 minutes. The solution was filtered using Millex filter (0.45 µm x 13 mm) providing a solution of approximately 300 g/L. Working solutions of approximately 150 g/L were prepared by reacting 500 µL of each sample solution with 250 µL of NED/HBr and 250 µL of sulphanilamide solution (outlined in appendix 3, section 3.2.1 of the 5 batch report) This solutions were then heated to 95 ºC for 15 minutes in a dry block to provide the working solutions.

Analysis of test samples
10 µL of the prepared sample solution is injected into the HPLC coupled mass spectrometry and analysed under the following chromatographic conditions:

Instrument:	Agilent 1200 series HPLC
Column:	Phenomenex Luna C18: 5µm internal diameter, 250 x 2.0 mm
Mobile Phase:	Phase A: 10mM ammonium acetate (+0.1% acetic acid) Phase B: Acetonitrile
Injection Volume:	10 µL
Flow Rate:	0.3 mL/min
Temperature:	40 ºC
Detection:	Mass spectrometry
Run Time:	25 minutes

	Time 0: %A:85 %B:15 Time 7: %A:80 %B:20 Time 12: %A:50 %B:50 Time 25: %A:50 %B:50
Retention Time:	N-nitrosoglyphosate at ~15.3 minutes

Findings

The following validation data was generated with respect to the specificity, linearity of response, (method) precision, and accuracy for the method when quantifying N-nitrosoglyphosate in the glyphosate active substance technical material.

Specificity
Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard, and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material (~15.3 minutes). Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to determination of N-nitrosoglyphosate content.

Linearity
The linearity of response was demonstrated by preparing 5 analytical standards at concentrations ranging from 56.88-334.57 µg/L (corresponding to 3.792E-5 to 2.23E-4 % w/w content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 10.94235x + 401.71181$ and a correlation coefficient (r) of 0.99017.

Accuracy
Recovery data was generated on samples of glyphosate technical material spiked with an analytical standard of NNG was spiked at concentrations of 55.11 µg/L (3.41E-5 % w/w), 153.51 µg/L (1.02E-4 % w/w) and 200.74 µg/L (1.34E-4 % w/w). The accuracy of the method was evaluated by the determination of the analyte content in the spiked samples (Accuracy = 100 x amount found / amount expected).The results obtained show a good accuracy within the acceptability limits as outlined by SANCO/3030/99 rev.5. The recovery rates fell within this range with 118.92 % for the 55.11 µg/L (3.41E-5 %) spiking solution, 98.57 % for the 153.51 µg/L (1.02E-4 %) spiking solution, and 88.97 % for the 200.74 µg/L (1.34E-4 %) spiking solution.

Note: The lowest recovery sample (0.0000341 % w/w) is measured outside the linear range of the calibration curve (0.00003792 – 0.000223 % w/w). An explanation should be provided.

Precision
The method precision was assessed through the seven-fold determination of N-nitrosoglyphosate within the test material through two independent experiments each performed by different analysts. The results obtained indicate a good precision of the data with H_r values of 0.84 and 0.71 for experiments one and two respectively (acceptability according to SANCO/3030/99/rev.5, RSD < RSD max given by modified Horwitz equation based on the theoretical amount in spiked samples).

LOQ
The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 0.86%) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. The LOQ of the method is 0.000038%

Analyte	**content (%w/w)**	**No of replicates**	**Mean recoveries%**	**RSD (%)**
NNG	0.000038	6	88.75	0.88

Conclusions
Supporting validation data have been provided for the use of analytical method AN161111117-B to quantify the N-nitrosoglyphosate content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The method has been shown to be validated for determination of N-nitrosoglyphosate content in the active substance technical material with acceptable accuracy and precision down to levels of 0.6 mg/kg. The validation data is therefore sufficient to support

the determination of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision with an LOQ 0.000038% in accordance with the criteria laid down in SANCO/3030/99 rev. 5.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-3 (b) Summary of validation data for determination of the impurity N-nitrosoglyphosate in the glyphosate active substance technical material as manufactured

Analyte	**LOQ (g/kg)**	**Accuracy**		**Repeatability % RSD (n)**	**Linearity**	**Specificity**
		Recovery Fortification Level (µg/L)	**Recoveries % range (mean)***			
N-nitrosoglyphosate	The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 0.88%; mean recovery88.75 %:) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. The LOQ of the method is 0.000038%	55.11 (3.41E-5 % w/w)	118.92 n=1	<u>Analyst 1:</u> Mean content: 0.76 mg/kg %RSD = 9.38 (7) Modified Horwitz value ($\%RSD_r$): 11.18 Horwitz ratio (H_r = %RSD/$\%RSD_r$): 0.84 As H_r < 1, precision is acceptable.	**Calibration plot:** y = 10.94235x + 401.71181 **Coefficient of determination:** R^2 = 0.99017 **Range:** 56.88-334.57 µg/L **(**3.792E-5-2.23E-4 % w/w) **Number of determinations:** 5 Standards	Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard, and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. The data demonstrates the specificity of the method to be acceptable.
		153.51 (1.02E-4 % w/w)	98.57 n=1	**Analyst 2:** Mean content: 0.76 mg/kg %RSD = 7.94 (7) Modified Horwitz value ($\%RSD_r$): 11.18 Horwitz ratio (H_r = %RSD/$\%RSD_r$): 0.71 As H_r < 1, precision is acceptable.		
		200.74 (1.34E-4 % w/w)	88.97 n=1			

Source 3

Report:	**KCA 4.1.1/001 - Study No.OS-012, [redacted] (2009)**
Title:	Determination of Active Content and Impurity Profile of Glyphosate
Document No:	OS-012
Guidelines:	CIPAC
GLP:	Yes
Acceptability	Yes (further data required)

Determination of formaldehyde

Principle of method

The formaldehyde content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection following derivatisation of the analyte with Hantzch reagent.
The identity of the formaldehyde is confirmed by the comparison of the retention time of the standard solution of the formaldehyde and the test item

Sample preparation
Hantzsch reagent was prepared by combining 150 g ammonium acetate, 3 mL acetic acid and 2 mL of acetyl acetate in a 1 L volumetric flask and diluting to volume with HPLC grade water.

11% NaOH solution was prepared by diluting 110 g of NaOH in 1000 mL of HPLC grade water.

Equal volumes of sample or standard solution were combined with Hantzsch reagent, shaken, and allowed to stand at ambient temperature for two hours prior to analysis to allow for spectroscopic detection of formaldehyde.

Specifity
Preparation of blank solution
The blank solution was prepared by the addition of 120 µL of 11% sodium hydroxide solution to a 10 mL volumetric flask which was then filled to the mark with HPLC grade water. 500 µL of this solution was then combined with 500 µL Hantzsch reagent. The solution then left for two hours at ambient temperature to provide the blank solution.

Linearity
Preparation of calibration standards
A stock solution of formaldehyde was prepared by weighing approximately 45 mg of the formaldehyde analytical standard (37% w/w) into a 10 mL volumetric flask. The volume was completed with water and the solution contents dissolved, producing a concentration of approximately 1000 mg/L.

Volumes of 200, 400, 600, 800, and 1000 µL were further diluted in 10 mL to produce a working range of 1.280 – 12.80 mg/L (0.035 – 0.35 % w/w) of formaldehyde.

Accuracy
Preparation of spiked solutions
Three solutions of test substance (batch AFS08/1973) were prepared by weighing approximately 40 mg of sample into a 10 mL volumetric flask. Samples were dissolved and spiked with derivatised formaldehyde at concentrations of 1.15 g/kg (0.115 % w/w), 1.19 g/kg (0.119 % w/w), and 1.55 g/kg (0.155 % w/w).

Test item
Preparation of test samples
Solutions of the test item were prepared by weighing approximately 40 mg of sample into 10 mL volumetric flasks. To each flask, 120 µL of 11% sodium hydroxide solution was added and made up to the mark with HPLC grade water. Samples were dissolved and then derivatised by combining equal volumes of sample and Hantzsch reagent and leaving at ambient temperature for 2 hours. This provided derivatised samples with a working concentration of 2000 mg/L of technical material.

Analysis of test samples

20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	LC-10ADVP Pump, SIL-10ADVP Autosampler, SPD-M10AVP PDA-Detector (Shimadzu)
Column:	Altima C18: 4.6 x 250 mm
Mobile Phase:	70:30 (v/v) water / acetonitrile
Injection Volume:	20 µL
Flow Rate:	1 mL/min
Temperature:	30 ºC
Detection:	412 (UV)
Run Time:	10 minutes
Retention Time:	Formaldehyde at ~5.9 minutes

Findings
The following validation has generated with respect to the specificity, linearity of response, (method) precision, and accuracy for the method when quantifying formaldehyde in the glyphosate active substance technical material.

Specificity
Representative chromatograms of the formaldehyde standard, of solvent blank and formaldehyde spiked test material (formaldehyde was present at levels lower than the LOD) were attained and compared to assess specificity. The retention time of the peak of the formaldehyde analytical standard matches that of the impurity peak from the active substance technical material (~5.9 minutes). Comparison of the response at different wave lengths shows there to be no interfering peaks coinciding with the retention time of the formaldehyde impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to determination of formaldehyde content.

Linearity
The linearity response was demonstrated by preparing six analytical standards of formaldehyde, ranging in concentration from 1.280 - 12.80 mg/L (corresponding to a concentration of 0.035 – 0.35 % w/w content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 137670x + 30105$ and a correlation coefficient (r) of 0.9999.

Accuracy

Recovery data was generated on samples of glyphosate technical material spiked with an analytical standard of formaldehyde. Formaldehyde was spiked at concentrations of 1.15 g/kg (0.115 % w/w), 1.19 g/kg (0.119 % w/w) and 1.60 g/kg (0.160 % w/w). The accuracy of the method was evaluated by the determination of the analyte content in the spiked samples. The recovery rates fell within this range with 106% for the 1.15 g/kg (0.115 % w/w) spiking solution, 109% for the 1.19 g/kg (0.119 % w/w) spiking solution, and 103% for the 1.60 g/kg (0.160 % w/w) spiking solution.

Precision:
The method precision could not be calculated in a meaningful way using samples of the glyphosate active substance technical material as the formaldehyde content in the technical material was <LOD in all cases.

However, an assessment of the method precision was performed by the sevenfold determination of a formaldehyde standard (10.24 mg/L). The results obtained indicate a good precision of the data (acceptability according to SANCO/3030/99/rev.5, RSD < RSD max given by modified Horwitz equation based on the theoretical amount in spiked samples). The associated %RSDs was 0.41 with a modified Horwitz value ($\%RSD_r$) of 0.94. This corresponded to a Horwitz ratios (H_r) of 0.43.

LOQ
For the LOQ, a standards solution of approximately 1.280mg/L (0.035%) each was injected seven times and mean area, standards deviation and coefficient of variation were determined.

The LOQ corresponds to the level for which the repeatability and the recovery are validated. The repeatability has been validated at the level 0.035%. However no measurement of the recovery has been performed.

Conclusions
Supporting validation data have been provided for the analytical method used to quantify the formaldehyde content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The validation data is therefore sufficient to support the determination of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method linearity, accuracy and precision with an LOQ of 0.035% in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. However, the LOQ corresponds to the level for which the repeatability and the recovery are validated. The LOQ proposed by applicant show a validated repeatability but no recovery has been perfpormed at this level. Therefore the LOQ cannot be validated without further data. Recovery data at this level 0.35 g/kg should be provided

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-2 (c)Summary of validation data for determination of the impurity formaldehyde in the glyphosate active substance technical material as manufactured

Analyte	**LOQ (g/kg)**	**Accuracy**		**Repeatability % RSD (n)**	**Linearity**	**Specificity**
		Recovery Fortification Level (g/kg)	**Recoveries % range (mean)**			
Formaldehyde	A standards solution of approximately 1.280mg/L each was injected seven times and mean area, standards deviation and coefficient of variation were determined. LOQ = 0.35g/kg (0.035% w/w)	1.15 (0.1157% w/w)	106 n=1	Mean content: 10.24 mg/L %RSD = 0.41 (7) Modified Horwitz value ($\%RSD_r$): 0.94 Horwitz ratio (H_r = $\%RSD/\%RSD_r$): 0.43 As H_r < 1, precision is acceptable.	**Calibration plot:** *y* = 137670x - 30105 **Coefficient of determination:** R^2 = 0.9999 **Range:** 1.28 – 12.80 mg/L **(**0.035 – 0.35% w/w content in the active substance) **Number of determinations:** 6 Standards	Example chromatograms of a solvent blank, analytical standards of each impurity, and the active substance technical material are available. Comparison of the solvent blank and analytical standards of the impurities show no significant peak interferences with formaldehyde. The retention time of the analytical standard matches that of the analyte peak from the active substance technical material (~5.9 min). The data is considered sufficient to demonstrate the specificity of the method with respect to formaldehyde
		1.19 (0.119% w/w)	109 n=1			
		1.55 (0.155% w/w)	103 n=1			

Determination of N-nitrosoglyphosate

Principle of method

The N-nitrosoglyphosate content in the glyphosate active substance technical material as manufactured was quantified using HPLC coupled UV-vis detection. The method was based on an FAO recommended procedure with adjustments to test facility equipment.

The N-nitrosoglyphosate is derivatised by reaction with HBr to form a nitrosyl cation. The nitrosyl cation then reactions with N-(1-Naphthyl) ethylenediamine and sulphanilamide to form a purple azo dye which is detectable at 480 nm.

The identity of NNG is confirmed by the comparison of the retention time of the standard solution of the NNG and the test item.

Derivatisation
1000 µL of sample or standard solution was pipetted into a Teflon capped test tube. To the tube, 250 µL 48% HBr and 250 µL sulfanilamide reagent (2.5 g sulphanilamide in 20 mL 48% HBr and 30 mL deionised water) were added and allowed to stand for five minutes. 500 µL NED reagent (4.35g NED in 400 µL deionised water and 500 µL 48% HBr) was added to bring the volume to 2 mL. The solution was then placed on a heat block at 95 ºC for 15 minutes to provide the derivatised working solution.

Linearity
Preparation of calibration standards
Calibration solutions were prepared by weighing approximately 10 mg of the analytical standard of N-nitroglyphosate into a 10 mL volumetric flask. The standard solution was dissolved in deionized water and the volumetric flask filled to the mark, providing a standard solution with ~1000 mg/L analytical standard

A ~20 mg/L working solution of N-nitroglyphosate was made by pipetting 200 µL of the 1000 mg/L standard solution into a 10 mL volumetric flask and diluting to volume. From this working solution, volumes of 50, 100, 200, 300, 400, 600, and 800 µL were diluted further in a total volume of 10 mL. Each solution was then derivatised by combining 5 mL of each standard solution with 2.5 mL of NED solution and 2.5 mL of sulfanilamide solution. This provided a derivatised standard working range of 0.047 – 0.748 mg/L (0.54 -8.59mg/kg).

Accuracy
Preparation of spiked solutions
Three solutions of test substance (Batch AFS08/1973) were prepared by weighing approximately 2000 mg of sample into a 10 mL volumetric flask. Samples were spiked at concentrations of 0.751 mg/kg, 0.854 mg/kg and 1.004 mg/kg N-nitroglyphosate

Test item
Preparation of test samples
2000 mg of each sample was weighed into a 10 mL volumetric flask. To each flask, 6 mL of 5N sodium hydroxide was added the solution shaken until the contents were dissolved. The flask was made to the 10 mL mark with MilliQ water. 5 mL of each test sample was transferred to a fresh 10 mL volumetric flask. 2.5 mL of each derivatisation solution was added to the flask (NED and sulfanilamide) to provide a total volume of 10 mL. The solution was placed in an aluminium heating block at 95 ºC for 15 minutes, providing the derivatised test item.

Analysis of test samples
10 µL of the prepared sample solution is injected into the HPLC coupled mass spectrometry and analysed under the following chromatographic conditions:

Instrument:	LC-10ADVP Pump, SIL-10ADVP autosampler, SPD-M10AVP PDA Detector
Column:	Altima C18 column: 250 x 4.6 mm
Mobile Phase:	Phase A: Acetonitrile Phase B: 50mM CH_3COONH_4 pH 6.4
Injection Volume:	30 µL
Flow Rate:	1 mL/min

Temperature:	40 ºC
Detection:	PDA at 480 nm
Run Time:	28 minutes Time 0: %A:25 %B:75 Time 12: %A:30 %B:70 Time 15: %A:40 %B:60 Time 20: %A:25 %B:75 Time 28: Stop
Retention Time:	N-nitrosoglyphosate at ~18.3 minutes

Findings

The following validation has generated with respect to the specificity, linearity of response, (method) precision, and accuracy for the method when quantifying N-nitrosoglyphosate in the glyphosate active substance technical material.

Specificity
The specificity was assessed by examination of the peak homogeneity and purity using a diode array detector. The examination of response at different wavelengths, comparison of UV spectra of the reference material and spiked samples, and determination of peak purity demonstrated no interfering compounds. For both the spiked test samples and the N-nitroglyphosate reference material, a retention time of ~18.3 minutes was observed. The data is therefore concluded to be sufficient to demonstrate the specificity of the method with respect to determination of N-nitrosoglyphosate content.

Linearity
The linearity of response was demonstrated by preparing 5 analytical standards at concentrations ranging from 0.047 – 0.748 mg/L (corresponding to 0.54 - 8.59mg/kg content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 141939x - 821.88$ and a correlation coefficient (r) of 0.9999.

Accuracy
Recovery data was generated on samples of glyphosate technical material spiked with an analytical standard of N-nitroglyphosate. N-nitroglyphosate was spiked at concentrations of 0.751 mg/kg, 0.854 mg/kg and 1.004mg/kg. The accuracy of the method was evaluated by the determination of the analyte content in the spiked samples. The recovery rates fell within this range with 94% for the 0.751 mg/kg spiking solution, 97% for the 0.854 mg/kg spiking solution, and 94% for the 1.004 mg/kg spiking solution.
Precision
The method precision was assessed through the seven-fold determination of a derivatised, N-nitroglyphosate standard (0.561 mg/L). A % RSD value of 0.44 and modified Horwitz value ($\%RSD_r$) of 1.46 were attained at this concentration of N-nitroglyphosate. The resulting Howtiz ratio ($H_r = 0.30$) is less than 1 as stipulated by SANCO/3030/99 rev. 5.

LOQ
For the LOQ, a standards solution of approximately 0.047mg/L (eq 0.00054 g/kg) each was injected six times and mean area, standards deviation and coefficient of variation were determined. The LOQ is 0.00054g/kg.
The LOQ corresponds to the level for which the repeatability and the recovery are validated. The repeatability has been validated at the level 0.035%. However no measurement of the recovery has been performed.

Conclusions
Supporting validation data have been provided for the analytical method used to quantify the N-nitrosoglyphosate content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The method has been shown to be validated for determination of N-nitrosoglyphosate content in the active substance technical material with acceptable accuracy and precision down to levels of ~0.5 mg/kg. The validation data is therefore sufficient to support the determination of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision with an LOQ of 0.00054g/kg in accordance with the criteria laid down in SANCO/3030/99 rev. 5
The LOQ corresponds to the level for which the repeatability and the recovery are validated. The LOQ proposed by applicant show a validated repeatability but no recovery has been perfpormed at this level. Therefore the LOQ cannot be validated without further data. Recovery data at this level 0.54mg/kg should be provided

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-3 (c)Summary of validation data for determination of the impurity N-nitrosoglyphosate in the glyphosate active substance technical material as manufactured

Analyte	**LOQ (g/kg)**	**Accuracy**		**Repeatability % RSD (n)**	**Linearity**	**Specificity**
		Recovery Fortification Level (mg/kg)	**Recoveries % range (mean)**			
N-nitroso-glyphosate	The limit of quantification was determined based on the sevenfold determination of the lowest concentration used for determining linearity. The RSD was determined to be 2.27%, (n=6) LOQ = 0.00054g/kg	0.751	94 n=1	Mean content: 0.561 mg/L (6.44mg/kg) %RSD = 0.44 (7) Modified Horwitz value ($\%RSD_r$): 1.46 Horwitz ratio (H_r = $\%RSD/\%RSD_r$) : 0.30 As H_r < 1, precision is acceptable.	**Calibration plot:** *y* = 141939x - 821.88 **Coefficient of determination:** R^2 = 0.9999 **Range:** 0.047 – 0.748 mg/L (0.54 - 8.59mg/kg) **Number of determinations:** 6 Standards	Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard, and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material (18.3 minutes). Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak.
		0.854	97 n=1			
		1.004	94 n=1			

						The data demonstrates the specificity of the method to be acceptable.

Source 4

Report:	**SSL04409: [redacted] (2010)**
Title:	KCA section 1/021: Glyphosate TC Analytical Profile of 5 Batches
Document No:	SSL04409
GLP:	Yes
Acceptability	Yes (further data required)

Determination of formaldehyde: FAO-method P25 (Monsanto Method No. AQC 678-86)

Principle of method

The formaldehyde content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection following derivatisation of the analyte with Hantzsch reagent.
The identity of the formaldehyde is confirmed by the comparison of the retention time of the standard solution of the formaldehyde and the test item

Preparation of Hantzsch reagent solution
The Hantzsch reagent was prepared by placing 15.032 g ammonium acetate, 0.3 mL acetic acid and 0.2 mL acetyl acetone in a 100 mL volumetric flask and diluting to volume with water

Preparation of calibration standards
A formaldehyde stock solution was made by dilution of 100 µL formaldehyde 37.9% w/w solution in 100 mL of water. This provided a stock solution of concentration 410.46 µg/mL of formaldehyde. The stock solution was further diluted to provide six standard solutions which were mixed 1:1 (v/v) with Hantzsch reagent to derivatise the standards. This provided a working range of 0.2052 – 12.31371 µg/mL (equivalent to 0.0041 – 0.25 % w/w) of formaldehyde.

Preparation of test samples
1g of glyphosate technical material was weighed into a 100 mL volumetric flask. 10 to 15 mL of water and 3 mL 11% sodium hydroxide solution were added to the flask and the sample dissolved by sonication for 5 minutes. The flask was the filled to the mark with water and the samples mixed 1:1 (v/v) with Hantzsch reagent solution and left to stand at ambient temperature for 2 hours to provide the test samples (0.005 g/mL)

Preparation of reagent blank
A reagent blank was prepared as described above, without the addition of any test item.

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Column:	Zorbax ODS XDB 5 µm, 150 x 4.6 mm
Mobile Phase:	20:80 (v/v) acetonitrile / water
Injection Volume:	50 µL
Flow Rate:	1.0 mL/min
Temperature:	40 ºC
Detection:	412 nm (UV)
Run Time:	12 minutes
Retention Time:	Formaldehyde at ~4.5 minutes

Findings

The following validation has generated with respect to the specificity, linearity of response, precision, and accuracy for the method when quantifying formaldehyde in the glyphosate active substance technical material.

Specificity
Representative chromatograms of a blank (internal standard only), formaldehyde analytical standard (0.43 µg/mL) and glyphosate active substance technical material. No interference is observed at the retention time of the analyte.

Linearity
The linearity of response was demonstrated by the two fold determination of six analytical standards at concentrations ranging from 0.2052 – 12.3137 µg/mL (equivalent to 0.0041 – 0.25 % w/w, *i.e.* 0.04 – 2.5 g/kg content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 1620155.5114x - 62594.2704$ and a correlation coefficient (r) of 0.9994. The data meets the criteria of acceptability according to SANCO/3030/99 rev. 5. The method linearity therefore considered to have been adequately addressed.

Accuracy
Recoveries data for fortified samples are not presented here. The analytical method being used to determine the formaldehyde content is FAO-method P25 (Method No. AQC 678-86). The method is referenced in the FAO Specifications and Evaluations for Plant Protection Products document for glyphosate (2000/2001) for determination of formaldehyde content in glyphosate technical material.

Precision
Method precision was evaluated by the 5-fold determination of formaldehyde content in a single batch of technical material (ERG24795-1). The mean content was determined to be 0.025 g/kg (0.0025 % w/w) with an associated %RSDs of 3.6. The modified Horwitz value ($\%RSD_r$) was 3.30, corresponding to Horwitz ratios (H_r) of 0.92. As the Horwitz ratios are not greater than 1 in any instance, the method precision at all spiking levels investigated is considered acceptable.

LOQ
The LOQ of the method is 0.025g/kg.

Analyte	**Content (g/kg)**	**No of replicates**	**Mean recoveries%**	**RSD (%)**
formaldehyde	0.025	5	98.4	3.6

Conclusions
Supporting validation data have been provided for the use of analytical method to quantify the formaldehyde content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. An assessment of the method precision, linearity, and accuracy were all found to be acceptable, the method precision is concluded to be adequate. The validation data is therefore sufficient to support the determination of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fit-for-purpose and no further consideration is needed.

Assessment and conclusion by RMS:. The data provided confirm the acceptability of the method in term of specificity, linearity and precision..
Concerning the accuracy, the Horrat value is above 1 (but <2) for the content 0.025g/kg. An explanation should be provided.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-2 (d)Summary of validation data for determination of the impurity formaldehyde in the glyphosate active substance technical material as manufactured

Analyte	**Impurity level (g/kg)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
Formaldehyde	0.025g/kg	-	Mean content = 0.025 g/kg %RSD: 3.6 (5) Modified Horwitz value: 3.30 Horwitz ratio (Hr = %RSD/%RSDr): 1.10 Hr >1.	**Calibration plot:** y = 1620155.5114x – 62594.2704 **Correlation coefficient:** R = 0.9994 **Range:** 0.2052 – 12.3137 µg/mL (corresponding to 4.1E-4 – 0.25 % w/w content in the active substance technical material **Number of determinations:** 6 Standards	Representative chromatograms of a blank (internal standard only), formaldehyde analytical standard (0.43 µg/mL), and glyphosate active substance technical material. The data demonstrates the specificity of the method to be acceptable.

Determination of N-nitrosoglyphosate

Principle of method

The N-nitrosoglyphosate content in the glyphosate active substance technical material as manufactured was quantified by ion chromatography with UV-vis detection.
The identity of NNG is confirmed by the comparison of the retention time of the standard solution of the NNG and the test item

Preparation of standard stock solutions
A stock solution was prepared by dissolving 55.6 mg of N-nitrosoglyphosate monosodium salt in 100 mL water containing trifluoro acetic acid 0.1% (v/v) and purified glyphosate (1 g/L). The stock solution was equivalent to ~500 µg/mL N-nitrosoglyphosate and labelled SSN I

A second solution, SSN II, was prepared by further dilution of 10 mL SSN I in 100 mL HPLC grade water/trifluoro acetic acid 0.1% v/v providing a concentration of ~50 µg/mL N-nitrosoglyphosate.

Preparation of calibration standards
Calibration solutions were prepared by further dilution of various volumes SSN II (200, 300, 400, 500, 1000, 1500 µL) in 100 mL HPLC grade water containing trifluoro acetic acid (0.1% v/v). This provided a working range of 0.1 – 0.78 µg/mL, equivalent to 0.00004 – 0.0003 % w/w of the technical material samples.

Preparation of test samples
Samples were prepared by weighing approximately 500 mg glyphosate technical material from each of the five batches in a 2 mL, screw capped centrifuge tube. 1.5 mL sodium hydroxide solution (2.2 mol/L) was added and the volume made to the mark with deionised water. Samples were placed in an orbital shaker until completely dissolved at which point 500 mg cation exchanger Dowex 50 WX8-100 (extracted with methanol) was added to the samples. The samples were shaken again for 5 minutes and the centrifuged for 5 minutes at 2000 rpm. The supernatant was subject to direct HPLC for measurement.

Analysis of test samples
50 µL of the prepared sample solution is injected into the ion chromatography system and analysed under the following chromatographic conditions:

Column:	PRP-X100 10µm, 250 x 4 mm + pre-column 10 x 4 mm
Mobile Phase:	Water + 0.075% trifluoro acetic acid
Injection Volume:	50 µL
Flow Rate:	1.5 mL/min
Temperature:	40 °C
Detection:	240 nm (UV)
Run Time:	12 minutes
Retention Time:	N-nitrosoglyphosate at ~7.0 – 7.8 minutes

Findings

The following validation data has been generated with respect to the specificity, linearity of response, precision, and accuracy for the method when quantifying N-nitrosoglyphosate in the glyphosate active substance technical material.

Specificity
Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard (0.20 µg/mL; 0.8 mg/kg), and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to quantification of N-nitrosoglyphosate content.

Linearity

The linearity of response was demonstrated by the two-fold determination of 6 analytical standards at concentrations ranging from 0.10 - 0.78 µg/mL (corresponding to 0.00004 – 0.0003% w/w). A representative linearity of response plot gives a calibration of plot equation of $y = 258896.1122x - 7817.8267$ and a correlation coefficient (r) of 0.9998. The data meets the criteria of acceptability according to SANCO/3030/99 rev. 5. The method linearity therefore considered to have been adequately addressed.

Accuracy
The recovery of the method is not demonstrated.

Precision
Method precision was evaluated by the 5-fold determination of formaldehyde content in a single batch of technical material (ERG24795-1). The mean content was determined to be 0.00061 g/kg (0.000061 % w/w) with an associated %RSDs of 6.69. The modified Horwitz value ($\%RSD_r$) was 5.78, corresponding to Horwitz ratios (H_r) of 0.86. As the Horwitz ratios are not greater than 1 in any instance, the method precision is considered acceptable.

LOQ
The LOQ of the method is 0.00061 g/kg.

Analyte	**Content (mg/kg)**	**No of replicates**	**Mean recoveries%**	**RSD (%)**
NNG	0.61	5	100.2	6.69

Conclusions
Supporting validation data have been provided for the use of analytical method to quantify the N-nitrosoglyphosate content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. An assessment of the method precision, linearity, and accuracy were all found to be acceptable, the method precision is concluded to be adequate. The validation data is therefore sufficient to support the determination of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fit-for-purpose and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method in term of specificity, linearity and precision with an LOQ of 0.61mg/kg.
Concerning accuracy, the Horrat value is above 1 (but <2) for the content 0.61mg/kg. An explantion should be provided**.**

Table 4.1.1-3 (d)Summary of validation data for determination of the impurity N-nitrosoglyphosate in the glyphosate active substance technical material as manufactured

Analyte	Impurity level (g/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
N-nitroso-glyphosate	0.61mg/kg regarding data of repeatability	-	Mean content = 0.61 mg/kg %RSD: 6.69 (5) Modified Horwitz value: 5.77 Horwitz ratio (Hr = %RSD/%RSDr): 0.86 Hr > 1.	**Calibration plot:** $y = 258898.1122x + 7817.8267$ **Correlation coefficient:** R: 0.9998 **Range:** 0.1034 – 0.7756 µg/mL (corresponding to 0.00004 – 0.0003 % w/w content in the active substance technical material) **Number of determinations:** 6 Standards	Representative chromatograms of a blank (solvent only), N-nitrosoglyphosate analytical standard and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. The data demonstrates the specificity of the method to be acceptable.

Source 5

Report:	**KCA Section 1/028 to 031: [redacted] (2015)**
Title:	Qualitative and Quantitative Profile of the test substance Glyphosate Technical JiangNan (Five Batch Analysis)
Document No:	RF.14613.030.067.14
Guidelines:	Commission Regulation (EU) No 283/2013 of 1 March 2013 SOP-M 1573 – Estudo de Cinco Bateladas (Five Batch Analysis) – Revision 05
GLP:	Yes
Acceptability	Yes

Determination of formaldehyde

Principle of method

The formaldehyde content in the glyphosate active substance technical material as manufactured was quantified by HPLC with UV-vis detection following derivatisation of the analyte with a Hantzsch reagent (ammonium acetate, acetic acid and acetylacetone). The identity of the formaldehyde is confirmed by H1-NMR and IR

Preparation of standard stock solutions

The Hantzsch reagent was prepared by mixture of 50.01245 g of ammonium acetate, 1 mL of acetic acid, 670 µL of acetylacetone and 330 mL of ultra-pure water in a volumetric flask of 500 mL.

Preparation of calibration standards

A stock solution of impurity was prepared by weighing of 32.89 mg of the Formaldehyde analytical standard in volumetric flask of 10 mL. The volume was completed with ultra pure water and the solution was stirred and sonicated until complete solubilization, producing the stock solution with 1213.64 mg/L of Formaldehyde analytical standard. An aliquot of 420 µL of this solution was diluted to a final volume of 10 mL with ultra pure water producing the Solution A with 50.97 mg/L of analytical standard. 6 Standard solutions were prepared from dilutions of the Solution A with ultra pure water.

Each one of these standard solutions was derivatized by combining of equal volumes (500 µL) of standard solution and reagent Hantzsch for two hours, producing the calibration solutions at concentrations of 1.02, 2.55, 4.08, 6.12, 8.16, 10.20 mg./.

Preparation of test samples
Solutions of the test substance were prepared by weighing of approximately 60.0 mg of sample in volumetric flask of 10 mL. In each one of these flasks were added 180 µL of sodium hydroxide solution (11 %) and the volumes of solutions were completed with ultra pure water, the solutions were stirred and sonicated until complete solubilization producing the solutions with approximately 6000 mg/L.

All these solutions were derivatized with the reagent Hantzsch by combining equal volumes (500 µL) of sample solution and reagent Hantzsch, the solutions reposed for two hours before analysis, producing final solutions with concentrations of 3000 mg/L.

Analysis of test samples
20 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1100 series HPLC
Column:	Phenomenex Luna C18, 250mm x 2mm, 5 µm
Mobile Phase:	Acetonitrile / Ultra pure water (20 : 80 % v/v)
Injection Volume:	20 µL
Flow Rate:	0.4 mL/min
Temperature:	40ºC
Detection:	412 nm (UV)
Run Time:	10 minutes
Retention Time:	Formaldehyde at ~5.0 minutes

Findings
The following validation has generated with respect to the specificity, linearity of response, precision, and accuracy for the method when quantifying formaldehyde in the glyphosate active substance technical material.

Specificity
Representative chromatograms are provided of a solvent blank, 6 analytical standards (ranging between 1.02-10.20 mg/L) and the glyphosate active substance technical material. The retention time of the peak of the formaldehyde analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the formaldehyde impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to quantification of formaldehyde content.

Linearity
The linearity of response was demonstrated by preparing 6 analytical standards at concentrations ranging from 1.02-10.20 mg/L (corresponding to 0.034-0.34 % w/w, *i.e.* 0.34-3.4 g/kg content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 439.33595x - 15.00612$ and a correlation coefficient (r) of 0.99980.

Accuracy

Recoveries data were generated on samples of the glyphosate active substance technical material spiked with an analytical standard of formaldehyde at levels of 2.04 ng/µL (0.7 g/kg), 4.08 ng/µL (1.3 g/kg) and 6.12 ng/µL (2.0 g/kg). The mean recovery for the 0.7 g/kg spiking level is 98.49 % and is therefore within the acceptable range of 75-125 % according to the criteria of SANCO/3030/99 rev. 4. The mean recoveries for the 1.3 g/kg and 2.0 g/kg spiking levels are 101.84 % and 101.85 % and are therefore within the acceptable range of 80-120 % according to the criteria of SANCO/3030/99 rev. 5.

Precision
The method precision could not be calculated in a meaningful way using samples of the glyphosate active substance technical material as the formaldehyde content in the technical material was <LOQ in all cases.

However, an assessment of the method precision was performed on samples of the technical material spiked with an analytical standard of formaldehyde and evaluated by seven replicates of sample determinations in duplicate. Overall, for both sets of seven replicates the mean content of the spiked sample material was determined to be 0.172% (1.72 g/kg) with a %RSD of 0.64. The modified Horwitz equation for the spike level tested gives a value of 5.21. The experimentally derived %RSD and the modified Horwitz value (%RSD_r) give a Howitz ratio (H_r) = 0.64/5.21 = 0.12. As $H_r < 1$, it is therefore proposed that the method precision at this level is adequate to supporting determination of formaldehyde content down to 1.72 g/kg.

LOQ
The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 4.08%) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution.

Analyte	**Mean content (g/kg)**	**No of replicates**	**Mean recoveries%**	**RSD (%)**
formaldehyde	0.34	6	92.94	4.08

Conclusions
Supporting validation data have been provided for the use of analytical method used to quantify the formaldehyde content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The method has been shown to be validated for determination of formaldehyde content in the active substance technical material with acceptable accuracy and precision down to levels of 1.72 g/kg. The validation data is therefore sufficient to support quantification of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision with an LOQ of 0.034% in accordance with the criteria laid down in SANCO/3030/99 rev. 5.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-2 (e)Summary of validation data for determination of the impurity formaldehyde in the glyphosate active substance technical material as manufactured ([redacted] source, glycine route)

Analyte	**LOQ (g/kg)**	**Recovery Fortification Level**	**Recoveries % range (mean)***	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
Formaldehyde	The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 4.8%, mean recovery%: 92.9%) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. LOQ 0.034%	2.03ng/µL (0.7g/kg)	98.49 n = 1	Analyst 1 Mean content = 1.72 g/kg %RSD: 0.50 (n=7) Modified Horwitz value: 3.49 Horwitz ratio (Hr = %RSD/%RSDr): 0.14 Hr < 1. Analyst 2 Mean content = 1.72 g/kg %RSD: 0.80 (n=7) Modified Horwitz value: 3.49 Horwitz ratio (Hr = %RSD/%RSDr): 0.23 Hr < 1.	**Calibration plot:** $y = 439.33595x - 15.00612$ **Correlation coefficient:** R = 0.99980 **Range:** 1.02-10.20 mg/L (corresponding to 0.034-0.34 % w/w content in the active substance technical material) **Number of determinations:** 6 Standards	Representative chromatograms are provided of a solvent blank, 5 analytical standards (ranging between 1.02-10.20 mg/L) and the glyphosate active substance technical material. The data demonstrates the specificity of the method to be acceptable.
		4.07 ng/µL (1.3g/kg)	101.84 n = 1			
		6.22ng/µL (2.0g/kg)	101.85 n = 1			

Determination of N-nitrosoglyphosate

Principle of method

The N-nitrosoglyphosate (NNG) content in the glyphosate active substance technical material as manufactured was quantified by HPLC with mass spectrometer detection at selected ion monitoring (SIM) mode following derivatisation of the analyte with a derivatization with NED/HBr and sulphanilamide.

Preparation of reagents
A solution of NED/HBr was prepared by dissolution of 0.45015 g of N-(1-naphthyl) ethylenediamine dihydrochloride in 40 mL of ultra-pure water. It was added 50 mL of bromidric acid 48% and the volume was completed to 100 mL with ultra-pure water.

A solution of Brij 35 (30%) was prepared by dissolution of 3.00019 g of Brij 35 to a final volume of 10 mL with ultra-pure water.

A solution of sulphanilamide was prepared by weighing of 1.00022 g of sulphanilamide in a volumetric flask of 100 mL, it was added 50 mL of ultra-pure water, 10 mL of concentrated chloridric acid, 3.4 mL of Brij 35 (30%) and the volume of solution was completed with ultra-pure water.

Preparation of standard stock solutions
A stock solution of impurity was prepared by weighing of 2.80 mg of the glyphosate N-nitroso mono sodium salt analytical standard in a volumetric flask of 10 mL. The volume was completed with ultra-pure water and the solution was stirred and sonicated until complete solubilization producing a stock solution with 275.80 mg/L of analytical standard.

The stock solution prepared above presents the concentration of the analytical standard as mono sodium salt and the analysis determines the concentrations of compound in the acid form. Thus, the concentration of stock solution was changed using the conversion factor (F_C) to acid form of analyte.
The conversion factor is calculated as showed below:

$F_C = MW_{NNG\ acid}/MW_{NNG\ mono\ sodium\ salt} = 198.07/220.07 = 0.9$

F_C = conversion factor of content NNG mono sodium salt to acid form = 0.9

Therefore, the concentration of stock solution of glyphosate N-Nitroso in the acid form is 248.22 $mg.L^{-1}$. An aliquot of 130 µL of this stock solution was diluted to 50 mL with ultra-pure water producing 645.37 µg/L of analytical standard.

Preparation of calibration standards
7 calibration standard solutions were prepared by taking volumes of the analytical standard solution (645.37 µg/L), and derivatization with NED/HBr and sulphanilamide.

Preparation of test samples
Solutions of the test substance were prepared by weighing of approximately 1500 mg of sample. After then, 5 mL of ultra-pure water were added and the solutions were sonicated by 5 minutes and filtered with Millex filter (0.45µm x 13 mm), producing solutions with approximately 300,000,000 µg/L.

Work solutions with approximately 150,000,000 µg/L of the test substance were prepared by reacting of 500 µL of each sample solution with 250 µL of NED/HBr and 250 µL of sulphanilamide solution; these solutions were heated to 95ºC for 15 minutes using the dry block.

Analysis of test samples
10 µL of the prepared sample solution is injected into the HPLC and analysed under the following chromatographic conditions:

Instrument:	Agilent 1200 series HPLC
Column:	Phenomenex Luna C18, 250mm x 2mm, 5 µm
Mobile Phase:	Solvent A: acetonitrile Solvent B: Solution of ammonium acetate (10 mM) acidified with 0.1% (v/v) of acetic acid
Injection Volume:	10 µL
Flow Rate:	0.3 mL/min
Temperature:	40ºC
Detection:	Mass spectrometer, monitored ion = 368 m/z
Run Time:	25 minutes
Retention Time:	N-nitrosoglyphosate at ~15.36 minutes

Findings
The following validation has generated with respect to the specificity, linearity of response, precision, and accuracy for the method when quantifying N-nitrosoglyphosate in the glyphosate active substance technical material.

Specificity
Representative chromatograms of a blank solvent, 7 analytical standards (ranging between 51.63-322.69 µg/L) and glyphosate active substance technical material have been provided. The retention time of the peak of the N-nitrosoglyphosate analytical standard matches that of the impurity peak from the active substance technical material. Comparison of the chromatograms show there to be no interfering peaks coinciding with the retention time of the N-nitrosoglyphosate impurity peak. Hence the data is concluded to be sufficient to demonstrate the specificity of the method with respect to quantification of N-nitrosoglyphosate content.

Linearity
The linearity of response was demonstrated by preparing 7 analytical standards at concentrations ranging from 51.63-322.69 µg/L (corresponding to 0.00003-0.00022 % w/w, i.e. 0.3-2.2 mg/kg content in the active substance technical material). A representative linearity of response plot gives a calibration of plot equation of $y = 22.94070x - 96.82837$ and a correlation coefficient (r) of 0.99936.

Accuracy
Recoveries data were generated on samples of the glyphosate active substance technical material spiked with an analytical standard of N-nitrosoglyphosate at levels of 132.32 g/mL (0.67 mg/kg), 191.37 g/mL (1.0 mg/kg) and 234.84 µg/L (1.3 mg/kg). The mean recoveries for the 0.67 mg/kg, 1.0 mg/kg and 1.3 mg/kg spiking levels are 99.55 %, 105.95 % and 100.65 % and are therefore within the acceptable range of 70-130 % according to the criteria of SANCO/3030/99 rev. 5.

Precision
The method precision could not be calculated in a meaningful way using samples of the glyphosate active substance technical material as the N-nitrosoglyphosate content in the technical material was <LOQ in all cases.

However, an assessment of the method precision was performed on samples of the technical material spiked with an analytical standard of N-nitrosoglyphosate and evaluated by seven replicates of sample determinations in duplicate. The mean content of the spiked sample material was determined to be 0.66mg/kg with a %RSD of 7.14. The modified Horwitz equation for the spike level tested gives a value of 10.85. The experimentally derived %RSD and the modified Horwitz value ($\%RSD_r$) give a Howitz ratio (H_r) = 7.14/10.85=0.68.

LOQ
The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 3.68%) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution.

Analyte	**Mean content (%w/w)**	**No of replicates**	**Mean recoveries%**	**RSD (%)**
NNG	0.000030%	6	103.3	3.68

Conclusions
Supporting validation data have been provided for the use of analytical method used to quantify the N-nitrosoglyphosate content in the glyphosate active substance technical material. The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision in accordance with the criteria laid down in

SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013. The method has been shown to be validated for determination of N-nitrosoglyphosate content in the active substance technical material with acceptable accuracy and precision down to levels of 0.885 mg/kg. The validation data is therefore sufficient to support quantification of the impurity in the 5-batch analysis and the proposed technical specification. Overall, the method is considered fully validated in accordance with SANCO/3030/99 rev. 5, and no further consideration is needed.

Assessment and conclusion by RMS: The data provided confirm the acceptability of the method specificity, linearity, accuracy and precision with an LOQ of 0.000030% in accordance with the criteria laid down in SANCO/3030/99 rev. 5 and the data requirements of Regulation (EC) No 283/2013.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Table 4.1.1-3 (e)Summary of validation data for determination of the impurity N-nitrosoglyphosate in the glyphosate active substance technical material as manufactured ([redacted] source, glycine route)

Analyte	**LOQ (mg/kg)**	**Recovery Fortification Level (mg/kg)**	**Recoveries % range (mean)***	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
N-nitrosoglyphosate (NNG)	The limit of quantification of the method was evaluated by injecting of six replicates (RSD: 3.68%; Mean recoveries:103. 3%) of a standard solution (Solution LQ) with the same concentration of the most diluted calibration solution. LOQ: 0.000030%	0.67	99.55 n = 1	Analyst 1 Mean content = 0.66mg/kg %RSD: 7.14 (n=7) Modified Horwitz value: 10.85 Horwitz ratio (Hr = %RSD/%RSDr): 0.68 Hr < 1. Analyst 2 Mean content = 0.845mg/kg %RSD: 2.96 (n=7) Modified Horwitz value: 11.0 Horwitz ratio (Hr = %RSD/%RSDr): 0.27 Hr < 1.	**Calibration plot:** $y = 22.94070x - 96.82837$ **Correlation coefficient:** R = 0.99936 **Range:** 51.63-322.69 µg/L (corresponding to 0.00003 – 0.00022 % w/w content in the active substance technical material) **Number of determinations:** 7 Standards	Representative chromatograms are provided of a solvent blank, 7 analytical standards (ranging between 51.63-322.69 µg/L) and the glyphosate active substance technical material. The data demonstrates the specificity of the method to be acceptable.
		1.0	105.95 n = 1			
		1.3	100.65 n = 1			

Albaugh

Analytical method for the determination of relevant impurities N-nitroso glyphosate (NNG) and Formaldehyde in the technical material for the notifier Albaugh are provided and reported below.

Data point:	J-CA 1.11/017
Report author	[redacted]
Report year	2020
Report title	Analysis of five batches of Glyphosate wet cake to determine the content of active ingredient and specified impurities, with associated method validation, in compliance with good laboratory practice
Report No	DNA5494
Document No	-
Guidelines followed in study	Not stated
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

Analytical method for the determination of Impurity 1 – N-nitroso glyphosate (NNG)

Principle of method

The assay of Impurity 1 (*N*-Nitroso Glyphosate) was performed using approximately 0.25g of each batch of Technical Material (wet cake). The mass of the Technical Material was accurately recorded, transferred to a 25mL volumetric flask and made to partial volume with 0.01M Sodium Hydroxide in Deionised Water (5 samples in duplicate). These solutions were sonicated for 5 minutes and made to final volume once cooled. These solutions were then used for assay by injecting each solution once into the HPLC-DAD under the following conditions:

HPLC-DAD Conditions:

Instrument:	Agilent 1200 Series HPLC-DAD
Mode:	Gradient Reverse Phase
Column:	Lichrospher 60 RP-Select B, 250mm x 4.0mm
Packing:	RP-Select B, 5µm
Eluent:	(A) Acetonitrile : (B) 2mMol Cetyl Trimethyl Ammonium Bromide (CTAB) in Water adjusted to pH 2.4 with Phosphoric acid
Wavelength:	245nm
Injection Volume:	100µl
Flow Rate:	1.0mL/minute
Column Temperature:	25ºC
Data Collection:	LabSolutions
Retention Times:	Approximately 6.3 minutes

Validation

Specificity:
In the Specificity chromatograms the Impurity 1 (NNG) eluted at 6.4 minutes. Other significant peaks were accounted for by assaying a solvent blank and reference standards for Glyphosate, Impurity 2, Impurity 3, Impurity 4, Impurity 5, Impurity 6, Impurity 7, Impurity 8, Impurity 9 and Impurity 10. There were no significant peaks present in these chromatograms at the same elution time as the Impurity 1.
The UV, MS, FTIR and NMR spectra for impurity 1 confirm the species identification.

Linearity:
The linearity was determined from twenty-four injections of twelve concentrations of standard ranging from 0.005 mg/L to 1.0mg/L (corresponding to 0.5 mg/kg to 100 mg/kg). The samples were prepared for analysis at a sample concentration of 10mg/mL. From the sample assay it is known that the samples contain no detectable Impurity 1 above the LOQ Level of 0.0045mg/L. Recovery Precision was performed at 4.5mg/Kg which therefore equates to a concentration of 0.045mg/L stabilised *N*-Nitroso Glyphosate (0.04050135mg/L as *N*-Nitroso Glyphosate), which falls within the limits of the linearity range. The correlation coefficient was 1.0000. The first order equation of the calibration curve is y=000004x+0.000898.

Sample Precision:
To show the Sample Precision, six samples of approximately 0.25g of Technical Material were weighed into a 25mL volumetric flask and made to partial volume with 0.01M Sodium Hydroxide in Deionised Water. The samples were sonicated for 5 minutes, made to volume with 0.01M Sodium Hydroxide in Deionised Water once cooled and injected into the HPLC-DAD. The sample used contained no detectable Impurity 1 above the LOQ of <0.45mg/Kg.

Recovery Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2):
From the sample assay it is known that the samples contain no detectable Impurity 1 above the LOQ Level of <0.45mg/Kg. Therefore the Recovery Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2) samples were prepared for analysis at 0.045mg/L using the certified reference standard material. This was achieved by weighing approximately 0.25g of sample into a 25mL volumetric flask, spiked with 125µL of 10mg/L Impurity 1 reference standard solution and made to partial volume with 0.01M Sodium Hydroxide in Deionised Water. The sample was sonicated for 5 minutes and made to volume with 0.01M Sodium Hydroxide in Deionised Water once cooled.
Six separate solutions were prepared for each assay in this way and then injected into the HPLC-DAD. The results are indicated in the table below.

Intermediate Precision:
The results obtained from the Repetitiveness Assay 1 and Repetitiveness Assay 2 were combined to show the Sample Precision of the method and percentage relative standard deviation (%RSD) and Grubbs Test Criteria for the 12 Measurements. The results are indicated in the table below.

80% Recovery Precision :
From the sample assay it is known that the technical samples contain no detectable Impurity 1 above the LOQ Level of 0.45mg/Kg. Therefore the 80% Recovery Precision samples were prepared for analysis at 0.036mg/L using the certified reference standard material. This was achieved by weighing approximately 0.25g of technical sample into a 25mL volumetric flask, spiked with 100µL of 10mg/L Impurity 1 reference standard solution and made to partial volume with 0.01M Sodium Hydroxide in Deionised Water. The sample was sonicated for 5 minutes and made to volume with 0.01M Sodium Hydroxide in Deionised Water once cooled. Six separate solutions were prepared in this way and then injected into the HPLC-DAD. The results obtained indicate a percentage recovery range of 99.78% to 108.8%, with a standard deviation of 3.230. The results are summarized in the table below.

120% Recovery Precision:
The 120% Recovery Precision samples were prepared for analysis at 0.054mg/L using the certified reference standard material. This was achieved by weighing approximately 0.25g of sample into a 25mL volumetric flask, spiked with 150µL of 100mg/L Impurity 1 reference standard solution and made to partial volume with 0.01M Sodium Hydroxide in Deionised Water. The sample was sonicated for 5 minutes and made to volume with 0.01M Sodium Hydroxide in Deionised Water once cooled. Six separate solutions were prepared in this way and then injected into the HPLC-DAD. The results obtained indicate a percentage recovery range of 103.6% to 106.7%, with a standard deviation of 1.242. The results are summarized in the table below.

LOQ Recovery:
The LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery with an acceptable RSD, is obtained. There is no detectable Impurity 1 in the technical samples above the LOQ level, therefore the LOQ Recovery was performed by spiking Impurity 1 onto a selected technical sample. This was achieved by weighing approximately 0.25g of technical sample into a 25mL volumetric flask, spiking with 125µL of 1.0mg/L Impurity 1 reference standard solution and making to partial volume with 0.01M Sodium Hydroxide in Deionised Water. The sample was sonicated for 5 minutes and made to volume with 0.01M Sodium Hydroxide in Deionised Water once cooled. Five separate solutions were prepared in this way and then injected into the HPLC-DAD. The results obtained indicate a percentage recovery range of 83.59% to 105.4% with a standard deviation of 7.885. The results are summarized in the table below.

Validation Summary Table – Impurity 1 (*N*-Nitroso Glyphosate)

Validation Parameter	Results Obtained	Acceptance Criteria under SANCO/3030/99 rev.5
Linearity	R^2 = 1.0000 n=12*2	R^2 = >0.99

	0.5 mg/kg to 100 mg/kg	
Sample Precision	<0.45mg/Kg	n/a no detectable Impurity 1 above the LOQ Level of <0.45mg/Kg
Recovery Precision at 4.5mg/Kg (Repetitiveness Assay 1)	%RSD = 1.775 Hr = 0.208 n = 6	Horwitz %RSD less than 8.53 Horrat (Hr) ≤ 1 at 4.575mg/Kg
Recovery Precision at 4.5mg/Kg (Repetitiveness Assay 2)	%RSD = 2.018 Hr = 0.238 n = 6	Horwitz %RSD less than 8.47 Horrat (Hr) ≤ 1 at 4.797mg/Kg
Recovery Precision at 4.5mg/Kg (Intermediate Precision)	%RSD = 3.068 Hr = 0.361 n = 12	Horwitz %RSD less than 8.50 Horrat (Hr) ≤ 1 at 4.686mg/Kg
Grubbs Test (maximum) G_n	G_n = 1.612	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
Grubbs Test (minimum) G_1	G_1 = 1.345	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
80% Recovery at 3.6mg/Kg	Mean Recovery = 104.4% %RSD = 3.093 Hr = 0.352 n = 6	Between 70%-130% Horwitz %RSD less than 8.78 Horrat (Hr) ≤ 1 at 3.759mg/Kg
100% Recovery at 4.5mg/Kg (Repetitiveness Assay 1)	Mean Recovery = 101.7% %RSD = 1.775 Hr = 0.208 n = 6	Between 70%-130% Horwitz %RSD less than 8.53 Horrat (Hr) ≤ 1 at 4.575mg/Kg
100% Recovery at 4.5mg/Kg (Repetitiveness Assay 2)	Mean Recovery = 106.6% %RSD = 2.018 Hr = 0.238 n = 6	Between 70%-130% Horwitz %RSD less than 8.47 Horrat (Hr) ≤ 1 at 4.780mg/Kg
120% Recovery at 5.6mg/Kg	Mean Recovery = 105.6% %RSD = 1.176 Hr = 0.143 n = 6	Between 70%-130% Horwitz %RSD less than 1.176 Horrat (Hr) ≤ 1 at 5.705mg/Kg
LOQ Recovery at 0.45mg/Kg	Mean Recovery = 93.74% %RSD = 8.411 Hr = 0.689 n = 5	Between 70%-130 Horwitz %RSD less than 12.21 Horrat (Hr) ≤ 1 at 0.422mg/Kg

Assessment and conclusion

Assessment and conclusion by applicant:

Assessment and conclusion by RMS: The analytical method for the determination of the impurity N-Nitroso Glyphosate in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5 with a LOQ of 0.45 mg/kg.

Analytical method for the determination of Impurity 2 – Formaldehyde

Principle of method

The assay of Impurity 2 (Formaldehyde) was performed using approximately 0.0625g of each batch of Technical Material (wet cake). The mass of the Technical Material was accurately recorded and transferred to a 25mL volumetric flask. 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride) was added and made to partial volume with Deionised Water.

The samples were sonicated for 5 minutes, made to full volume with Deionised Water and allowed to stand for 2 hours to fully derivatize, and injected into the HPLC-PDA under the following conditions:

HPLC-PDA Conditions:

Instrument:	Shimadzu HPLC-PDA
Mode:	Isocratic Reverse Phase
Column:	Grace Alltima C8, (250mm x 4.6mm)
Packing:	C8, 5µm
Eluent:	65% Acetonitrile 35% Deionised Water adjusted to pH3 with Phosphoric Acid
Wavelength:	265nm
Injection Volume:	100µL
Flow Rate:	1.0mL/minute
Column Temperature:	25ºC
Data Collection:	LabSolutions
Retention Times:	Approximately 6.3 minutes

Validation

Specificity:
In the Specificity chromatograms derivatized Impurity 2 (formaldehyde) eluted at 6.3 minutes. Other significant peaks were accounted for by assaying a solvent blank and reference standards for Glyphosate, Impurity 1, Impurity 3, Impurity 4, Impurity 5, Impurity 6, Impurity 7, Impurity 8, Impurity 9, and Impurity 10. There were no significant peaks present in these chromatograms at the same elution time as the Impurity 2. This demonstrates that there were no analyte interferences.
The UV, MS, FTIR and NMR spectra for impurity 2 confirm the species identification.

Linearity:
The linearity was determined from thirty-six injections of eighteen concentrations of standard ranging from 0.025mg/L to 10mg/L (corresponding to 0.01 g/kg to 4 g/kg). The samples were prepared for analysis at a sample concentration of 2.5mg/mL. From the sample assay it is known that the technical samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2. This therefore equates to a concentration range of 0.0425mg/L and 0.5mg/L, which falls within the limits of the linearity range. The correlation coefficient was 0.9996. The first order equation of the calibration curve is y=0000091x+0.0168.

Low Sample Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2)
To show the Low Sample Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2), six samples of approximately 0.0625g of Technical Material were weighed into a 25mL volumetric flask. 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride) was added and made to partial volume with Deionised Water. The samples were sonicated for 5 minutes, made to full volume with Deionised Water and allowed to stand for 2 hours to fully derivatize, and injected into the HPLC-PDA. The results are indicated in the table below.

Intermediate Precision:
The results obtained from the Repetitiveness Assay 1 and Repetitiveness Assay 2 were combined to show the Sample Precision of the method and percentage relative standard deviation (%RSD) and Grubbs Test Criteria for the 12 Measurements. The results are indicated in the table below.

High Sample Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2):
To show the High Sample Precision (Repetitiveness Assay 1 & Repetitiveness Assay 2), six samples of approximately 0.0625g of Technical Material were weighed into a 25mL volumetric flask. 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride) was added and made to partial volume with Deionised Water. The samples were sonicated for 5 minutes, made to full volume with Deionised Water and allowed to stand for 2 hours to fully derivatize, and injected into the HPLC-PDA. The results are indicated in the table below.

Low 80% Recovery:

From the sample assay it is known that the samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2 This equates to between 0.0425mg/L and 0.5mg/L as the samples were made at 2.5mg/mL concentration. Therefore a Recovery performed at 80% of the Low Impurity 2 content, would equate to 0.034mg/L, equivalent to 0.0136%. Hence the Low 80% Recovery samples were prepared for analysis by spiking technical samples with 0.034mg/L using the certified reference standard material. This was achieved by weighing approximately 0.0625g of sample into a 25mL volumetric flask, spiking with 340µL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride), and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained indicate a percentage recovery range of 97.43% to 99.71%, with a standard deviation of 0.880. The results are summarized in the table below.

Low 100% Recovery:

From the sample assay it is known that the samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2. This equates to between 0.0425mg/L and 0.5mg/L as the samples were made at 2.5mg/mL concentration. Therefore the Low 100% Recovery samples were prepared for analysis by spiking samples of with 0.0425mg/L using the certified reference standard material. This was achieved by weighing approximately 0.0625g of technical sample into a 25mL volumetric flask, spiking with 425µL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride), and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained indicate a percentage recovery range of 98.51% to 110.1%, with a standard deviation of 4.252. The results are summarized in the table below.

Low 120% Recovery :

From the sample assay it is known that the samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2, this equates to between 0.0425mg/L and 0.5mg/L as the samples were made at 2.5mg/mL concentration. Therefore a Recovery performed at 120% of the Low Impurity 2 content, would equate to 0.051mg/L, equivalent to 0.0204g/Kg. Hence the Low 120% Recovery samples were prepared for analysis by spiking technical samples of with 0.051mg/L using the certified reference standard material. This was achieved by weighing approximately 0.0625g of sample into a 25mL volumetric flask, spiking with 510µL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride), and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained indicate a percentage recovery range of 101.5% to 105.9%, with a a standard deviation of 1.595. The results are summarized in the table below.

High 80% Recovery:

From the sample assay it is known that the samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2 which equates to between 0.0425mg/L and 0.5mg/L as the samples were made at 2.5mg/mL concentration. Therefore a Recovery performed at 80% of the High Impurity 2 content, would equate to 0.4mg/L, equivalent to 0.16g/Kg. Hence the High 80% Recovery samples were prepared for analysis by spiking samples with 0.4mg/L using the certified reference standard material. This was achieved by weighing approximately 0.0625g of sample DNA5400/8 (wet cake) into a 25mL volumetric flask, spiking with 4.0mL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride), and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained indicate a percentage recovery range of 90.47% to 95.48%, with a standard deviation of 1.795. The results are summarized in the table below.

High 100% Recovery:

From the sample assay it is known that the samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2. This equates to between 0.0425mg/L and 0.5mg/L as the samples were made at 2.5mg/mL concentration. Therefore the High 100% Recovery samples were prepared for analysis by spiking technical samples with 0.5mg/L using the certified reference standard material. This was achieved by weighing

approximately 0.0625g of sample into a 25mL volumetric flask, spiking with 5.0mL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride), and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained indicate a percentage recovery range of 80.52% to 85.68%, with a standard deviation of 2.154. The results are summarized in the table below.

High 120% Recovery:

From the sample assay it is known that the samples contain between approximately 0.017g/Kg and 0.2g/Kg Impurity 2. This equates to between 0.0425mg/L and 0.5mg/L as the samples were made at 2.5mg/mL concentration. Therefore a Recovery performed at 120% of the High Impurity 2 content, would equate to 0.6mg/L, equivalent to 0.24g/Kg. Hence the High 120% Recovery samples were prepared for analysis by spiking samples of DNA5400/8 (wet cake) with 0.6mg/L using the certified reference standard material. This was achieved by weighing approximately 0.0625g of sample into a 25mL volumetric flask, spiking with 6.0mL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride), and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained indicate a percentage recovery range of 86.83% to 92.59%, with a standard deviation of 2.248. The results are summarized in the table below.

LOQ Recovery:

The LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery with an acceptable RSD, is obtained. Impurity 2 is present in the samples above the LOQ level and therefore the LOQ Recovery was performed by spiking Impurity 2 onto the solvent blank. Hence the LOQ Recovery samples were prepared for analysis at 0.025mg/L using the certified reference standard material. This was achieved by spiking 250µL of 2.5mg/L Impurity 2 spiking standard solution and 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride) into a 25mL volumetric flask, and making to partial volume with Deionised Water. The sample was sonicated for 5 minutes, made to volume with Deionised Water once cooled and allowed to stand for 2 hours to fully derivatize. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results obtained at 0.01 g/kg indicate a percentage recovery range of 92.72% to 98.64% with a standard deviation of 2.350. The results are summarized in the table below.

Stability and Reproducibility of the Derivatized Method:

Due to the nature of the compound, Impurity 2 is not visible by UV on a standard method. Therefore, it was necessary to use a Derivatization method to produce a viable method that could produce a visible peak and meet regulatory requirements. The SANCO rev. 5 guidelines require additional evidence when the method validated is a Derivatization method. The method must be shown to be stable, reproducible and appropriate for the amount of Derivatization agent used.

Reproducibility of the Derivatization Method

The Low 100% Recovery shows that the Impurity 2 Derivatization method is reproducible. Six spiked samples were prepared and analysed according to the Derivatization method. The samples were spiked with underivatized Impurity 2 reference standard and then derivatized using the Derivatizing agent. These results produced a %RSD of 4.164. This meets the SANCO rev. 5 guideline of a Horwitz %RSD less than 6.98 for a recovery at 0.0174g/Kg. Therefore, the Derivatization method is considered reproducible.

Stability of the Derivatization Method

To show the stability of the Derivatization method, duplicate samples were prepared on two separate days and analysed together. To achieve this, two sets of duplicate samples of approximately 0.0625g of Technical Material were weighed into a 25mL volumetric flask. 250µL Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride) was added and made to partial volume with Deionised Water. The samples were sonicated for 5 minutes, made to full volume with Deionised Water and allowed to stand for 2 hours to fully derivatize. The samples were injected into the HPLC-PDA. The percentage change from the sample content ranged from 0.23% to 3.14%. There was no significant difference between the samples. Therefore, the Derivatization method is considered stable.

Suitability of the Amount of Derivatizing Agent Used for the Derivatization Method

To show the amount of Derivatization agent used was appropriate for the method, recovery spikes were prepared with varying amounts of the Derivatizing agent. To achieve this, samples of approximately 0.0625g of Technical Material were weighed into a 25mL volumetric flask. An appropriate amount of Derivatization reagent (a 10mg/mL concentration solution of *o*-(2,3,4,5,6-pentafluorobenzyl) Hydroxylamine Hydrochloride) was added and made to partial volume with Deionised Water. The samples were sonicated for 5 minutes, made to full volume with Deionised Water and allowed to stand for 2 hours to fully derivatize. The samples were injected into the HPLC-PDA. The Impurity 2 method uses 1% Derivatization agent to derivatize Impurity 2. When using less Derivatizing agent the results show that not all of the Impurity 2 is derivatized. When using more Derivatizing agent, the results are consistent with the 1% Derivatizing agent, showing that all the Impurity 2 has been derivatized. Therefore the amount of Derivatizing agent used in the Impurity 2 methodology is appropriate.

Validation Summary Table – Impurity 2 (Formaldehyde)

Validation Parameter	Results Obtained	Acceptance Criteria under SANCO/3030/99 rev.5
Linearity	R^2 = 0.9996 n=18*2 0.01 g/kg to 4 g/kg	R^2 = >0.99
Low Sample Precision (Repetitiveness Assay 1)	%RSD = 0.779 Hr = 0.112 n = 6	Horwitz %RSD less than 6.99 Horrat (Hr) ≤ 1 at 0.0172g/Kg
Low Sample Precision (Repetitiveness Assay 2)	%RSD = 2.980 Hr = 0.427 n = 6	Horwitz %RSD less than 6.98 Horrat (Hr) ≤ 1 at 0.0173g/Kg
Low Sample Precision (Intermediate Precision)	%RSD = 2.086 Hr = 0.299 n = 12	Horwitz %RSD less than 6.98 Horrat (Hr) ≤ 1 at 0.0172g/Kg
Low Grubbs Test (maximum) G_n	G_n = 1.980	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
Low Grubbs Test (minimum) G_1	G_1 = 1.462	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
High Sample Precision (Repetitiveness Assay 1)	%RSD = 2.338 Hr = 0.485 n = 6	Horwitz %RSD less than 4.82 Horrat (Hr) ≤ 1 at 0.202g/Kg
High Sample Precision (Repetitiveness Assay 2)	%RSD = 2.789 Hr = 0.578 n = 6	Horwitz %RSD less than 4.82 Horrat (Hr) ≤ 1 at 0.201g/Kg
High Sample Precision (Intermediate Precision)	%RSD = 2.457 Hr = 0.509 n = 12	Horwitz %RSD less than 4.82 Horrat (Hr) ≤ 1 at 0.202g/Kg
High Grubbs Test (maximum) G_n	G_n = 1.739	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
High Grubbs Test (minimum) G_1	G_1 = 1.579	Grubbs Test Criteria for 12 Measurements = ≤ 2.412 at 97.5% Confidence
Low 80% Recovery at 0.0136g/Kg	Mean Recovery = 98.93% %RSD = 0.890 Hr = 0.123 n = 6	Between 70%-130% Horwitz %RSD less than 7.25 Horrat (Hr) ≤ 1 at 0.0135g/Kg
Low 100% Recovery at 0.017g/Kg	Mean Recovery = 102.1% %RSD = 4.164 Hr = 0.597 n = 6	Between 70%-130% Horwitz %RSD less than 6.98 Horrat (Hr) ≤ 1 at 0.0174g/Kg
Low 120% Recovery at 0.0204g/Kg	Mean Recovery = 103.5% %RSD = 1.541	Between 70%-130% Horwitz %RSD less than 6.77

	Hr = 0.227 n = 6	Horrat (Hr) ≤ 1 at 0.0211g/Kg
High 80% Recovery at 0.16g/Kg	Mean Recovery = 92.16% %RSD = 1.947 Hr = 0.385 n = 6	Between 75%-125% Horwitz %RSD less than 5.06 Horrat (Hr) ≤ 1 at 0.147g/Kg
High 100% Recovery at 0.2g/Kg	Mean Recovery = 82.88% %RSD = 2.599 Hr = 0.523 n = 6	Between 75%-125% Horwitz %RSD less than 4.97 Horrat (Hr) ≤ 1 at 0.166g/Kg
High 120% Recovery at 0.24g/Kg	Mean Recovery = 88.74% %RSD = 2.534 Hr = 0.530 n = 6	Between 75%-125% Horwitz %RSD less than 4.78 Horrat (Hr) ≤ 1 at 0.213g/Kg
LOQ Recovery at 0.01g/Kg	Mean Recovery = 96.53% %RSD = 2.434 Hr = 0.319 n = 6	Between 70%-130% Horwitz %RSD less than 7.62 Horrat (Hr) ≤ 1 at 0.00965g/Kg

Assessment and conclusion

Assessment and conclusion by applicant:

Assessment and conclusion by RMS: The analytical method for the determination of the impurity Formaldehyde in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5 with a LOQ of 0.01 g/kg.

Sinon ([redacted] Source and [redacted] source)

[redacted] source:

Analytical method for the determination of relevant impurities N-nitroso glyphosate (NNG) and Formaldehyde in the technical material for the [redacted] source are provided and reported below.

Data point:	J-CA 4.1.1/023
Report author	[redacted]
Report year	2020
Report title	Five Batches Analysis of Technical Grade Active Ingredient (TGAI) Glyphosate
Report No	SNCL Report No. SB03
Document No	-
Guidelines followed in study	Not stated
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes (further data required)
Category study in AIR 5 dossier (L docs)	Category 1

Analytical method for the determination of relevant impurity NNG in technical material (method LCN02)

Principle of method

N-nitrosoglyphosate (*N*-nitrosoglyphosate aniline salt) reacts with HBr to form nitrosyl cation. Nitrosyl cation then reacts with *N*-(1-naphthyl)ethylenediamine (NED) and sulfanilamide to form a purple azo dye which is detectable at 540-550 nm. The content is determined by high performance liquid chromatography technique.

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

HPLC system:	Agilent 1100 series LC with a DAD detector and autosample injector, or equivalent
Column:	Nucleosil 10 µ SB 100A, 4.6 mm × 250 mm
Column temperature:	25 °C
Mobile phase:	Isocratic: 0.02M Potassium dihydrogen phosphate (adjust to pH= 2.0 ± 0.1with phosphoric acid)
Flow rate:	1.5 mL/min
Injection volume:	100 µL
Detector:	DAD
Detection wavelength	540 nm
Retention time:	Approx. 9.3 min

Specificity

The specificity of the analytical method is demonstrated from the overlapping DAD spectrum of NNG analytical standards and NNG in glyphosate technical sample, the peak purities match for NNG was 996.0258, indicated they are spectrometrically pure.
Besides, chromatograms of a standard solution, a blank solution and a fortified sample are provided. There was no interference peak at the retention time of NNG. Thus the method is specific to determine NNG.

The identification of NNG analytical standards was confirmed by IR spectrum, UV-Vis spectrum, LC-MS, ^{1}H and ^{13}C-NMR spectrum.

Linearity

Linearity was determined with at five standard levels containing NNG after derivertization with NED/HBr solution and sulfanilamide solution with correlation coefficient > 0.99.

Table 4.1.1-46: Linearity data for NNG

Analyte	**Calibration ranges**	**Calibration curve**	**Correlation coefficient (r)**
NNG	0.02 – 0.58 mg/L (corresponding to 0.13 – 3.85 mg/kg)	y = 419.7533 x – 2.9658	0.9990

Accuracy

The recovery test for the impurity NNG was performed by standard addition method. One solution of one batch of the test substance (batch no. SNGA1910010) was derivertized with NED/HBr and sulfanilamide solution and spiked with three different levels of analytical standard solutions (after derivertization).

Table 4.1.1-47: Accuracy data for NNG in [redacted] source

Analyte	Fortification level (mg/L)[1]	Nominal concentration (mg/L)	No of replicates	Mean recovery (%)	RSD (%)
NNG	0.14 (0.93 mg/kg)	0.10	2	119.57	N/A
	0.29 (1.93 mg/kg)	0.17	2	97.99	N/A
	0.43 (2.87 mg/kg)	0.24	2	97.69	N/A
	Total sum		6	105.08	11.940

N/A: not applicable
[1]Fortification levels in parentheses are converted to g/kg based on values given in study report

Repeatability (precision)
To evaluate sample repeatability a single batch of glyphosate (batch no. SNGA1910010) was derivatized with NED/HBr and sulfanilamide solution and analysed six times.

Table 4.1.1-48: Repeatability data for NNG in [redacted] source

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
NNG	0.00013	6	2.95	0.286

[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

Limit of Quantification (Limit of Detection)

The LOQ corresponds to the level for which the repeatability and the recovery are validated. Only the repeatability is validated at level 1.3 mg/kg. The recovery at this level shoud be provided to validate the LOQ.

Assessment and conclusion

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate impurities in glyphosate technical material ([redacted] source) as manufactured was not previously evaluated at EU level. It was performed under GLP and meets the criteria stated in the current guidance document SANCO/3030/99 rev. 5. No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method for the determination of the impurity NNG in glyphosate technical material has been partially validated in accordance with SANCO/3030/99 rev. 5 for the specificity, the linearity, the accuracy and the repeatability with a LOQ of 1.03 mg/kg. Indeed, repeatability data should be provided to validate the LOQ at 0.93 mg/kg.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

Analytical method for the determination of relevant impurity formaldehyde in technical material (method LCF01)

Principle of method

Formaldehyde in the technical substance was derivatized with Hantzsch reagent. The resulting derivative, diacetyldihydrolutidine or DDL is quantified by use of HPLC-DAD. Quantification is performed using peak area responses from a UV detector and external standards calibration.

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

HPLC system:	Agilent 1100 series LC with a DAD detector and autosample injector, or equivalent.
Column:	Lichrospher 100 RP-18 e (5 µm) L:12.5 cm, ID: 4.0 mm
Mobile phase:	Isocratic: 80 % deionized water + 20 % acetonitrile
Flow rate:	1.0 mL/min
Injection volume:	50 µL
Detector:	DAD
Signal	412 nm
Retention time:	Approx. 3.5 min

Specificity

The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the test substance. The derivatization reaction used in this method is so selective for formaldehyde that other expected components are not expected to affect this determination.
Besides, chromatograms of a standard solution, a blank solution and a fortified sample are provided. There was no interference peak at the retention time of the analyte.

The identification of formaldehyde analytical standards was confirmed by IR spectrum, UV-Vis spectrum, GC-MS, ^{1}H and ^{13}C-NMR spectrum.

Linearity

Linearity was determined with at five standard levels containing formaldehyde standard derivertized with Hantzsch reagent solution with correlation coefficient > 0.99.

Table 4.1.1-49: Linearity data for formaldehyde

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Formaldehyde	0.06 – 0.89 mg/L (corresponding to 0.012 – 0.18 g/kg)	y = 383.2249 x + 0.6674	1.0000

Accuracy

The recovery test for the impurity formaldehyde was performed by standard addition method. One solution of one batch of the test substance (batch no. SNGA1910010) was derivatized with Hantzsch reagent solution and spiked with three different levels of analytical standard solutions (after derivatization).

Table 4.1.1-50: Accuracy data for formaldehyde in [redacted] source

Analyte	Fortification level (mg/L)[1]	Nominal concentration (mg/L)	No of replicates	Mean recovery (%)	RSD (%)
Formaldehyde	0.11 (0.02 g/kg)	0.06	2	105.40	N/A
	0.22 (0.04 g/kg)	0.11	2	98.40	N/A
	0.44 (0.09 g/kg)	0.22	2	99.11	N/A

	Total sum	6	100.97	3.816

N/A: not applicable
[1]Fortification levels in parentheses are converted to g/kg based on values given in study report

Repeatability (precision)

To evaluate sample repeatability a single batch of glyphosate (batch no. SNGA1910010) was derivertized with Hantzsch reagent and analysed six times.

Table 4.1.1-51: Repeatability data for formaldehyde in [redacted] source

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	0.0044	6	0.99	0.163

[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

Limit of Quantification (Limit of Detection)

The LOQ corresponds to the level for which the repeatability and the recovery are validated. Only the repeatability is validated at level 0.044 mg/kg. The recovery at this level shoud be provided to validate the LOQ.

Assessment and conclusion

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate impurities in glyphosate technical material ([redacted] source) as manufactured was not previously evaluated at EU level. It was performed under GLP and meets the criteria stated in the current guidance document SANCO/3030/99 rev. 5. No deviations with the applied test guidelines were reported. The method is suitable for the determination of active substance glyphosate in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method for the determination of the impurity formaldehyde in glyphosate technical material has been partially validated in accordance with SANCO/3030/99 rev. 5 for the specificity, the linearity, the accuracy and the repeatability with a LOQ of 0.04 g/kg.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

[redacted] source:

Analytical method for the determination of relevant impurities N-nitroso glyphosate (NNG) and Formaldehyde in the technical material for the [redacted] source are provided and reported below.

Data point:	J-CA 4.1.1/024
Report author	[redacted]
Report year	2017
Report title	5-Batch analysis of glyphosate TGAI in accordance with regulation (EC) No. 1107/2009, referencing SANCO 3030/99 rev. 4
Report No	AN16111117
Document No	-
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

Analytical method for the determination of relevant impurity NNG in technical material (method AN16111117-B)

Principle of method

NNG in the technical substance was quantified by use of ion chromatography with UV detection.
Liquid scintillation was used for the preparation of samples.

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

HPLC system:	Agilent 1100
Column:	Dionex IonPac AS 11 HC 250×4.0 mm with Dionex IonPac AG 11-HC guard column
Column temperature:	25 °C
Mobile phase:	Isocratic: 50 mM sodium carbonate in water
Flow rate:	1.75 mL/min
Injection volume:	100 µL
Detection:	UV detection at 244 nm
Retention time:	Approx. 8.0 min

The spectral identities of NNG, analytical standard and five batches of Glyphosate technical samples were characterized in terms of the LC mass spectra, GC mass spectra, infrared (IR) spectra, ultraviolet/visible (UV/VIS) spectra and nuclear magnetic resonance (NMR) spectra. The obtained spectra indicate consistent with NNG chemical structure.

Specificity

Representative chromatograms of a standard sample, of glyphosate TGAI production batches and a blank solution (solvent only) were provided.
No interference was observed. The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the samples. There was no impurities or interferences co-eluted with the analyte of interest.

Linearity

A plot of peak area ratio of NNG to internal standard vs. concentration of NNG in the samples demonstrated acceptable linearity, indicated by a correlation coefficient of 1.0000.

Table 4.1.1-52: Linearity data for NNG

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
NNG	0.05 – 0.50 mg/L (equivalent to 0.20 – 2.00 mg/kg) n=7	y = 1.73077 x + 0.00310	1.0000

Accuracy

The recovery test for the impurity NNG was performed by standard addition method. Solution of one batch of the test substance (batch no. XF20161125) was spiked with three different levels.

Table 4.1.1-53: Accuracy data for NNG in [redacted] source

Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
NNG	0.60	5	96.0	6.5
	1.04	5	99.2	3.4
	1.24	5	105.2	3.2

Repeatability (precision)

The system precision (%RSD) of standard solution of 0.1 mg/L (0.40 mg/kg) was found to be 2.3. To evaluate sample repeatability a single batch of glyphosate (batch no. XF20161125) was analysed eight times.

Table 4.1.1-54: Repeatability data for NNG in [redacted] source

Analyte	Mean content (mg/kg)	No of replicates	RSD (%)	Horrat value (H_r)[1]
NNG	< LOQ (0.6)	8	N/A	N/A
	0.60	5	6.5	0.560[2]
	1.04	5	3.4	0.318[2]
	1.24	5	3.2	0.308[2]

N/A: not applicable

[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

[2] Since NNG is not detected in technical material, the precision data at three fortification levels in accuracy tests are included

Limit of Quantification

The LOQ is defined as the lowest spiking concentration of NNG in accuracy test, at which an acceptable mean recovery with an acceptable RSD according to SANCO/3030/99 rev. 5. The LOQ of NNG is 0.6 mg/kg.

Assessment and conclusion

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate impurities in glyphosate technical material ([redacted] source) was not previously evaluated 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 with the applied test guidelines were reported. The method is suitable for the determination of glyphosate impurities in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method for the determination of the impurity NNG in glyphosate technical material has been satisfactorily validated in accordance with SANCO/3030/99 rev. 5 with a LOQ of 0.6 mg/kg.

Analytical method for the determination of relevant impurity formaldehyde in technical material (method AN16111117-A)

Principle of method

Formaldehyde in glyphosate technical samples is quantified employing HPLC analysis following derivatisation with 2,4-Dinitrophenylhydrazine using HPLC with UV detection at 240 nm. Internal standardisation is employed using acetaldehyde.

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

HPLC system:	Agilent 1100 series HPLC
Column:	Hypersil ODS, 125 × 4.6 mm, 5 µm
Column temperature:	Ambient
Mobile phase:	Isocratic: 50/50 (v/v) Water / Acetonitrile
Flow rate:	1.2 mL/min
Injection volume:	20 µL
Detection:	UV at 240 nm
Retention time:	Approx. 3.8 min

The spectral identities of formaldehyde, analytical standard and five batches of Glyphosate technical samples were characterized in terms of the LC mass spectra, GC mass spectra, infrared (IR) spectra, ultraviolet/visible (UV/VIS) spectra and nuclear magnetic resonance (NMR) spectra. The obtained spectra indicate consistent with formaldehyde chemical structure.

Specificity

Representative chromatograms of a standard sample (formaldehyde), of glyphosate TGAI production batches, a single blank (internal standard only) were provided.

No interference was observed. The analytical method was found to demonstrate sufficient resolution of the components of interest from other peaks present in the samples. There was no impurities or interferences co-eluted with the analyte of interest.

Linearity

A plot of peak area ratio of formaldehyde to internal standard vs. concentration of formaldehyde in the samples demonstrated acceptable linearity, indicated by a correlation coefficient of 0.9988.

Table 4.1.1-55: Linearity data for formaldehyde

Analyte	Calibration ranges	Calibration curve	Correlation coefficient (r)
Formaldehyde	0.43 – 3.03 mg/L (equivalent to 0.043 – 0.30 % w/w) n=7	y = 1.49486 x – 0.00145	0.9988

Accuracy

The recovery test for the impurity formaldehyde was performed by standard addition method. Solution of one batch of the test substance (batch no. XF20161130) was spiked with three different levels.

Table 4.1.1-56: Accuracy data for formaldehyde in [redacted] source

Analyte	Fortification level (% w/w)	No of replicates	Mean recovery (%)	RSD (%)
Formaldehyde	0.08	5	86.0	4.7
	0.17	5	85.5	0.9
	0.21	5	85.7	1.5

Repeatability (precision)

The system precision (%RSD) of standard solution of 0.87 mg/L (0.087 % w/w) was found to be 0.8. To evaluate sample repeatability a single batch of glyphosate (batch no. XF20161130) was analysed eight times.

Table 4.1.1-57: Repeatability data for formaldehyde in [redacted] source

Analyte	Mean content (% w/w)	No of replicates	RSD (%)	Horrat value (H_r)[1]
Formaldehyde	< LOQ (0.08)	8	N/A	N/A
	0.08	5	4.7	1.199[2,3]
	0.17	5	0.9	0.257[2]
	0.21	5	1.5	0.442[2]

N/A: not applicable

[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

[2] Since formaldehyde is not detected in technical material, the precision data at three fortification levels in accuracy tests are included

[3] H_r value at low fortification level is higher than 1 due to the fact that sample preparation includes a derivatisation stage

Limit of Quantification

The LOQ is defined as the lowest spiking concentration of formaldehyde in accuracy test, at which an acceptable mean recovery with an acceptable RSD according to SANCO/3030/99 rev. 5. The LOQ of formaldehyde is 0.08 % w/w (0.8 g/kg).

Assessment and conclusion

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate impurities in glyphosate technical material ([redacted] source) was not previously evaluated 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 with the applied test guidelines were reported. The method is suitable for the determination of glyphosate impurities in glyphosate technical material.

Assessment and conclusion by RMS: The analytical method for the determination of the impurity formaldehyde in glyphosate technical material has been partially validated in accordance with SANCO/3030/99 rev. 5 for the specificity, the linearity, the accuracy and the repeatability with a LOQ of 0.8 g/kg.

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. Moreover, the specificity of the derivatised agent has been demonstrated in the FAO method.

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B.5.1.2 Methods for risk assessment

B.5.1.2.1 Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Overview table for analytical methods in in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

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
CA 6.1/002	[redacted] 2012 Report No. MSL0023608	Storage stability of residues of Glyphosate and AMPA in citrus fruit	AG-ME-1294-01 [redacted], 2016 Report No. MSL0027298	LC-MS/MS *LOQ 0.05 mg/kg, 0.05-5 mg/kg*	Yes		Y
CA 6.3.1/002	[redacted] 2016 Report No. S15-00018	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in tree nuts (outdoor) at 2 sites in Southern Europe 2015	AG-ME-1294-01 [redacted] 2016 Report No. S15-00018	LC-MS/MS *LOQ 0.05 mg/kg, 0.05-0.5 mg/kg*			
CA 6.3.1/006	[redacted] 2016 Report No. S15-00019	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in apricots (outdoor) at 4 sites in Southern Europe 2015	AG-ME-1294-01 [redacted] 2016 Report No. S15-00019	LC-MS/MS *LOQ 0.05 mg/kg, 0.05-0.5 mg/kg*			
CA 6.3.1/009	[redacted] 2013 Report No. S12-03071	Determination of residues of glyphosate in stone fruit following one application of glyphosate SL 360 g/L (CA2705) in northern and southern France, in 2012	AG-ME-1294-01 [redacted] 2013 Report No. S12-03071	LC-MS/MS *LOQ 0.05 mg/k, g 0.05-0.5 mg/kg*			

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
CA 4.1.2/114 (CA 6.3.1/010)	[redacted] 2016 Report No. S15-00491	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 2 sites in Germany 2015	AG-ME-1294-01 [redacted] 2015 Report No. S14-05172	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/114 (CA 6.3.1/011)	[redacted] 2015 Report No. S14-04157	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 4 sites in Northern France	AG-ME-1294-01 [redacted] 2015 Report No. S14-05172	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/114 (CA 6.3.1/012)	[redacted] 2015 Report No. S14-04158	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 3 sites in Germany and 2 sites in Spain 2014	AG-ME-1294-01 [redacted] 2015 Report No. S14-05172	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/114 (CA 6.3.1/013)	[redacted] 2015 Report No. S14-04226	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 4 sites in Southern Europe 2014	AG-ME-1294-01 [redacted] 2015 Report No. S14-05172	LC-MS/MS *LOQ 0.05 mg/kg 0.05 - 0.5 mg/kg*			
CA 4.1.2/134 (CA 6.3.1/019)	[redacted] 2015 Report No. S15-00469	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in kiwi fruit (outdoor) at 2 sites in Southern Europe 2015	AG-ME-1294-01 [redacted] 2015 Report No. S15-00469	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			

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
CA 4.1.2/135 (CA 6.3.1/020)	[redacted] 2015 Report No. S14-04159	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in bananas (outdoor) at 4 sites in Spain (Canary Islands) 2014	AG-ME-1294-01 [redacted] 2015 Report No. S14-04159	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/001)	[redacted], 2012 Report No. S11-00258	Determination of residues of glyphosate and AMPA after one application of MON 52276 in potatoes (outdoor) at 4 sites in France, Germany and Italy 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/002)	[redacted] 2012 Report No. S11-00259	Determination of residues of glyphosate and AMPA after one application of MON 52276 in carrots (outdoor) at 4 sites in France, Spain and Poland 2011	AG-ME-1294-01 [redacted], 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/003)	[redacted] 2012 Report No. S11-00260	Determination of residues of glyphosate and AMPA after one application of MON 52276 in bulb onions (outdoor) at 4 sites in France, Spain and Bulgaria 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/004)	[redacted] 2012 Report No. S11-00267	Determination of residues of glyphosate and AMPA after one application of MON 52276 in tomato (outdoor) at 2 sites in Hungary and Germany 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			

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
CA 4.1.2/113 (CA 6.3.2/005)	[redacted] 2012 Report No. S11-00261	Determination of residues of glyphosate and AMPA after one application of MON 52276 in cucumber and zucchini (outdoor) at 3 sites in Italy, France and Germany 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/006)	[redacted] 2012 Report No. S11-00263	Determination of residues of glyphosate and AMPA after one application of MON 52276 in cauliflower (outdoor) at 4 sites in France, Hungary, Bulgaria and Italy 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/007)	[redacted] 2012 Report No. S11-00262	Determination of residues of glyphosate and AMPA after one application of MON 52276 in head cabbage (outdoor) at 4 sites in Hungary, France (North), Spain and Bulgaria 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/008)	[redacted] 2012 Report No. S11-00264	Determination of residues of glyphosate and AMPA after one application of MON 52276 in leaf and head lettuce (outdoor) at 4 sites in France, Spain, UK and Germany 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			

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
CA 4.1.2/113 (CA 6.3.2/009)	[redacted] 2012 Report No. S11-00265	Determination of residues of glyphosate and AMPA after one application of MON 52276 in leek (outdoor) at 4 sites in France, United Kingdom, Bulgaria and Italy 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.2/010)	[redacted] 2012 Report No. S11-00266	Determination of residues of glyphosate and AMPA after one application of MON 52276 in sugar beet (outdoor) at 2 sites in Spain and Italy 2011	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.3/001)	[redacted] 2016 Report No. S15-00482	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in carrots (outdoor) at 4 sites in Southern Europe 2015	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/136 (CA 6.3.3/002)	[redacted] 2016 Report No. S15-00467	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in radish (outdoor) at 2 sites in Southern Europe 2015	AG-ME-1294-01 [redacted] 2016 Report No. S15-00467	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.3/003)	[redacted] 2016 Report No. S15-00466	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in bulb onions (outdoor) at 4 sites in Southern and 2 sites in Northern Europe 2015	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			

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
CA 4.1.2/113 (CA 6.3.3/004)	[redacted] 2016 Report No. S15-00465	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in tomato (outdoor) at 4 sites in Southern Europe 2015	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.3/005)	[redacted] 2016 Report No. S15-00464	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in cucumber (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/137 (CA 6.3.3/006)	[redacted] 2016 Report No. S15-00463	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in courgette (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015	AG-ME-1294-01 [redacted] 2016 Report No. S15-00463	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/113 (CA 6.3.3/007)	[redacted] 2016 Report No. S15-00460	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in head lettuce (outdoor) at 4 sites in Southern and 2 sites in Northern Europe 2015	AG-ME-1294-01 [redacted] 2012 Report No. S11-03331	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/138 (CA 6.3.3/008)	[redacted] 2016 Report No. S15-00459	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in parsley (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015	AG-ME-1294-01 [redacted] 2016 Report No. S15-00459	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			

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
CA 4.1.2/139 (CA 6.3.3/009)	[redacted] 2016 Report No. S15-00461	Determination of residues of glyphosate and its metabolite AMPA after oneapplication of MON 79351 in green beans (outdoor) at 4 sites in Southern and 4 sites in Northern Europe 2015	AG-ME-1294-01 [redacted] 2016 Report No. S15-00461	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/123 (CA 6.1/013)	[redacted] 1989 Report No. WRC 89-22	Storage stability validation for ICIA 0224 in raw agricultural commodities	Method RRC 85-34 [redacted] 1989 Report No. WRC 89-22	HPLC-FD after derivatisation *LOQ 0.4 for soybean seed and straw or 0.2 mg/kg for wheat grain 0.1-1.0 mg/kg*	No	Fit for purpose	Y
CA 4.1.2/144 (CA 6.5.3/001)	[redacted] 1986 Report No. MSL-6194	Determination of Glyphosate and Aminomethylphosphonic acid residues in citrus fruit and process fractions following postdirected treatment with Roundup herbicide	N/A [redacted] 1986 Report No. MSL-6194	GLC-FPD after derivatisation *no LOQ validated*	No	-	No
CA 4.1.2/145 (CA 6.5.3/002)	[redacted] 1975 Report No. 377	CP 57573, Residue and metabolism part 27: Determination of CP 67573 and CP 50435 residues in citrus process fractions	N/A [redacted] 1975 Report No. 377	GLC-FPD after derivatisation *No LOQ validated*	No	-	No
CA 4.1.2/124 (CA 6.1/015, CA 6.4.1/002)	[redacted] 1987 [redacted] 87-43	Magnitude of SC-0224 Residues in Eggs and Poultry	N/A [redacted] 1987 [redacted] 87-43	HPLC after derivatisation *no LOQ validated*	No	-	No

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
CA 4.1.2/125 (CA 6.1/016, CA 6.4.2/003)	[redacted], 1987 [redacted] 87-44	Magnitude of SC-0224 Residues in Meat and Milk	N/A [redacted] 1987 [redacted] 87-44	HPLC after derivatisation *no LOQ validated*			
CA 4.1.2/118	[redacted]	Analytical method for the determination of glyphosate and degradate residues in various crop matrices using LC/MS/MS	method DuPont-15444	LC-MS/MS LOQ 0.05mg/kg 0.05 -0.5mg/kg	Yes		Y
CA 4.1.2/118 (CA 6.1/004 ;))	[redacted] 2009 Report No. DuPont-20094	Stability of Glyphosate and metabolites in corn green plant, forage, grain, and stover containing the GAT and ZM-HRA genes during frozen storage	Method Dupont-15444 [redacted] 2007 Report No. DuPont-15444 Revision No. 1	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*	No	Fit for purpose	Y
CA 4.1.2/118 (CA 6.1/005)	[redacted] 2009 Report No. DuPont-17573	Stability of Glyphosate, N-Acetylglyphosate, Aminomethyl phosphonic acid and N-Acetyl AMPA in GAT soybean forage, seed, and hay stored frozen	Method Dupont-15444 [redacted] 2007 Report No. DuPont-15444 Revision No. 1	LC-MS/MS *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/118 (CA 6.1/006)	[redacted] 2007 Report No. DuPont-17379	Stability of Glyphosate, N-Acetylglyphosate and Aminomethyl phosphonic acid in GAT corn forage, grain, and stover, stored frozen	Method Dupont-15444 [redacted] 2007 Report No. DuPont-15444 Revision No. 1	CA 4.1.2/118 (CA 6.1/006)			

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
CA 4.1.2/119	[redacted] 2014 Report No. S13 04580	Glyphosate – Validation of Analytical Method GRM067.01A for the Determination of Residues of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Crop Matrices	LC-MS/MS after derivatisation with FMOC (Syngenta method GRM067.01A)	LOQ 0.05mg/kg 0.05- 0.5mg/kg	No	The demonstration of the derivatisation efficiency is missing and should be provided during peer reviewed to validate the method	No.
CA 4.1.2/128 (CA 6.3.1/007)	[redacted] 2014 Report No. S13-03427	Glyphosate - Residue study on cherry in Spain and Italy in 2013	Syngenta method GRM067.01A [redacted] 2014 Report No. S13-03427	LC-MS/MS after derivatisation *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/129 (CA 6.3.1/008)	[redacted] 2014 Report No. S13-03233	Glyphosate - Residue study on plum in Italy in 2013	Syngenta method GRM067.01A [redacted] 2014 Report No. S13-03233	LC-MS/MS after derivatisation *LOQ 0.05 mg/kg 0.05-0.5 mg/kg*			
CA 4.1.2/122 (CA 6.1/010)	[redacted]	Storage stability of residues of N-(phosphonomethyl) glycine and trimethylsulphonium cation in banana	Residue Analytical Method 245/02 ([redacted], 1994) for the analysis of banana	GC-MSD after derivatisation No LOQ validated Range not applicable	No	Fir for purpose	Y

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
CA 4.1.2/132 (CA 6.3.1/016)	[redacted] 1996 Report No. RJ 2217B	Glyphosate-trimesium: Residue levels in olives from trials carried out in Greece during 1995	analytical method RR92-042B RES for olive samples N/A	GC-MSD after derivatisation *no LOQ validated*			
CA 4.1.2/133 (CA 6.3.1/017)	[redacted] 1996 Report No. RJ 2218B	Glyphosate-trimesium: Residue levels in olives from trials carried out in Italy during 1995	[redacted] 1996 Report No. RJ 2217B N/A	GC-MSD after derivatisation *No LOQ validated*			
CA 4.1.2/121	[redacted] 2007 Report No. DuPont-20009	Analytical method for the determination of N-acetyl-glyphosate and other analytes in various animal matrices using LC/MS/MS	LC-MS/MS with labelled internal standard (Method DuPont-20009)	LOQ:0.025mg/kg 0.025 to 0.25mg/kg	No	-	No
CA 4.1.2/115	[redacted] 2015 FCS-0703V	Validation of the analytical method DFG Method 405 for the determination of Glyphosate and its metabolite AMPA in various plant materials	DFG method 405	HPLC-FD LOQ 0.05 mg/kg 0.05-0.5 mg/kg	Y	-	Y

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
CA 4.1.2/116	[redacted] 2008 FCS-0703V	1st Amendment to final report Validation of the analytical method DFG Method 405 for the determination of Glyphosate and its metabolite AMPA in various plant materials	DFG method 405	HPLC-FD LOQ 0.05 mg/kg 0.05-0.5 mg/kg			
CA 4.1.2/117	[redacted] 1985 MSL 4268	Validation of a new residue method for the analysis of glyphosate and aminomethylphosphonic acid (AMPA) – a round-robin study	DFG method 405	HPLC-FD LOQ 0.05 mg/kg 0.05-0.5 mg/kg	Y	-	Y
CA 4.1.2/131 (CA 6.3.1/015, CA 6.5.3/004)	[redacted] 1996 Report No. MLL 30469	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with Roundup herbicide. Spanish field trials, 1995	N/A (DFG Method 405) [redacted] 1996 Report No. MLL 30469	HPLC-FD *LOQ 0.05 mg/kg for grapes 0.05-0.5 mg/kg Not validated for Olive oil*	Y for grapes	-	Y for grapes
CA 4.1.2/131 (CA 6.5.3/005)	[redacted] 1993 Report No. MLL 30319	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with MON 65040 herbicide. Italian field trials, 1993	N/A (DFG Method 405) [redacted]1996 Report No. MLL 30469				
CA 4.1.2/131 (CA 6.5.3/006)	[redacted] 1992 Report No. MLL 30297	Residues of glyphosate/AMPA in olives and olive oil following use of Sting SE - Spanish field trials 1990/1992.	N/A (DFG Method 405) [redacted] 1996 Report No. MLL 30469				
CA 4.1.2/117 (CA 6.3.1/014)	[redacted] 1989 Report No. MLL 30227	Glyphosate and AMPA residues in grapes following MON 8755 (Arcade) herbicide applications in vineyards. German field trials 1988	N/A (DFG Method 405) [redacted] 1985 Report No MSL 4268				

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
CA 4.1.2/146 (CA 6.5.3/003)	[redacted] 1988 Report No. MSL-7877	Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide	N/A (DFG Method 405) [redacted] 1988 Report No. MSL-7877	HPLC-FD *LOQ 0.1 mg/kg for potato whole tuber* *Range between 0.05-0.2 mg/kg for potato whole tuber* *Not validated for other matrices*	Y for potato whole tuber only	-	Y only for potato whole tuber
CA 4.1.2/115 CA 4.1.2/116 (CA 6.1/003)	[redacted] 2010 Report No. FCS-0707	Storage stability of residues of Glyphosate and AMPA in various plant materials	DFG Method 405 [redacted] 2007 Report No. FCS-0703V [redacted] 2008 Report No. FCS-0703V	HPLC-FD *LOQ 0.05 mg/kg* *0.05-0.5 mg/kg*	No	Fit for purpose	Y
CA 4.1.2/115 CA 4.1.2/116 (CA 6.1/007)	[redacted] 1997 Report No. IF-94/13882-00	Determination of the Storage Stability of Glyphosate in Beans, Oilseed Rape and Linseed	DFG Method 405 [redacted], 2007 Report No. FCS-0703V [redacted] 2008 Report No. FCS-0703V	HPLC-FD *LOQ 0.05 mg/kg* *0.05-0.5 mg/kg*	No	Fit for purpose	Y

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
CA 4.1.2/117 (CA 6.1/008)	[redacted] 1993 Report No. 91210	Determination of glyphosate in soybean raw agricultural commodities (RAC) stability report	N/A (DFG Method 405) [redacted] 1985 Report No MSL 4268	HPLC-FD *LOQ 0.05 mg/kg* *0.05-5.0 mg/kg*	No	Fir for purpose	Y
CA 4.1.2/117 (CA 6.1/009)	[redacted], 1993 Report No. 91212	Determination of glyphosate in pasture grasses stability report	N/A (DFG Method 405) [redacted], 1985 Report No MSL 4268	HPLC-FD *LOQ 0.05 mg/kg* *0.05-5.0 mg/kg*	No	Fit for purpose Only for glyphosate	Y
CA 4.1.2/115 CA 4.1.2/116 (CA 6.1/011)	[redacted] 1995 Report No. 303614	Storage Stability of Glyphosate and AMPA in Wheat Grain and Straw and in Rye Grain and Straw	DFG Method 405 [redacted] 2007 Report No. FCS-0703V [redacted] 2008 Report No. FCS-0703V	HPLC-FD with post-column derivatisation *LOQ 0.05 mg/kg* *0.05-0.5 mg/kg*	No	Fit for purpose	Y

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
CA 4.1.2/115 CA 4.1.2/116 (CA 6.1/012)	[redacted], 1991 Report No. MSL10843	Storage stability of glyphosate residues in crop commodities	DFG Method 405 [redacted] 2007 Report No. FCS-0703V [redacted] 2008 Report No. FCS-0703V [redacted] 1985 Report No MSL 4268	HPLC-FD with post-column derivatisation *LOQ 0.05 mg/kg* *0.05-0.5 mg/kg*	No	Fit for purpose	Y
CA 4.1.2/120	[redacted] 1988	Validation of an analytical determination of glyphosate residues in animal tissues	DFG method 405	HPLC-FD with post-column derivatisation HPLC – FD with post column derivatisation LOQ is 0.05 mg/kg	No	-	No
CA 4.1.2/140 (CA 6.4.1/003)	[redacted] 1987 Report No [redacted]-6676	Residue determination of Glyphosate and AMPA in laying hen tissues and eggs following a 28 day feeding study	N/A (DFG Method 405) For tissue: [redacted] 1988 Report No. MSL-7358 For chicken chow: [redacted] 1987 Report No [redacted]-6676		No	-	No

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
CA 4.1.2/141 (CA 6.4.2/002)	[redacted] 1987 Report No [redacted]-6729	Residue determination of Glyphosate and AMPA in dairy cow tissues and milk following a 28 day feeding study	N/A (DFG Method 405) For tissue: [redacted], 1988 Report No. MSL-7358 For cow chow: [redacted] 1987 Report No [redacted]-6729		No	-	No
CA 4.1.2/142 (CA 6.4.3/001)	[redacted] 1987 Report No. [redacted]-6627	Residue determination of Glyphosate and AMPA in swine tissues following a 28-day feeding study	N/A (DFG Method 405) For tissue: [redacted] 1988 Report No. MSL-7358 For pig chow: [redacted]1987 Report No [redacted]-6729	HPLC-FD with post-column derivatisation no LOQ validated	No	Fit for purpose	Y

The analytical method AG-ME-1294-01 (see monitoring part for full validation) is used for the determination of glyphosate residue in all studies below. The method is independently validated with an LOQ of 0.05 mg/kg for both glyphosate and AMPA in all tested matrix groups (high oil, high acid, high water containing and dry commodities). The method is considered as validated.

Data point	Report authors	Report year	Report number	Report title	Test facility
CA 6.1/002	[redacted]	2012	MSL0023608	Storage stability of residues of Glyphosate and AMPA in citrus fruit	Monsanto Company Environmental Sciences Technology Center 800 N. Lindbergh Blvd. St. Louis, MO 63167
CA 6.3.1/002	[redacted]	2016	S15-00018	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in tree nuts (outdoor) at 2 sites in Southern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/006	[redacted]	2016	S15-00019	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in apricots (outdoor) at 4 sites in Southern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/009	[redacted]	2013	S12-03071	Determination of residues of glyphosate in stone fruit following one application of glyphosate SL 360 g/L (CA2705) in northern and southern France, in 2012	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/010	[redacted]	2016	S15-00491	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 2 sites in Germany 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/011	[redacted]	2015	S14-04157	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 4 sites in Northern France	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/012	[redacted]	2015	S14-04158	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 3 sites in Germany and 2 sites in Spain 2014	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/013	[redacted]	2015	S14-04226	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 4 sites in Southern Europe 2014	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.1/019	[redacted]	2016	S15-00469	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in kiwi fruit (outdoor) at 2 sites in Southern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany

Data point	Report authors	Report year	Report number	Report title	Test facility
CA 6.3.1/020	[redacted]	2015	S14-04159	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in bananas (outdoor) at 4 sites in Spain (Canary Islands) 2014	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/001	[redacted]	2012	S11-00258	Determination of residues of glyphosate and AMPA after one application of MON 52276 in potatoes (outdoor) at 4 sites in France, Germany and Italy 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/002	[redacted]	2012	S11-00259	Determination of residues of glyphosate and AMPA after one application of MON 52276 in carrots (outdoor) at 4 sites in France, Spain and Poland 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/003	[redacted]	2012	S11-00260	Determination of residues of glyphosate and AMPA after one application of MON 52276 in bulb onions (outdoor) at 4 sites in France, Spain and Bulgaria 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/004	[redacted]	2012	S11-00267	Determination of residues of glyphosate and AMPA after one application of MON 52276 in tomato (outdoor) at 2 sites in Hungary and Germany 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/005	[redacted]	2012	S11-00261	Determination of residues of glyphosate and AMPA after one application of MON 52276 in cucumber and zucchini (outdoor) at 3 sites in Italy, France and Germany 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/006	[redacted]	2012	S11-00263	Determination of residues of glyphosate and AMPA after one application of MON 52276 in cauliflower (outdoor) at 4 sites in France, Hungary, Bulgaria and Italy 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/007	[redacted]	2012	S11-00262	Determination of residues of glyphosate and AMPA after one application of MON 52276 in head cabbage (outdoor) at 4 sites in Hungary, France (North), Spain and Bulgaria 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.2/008	[redacted]	2012	S11-00264	Determination of residues of glyphosate and AMPA after one application of MON 52276 in leaf and head lettuce (outdoor) at 4 sites in France, Spain, UK and Germany 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.2.3/009	[redacted]	2012	S11-00265	Determination of residues of glyphosate and AMPA after one application of MON 52276 in leek (outdoor) at 4 sites in France, United Kingdom, Bulgaria and Italy 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany

Data point	Report authors	Report year	Report number	Report title	Test facility
CA 6.3.2/010	[redacted]	2012	S11-00266	Determination of residues of glyphosate and AMPA after one application of MON 52276 in sugar beet (outdoor) at 2 sites in Spain and Italy 2011	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/001	[redacted]	2016	S15-00482	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in carrots (outdoor) at 4 sites in Southern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/002	[redacted]	2016	S15-00467	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in radish (outdoor) at 2 sites in Southern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/003	[redacted]	2016	S15-00466	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in bulb onions (outdoor) at 4 sites in Southern and 2 sites in Northern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/004	[redacted]	2016	S15-00465	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in tomato (outdoor) at 4 sites in Southern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/005	[redacted]	2016	S15-00464	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in cucumber (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/006	[redacted]	2016	S15-00463	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in courgette (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/007	[redacted]	2016	S15-00460	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in head lettuce (outdoor) at 4 sites in Southern and 2 sites in Northern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/008	[redacted]	2016	S15-00459	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in parsley (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany
CA 6.3.3/009	[redacted]	2016	S15-00461	Determination of residues of glyphosate and its metabolite AMPA after oneapplication of MON 79351 in green beans (outdoor) at 4 sites in Southern and 4 sites in Northern Europe 2015	Eurofins Agroscience Services GmbH Carl-Goerdeler-Weg 5 D-21684 Stade Germany

Principle of method

Glyphosate and AMPA were isolated from crop matrices by high speed blender extraction using 100 mL of 0.1% formic acid in water and 100 mL of dichloromethane. Following centrifugation, an aliquot of the aqueous phase extract was mixed with isotopically labelled glyphosate and AMPA internal standards then passed through solid phase extraction media for final clean-up. Glyphosate and AMPA residues were determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) was 0.05 mg/kg for both analytes for all crops.

Validation data are reported below:

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.1/002	[redacted]	2012	MSL0023608	For both substance, chromatogram of standards solution, of control sample, and fortified sample at 10xLOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate: Y=ax+b ; Range :0.0025 – 0.6ppm (n>5, R>0.99) AMPA: Y=ax+b Range : 0.025-0.6ppm (n>5 ; R>0.99)	
CA 6.3.1/002	[redacted]	2016	S15-00018	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.011mg/kg – 2.2mg/kg (n>5, R>0.99)	
CA 6.3.1/006	[redacted]	2016	S15-00019	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.011mg/kg – 2.2mg/kg (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate	citrus fruit	0.5	5	86,4	90	88,2	1,71
AMPA		0.5	5	85,2	88.2	86,8	1,57

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate (168-> 63m/z)	Hazelnut - Nutmeat	0,05	3	98	100	99,3	1,16
		0,50	3	91	98	95,3	3,97
AMPA (110->63m/z)		0,05	3	90	98	93,3	4,46
		0,5	3	96	101	98,7	2,55

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Apricot (without stone)	0,05	3	81	89	85,7	4,86
		0,50	3	76	86	81,3	6,19
AMPA (110->63m/z)		0,05	3	87	89	87,7	1,32
		0,5	3	82	88	85,3	3,58

<table>
<tr><th>Data point</th><th>Report authors</th><th>Report year</th><th>Report number</th><th>Specificity</th><th>Linearity</th><th>Recovery</th></tr>
<tr><td>CA 6.3.1/009</td><td>[redacted]</td><td>2013</td><td>S12-03071</td><td>For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.</td><td>Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/mL – 125ng/mL (n>5, R>0.99</td><td>
<table>
<tr><th>Analyte</th><th>Matrix</th><th>fortification level (mg/kg)</th><th>n</th><th colspan="2">recovery range (%)</th><th>mean recovery (%)</th><th>RSD (%)</th></tr>
<tr><td>glyphosate ((168-> 63m/z)</td><td rowspan="4">plums fruits</td><td>0,05</td><td>5</td><td>80</td><td>86</td><td>82,0</td><td>3,11</td></tr>
<tr><td></td><td>0,5</td><td>5</td><td>80</td><td>100</td><td>85,4</td><td>9,85</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,05</td><td>5</td><td>79</td><td>90</td><td>84,2</td><td>4,71</td></tr>
<tr><td>0,5</td><td>5</td><td>83</td><td>100</td><td>86,8</td><td>8,52</td></tr>
</table>
</td></tr>
<tr><td>CA 6.3.1/010</td><td>[redacted]</td><td>2016</td><td>S15-00491</td><td>For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.</td><td>Glyphosate and AMPA: Y=ax+b ; Range :0.011mg/kg – 2.2mg/kg (n>5, R>0.99</td><td>
<table>
<tr><th>Analyte</th><th>Matrix</th><th>fortification level (mg/kg)</th><th>n</th><th colspan="2">recovery range (%)</th><th>mean recovery (%)</th><th>RSD (%)</th></tr>
<tr><td>glyphosate ((168-> 63m/z)
glyphosate</td><td rowspan="4">grape</td><td>0,05</td><td>1</td><td>71</td><td>71</td><td>71,0</td><td>-</td></tr>
<tr><td></td><td>0,50</td><td>1</td><td>71</td><td>71</td><td>71,0</td><td>-</td></tr>
<tr><td>AMPA (110->63m/z)
AMPA</td><td>0,050</td><td>1</td><td>82</td><td>82</td><td>82,0</td><td>-</td></tr>
<tr><td></td><td>0,50</td><td>1</td><td>83</td><td>83</td><td>83,0</td><td>-</td></tr>
</table>
</td></tr>
<tr><td>CA 6.3.1/011</td><td>[redacted]</td><td>2015</td><td>S14-04157</td><td>For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.</td><td>Glyphosate and AMPA: Y=ax+b ; Range :0.01mg/kg – 1.0mg/kg (n>5, R>0.99)</td><td>
<table>
<tr><th>Analyte</th><th>Matrix</th><th>fortification level (mg/kg)</th><th>n</th><th colspan="2">recovery range (%)</th><th>mean recovery (%)</th><th>RSD (%)</th></tr>
<tr><td>glyphosate ((168-> 63m/z)
glyphosate</td><td rowspan="4">grapes (bunches)</td><td>0,05</td><td>1</td><td>84</td><td>84</td><td>84,0</td><td>-</td></tr>
<tr><td></td><td>0,50</td><td>3</td><td>84</td><td>86</td><td>85,0</td><td>1,18</td></tr>
<tr><td>AMPA (110->63m/z)
AMPA</td><td>0,050</td><td>1</td><td>91</td><td>91</td><td>91,0</td><td>-</td></tr>
<tr><td></td><td>0,50</td><td>3</td><td>89</td><td>93</td><td>90,3</td><td>2,56</td></tr>
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</td></tr>
</table>

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.1/012	[redacted]	2015	S14-04158	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.01mg/kg – 1.0mg/kg (n>5, R>0.99)	
CA 6.3.1/013	[redacted]	2015	S14-04226	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.01mg/kg – 1.0mg/kg (n>5, R>0.99)	
CA 6.3.1/019	[redacted]	2016	S15-00469	For both substance and matrices, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.011mg/kg – 2.2mg/kg (n>5, R>0.99)	

Recovery

Analyte	*Matrix*	*fortification level (mg/kg)*	*n*	*recovery range (%)*		*mean recovery (%)*	*RSD (%)*
glyphosate ((168-> 63m/z)	grapes (bunches)	0,05	1	88	88	88,0	-
		0,50	1	87	87	87,0	-
AMPA (110->63m/z)		0,050	1	93	93	93,0	-
		0,50	1	96	96	96,0	-

Analyte	*Matrix*	*fortification level (mg/kg)*	*n*	*recovery range (%)*		*mean recovery (%)*	*RSD (%)*
glyphosate ((168-> 63m/z)	grapes (bunches)	0,05	1	93	93	93,0	-
		0,50	1	90	90	90,0	-
AMPA (110->63m/z)		0,050	1	93	93	93,0	-
		0,50	1	91	91	91,0	-

Analyte	*Matrix*	*fortification level (mg/kg)*	*n*	*recovery range (%)*		*mean recovery (%)*	*RSD (%)*
glyphosate (168-> 63m/z)	Kiwi (peel)	0,05	3	87	91	89,7	2,58
		0,50	3	87	89	88,3	1,31
AMPA (110->63m/z)		0,050	3	85	90	88,3	3,27
		0,50	3	87	88	87,3	0,66
Glyphosate (168-> 63m/z)	Kiwi (pulp)	0,05	3	91	92	91,3	0,63
		0,50	3	87	89	88,0	1,14
AMPA (110->63m/z)		0,050	3	85	92	89,3	4,24
		0,50	3	89	92	91,0	1,90

<table>
<tr><th>Data point</th><th>Report authors</th><th>Report year</th><th>Report number</th><th>Specificity</th><th>Linearity</th><th>Recovery</th></tr>
<tr>
<td>CA 6.3.1/020</td>
<td>[redacted]</td>
<td>2015</td>
<td>S14-04159</td>
<td>For both substance and matrice whole plant, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance

Chromatograms for other matrices are not available in the study</td>
<td>Glyphosate and AMPA: Y=ax+b ; Range :0.01mg/kg – 1.0mg/kg (n>5, R>0.99</td>
<td>
<table>
<tr><th>Analyte</th><th><u>Matrix</u></th><th><u>fortification level (mg/kg)</u></th><th><u>n</u></th><th colspan="2"><u>recovery range (%)</u></th><th><u>mean recovery (%)</u></th><th><u>RSD (%)</u></th></tr>
<tr><td rowspan="2">glyphosate ((168->63m/z)</td><td rowspan="4">banana(whole fruit)</td><td>0,05</td><td>3</td><td>96</td><td>106</td><td>100,7</td><td>5,00</td></tr>
<tr><td>0,50</td><td>3</td><td>84</td><td>86</td><td>85,3</td><td>1,35</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,050</td><td>3</td><td>83</td><td>92</td><td>87,0</td><td>5,27</td></tr>
<tr><td>0,50</td><td>3</td><td>80</td><td>86</td><td>82,7</td><td>3,70</td></tr>
<tr><td rowspan="2">glyphosate ((168->63m/z)</td><td rowspan="4">Banana pulp</td><td>0,05</td><td>1</td><td>91</td><td>91</td><td>91,0</td><td>-</td></tr>
<tr><td>0,50</td><td>1</td><td>90</td><td>90</td><td>90,0</td><td>-</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,050</td><td>1</td><td>92</td><td>92</td><td>92,0</td><td>-</td></tr>
<tr><td>0,50</td><td>1</td><td>85</td><td>85</td><td>85,0</td><td>-</td></tr>
<tr><td rowspan="2">glyphosate ((168->63m/z)</td><td rowspan="4">banana peel</td><td>0,05</td><td>1</td><td>84</td><td>84</td><td>84,0</td><td>-</td></tr>
<tr><td>0,50</td><td>1</td><td>86</td><td>86</td><td>86,0</td><td>-</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,050</td><td>1</td><td>83</td><td>83</td><td>83,0</td><td>-</td></tr>
<tr><td>0,50</td><td>1</td><td>84</td><td>84</td><td>84,0</td><td>*</td></tr>
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</td>
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<tr>
<td>CA 6.3.2/001</td>
<td>[redacted]</td>
<td>2012</td>
<td>S11-00258</td>
<td>For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.</td>
<td>Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99</td>
<td>
<table>
<tr><th>Analyte</th><th><u>Matrix</u></th><th><u>fortification level (mg/kg)</u></th><th><u>n</u></th><th colspan="2"><u>recovery range (%)</u></th><th><u>mean recovery (%)</u></th><th><u>RSD (%)</u></th></tr>
<tr><td rowspan="2">Glyphosate ((168-> 63m/z)</td><td rowspan="4">potatoes tuber</td><td>0,05</td><td>1</td><td>88</td><td>88</td><td>88,0</td><td>-</td></tr>
<tr><td>0,50</td><td>1</td><td>88</td><td>88</td><td>88,0</td><td>-</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,050</td><td>1</td><td>85</td><td>85</td><td>85,0</td><td>-</td></tr>
<tr><td>0,50</td><td>1</td><td>87</td><td>87</td><td>87,0</td><td>-</td></tr>
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Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.2/002	[redacted]	2012	S11-00259	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate (168-> 63m/z)	carrots	0,05	1	97	97	97,0	-
		0,50	1	91	91	91,0	-
AMPA (110->63m/z)		0,050	1	93	93	93,0	-
		0,50	1	90	90	90,0	-

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.2/003	[redacted]	2012	S11-00260	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	bulb onion	0,05	1	92	92	92,0	-
		0,50	1	91	91	91,0	-
AMPA (110->63m/z)		0,050	1	89	89	89,0	-
		0,50	1	88	88	88,0	-

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.2/004	[redacted]	2012	S11-00267	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Tomato	0,05	1	90	90	90,0	-
		0,50	1	87	87	87,0	-
AMPA (110->63m/z)		0,050	1	90	90	90,0	-
		0,50	1	88	88	88,0	-

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.2/005	[redacted]	2012	S11-00261	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance. Chromatograms for cucumber is not provided in the study	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	
CA 6.3.2/006	[redacted]	2012	S11-00263	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	
CA 6.3.2/007	[redacted]	2012	S11-00262	For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
Glyphosate (168-> 63m/z)	Zucchini	0,05	1	92	92	92,0	-
		0,50	1	88	88	88,0	-
AMPA (110->63m/z)		0,050	1	90	90	90,0	-
		0,50	1	90	90	90,0	-
glyphosate ((168-> 63m/z)	cucumber	0,05	1	90	90	90,0	-
		0,50	1	87	87	87,0	-
AMPA (110->63m/z)		0,050	1	87	87	87,0	-
		0,50	1	90	90	90,0	-

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	cauliflower	0,05	1	95	95	95	-
		0,5	1	90	90	90	-
AMPA (110->63m/z)		0,05	1	84	84	84	-
		0,5	1	89	89	89	-

	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Head cabbage	0,05	1	87	87	87	-
		0,5	1	87	87	87	-
AMPA (110->63m/z)		0,05	1	91	91	91	-
		0,5	1	90	90	90	-

<table>
<tr><th>Data point</th><th>Report authors</th><th>Report year</th><th>Report number</th><th>Specificity</th><th>Linearity</th><th>Recovery</th></tr>
<tr><td>CA 6.3.2/008</td><td>[redacted]</td><td>2012</td><td>S11-00264</td><td>For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.

Chromatograms of lettuce head is not available in the study</td><td>Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99</td><td>
<table>
<tr><th>Analyte</th><th>Matrix</th><th>fortification level (mg/kg)</th><th>n</th><th colspan="2">recovery range (%)</th><th>mean recovery (%)</th><th>RSD (%)</th></tr>
<tr><td rowspan="2">glyphosate ((168-> 63m/z)</td><td rowspan="4">lettuce leaves</td><td>0,05</td><td>1</td><td>91</td><td>91</td><td>91</td><td>-</td></tr>
<tr><td>0,5</td><td>1</td><td>86</td><td>86</td><td>86</td><td>-</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,05</td><td>1</td><td>86</td><td>86</td><td>86</td><td>-</td></tr>
<tr><td>0,5</td><td>1</td><td>85</td><td>85</td><td>85</td><td>-</td></tr>
<tr><td rowspan="2">glyphosate ((168-> 63m/z)</td><td rowspan="4">lettuce head</td><td>0,05</td><td>1</td><td>93</td><td>93</td><td>93</td><td>-</td></tr>
<tr><td>0,5</td><td>1</td><td>88</td><td>88</td><td>88</td><td>-</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,05</td><td>1</td><td>85</td><td>85</td><td>85</td><td>-</td></tr>
<tr><td>0,5</td><td>1</td><td>84</td><td>84</td><td>84</td><td>-</td></tr>
</table>
</td></tr>
<tr><td>CA 6.2.3/009</td><td>[redacted]</td><td>2012</td><td>S11-00265</td><td>For both substance, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.</td><td>Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99</td><td>
<table>
<tr><th>Analyte</th><th>Matrix</th><th>fortification level (mg/kg)</th><th>n</th><th colspan="2">recovery range (%)</th><th>mean recovery (%)</th><th>RSD (%)</th></tr>
<tr><td rowspan="2">glyphosate ((168-> 63m/z)</td><td rowspan="4">Leek</td><td>0,05</td><td>1</td><td>90</td><td>90</td><td>90</td><td>-</td></tr>
<tr><td>0,5</td><td>1</td><td>89</td><td>89</td><td>89</td><td>-</td></tr>
<tr><td rowspan="2">AMPA (110->63m/z)</td><td>0,05</td><td>1</td><td>89</td><td>89</td><td>89</td><td>-</td></tr>
<tr><td>0,5</td><td>1</td><td>87</td><td>87</td><td>87</td><td>-</td></tr>
</table>
</td></tr>
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Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.2/010	[redacted]	2012	S11-00266	For both substance and for each matrices, chromatogram of standards solution, of control sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :1.25ng/L - 250ng/L (n>5, R>0.99	
CA 6.3.3/001	[redacted]	2016	S15-00482	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	
CA 6.3.3/002	[redacted]	2016	S15-00467	For both substance and matrices, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	sugar beet (leaves and top)	0,05	1	96	96	96	-
		0,5	1	93	93	93	-
AMPA (110->63m/z)		0,05	1	94	94	94	-
		0,5	1	87	87	87	-
glyphosate ((168-> 63m/z)	sugar beet (roots)	0,05	1	91	91	91	-
		0,5	1	90	90	90	-
AMPA (110->63m/z)		0,05	1	93	93	93	--
		0,5	1	89	89	89	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	carrot (roots)	0,05	1	82	82	82	-
glyphosate		0,5	1	72	72	72	-
AMPA (110->63m/z)		0,05	1	88	88	88	-
AMPA		0,5	1	84	84	84	-

	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z) 7	radish roots	0,05	3	91	107	100,67	8,45
		0,5	3	79	86	81,67	4,64
AMPA (110->63m/z) 7		0,05	3	83	87	85,33	2,44
		0,5	3	84	92	88,67	4,70
glyphosate ((168-> 63m/z) 7	radish leaves	0,05	3	78	87	83,67	5,90
		0,5	3	77	82	79,33	3,17
AMPA (110->63m/z) 7		0,05	3	80	83	81,67	1,87
		0,5	3	82	92	87,33	5,76

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.3/003	[redacted]	2016	S15-00466	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	
CA 6.3.3/004	[redacted]	2016	S15-00465	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	
CA 6.3.3/005	[redacted]	2016	S15-00464	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Onion bulbs	0,05	1	90	90	90,00	-
glyphosate		0,5	1	82	82	82,00	-
AMPA (110->63m/z)		0,05	1	93	93	93,00	-
AMPA		0,5	1	83	83	83,00	-

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Tomato fruits	0,05	1	86	86	86,00	-
glyphosate		0,5	1	81	81	81,00	-
AMPA (110->63m/z)		0,05	1	84	84	84,00	-
AMPA		0,5	1	89	89	89,00	-

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Cucumber	0,05	1	90	90	90	-
glyphosate		0,5	1	84	84	84	-
AMPA (110->63m/z)		0,05	1	92	92	92	-
AMPA		0,5	1	90	90	90	-

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.3/006	[redacted]	2016	S15-00463	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	
CA 6.3.3/007	[redacted]	2016	S15-00460	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	
CA 6.3.3/008	[redacted].	2016	S15-00459	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	courgette (fruits)	0,05	3	79	83	81,67	2,83
glyphosate		0,5	3	83	87	85,00	2,35
AMPA (110->63m/z)		0,05	3	77	85	81,00	4,94
AMPA		0,5	3	85	92	88,33	3,98

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Head lettuce	0,05	1	91	91	91,00	-
		0,5	1	87	87	87,00	-
AMPA (110->63m/z)		0,05	1	89	89	89,00	-
		0,5	1	89	89	89,00	-

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	Parsley leaves	0,05	3	90	95	93,00	2,84
		0,5	3	81	90	84,33	5,85
AMPA (110->63m/z)		0,05	3	85	89	87,33	2,38
		0,5	3	90	91	90,67	0,64

Data point	Report authors	Report year	Report number	Specificity	Linearity	Recovery
CA 6.3.3/009	[redacted]	2016	S15-00461	For both substance, chromatogram of standards solution, of untreated sample, and fortified sample at LOQ are provided. No interfrence is observed at the retention time of each substance.	Glyphosate and AMPA: Y=ax+b ; Range :0.11mg/kg – 2.2mg/kg (n>5, R>0.99	

Analyte	Matrix	fortification level (mg/kg)	n	recovery range (%)		mean recovery (%)	RSD (%)
glyphosate ((168-> 63m/z)	green beans (pods)	0,05	3	90	96	92,33	3,48
		0,5	3	87	97	90,33	6,39
AMPA (110->63m/z)		0,05	3	93	100	95,33	4,24
		0,5	3	88	94	91,33	3,34

Conclusion
The method AG-ME-1294-01 was successfully validated for the analysis of residues of glyphosate and AMPA in potato (tubers), carrot (roots), onion (bulbs), cucumber (fruit), cabbage (heads), cauliflower (heads), lettuce (leaves), leek (plants), tomato (fruit), cereal (grain), sunflower (seeds), grape (bunches), apricot (fruit without stone), plum (fruit), green beans (pod), courgette (fruit), radish (tops (leaves), root), parsley (leaves), hazelnut (nutmeat), kiwi (peel, pulp) and banana (whole fruit) at 0.05 mg/kg (LOQ) and 0.5 mg/kg and fulfils the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000)

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level for some of the presented matrices. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4).The method is fit for purpose to support the residue studies concerned.

Assessment and conclusion by RMS: All studies have been performed in the same laboratory except the study [redacted] 2012. Therefore, the validation data can be compiled. The analytical method for the determination of glyphosate and AMPA fulfill the requirement of the guidance document SANCO 3029/99 rev.4 for high water, acidic and high oil matrices with an LOQ of 0.05 mg/kg

Concerning the study report [redacted] 2012, the validation data are in agreement with the guidance document SANCO 3029/99 rev.4. for acidic matrices with an LOQ of 0.05mg/kg.

The extraction solvent used is 100 mL 0.1% formic acid in water + 100 mL methylene chloride, consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It si not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

The method can be considered as validated.

Data point	CA 4.1.2/123 (CA 6.1/013)
Report authors	[redacted]
Report year	1989
Report title	Storage stability validation for ICIA 0224 in raw agricultural commodities
Report No	WRC 89-22
Document No	RIP9500028
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Not enough recoveries • No details on linearity is given • Matrix effects not assessed • Derivatisation efficiency not reported • Stability of the analytes in sample extract not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	ICI Americas Inc Richmond 94804-0023

Principle of the method
In this study, glyphosate and AMPA were analysed with method RRC 85-34. This method was broadly used and validated for a variety of matrices (high water, high acid, dry, high oil, difficult).
Samples of sorghum grain, wheat grain, soybean seeds and soybean straw were extracted with water. The extracts were cleaned up using a cation exchange column, the analytes were collected separately and converted to f1uorescing derivatives with 9-fluorenylmethyl chloroformate. Derivatives were determined by HPLC using an anion exchange column with fluorescence detection.

Chromatographic conditions:

HPLC system:	HPLC system capable of pulse-free operation at 1500 psi, equipped with a sensitive fluorescence detector (Perkin Elmer LC-10 Fluoromonitor)
HPLC column:	Aninon exchange column, Ultrasil AX, 25 cm × 4.6 mm, 5 or 10 µ or equivalent
Column temperature:	Ambient
Mobile phase:	Composition of mobile phase depends on the HPLC equipment available. An average sample is given in the report with the following composition: Mixture containing 11% pH 2.5 buffer in 22% acetonitrile for Glyphosate (pH 2.5 buffer: 970 mL of water with 20 mL glacial acetic acid and 10 mL phosphoric acid (86%)) Mixture containing 10 % pH 5 buffer with 90% of 22% acetonitrile solution to effect a 0.01M buffer solution for AMPA (pH 5 buffer: 13.61 g of KH_2PO_4, 600 mL of water and 400 mL of acetonitrile)
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Retention time:	Glyphoste: Approximately 19 min AMPA: Approxiately 13 min

Detection:	Excitation wavelength: 254 nm Emission wavelength: 300-315 nm

Findings

Recoveries (accuracy)

Samples of sorghum grain, soybean seed and straw and wheat grain prepared at 0 day storage were analysed for the concentration of the glyphosate and AMPA using the mentioned analytical method. Glyphosate and AMPA were analysed at nominal concentration levels of 1.0 mg/kg for wheat grain, soybean seeds, soybean straw, and at 5.0 mg/kg for sorghum grain. The recovery results are shown in the table below. The recoveries were in acceptable range of 70 – 110 %.

Further fortification of untreated control samples were obtained at 0.1 mg/kg and 0.2 mg/kg for the detection of glyphosate and 0.1 mg/kg, 0.2 mg/kg and 0.4 mg/kg for the detection of AMPA. The recovery data of these fortification samples were not provided within the report.

Table 5.1-1: Recovery results of glyphosate and AMPA in day 0 samples used for storage stability study (t=0)

Commodity	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Sorghum grain	Glyphosate	5.0	93	N/A	N/A	N/A	1
	AMPA	5.0	83	N/A	N/A	N/A	1
Soybean seed	Glyphosate	1.0	88 – 106	100	10	10	3
	AMPA	1.0	87 – 92	90	2.5	2.8	3
Soybean straw	Glyphosate	1.0	96 – 105	100	4.6	4.6	3
	AMPA	1.0	70 – 77	74	3.5	4.8	3
Wheat grain	Glyphosate	1.0	83 – 98	92	7.9	8.6	3
	AMPA	1.0	95 – 100	98	2.5	2.6	3

[1] Corrected for contamination in untreated control sample

N/A Not applicable

Recovery data, obtained during the validation of method RRC 85-34 performed in the same laboratory was presented in Appendix A of the report. A summary of the recovery results for soybean (seed and straw) and wheat (grain) is given in the table below.

Table 5.1-2: Recovery results for glyphosate and AMPA in soybean and wheat

Commodity	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Soybean seed	Glyphosate	0.1	112	112	N/A	N/A	1
		0.2	113	113	N/A	N/A	1
		0.4	99 – 112	105	5.3	5.0	5

Table 5.1-2: Recovery results for glyphosate and AMPA in soybean and wheat

Commodity	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		1.0	101 – 107	103	2.9	2.8	4
	AMPA	0.1	97	97	N/A	N/A	1
		0.2	99	99	N/A	N/A	1
		0.4	84 – 107	94	8.8	9.4	5
		1.0	85 – 92	89	3.0	3.3	4
Soybean straw	Glyphosate	0.2	105	105	N/A	N/A	1
		0.4	80 – 99	90	7.6	8.4	6
		1.0	95 – 99	96	2.3	2.4	3
	AMPA	0.2	93	93	N/A	N/A	1
		0.4	70 – 98	81	9.8	12.0	6
		1.0	64 – 84	77	11.3	14.6	3
Wheat grain	Glyphosate	0.2	68 – 126	88	23.5	26.8	5
		0.4	83	83	N/A	N/A	1
		0.5	84 – 103	91	10.4	11.5	3
		1.0	65	65	N/A	N/A	1
	AMPA	0.2	80 – 102	88	9.7	10.9	5
		0.4	80	80	N/A	N/A	1
		0.5	80 – 100	92	10.6	11.5	3
		1.0	95	95	N/A	N/A	1

N/A Not applicable

Specificity

Chromatograms of standards solution, unfortified samples (Sorghum grain, Soybean seed, Soybean straw, Wheat grain), fortified sample for AMPA and glyphosate are provided. No interference (below 30xof LOQ) are observed at the retention time of each analyte.

Linearity

For glyphosate and AMPA, two calibration standard concentrations of 0.05 µg/mL to 0.2 µg/mL were used. Further information on linearity is not reported in the study.

Repeatability (Precision)

The relative standard deviations (RSDs) of all recoveries were below 20 %, except for the initial validation in in wheat grain for glyphosate, where the average RSD was slightly above 20 %. As the exceedance is very low the method is still considered as valid and complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection

The limit of quantification (LOQ) is not reported in the study, however the detection limit for glyphosate in crops used in this study was 0.1 – 0.2 mg/kg and for AMPA was 0.1 mg/kg.

Interference
No significant interference was observed at the retention time of the analytes.

Matrix effects
Not directly assessed. However, matrix blank sample did not show any peak at the retention time of interest.

Stability of glyphosate and AMPA in sample extracts
Stability of the analytes in sample extracts was not assessed. However, it was shown that the test materials were stable in the test matrices for duration of the storage stability study.

Conclusion
The analytical method was developed for the determination of glyphosate and AMPA in different raw agricultural commodities. The method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate and AMPA in different plant matrices.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate and AMPA was not previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) in most aspects (deficits: not enough validation data, limited linearity data, matrix effects and stability of the analytes in sample extract not assessed). Nevertheless, the method is considered as fit-for-purpose to support the storage stability study concerned.

Assessment and conclusion by RMS: The analytical method does not meet the requirement of the guidance document SANCO 3029/99 re.4.

The number of sample by fortification level is too low. However, the validation can be compiled as soybean seed, soybean straw and wheat grain are considered as dry matrices. Except the fortification level 0.1 mg/kg where number of sample is < 5, the other fortification levels meet the requirement. The accuracy are in the acceptable limit.
The linearity range are provided in µg/L this does not allow to verify that fortification levels are in the linearity range or if dilution was performed.
The matrix effect was not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed.

The derivatisation efficiency was not demonstrated.

The specificity of the method is acceptable.

In conclusion, the validation data do not fulfill the requirement of the guidance document SANCO 3029/99 rev.4. However, as the objective of the residue study is to validate the stability of the sample at targeted concentrations, we can consider the validation data provided for the method as sufficient to validate the content of glyphosate and AMPA at 1.0 mg/kg for wheat grain, soybean seeds, soybean straw, and at 5.0 mg/kg for sorghum grain

Data point	CA 4.1.2/144 (CA 6.5.3/001)
Report author	[redacted]
Report year	1986
Report title	Determination of Glyphosate and Aminomethylphosphonic acid residues in citrus fruit and process fractions following postdirected treatment with Roundup herbicide
Report No	MSL-6194
Document No	Not applicable

Guidelines followed in study	OECD GLP FAO Guidelines
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • All analytical data, beside an overview of recovery results, was reported in [redacted], 1975 (CA 6.5.3/002)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Not available

Data point	CA 4.1.2/145 (CA 6.5.3/002)
Report author	[redacted]
Report year	1975
Report title	CP 57573, Residue and metabolism part 27: Determination of CP 67573 and CP 50435 residues in citrus process fractions
Report No	377
Document No	Not applicable
Guidelines followed in study	No test guidelines cited in the report
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Limited information on calibration • RSD ≥20% for some matrices • Average recovery values not between 70% and 110% for some matrices • Matrix effects were not assessed Stability in extracts was not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Not available

An analytical method was developed to determine glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in the peel and juice of citrus fruits and other processed fractions. Analytes were extracted by aqueous extraction, followed by ion exchange chromatography and derivatisation to the N-trifluoroacetyl methyl esters. Determination was done using a phosphorus specific flame photometric detector.

Principle of the method
Samples were grinded with an electric food chopper, where required and extracted with deionized water before ion exchange chromatography on A-101D. In case of oil samples a water/chloroform (50/50) extraction was done before the aqueous layer was analysed further.
After elution and further charcoal treatment, where required, samples were evaporated to dryness and dissolved in deionized water. Dissolved samples were subjected to AG 50W-X8 chromatography to separate glyphosate and AMPA followed by derivatisation with O-methyl-N,N´-dicyclohexyl pseudourea. In case of feed meal samples a further clean-up step could have been necessary. Samples were analysed by GLC-FPD.

Findings
Recoveries

Samples were spiked with the analytes at fortification levels at of 0.05 mg/kg (LOQ), 0.1 mg/kg, 0.2 mg/kg and 0.4 mg/kg. Prewash water and after wash water samples were fortified with 0.025 mg/kg, 0.05 mg/kg and 0.1 mg/kg. The detailed results are given in the table below.

Table 5.1-3: Recovery results of glyphosate and AMPA in processed fractions of citrus fruit

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Juice	Glyphosate	0.1	55 – 104	71	14	19	22
		0.2	50 – 89	64	9.9	16	21
	AMPA	0.1	53 – 106	75	14	19	24
		0.2	53 – 99	72	16	22	24
Peel	Glyphosate	0.05	59 – 98	80	20	25	4
		0.1	52 – 95	71	11	16	22
		0.2	53 – 80	66	8.4	13	21
	AMPA	0.05	55 – 95	66	19	29	4
		0.1	41 – 110	67	16	24	21
		0.2	44 – 99	66	14	21	19
Press liquor	Glyphosate	0.05	106	106	N/A	N/A	1
		0.1	71 – 92	80	8.3	10	6
		0.2	56 – 85	74	10	14	8
		0.4	73	73	N/A	N/A	1
	AMPA	0.05	108	108	N/A	N/A	1
		0.1	80 – 106	93	9.2	9.9	7
		0.2	57 – 102	81	16	19	8
		0.4	85	85	N/A	N/A	1
Feed meal	Glyphosate	0.05	106	106	N/A	N/A	1
		0.1	59 – 93	76	13	18	10
		0.2	58 – 86	72	10	14	7
	AMPA	0.1	48 – 98	63	15	23	10
		0.2	43 – 65	56	7	13	8
Oil	Glyphosate	0.05	77 – 87	82	6.7	8.2	2
		0.1	56 – 80	67	8.2	12	8
		0.2	66	66	N/A	N/A	1
	AMPA	0.05	77 – 80	78	2.3	2.9	2
		0.1	76 – 95	83	6.8	8.1	8

Table 5.1-3: Recovery results of glyphosate and AMPA in processed fractions of citrus fruit

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
		0.2	96	96	N/A	N/A	1
Molasses	Glyphosate	0.1	58 – 81	71	9.7	13.7	5
		0.2	58 – 70	66	5.2	7.8	5
	AMPA	0.1	71 – 96	87	9.8	11	5
		0.2	61 – 86	77	12	15	5
Grapefruit pulp, rag, seed	Glyphosate	0.1	83 – 95	89	8.3	9.4	2
		0.2	80 – 94	87	10	12	2
	AMPA	0.1	91 – 97	94	4.2	4.5	2
		0.2	76 – 85	81	6.3	7.8	2
Finisher pulp	Glyphosate	0.1	81 – 115	95	18	19	3
		0.2	72 – 93	81	11	13	3
	AMPA	0.1	96 – 112	103	8.4	8.1	3
		0.2	87 – 88	88	0.6	0.7	3
Peel frits	Glyphosate	0.1	57 – 71	62	8.0	13	3
		0.2	56 – 63	59	3.6	6.0	3
	AMPA	0.1	62 – 69	64	3.7	5.8	3
		0.2	68 – 90	80	11	14	3
Citrus pulp	Glyphosate	0.1	86 – 89	88	2.1	2.4	3
		0.2	73 – 83	78	5.0	6.4	3
	AMPA	0.1	79 – 83	80	2.6	3.2	3
		0.2	879 – 86	81	3.8	4.7	3
Oil emulsion water	Glyphosate	0.1	93 – 96	94	1.8	1.9	3
		0.2	69 – 90	78	11	14	3
	AMPA	0.1	77 – 97	88	10	11	3
		0.2	75 – 89	82	7.2	8.8	3
Pre-wash water	Glyphosate	0.03	74 – 97	88	12	14	3
		0.05	82 – 93	88	5.9	6.6	3
	AMPA	0.03	63 – 93	79	16	20	3
		0.05	73 – 84	77	5.6	7.2	3
After wash water	Glyphosate	0.03	91 – 96	94	3.1	3.3	3
		0.05	96	96	N/A	N/A	1

Table 5.1-3: Recovery results of glyphosate and AMPA in processed fractions of citrus fruit

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
		0.1	82 – 96	89	10	11	2
	AMPA	0.03	84 – 90	88	3.5	3.9	3
		0.05	90	90	N/A	N/A	1
		0.1	84 – 90	87	4.2	4.9	2

1 Values for matrices from orange, lemon and grapefruit were combined.
2 Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.
N/A Not applicable

Specificity
For each matrix, chromatograms of standards solution, of control sample, of fortified sample and treated sample. No interference (below 30% of LOQ) is observed at the retention time of AMPA and glyphosate. For glyphosate and AMPA no interferences from the specimen matrices were detected by GLC-FPD.

Linearity
Calibration curve constants based on the injection of standards. Three to nine amount response pairs were used for each standard curve. Constants were reported in the study. The equation of the curve is $Y=A(x)^B$. The concentration range is not available.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were for most matrices and fortification level <20 %. Slight extensions were identified for glyphosate in peel and AMPA in juice, peel and feed meal.

Accuracy
Acceptable mean recovery values at LOQ and higher fortification level between 70 % and 110 % for glyphosate and AMPA were found for press liquor, samples from pulp, rag and seed, finisher pulp, citrus pulp, oil emulsion water, pre-wash water and after wash water. In addition, results between 70 % and 110 % could be identified for low fortification levels for glyphosate in juice, peel, feed meal, oil and molasses and for AMPA for juice, oil and molasses. Slightly lower recovery rates were found for glyphosate for molasses and peel frits and for AMPA in peel, feed meal and peel frits. And for glyphosate at higher fortification levels for the matrices juice, peel and oil.

Limit of Quantification and Detection
The limit of detection was stated to be 0.025 mg/kg for prewash water and after wash water and 0.05 mg/kg for all other sample materials for both analytes.

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

Matrix effects
Not assessed.

Stability of glyphosate and AMPA in sample extracts
Stability of the analytes in sample extracts was not assessed.

Conclusion

The analytical method was validated for the determination of glyphosate and AMPA in processed fractions of citrus fruit at a detection limit of 0.05 mg/L and additionally at 0.025 mg/kg for water samples. The analytical method fulfils the major European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000).

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate and AMPA was not previously evaluated at EU level. It was not performed under GLP and shows major deficits to current requirements (EU guideline SANCO/3029/99 rev. 4). Nevertheless, important points like number of recoveries and fortification level, specificity, linearity and accuracy were fulfilled in most aspects. Therefore, the method is considered as fit-for-purpose to support the residue studies concerned.

Assessment and conclusion by RMS: The analytical method does not meet the requirement of the guidance document SANCO 3029/99 re.4.

The number of sample to demonstrate the recovery for each matrix is not sufficient to validate the method. Moreover, the recoveries obtained for glyphosate in Peel fruit and for AMPA in feed meal are not in the acceptable range.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

The extraction solvent used is water that is identical to the extraction solvent used in metabolisme studies(see extraction efficiency part p660).

For oil samples the extraction solvent is water/chloroform (50/50), consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

In conclusion, the method cannot be considered as validated according to SANCO 3029/99 rev. 3 .

Data point	CA 4.1.2/118
Report author	[redacted]
Report year	2007
Report title	Analytical method for the determination of glyphosate and degradate residues in various crop matrices using LC/MS/MS
Report No	DuPont-15444 Revision No. 1
Document No	ASB2008-2635
Guidelines followed in study	EPA OPPTS 860.1340 SANCO/825/00 rev. 7
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	Yes
Category study in AIR 5 dossier (L docs)	Category 2a (with relevance for analytical methods)

Test facility	E. I. du Pont de Nemours and Company DuPont Crop Protection Global Technology Division Stine-Haskell Research Center Newark, Delaware 19714-0030

Principle of method

The analytical method DuPont-15444 was validated for the determination of residues of glyphosate, *N*-acetylglyphosate, AMPA, and *N*-acetyl AMPA in matrices with high water content (plum, corn grain, corn and soybean forage), high oil content (soybean seed, corn oil, soybean oil), dry crops (soybean hay) and one acidic fruit (lime).

This method was used within several residue studies; an overview of the relevant studies is given in the table below.

Table 5.1-4: Overview on residue studies

Data point	Report authors	Report year	Report number	Report title
CA 6.1/004	[redacted]	2009	DuPont-20094	Stability of Glyphosate and metabolites in corn green plant, forage, grain, and stover containing the GAT and ZM-HRA genes during frozen storage
CA 6.1/005	[redacted]	2009	DuPont-17573	Stability of Glyphosate, N-Acetylglyphosate, Aminomethyl phosphonic acid and N-Acetyl AMPA in GAT soybean forage, seed, and hay stored frozen
CA 6.1/006	[redacted]	2007	DuPont-17379	Stability of Glyphosate, N-Acetylglyphosate and Aminomethyl phosphonic acid in GAT corn forage, grain, and stover, stored frozen

Glyphosate, *N*-acetylglyphosate, AMPA, and *N*-acetyl AMPA were extracted from plant tissue and solid process fraction matrices of various crops and diluted to aqueous acid (0.1 % formic acid or 0.025 N hydrochloric acid)/ methanol (96/4, v/v) using a probe homogeniser. Dilute hydrochloric acid was substituted for formic acid to increased acidity for more efficient extraction of *N*-acetylglyphosate from corn flour and meal process fractions. Three extractions were made for quantitative recovery of analytes and to eliminate moisture content in the matrix as a recovery factor. Additional extraction solution volumes were necessary for stover, hay, and hulls because of the lower moisture content in those matrices.

Purification of glyphosate, *N*-acetylglyphosate, and *N*-acetyl AMPA in solid matrix sample extracts:
An aliquot of extract was partitioned with methylene chloride and the aqueous fraction is recovered and filtered (0.2 - 1.0 µm) to remove particulates. Approximately 10 mL of the aqueous fraction was collected following filtration through a C_{18} SPE cartridge. An aliquot of the eluate collected from the C_{18} SPE was diluted and applied to a MAX SPE cartridge. The analytes were eluted from the MAX sorbent in 1 % TFA in methanol/ water (9/1, v/v) solution following several solution rinses. The MAX eluate was evaporated to dryness and re-dissolved in aqueous 0.02 M phosphoric acid, filtered, and analysed for glyphosate and *N*-acetylglyphosate. A minor modification was made to this procedure for the analysis of soybean samples. For soybean seed and meal following partitioning, extract samples were heated in a steam bath for approximately 15 minutes to precipitate additional material in the extract prior to particulate filtration.

Purification of AMPA in solid matrix sample extracts:
A second aliquot of the eluate collected from the C_{18} SPE described above was processed through a MCX SPE cartridge, diluted, filtered and analysed for AMPA. A separate analyte purification procedure was required for AMPA due to low recoveries using MAX SPE purification.

Analysis of Glyphosate, *N*-acetylglyphosate, AMPA, and *N*-acetyl AMPA in oil samples:
An aliquot of the sample was diluted with methylene chloride and the analytes are liquid-liquid partitioned into 0.02 M aqueous phosphoric acid. The sample was partitioned twice for quantitative transfer of the analytes. Centrifugation was used to define phase separation in each partition.

All final extracts were filtered (0.2 µm) prior to LC-MS/MS analysis with positive electrospray ionisation (ESI^+) in the selected reaction mode using a phenyl-hexyl column. An Applied Biosystems/MDS SCIEX API 4000 mass spectrometer was used instead of the Quattro Premier for a second lab tryout. Injection volumes were increased to compensate for decreased mass spectrometer sensitivity. The reported results are based on calibration with an internal standard for glyphosate and AMPA.

Chromatographic conditions:

HPLC-system:	Agilent HP1100: G1322A vacuum degasser, G1311A quaternary pump, G1367A chilled autosampler, G1330A chiller, G1316A column compartment
MS System:	Waters Quattro Premier triple quadrupole mass spectrometer, ESI interface, MassLynx version 4 SP4 software
Guard column (optional):	Waters Nova-Pak® Sentry C_{18} (3.9 mm i.d. x 20 mm, 4 µm diameter particle) Guard column should not be used if peak broadening occurs.
Column:	Phenomenex Luna® Phenyl-Hexyl (15.0 cm x 4.6 mm i.d. 3 µm diameter particle)
Column temperature:	40°C
Injection volume:	25 µL (may be varied to correct for MS sensitivity)
Mobile phase:	A: aqueous 0.2 M formic acid B: methanol
Flow rate:	0.35 – 0.5 mL/min
Retention time:	Glyphosate: 5.3 min *N*-acetylglyphosate: 7.4 min AMPA: 4.6 min *N*-acetyl AMPA: 7.1 min
Scan type:	Positive Ion MRM
Ion source:	Electronspray (ESI)

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Dwell (secs)	Cone (volts)	Collision Energy (eV)
Glyphosate	170	88	0.10	14	9
	170	60	0.10	14	17
N-acetylglyphosate	212	88	0.10	17	17
	212	170	0.10	17	10
1,2-$^{13}C^{15}N$-glyphosate	173	91	0.10	14	9
AMPA	112	30	0.30	12	8
N-acetyl AMPA	154	30	0.10	14	15
	154	112	0.10	14	9
$^{13}C^{15}N$-AMPA	114	32	0.30	12	8

Findings

Recoveries

The method proved to be suitable to determine glyphosate, *N*-acetylglyphosate, AMPA and *N*-acetyl AMPA in high water content (plum, corn grain, corn and soybean forage), high oil content (soybean seed, corn oil, soybean oil), dry crops (soybean hay) and one acidic fruit (lime). Flour, grits, starch and meal from corn and meal and hulls from soybean were analysed for glyphosate, *N*-acetylglyphosate and AMPA. Samples were spiked with the analytes at the LOQ and 10 x LOQ levels. Corn stover and corn forage samples were additionally spiked with 10

and 5.0 mg/kg *N*-acetyl AMPA, respectively. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Plums (DuPont-15444)	Glyphosate	0.05	90 – 99	96	3.5	3.7	5
		0.5	85 – 96	91	4.3	4.7	5
		Overall	85 – 99	93	4.4	4.7	10
	N-acetyl-glyphosate	0.05	91 – 110	102	9.2	9.0	5
		0.5	82 – 100	93	7.9	8.5	5
		Overall	82 – 110	97	9.4	9.6	10
	AMPA	0.05	88 – 108	95	7.9	8.3	5
		0.5	92 – 112	100	9.1	9.1	5
		Overall	88 – 112	97	8.4	8.6	10
	N-acetyl AMPA	0.05	98 – 109	102	4.8	4.7	5
		0.5	89 – 112	100	8.2	8.2	5
		Overall	89 – 112	101	6.4	6.3	10
Limes (DuPont-15444)	Glyphosate	0.05	88 – 110	100	8.4	8.5	5
		0.5	90 – 107	99	6.4	6.5	5
		Overall	88 – 110	99	7.1	7.1	10
	N-acetyl-glyphosate	0.05	78 – 99	86	9.0	11	5
		0.5	85 – 98	91	5.6	6.1	5
		Overall	78 – 99	89	7.6	8.6	10
	AMPA	0.05	85 – 104	95	7.5	7.8	5
		0.5	90 – 101	98	4.8	4.9	5
		Overall	85 – 104	97	6.1	6.3	10
	N-acetyl AMPA	0.05	79 – 105	94	11	12	5
		0.5	95 – 121	107	10	9.5	5
		Overall	79 – 121	101	12	12	10
Corn forage (DuPont-15444)	Glyphosate	0.05	72 – 100	83	9.6	12	13
		0.5	66 – 100	80	9.7	12	12
		Overall	66 – 100	82	9.6	12	25
	N-acetyl-glyphosate	0.05	75 – 97	90	6.4	7.1	13
		0.5	71 – 96	86	7.0	8.1	12

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	71 – 97	88	6.9	7.8	25
	AMPA	0.05	87 – 117	98	9.7	9.9	10
		0.5	83 – 98	91	4.6	5.0	10
		Overall	83 – 117	94	8.1	8.6	20
	N-acetyl AMPA	0.05	69 – 122	87	13	15	22
		0.5	73 – 91	82	N/A	N/A	2
		5.0	72 – 112	91	9.0	9.9	21
		Overall	69 – 122	88	11	13	45
Corn grain (DuPont-15444)	Glyphosate	0.05	71 – 95	77	7.2	9.3	12
		0.5	70 – 97	79	11	13	12
		Overall	70 – 97	78	8.9	11	24
	N-acetyl-glyphosate	0.05	78 – 98	87	6.4	7.3	12
		0.5	83 – 98	89	4.7	5.3	12
		Overall	78 – 98	88	5.6	6.3	24
	AMPA	0.05	97 – 129	109	8.8	8.1	12
		0.5	83 – 106	97	7.7	7.9	12
		Overall	83 – 129	103	10	10	24
	N-acetyl AMPA	0.05	74 – 100	86	8.5	9.9	11
		0.5	77 – 97	83	5.8	6.9	10
		Overall	74 – 100	85	7.3	8.6	21
Corn stover (DuPont-15444)	Glyphosate	0.05	73 – 91	82	6.6	8.0	10
		0.5	76 – 88	83	3.7	44.5	10
		Overall	73 – 91	82	5.2	6.4	20
	N-acetyl-glyphosate	0.05	81 – 97	91	4.7	5.2	10
		0.5	84 – 102	90	4.8	5.3	10
		Overall	81 – 102	91	4.6	5.1	20
	AMPA	0.05	86 – 106	97	5.7	5.9	10
		0.5	76 – 100	90	8.9	9.9	10
		Overall	76 – 106	94	8.1	8.7	20
	N-acetyl AMPA	0.05	66 – 98	83	7.5	9.1	24
		0.5	85 – 89	87	N/A	N/A	2

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		10	75 – 97	90	5.9	6.6	17
		Overall	66 – 98	86	7.5	8.7	43
Corn oil (DuPont-15444)	Glyphosate	0.05	93 – 106	99	3.7	3.7	10
		0.5	95 – 107	101	4.3	4.3	10
		Overall	93 – 107	100	4.0	4.0	20
	N-acetyl-glyphosate	0.05	93 – 101	99	2.3	2.3	10
		0.5	91 – 103	100	3.6	3.6	10
		Overall	91 – 103	99	3.0	3.0	20
	AMPA	0.05	77 – 130	102	19	19	10
		0.5	77 – 99	91	6.1	6.8	10
		Overall	77 – 130	96	15	16	20
	N-acetyl AMPA[2]	0.05	96 – 109	101	5.8	5.7	6
		0.5	97 – 105	101	3.1	3.0	6
		Overall	96 – 109	101	4.4	4.4	12
Corn flour (DuPont-15444)	Glyphosate	0.05	83 – 101	91	8.4	9.2	5
		0.5	69 – 93	79	11	14	5
		Overall	69 – 101	85	11	13	10
	N-acetyl-glyphosate	0.05	80 – 95	85	6.4	77.5	5
		0.5	72 – 91	82	7.4	79.0	5
		Overall	72 – 95	84	6.7	8.0	10
	AMPA	0.05	74 – 100	87	9.3	11	5
		0.5	71 – 81	76	3.6	4.7	5
		Overall	71 – 100	82	8.7	11	10
	N-acetyl AMPA	Not determined					
Corn grits (DuPont-15444)	Glyphosate	0.05	80 – 93	86	5.2	6.1	5
		0.5	74 – 99	82	10	12	5
		Overall	74 – 99	84	8.0	9.5	10
	N-acetyl-glyphosate	0.05	79 – 83	81	1.7	2.1	5
		0.5	75 – 106	85	13	15	5
		Overall	75 – 106	83	9.0	11	10
	AMPA	0.05	87 – 94	89	2.9	3.2	5

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.5	77 – 83	80	2.6	3.2	5
		Overall	77 – 94	85	5.7	6.7	10
	N-acetyl AMPA	Not determined					
Corn starch (DuPont-15444)	Glyphosate	0.05	74 – 83	78	4.2	5.4	5
		0.5	71 – 88	80	6.8	6.8	5
		Overall	71 – 88	79	5.4	6.9	10
	N-acetyl-glyphosate	0.05	90 – 99	95	3.3	3.5	5
		0.5	93 – 95	94	0.8	0.9	5
		Overall	90 – 99	94	2.3	2.5	10
	AMPA[3]	0.05	94 – 103	99	3.9	4.0	5
		0.5	88 – 94	92	2.5	2.7	5
		Overall	88 – 103	95	4.6	4.8	10
	N-acetyl AMPA	Not determined					
Corn meal (DuPont-15444)	Glyphosate	0.05	83 – 116	99	12	12	5
		0.5	85 – 100	92	7.0	7.6	5
		Overall	83 – 116	96	9.9	10	10
	N-acetyl-glyphosate	0.05	65 – 91	80	11	14	5
		0.5	78 – 84	81	2.5	3.1	5
		Overall	65 – 91	81	7.5	9.3	10
	AMPA	0.05	80 – 113	99	15	15	5
		0.5	74 – 91	81	8.0	9.9	5
		Overall	74 – 113	90	15	16	10
	N-acetyl AMPA	Not determined					
Soybean forage (DuPont-15444)	Glyphosate	0.05	86 – 124	98	15	15	8
		0.5	89 – 103	94	5.0	5.3	7
		Overall	86 – 124	96	11	12	15
	N-acetyl-glyphosate	0.05	84 – 108	91	7.8	8.6	8
		0.5	80 – 100	93	8.8	9.5	7
		Overall	80 – 108	92	8.0	8.8	15

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	AMPA	0.05	72 – 105	90	9.1	10	8
		0.5	72 – 99	85	11	13	7
		Overall	72 – 105	87	10	11	15
	N-acetyl AMPA	0.05	77 – 96	86	7.3	8.5	6
		0.5	74 – 96	85	6.2	7.3	10
		Overall	74 – 96	85	6.4	7.5	16
Soybean seed (DuPont-15444)	Glyphosate	0.05	78 – 91	85	5.8	6.8	5
		0.5	72 – 85	78	5.5	7.1	5
		Overall	72 – 91	82	6.5	8.0	10
	N-acetyl-glyphosate	0.05	94 – 100	97	2.2	2.3	5
		0.5	92 – 99	95	3.0	3.1	5
		Overall	92 – 100	96	2.8	2.9	10
	AMPA	0.05	77 – 108	94	12	13	5
		0.5	73 – 85	78	5.4	6.8	5
		Overall	73 – 108	86	12	14	10
	N-acetyl AMPA	0.05	66 – 113	90	12	13	14
		0.5	70 – 95	79	9.3	12	8
		Overall	66 – 113	86	12	14	22
Soybean hay (DuPont-15444)	Glyphosate[4]	0.05	83 – 107	94	9.2	9.8	5
		0.5	76 – 86	80	4.1	5.2	5
		Overall	76 – 107	87	9.9	11	10
	N-acetyl-glyphosate[4]	0.05	87 – 105	94	7.8	8.3	5
		0.5	84 – 88	86	1.6	1.9	5
		Overall	84 – 105	90	7.0	7.8	10
	AMPA[4]	0.05	87 – 105	99	8.0	8.0	5
		0.5	84 – 88	79	4.4	5.6	5
		Overall	84 – 105	89	12	13	10
	N-acetyl AMPA	0.05	61 – 92	74	7.4	10	23
		0.5	71 – 83	78	5.0	6.4	6
		Overall	61 – 92	75	7.0	9.4	29
Soybean oil	Glyphosate	0.05	91 – 105	99	5.4	5.5	5

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
(DuPont-15444)		0.5	83 – 101	93	8.6	9.2	5
		Overall	83 – 105	96	7.4	7.8	10
	N-acetyl-glyphosate	0.05	92 – 97	94	1.9	2.0	5
		0.5	96 – 101	98	1.9	1.9	5
		Overall	92 – 101	96	2.7	2.9	10
	AMPA	0.05	98 – 118	107	9.6	9.0	5
		0.5	94 – 96	95	0.8	0.9	5
		Overall	94 – 118	101	8.8	8.7	10
	N-acetyl AMPA	0.05	96 – 108	100	5.7	5.7	4
		0.5	99 – 105	102	2.9	2.9	4
		Overall	96 – 108	101	4.4	4.4	8
Soybean meal (DuPont-15444)	Glyphosate	0.05	87 – 102	93	6.0	6.5	5
		0.5	77 – 81	79	1.5	1.9	5
		Overall	77 – 102	86	8.7	10.1	10
	N-acetyl-glyphosate	0.05	75 – 97	89	8.2	9.3	5
		0.5	88 – 100	93	5.0	5.3	5
		Overall	75 – 100	91	6.8	7.5	10
	AMPA	0.05	76 – 90	84	5.3	6.3	5
		0.5	72 – 76	74	1.6	2.1	5
		Overall	72 – 90	79	6.3	8.0	10
	N-acetyl AMPA	Not determined					
Soybean hulls (DuPont-15444)	Glyphosate	0.05	72 – 93	84	9.7	12	5
		0.5	71 – 78	75	2.8	3.7	5
		Overall	71 – 93	79	8.2	10	10
	N-acetyl-glyphosate	0.05	93 – 104	99	5.1	5.1	5
		0.5	95 – 102	100	2.9	2.9	5
		Overall	93 – 104	99	3.9	3.9	10
	AMPA	0.05	84 – 96	88	5.4	6.1	5
		0.5	72 – 84	80	4.8	5.9	5
		Overall	72 – 96	84	6.2	7.4	10

Table 5.1-5: Recovery results for glyphosate, *N*-acetyl-glyphosate, AMPA, *N*-acetyl AMPA residues in commodities of plant origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	N-acetyl AMPA	Not determined					

[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 recovery values as given in the report.

[2]

[3] AMPA results are average of 2 injections of the same extracts (25 µL and 50 µL injection volumes)

[4] Average of 2 analyses of same extract (repurified)

N/A Not applicable

Specificity

The method allows the determination of glyphosate and the metabolites *N*-acetyl-glyphosate, AMPA and N-acetyl-AMPA using HPLC-MS/MS and is highly specific for the analytes detected. No interference was observed at the retention times of interest. Therefore, no confirmatory technique is required.

Linearity

Calibration standards were prepared in glyphosate free-acid equivalent concentrations from dilutions of fortification standards or individual stock standards. The use of the glyphosate and AMPA stable isotopes as internal standards in calibration standards and extract solutions is recommended to normalise recoveries for matrix effects and SPE purification performance for sample analysis. Generally, five calibration solutions were analysed for quantitative LC-MS/MS analysis. Calibration standards typically yielded a linear response (r^2 >0.99) for calibration standard response factors (peak area/concentration) over the range of 0.5 to 20 ng/mL for glyphosate and *N*-acetyl-glyphosate or 0.5 to 100 ng/mL for AMPA corresponding to 50% of LOQ and 120% of the final extract. Representative calibration curves for each analyte were conducted using calibration standards from validation sets including expanded range of 0.5 to 100 ng/mL.

Repeatability (Precision)

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

Accuracy

Mean recoveries obtained at each level of fortification and overall for each matrix were in the range 70 – 110 % in the method validation for glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl AMPA. The accuracy of the method is within the limits specified by current EU guidance.

Limit of Quantification and Detection

The limit of quantification (LOQ), defined as the lowest fortification level at which average recoveries of 70 - 120 % and a RSD < 20 % are achieved, was 0.05 mg/kg for glyphosate, *N*-acetyl-glyphosate, and AMPA in all matrices. Acceptable recoveries were achieved at the lowest fortification level. The limit of detection (LOD) is defined by applicant as the analyte concentration in matrix with a response equivalent to a signal-to-noise ratio of approximately 3 to 1. The LOD estimated in this method was 0.004 mg/kg for glyphosate, 0.006 mg/kg for *N*-acetyl-glyphosate, 0.007 mg/kg for AMPA, and 0.006 mg/kg for *N*-acetyl AMPA. Variation in the LOD was observed and each laboratory using this method should estimate an LOD value.

Interference

The chromatograms of a control sample did not reveal any significant interferences (>30% LOQ), which would interfere with the determination of the analytes.

Matrix effects
Matrix effects on detection were generally corrected by the use of response ratio of analyte to radiolabelled internal standard compensating for any difference in response between sample and standard.

Stability of analytes in sample extracts
Not assessed.

Extraction efficiency
The extraction procedure developed in a DuPont gat pilot corn 14C metabolism study (Dupont-12832, [redacted] 2004) for the extraction of glyphosate and degradates residues was applied to this residue analytical method.

Conclusion
The analytical method does mainly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate and degradate residues in crop matrices.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and mainly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the feeding study concerned.

Assessment and conclusion by RMS:
The linearity, specificity, recovery and repeatability are in agreement with the SANCO/3029/99 rev.4 . The extraction solvent used is the same as the one used in the metabolism studies.

This method was used within several residue studies : CA 6.1/004, CA 6.1/005 and CA 6.1/006. The test facility of these studies is not the same as the one used to validate the analytical method. Moreover the N-aceyl-AMPA was not validated for several matrices. Therefore the validation reported in the analytical report Dupont-15444 revision n°1 (CA 4.1.2/118) cannot be considered enough for the residue studies.

In the residue study reports it is indicated that the validation data are available for glyphosate, N-acetylglyphosate, and AMPA in corn forage and grain in the study DUPONT-16701 (ABC number 49678) and for N-acetyl AMPA in corn green plant, forage and grain in the study report DuPont-20122 (ABC Number 50165). These study reports were not submitted.

However, validation data of analytical method are available in the stability residue studies. These data are presented below by RMS.

Data point	CA 6.1/004, 6.1/005, 61/006
	CA 6.1/004
Report author	[redacted]
Report year	2009
Report title	STABILITY OF GLYPHOSATE AND METABOLITES IN CORN GREEN PLANT, FORAGE, GRAIN, AND STOVER CONTAINING THE GAT AND ZM-HRA GENES DURING FROZEN STORAGE
Report No	DuPont-20094
Document No	/
Guidelines followed in study	EPA OPPTS 860.1340 SANCO/3029/99
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	Fit for purpose
Test facility	ABC Laboratories, Inc. 7200 East ABC Lane Columbia, MO 65202

Report author	[redacted]
Report year	2009
Report title	STABILITY OF GLYPHOSATE, N-ACETYLGLYPHOSATE, AMINOMETHYL PHOSPHONIC ACID AND N-ACETYL AMPA IN GAT SOYBEAN FORAGE, SEED, AND HAY STORED FROZEN
Report No	DuPont-17573
Document No	/
Guidelines followed in study	EPA OPPTS 860.1340 SANCO/3029/99
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Fit for purpose
Test facility	ABC Laboratories, Inc. 7200 East ABC Lane Columbia, MO 65202

Report author	[redacted]
Report year	2007
Report title	STABILITY OF GLYPHOSATE, N-ACETYLGLYPHOSATE AND AMINOMETHYL PHOSPHONIC ACID IN GAT CORN FORAGE, GRAIN, AND STOVER, STORED FROZEN
Report No	DuPont-17379
Document No	/
Guidelines followed in study	EPA OPPTS 860.1340 SANCO/3029/99
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Fit for purpose
Test facility	ABC Laboratories, Inc. 7200 East ABC Lane Columbia, MO 65202

Principle of method
Stability samples were analyzed for glyphosate, N-acetylglyphosate, AMPA, and N-acetyl AMPA using procedures described in the analytical method based on DuPont Report No. DuPont-15444, "Analytical Method for the Determination of Glyphosate and Relevant Metabolite Residues in Various Crop Matrices Using LC/MS/MS" with modifications. The modifications have been done on the spectrometry conditions. These conditions had been optimized in order to monitor only the mass transition showing the least amount of noise and interference.

Recovery for soybean stover residues (high water matrix)

Compound	Fortification level mg/kg	Mean Recoveries % N=4	RSD %
Glyphosate	0.05	106	9.8
N-acetylglyphosate	0.05	99	7.4
AMPA	0.05	90	10.5

N-acetyl AMPA	0.05	89	6.6

Recovery for soybean forage residues (high ater matrix)

Compound	Fortification level mg/kg	Recoveries %	RSD %
Glyphosate	0.05	100; 90	/
	0.5	92; 92	/
N-acetylglyphosate	0.05	101; 101	/
	0.5	83; 85	/
AMPA	0.05	103; 110	/
	0.5	92; 83	/
N-acetyl AMPA	0.05	92; 96	/
	0.5	96; 82	/

Recovery for soybean seed residues (high oil matrix)

Compound	Fortification level mg/kg	Recoveries %	RSD %
Glyphosate	0.05	77; 63	/
	0.5	76; 80	/
N-acetylglyphosate	0.05	77; 87	/
	0.5	77; 74	/
AMPA	0.05	121; 107	/
	0.5	97; 73	/
N-acetyl AMPA	0.05	96; 100	/
	0.5	95; 91	/

Recovery for soybean hay residues (dry matrix)

Compound	Fortification level mg/kg	Recoveries %	RSD %
Glyphosate	0.05	109; 99	/
	0.5	90; 92	/
N-acetylglyphosate	0.05	92; 95	/
	0.5	84; 81	/
AMPA	0.05	73; 74	/
	0.5	63; 84	/
	0.05*	97; 79	
	0.5*	76; 88	
N-acetyl AMPA	0.05	86; 110	/
	0.5	77; 75	/

* This data obtained using YMC ODS-AQ column.

Recovery for corn forage residues (high water matrix)

Compound	Fortification level mg/kg	Recoveries %	RSD %
Glyphosate	0.05	101; 93	/
	0.5	105; 102	/
N-acetylglyphosate	0.05	97; 103	/
	0.5	122; 99	/
AMPA	0.05	86; 88	/
	0.5	94; 87	/

Recovery for corn grain residues (dry matrix)

Compound	Fortification level mg/kg	Recoveries %	RSD %
Glyphosate	0.05	116; 93	/
	0.5	100; 101	/
N-acetylglyphosate	0.05	91; 83	/
	0.5	108; 107	/
AMPA	0.05	101; 93	/
	0.5	105; 102	/

Recovery for corn stover residues (high water matrix)

Compound	Fortification level mg/kg	Recoveries %	RSD %
Glyphosate	0.05	85; 80	/
	0.5	111; 112	/
N-acetylglyphosate	0.05	105; 104	/
	0.5	103; 103	/
AMPA	0.05	85; 80	/
	0.5	111; 112	/

Linearity
Calibration standards yielded a linear response (r^2 >0.99) for calibration standard response factors (peak area/concentration) over the range of 2 to 50 ng/mL for glyphosate and N-acetyl-glyphosate or 2 to 50 ng/mL for AMPA and N-acetyl AMPA.

Interference
The chromatograms of a control sample did not reveal any significant interferences (>30% LOQ), which would interfere with the determination of the analytes.

Assessment and conclusion by RMS:
The validation data show several deficiencies. The equivalence of linearity range in mg/kg is missing that does not allow to verify that fortification levels are in the linearity range.
The matrix effect was not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed.

In conclusion, the validation data do not fulfil the requirement of the guidance document SANCO 302999 rev.4/. However, as the objective of the residue stability study is to demonstrate that the concentration targeted is not modified during storage, we can consider the validation data provided for the method as sufficient to validate the content of glyphosate, N-acetylglyphosate, AMPA or n-acetyl AMPA at 0.05 mg/kg in matrices tested in these residue studies.

Determination of glyphosate and AMPA in crop matrices with 9-fluorenylmethyl chloroformate (FMOC) derivatisation and LC-MS/MS

Data point	CA 4.1.2/119
Report authors	[redacted]
Report year	2014
Report title	Glyphosate – Validation of Analytical Method GRM067.01A for the Determination of Residues of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Crop Matrices
Report No	S13-04580
Document No	Not applicable
Guidelines followed in study	ENV/JM/MONO(2007)17 EPA OCSPP 860.1340 (1996) Council Directive 1107/2009

	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 OECD GLP
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	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Eurofins Agroscience Services Chem Ltd Slade Lane Wilson Melbourne Derbyshire, DE73 8AG, UK

Data point	CA 4.1.2/128 (CA 6.3.1/007)
Report author	[redacted]
Report year	2014
Report title	Glyphosate - Residue study on cherry in Spain and Italy in 2013
Report No	S13-03427
Document No	A12798QA_10349
Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4
Deviations from current test guideline	None (SANCO/3029/99 rev. 4)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK

Data point	CA 4.1.2/129 (CA 6.3.1/008)
Report author	[redacted]
Report year	2014
Report title	Glyphosate - Residue study on plum in Italy in 2013
Report No	S13-03233
Document No	A12798QA_10347
Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4
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	NO

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test Facility	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK

A validation of the analytical method GRM067.01A for the determination of residues of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in apple (high water content), sunflower seed (high oil content), dried beans (high protein content), cereal grain (high starch content), whole orange (high acid content) and cereal straw (dry commodity) was performed in study S13-04580.
For the use in cherry (fruit) and plum (fruit) the method validation was carried out within the studies S13-03427 and S13-03233, respectively.

An overview of the residue studies, for which method GRM067.01A was used, is given in the table below.

Table 5.1-6: Overview on residue studies

Data point	Report authors	Report year	Report number	Test facility	Report title
CA 6.3.1/001	[redacted]	2014	S13-02531	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK	Glyphosate - Residue study on mandarin oranges in Spain in 2013
CA 6.3.1/003	[redacted]	2014	S13-03425	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK	Glyphosate - Residue study on apple in the United Kingdom and Germany in 2013
CA 6.3.1/004	[redacted]	2014	S13-03426	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK	Glyphosate - Residue study on apple in Spain and Italy in 2013
CA 6.3.1/007	[redacted]	2014	S13-03427	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK	Glyphosate - Residue study on cherry in Spain and Italy in 2013
CA 6.3.1/008	[redacted]	2014	S13-03233	Eurofins Agroscience Services Ltd Slade Lane Wilson Melbourne Derbyshire DE73 8AG UK	Glyphosate - Residue study on plum in Italy in 2013

Principle of the method

Glyphosate and AMPA were isolated from crop matrices by maceration using deionised water (80 mL) and dichloromethane (30 mL). Following centrifugation, derivatisation of an aliquot of the aqueous phase extraction was performed with 9-fluorenylmethyl chloroformate (FMOC) by mixing an aliquot (1mL) of the aqueous layer along with borate buffer (1mL) and FMOC-Cl derivatisation agent (1mL, 20mg/mL) followed by incubation at room temperature for at least 30 minutes. Samples were purified by partition with dichloromethane.
Glyphosate-FMOC and AMPA-FMOC were determined by liquid chromatography with mass spectrometer (LC-MS/MS) in positive multiple reaction monitoring (MRM) mode, monitoring one primary transition ion and two confirmatory transition ions (glyphosate-FMOC: quantifier: 392→170, qualifier: 392→88 and 392→179; AMPA-FMOC: quantifier: 334→156, qualifier: 334→179 and 334→112). The analytes were quantified using an external standardisation procedure and single point calibration. The limit of quantification (LOQ) is 0.05 mg/kg for both analytes for all crops.

Instrumentation and Chromatographic Conditions:
The final extracts were analysed for glyphosate-FMOC and AMPA-FMOC using a HPLC coupled to mass spectrometer with electrospray nebuliser. HPLC and mass spectral operating conditions are summarized in the following table.

HPLC-MS/MS:	Agilent Series 1200 HPLC or 1100 HPLC AB-Sciex API 5500 QTrap mass spectrometer or AB-Sciex API 4000 mass spectrometer					
Column:	Ascentis Express C18 (50 x 2.1 mm, 2.7 µm)					
Column oven temperature:	Ambient					
Injection volume:	20 µL					
Mobile phase:	Solvent A: 10 mM ammonium acetate in water Solvent B: Methanol					
Flow rate:	0.4 mL/min					
Retention time:	Glyphosate-FMOC: ~ 3.0 min (API 5500), ~ 3.4 min (API 4000) AMPA-FMOC: ~ 5.5 min (API 5500), ~ 4.9 min (API 4000)					
Scan type:	Positive					
Ion source:	ESI					
Ion Spray Voltage (IS):	5500 V		Ion Spray turbo heater (TEM):	450 °C		
Curtain gas (CUR):	40 (arbitrary units) (API 5500) 35(arbitrary units) (API 4000)		Gas flow 1 (GS1):	45 (arbitrary units)		
Collision Gas (CAD):	4 (arbitrary units)		Gas flow 2 (GS2):	50 (arbitrary units)		
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Entrance potential (EP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions						
Glyphosate-FMOC	392	170	80	8	19	14
AMPA-FMOC	334	156	75	7	13	13
Confirmatory ions						
Glyphosate-FMOC	392	88	80	8	32	7
AMPA-FMOC	334	179	75	7	26	14
Confirmatory ions						

Glyphosate-FMOC	392	179	80	8	32	13
AMPA-FMOC	334	112	75	7	20	8

The conversion factor for glyphosate to glyphosate-FMOC was 0.43 (mol.wt. glyphosate, 169.07 / mol. wt. glyphosate-FMOC, 391.31) and for AMPA to AMPA-FMOC 0.33 (mol.wt. AMPA, 111.04 / mol.wt. AMPA-FMOC, 333.28).

Findings

Recoveries

Recoveries were obtained for the analysis of residues of glyphosate and AMPA in apple, sunflower seed, dried beans, cereal grain, whole orange and cereal straw. The validation included analysis of five replicates fortified at the LOQ and at least 10 x LOQ for each matrix.

The recoveries for glyphosate and AMPA are summarized in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-7: Recovery results of glyphosate and AMPA for commodities of plant origin

Crop (study)	Commo-dity	Analyte	Target ion	Forti-fication level (mg/kg)	Recovery[1]				
					Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
Apple (S13-04580)	Fruit	Glypho-sate	392→170	0.05	87 – 90	89	1.1	1.3	5
				0.5	97 – 99	98	1.0	1.0	5
				Overall	87 – 99	93	5.1	5.4	10
			392→88	0.05	87 – 99	94	4.4	4.8	5
				0.5	97 – 105	100	3.5	3.5	5
				Overall	87 – 105	97	5.1	5.2	10
			392→179	0.05	86 – 94	89	3.0	3.4	5
				0.5	100 – 103	101	1.2	1.2	5
				Overall	86 – 103	95	6.6	6.9	10
		AMPA	334→156	0.05	88 – 99	93	4.3	4.6	5
				0.5	81 – 96	90	5.6	6.2	5
				Overall	81 – 99	92	4.9	5.3	10
			334→179	0.05	83 – 91	87	3.5	4.0	5
				0.5	81 – 92	89	4.4	5.0	5
				Overall	81 – 92	88	3.9	4.4	10
			334→112	0.05	82 – 100	93	6.5	7.1	5
				0.5	82 – 96	90	5.2	5.8	5
				Overall	82 – 100	91	5.7	6.3	10
Sunflower (S13-04580)	Seed	Glypho-sate	392→170	0.05	95 – 115	104	8.5	8.2	5
				20	95 – 108	103	5.2	5.1	5
				Overall	95 – 115	103	6.7	6.5	10
			392→88	0.05	99 – 111	105	4.8	4.6	5

Table 5.1-7: Recovery results of glyphosate and AMPA for commodities of plant origin

Crop (study)	Commo-dity	Analyte	Target ion	Forti-fication level (mg/kg)	Recovery[1]				
					Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
				20	102 – 107	104	2.6	2.5	5
				Overall	99 – 111	105	3.7	3.5	10
			392→179	0.05	97 – 111	105	5.4	5.1	5
				20	104 – 112	108	3.4	3.1	5
				Overall	97 – 112	107	4.6	4.3	10
		AMPA	334→156	0.05	76 – 83	80	2.8	3.5	5
				1.0	96 – 101	98	2.3	2.4	5
				Overall	76 – 101	89	9.9	11.1	10
			334→179	0.05	66 – 80	74	5.4	7.3	5
				1.0	89 – 99	94	4.0	4.3	5
				Overall	66 – 99	84	11.3	13.4	10
			334→112	0.05	71 – 93	82	8.4	10.2	5
				1.0	95 – 103	100	3.6	3.6	5
				Overall	71 – 103	91	10.9	12.0	10
Beans (S13-04580)	Dry beans	Glypho-sate	392→170	0.05	103 – 113	109	3.9	3.6	5
				10	94 – 101	98	3.1	3.2	5
				Overall	94 – 113	104	6.9	6.6	10
			392→88	0.05	97 – 115	107	6.5	6.1	5
				10	91 – 101	97	3.7	3.8	5
				Overall	91 – 115	102	7.3	7.1	10
			392→179	0.05	102 – 111	107	3.4	3.2	5
				10	93 – 100	98	2.9	3.0	5
				Overall	93 – 111	102	5.7	5.6	10
		AMPA	334→156	0.05	78 – 86	82	3.1	3.8	5
				0.5	91 – 94	93	1.1	1.2	5
				Overall	78 – 94	87	6.0	6.9	10
			334→179	0.05	88 – 92	90	1.8	2.0	5
				0.5	89 – 94	93	2.2	2.3	5
				Overall	88 – 94	91	2.5	2.8	10
			334→112	0.05	75 – 85	81	4.3	5.3	5
				0.5	90 – 99	93	3.7	4.0	5

Table 5.1-7: Recovery results of glyphosate and AMPA for commodities of plant origin

Crop (study)	Commo-dity	Analyte	Target ion	Forti-fication level (mg/kg)	Recovery[1]				
					Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
				Overall	75 – 99	87	7.5	8.6	10
Cereal (S13-04580)	Grain	Glypho-sate	392→170	0.05	90 – 97	93	3.1	3.4	5
				20	80 – 92	87	4.3	5.0	5
				Overall	80 – 97	90	4.8	5.4	10
			392→88	0.05	89 – 96	92	3.1	3.4	5
				020	79 – 92	87	5.1	5.9	5
				Overall	79 – 96	90	4.7	5.2	10
			392→179	0.05	89 – 98	93	3.5	3.8	5
				020	78 – 92	86	5.1	5.9	5
				Overall	78 – 98	90	5.5	6.2	10
		AMPA	334→156	0.05	80 – 85	82	1.9	2.3	5
				1.0	93 – 102	98	3.8	3.9	5
				Overall	80 – 102	90	8.7	9.7	10
			334→179	0.05	74 – 80	77	2.4	3.2	5
				1.0	94 – 99	97	2.0	2.1	5
				Overall	74 – 99	87	10.7	12.4	10
			334→112	0.05	81 – 98	87	6.6	7.5	5
				1.0	90 – 98	93	3.0	3.2	5
				Overall	81 – 98	90	5.8	6.4	10
Orange (S13-04580)	Whole fruit	Glypho-sate	392→170	0.05	81 – 88	84	2.9	3.5	5
				0.5	62 – 83	78	9.0	11.5	5
				Overall	62 – 88	81	7.0	8.7	10
			392→88	0.05	73 – 88	80	5.4	6.7	5
				0.5	58 – 82	76	10.1	13.3	5
				Overall	58 – 88	78	8.0	10.2	10
			392→179	0.05	77 – 92	83	5.6	6.7	5
				0.5	56 – 81	75	10.9	14.4	5
				Overall	56 – 92	79	9.2	11.6	10
		AMPA	334→156	0.05	70 – 79	74	3.8	5.1	5
				0.5	74 – 77	76	1.2	1.6	5
				Overall	70 – 79	75	2.9	3.8	10

Table 5.1-7: Recovery results of glyphosate and AMPA for commodities of plant origin

Crop (study)	Commo-dity	Analyte	Target ion	Forti-fication level (mg/kg)	Recovery[1]				
					Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
			334→179	0.05	71 – 81	75	3.9	5.2	5
				0.5	75 – 77	76	1.1	1.4	5
				Overall	71 – 81	76	2.8	3.6	10
			334→112	0.05	72 – 84	78	5.1	6.6	5
				0.5	71 – 79	75	2.9	3.9	5
				Overall	71 – 84	76	4.2	5.6	10
Cereal (S13-04580)	Straw	Glypho-sate	392→170	0.05	93 – 106	101	4.9	4.9	5
				40	79 – 99	88	7.5	8.5	5
				Overall	79 – 106	95	9.0	9.5	10
			392→88	0.05	97 – 109	102	5.2	5.1	5
				40	86 – 96	91	4.0	4.3	5
				Overall	86 – 109	97	7.1	7.3	10
			392→179	0.05	104 – 112	107	3.1	2.9	5
				40	81 – 99	88	6.7	7.6	5
				Overall	81 – 112	98	10.9	11.1	10
		AMPA	334→156	0.05	81 – 91	86	3.6	4.2	5
				2.0	98 – 107	101	3.6	3.6	5
				Overall	81 – 107	94	8.8	9.4	10
			334→179	0.05	64 – 81	75	6.5	8.6	5
				2.0	99 – 107	103	3.0	3.0	5
				Overall	64 – 107	89	15.3	17.2	10
			334→112	0.05	71 – 101	82	12.7	15.5	5
				2.0	99 – 104	102	1.9	1.9	5
				Overall	71 – 104	92	13.3	14.5	10
Cherry, sweet (study S13-03427)	Fruit	Glypho-sate	392→170	0.05	96 – 102	98	2.3	2.3	5
				0.5	99 – 105	103	2.5	2.4	5
				Overall	96 – 105	101	3.2	3.2	10
			392→88	0.05	96 – 104	100	3.4	3.3	5
				0.5	99 – 102	100	1.5	1.5	5
				Overall	96 – 104	100	2.5	2.4	10
			392→179	0.05	96 – 100	98	1.5	1.5	5

Table 5.1-7: Recovery results of glyphosate and AMPA for commodities of plant origin

Crop (study)	Commo-dity	Analyte	Target ion	Forti-fication level (mg/kg)	Recovery[1]				
					Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
				0.5	96 – 102	100	2.3	2.3	5
				Overall	96 – 102	99	2.0	2.0	10
		AMPA	334→156	0.05	81 – 87	84	2.6	3.1	5
				0.5	82 – 87	84	2.3	2.7	5
				Overall	81 – 87	84	2.3	2.8	10
			334→179	0.05	78 – 84	81	2.6	3.2	5
				0.5	80 – 86	83	2.4	2.9	5
				Overall	78 – 86	82	2.8	3.4	10
			334→112	0.05	80 – 86	82	2.7	3.3	5
				0.5	81 – 87	84	2.4	2.9	5
				Overall	80 – 87	83	2.5	3.0	10
Plum (study S13-03233)	Fruit	Glypho-sate	392→170	0.05	102 – 109	104	2.8	2.7	5
				0.5	97 – 103	100	2.2	2.2	5
				Overall	97 – 109	102	3.2	3.1	10
			392→88	0.05	98 – 112	104	5.3	5.1	5
				0.5	99 – 104	100	2.1	2.1	5
				Overall	98 – 112	102	4.3	4.2	10
			392→179	0.05	96 – 106	100	4.0	4.0	5
				0.5	100 – 103	101	1.2	1.2	5
				Overall	96 – 106	101	2.8	2.8	10
		AMPA	334→156	0.05	93 – 99	95	2.5	2.6	5
				0.5	92 – 96	93	1.7	1.8	5
				Overall	92 – 99	94	2.2	2.3	10
			334→179	0.05	92 – 102	96	3.9	4.1	5
				0.5	92 – 96	93	1.7	1.8	5
				Overall	92 – 102	95	3.1	3.3	10
			334→112	0.05	91 – 103	98	4.7	4.8	5
				0.5	93 – 96	94	1.1	1.2	5
				Overall	91 – 103	96	3.7	3.8	10

[1] Residues of glyphosate and AMPA in blank / control matrix were less than 30 % of the limit of quantitation. Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.

Specificity
LC-MS/MS with a quantifier ion and a qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity
Matrix matched standard solutions for apple, dried beans, cereal grain, and whole orange containing glyphosate-FMOC and AMPA-FMOC at concentrations ranging from 0.00075 µg/mL to 0.1 µg/mL (corresponding to 26% of LOQ for glyphosate and 20% of LOQ for AMPA - 34*LOQ for glyphosate and 27*LOQ for AMPA) were analysed by LC-MS/MS. For sunflower seed and cereal straw matrix matched standard solutions containing glyphosate-FMOC and AMPA-FMOC at concentrations ranging from 0.0004 µg/mL to 0.1 µg/mL (corresponding to 28% LOQ for glyphosate and 21% LOQ for AMPA - 71*LOQ for glyphosate and 53*LOQ for AMPA) were analysed. The detector response was plotted against standard concentration.
A linear detector response was observed for each analyte and matrix combination for all three transitions monitored. Correlation coefficients (R^2) of ≥0.9966 for glyphosate-FMOC and ≥0.9973 for AMPA-FMOC were obtained.

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

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for all the analysed matrices Therefor these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all matrices investigated. The limit of detection (LOD) for glyphosate and AMPA in all matrices was estimated for primary and confirmatory transitions and in all cases was equal or less than 30 % of the LOQ.

Interference
Residues of glyphosate and AMPA measured were lower than 30 % of the limit of quantification (LOQ) in all of the control and reagent blank samples used in this study for primary and confirmatory transitions.

Matrix effects
Insignificant matrix were observed for glyphosate-FMOC in apple, sunflower seed, cereal grain, whole orange, cherry and plum. However, significant (>20 %) matrix effects were observed for glyphosate-FMOC in dried beans and cereal straw, therefore matrix matched calibration standards should be used for these matrices.
Insignificant matrix effects were observed for AMPA-FMOC in apple, sunflower seed, dried beans, cereal grain, whole orange and plum matrices; however significant (>20 %) matrix effects were observed for AMPA-FMOC in cereal straw and cherry, therefore matrix matched calibration standards should be used for this matrix.
Matrix matched standards were used for all matrices in this study.

Stability of analytes in sample extracts
Extract stability was assessed for apple and sunflower seed extracts by measuring each five samples fortified at the LOQ after stored refrigerated for 8 days (sunflower seed) or 11 days (apple). Glyphosate and AMPA recovery levels were calculated using the primary transition only. The mean recoveries were within 70 – 110 % with relative standard deviations of ≤20 % for glyphosate and AMPA in the matrices assessed. It is therefore considered that sunflower seed extracts are stable for up to 8 days and apple extracts are stable for up to 11 days when stored refrigerated.

Conclusion
The analytical method is considered valid for the determination of residues of glyphosate and AMPA in crops at the LOQ of 0.05 mg/kg. The method has been validated according to the EU guidelines SANCO/3029/99 Rev.4 and SANCO/825/00 Rev. 8.1. The method validation also complies with US EPA guideline OPPTS 860.1340 and OECD guidance document ENV/JM/MONO (2007) 17.

Assessment and conclusion by applicant:

The validation of the method for analysis of glyphosate 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 fit for purpose to support the residue studies concerned.

Assessment and conclusion by RMS:
The specificity, linearity, recovery and repeatability are in agreement with the SANCO 3029/99 rev.4 for apple, sunflower seed, cereal grain, whole orange, cherry and plum.

However, the derivatisation efficiency was not demonstrated. This should be provided during the peer reviewed

The extraction solvent used is 80 mL 0.1% formic acid in water + 30 mL methylene chloride, consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It si not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Therefore, the analytical method cannot be considered as validated without the demonstration of the derivatisation efficiency.

Determination of glyphosate in crop matrices by derivatisation with heptafluorobutanol and trifluoroacetic anhydride followed by GC-MSD

Data point	CA 4.1.2/122 (CA 6.1/010)
Report authors	[redacted]
Report year	1996
Report title	Storage stability of residues of *N*-(phosphonomethyl) glycine and trimethylsulphonium cation in banana
Report No	RJ 2161B
Document No	Not applicable
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effects not assessed • Data on linearity is missing • Storage stability in extracts was not addressed
Previous evaluation	Not 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	ZENECA Agrochemicals, Dietary Exposure Section, Jealott's Hili Research Station, Bracknell, Berkshire RG42 6ET, UK.

Data point	CA 4.1.2/132 (CA 6.3.1/016)
Report author	[redacted]
Report year	1996
Report title	Glyphosate-trimesium: Residue levels in olives from trials carried out in Greece during 1995

Report No	RJ 2217B
Document No	Not applicable
Guidelines followed in study	EEC Registration Directive 91/414/EEC Annex III
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effects not assessed • Data on linearity is missing • Storage stability in extracts was not addressed
Previous evaluation	Not 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	ZENECA Agrochemicals, Dietary Exposure Section, Jealott's Hili Research Station, Bracknell, Berkshire RG42 6ET, UK.

Data point	CA 4.1.2/133 (CA 6.3.1/017)
Report author	[redacted]
Report year	1996
Report title	Glyphosate-trimesium: Residue levels in olives from trials carried out in Italy during 1995
Report No	RJ 2218B
Document No	Not applicable
Guidelines followed in study	EEC Registration Directive 91/414/EEC Annex III
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effects not assessed • Data on linearity is missing • Storage stability in extracts was not addressed
Previous evaluation	Not 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	ZENECA Agrochemicals, Dietary Exposure Section, Jealott's Hili Research Station, Bracknell, Berkshire RG42 6ET, UK.

The determination of glyphosate (present as anion after application of glyphosate-trimesium) was done by derivatisation with heptafluorobutanol and trifluoroacetic anhydride followed by gas chromatography. This principle was used in Residue Analytical Method 245/02 ([redacted] 1994) for the analysis of banana (peel plus flesh) and in the analytical method RR92-042B RES for olive samples.

An overview of the residue studies, which used this principle, is given in the table below.

Table 5.1-8: Overview on residue studies

Data point	Report authors	Report year	Report number	Report title
CA 6.1/010	[redacted]	1996	RJ 2161B	Storage stability of residues of *N*-(phosphonomethyl) glycine and trimethylsulphonium cation in banana
CA 6.3.1/016	[redacted]	1996	RJ2217B	Glyphosate-trimesium: Residue levels in olives from trials carried out in Greece during 1995
CA 6.3.1/017	[redacted]	1996	RJ2218B	Glyphosate-trimesium: Residue levels in olives from trials carried out in Italy during 1995

Principle of the method
Glyphosate was extracted from the samples by maceration with water. The extracts were then cleaned-up by partitioning with chloroform followed by cation exchange chromatography. An aliquot of the glyphosate-containing fraction was then derivatised with heptafluorobutanol and trifluoroacetic anhydride. The glyphosate derivative was analysed by gas chromatography with mass selective detection (GC-MSD). Residues were quantified by external standardization.

Findings
Recoveries (accuracy)
Samples of banana whole fruit (peel plus flesh) from storage day 0 , 6 and 12 months (n=2 by interval) were analysed for the concentration of the glyphosate using the analytical method. The recovery results are shown in the table below. Recovery values were in acceptable range of 70 – 110 %.

Table 5.1-9: Recovery results of glyphosate in freshly fortified samples at different storage intervals

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Banana whole fruit (peel plus flesh)	Glyphosate	0.5	71 – 91	80	8	10	6

[1] Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.

In the case of olive samples, recoveries at LOQ (0.05 mg/kg) and 10x LOQ (0.50 mg/kg) were analysed. The results are shown in the table below.

Table 5.1-10: Recovery results of glyphosate in freshly fortified samples

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Recovery (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Olives (RJ2217B)	Glyphosate	0.05	68, 79	73	10	2
		0.05, 0.10	75, 91	83	/	2
		0.10, 0.25	87, 80	84	/	2

Table 5.1-10: Recovery results of glyphosate in freshly fortified samples

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Recovery (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
		0.20, 0.40	71, 65	68	/	2
		Overall	-	77	12	8
Olives (RJ2218B)	Glyphosate	0.05	99, 79	89	/	2
		0.05	109, 101	105	/	2
		0.05, 0.50	93, 77	85	/	2
		0.05, 0.50	76, 77	77	/	2
		Overall	-	89	/	8

[1] Calculations of overall mean and RSDs were performed using excel with individual recovery values as given in the report.

Specificity
A confirmatory method is not considered necessary and is not specifically required for data generation methods.

Linearity
Not assessed

Repeatability (Precision)
The relative standard deviations (RSDs) of recoveries was below 20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was not reported, but according to the study limit of determination was 0.05 mg/kg. Limit of detection (LOD) was not reported.

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

Matrix effects
Not assessed.

Stability of glyphosate in sample extracts
Stability of the analytes in sample extracts was not assessed.

Conclusion
The analytical method was used for the determination of glyphosate in banana and olives. The method validation meets criteria set in SANCO/3029/99 rev. 4, in several relevant points and is considered as fit-for-purpose for the determination of glyphosate in different plant matrices.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate and AMPA was not previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) in several aspects (deficits: linearity information missing, matrix effects and stability of analyte in sample extract not assessed). Nevertheless, the method is considered as fit-for-purpose to support the storage stability study concerned as the presented analytical data show good performance of the method.

Assessment and conclusion by RMS:
The validation data available show several deficiencies. The linearity range is missing, the matrix effect and the deritivatisation efficiency were not demonstrated.

However, the recoveries for glyphosate in banana and olive are in the acceptable range and show that the recovery is acceptable in the range of concentration 0.05 to 0.5 mg/kg.. As the objective of the residue study is to validate the stability of the sample at targeted concentrations, we consider that data available for the method are sufficient to validate for the content of glyphosate at 0.05 mg/kg in olive and 0.5 mg/kg for banana.

Determination of glyphosate and AMPA in crop commodities by post-column derivatisation with OPA (Method DFG Method 405).

Information on the study

Data point:	CA 4.1.2/115
Report author	[redacted]
Report year	2007
Report title	Validation of the analytical method DFG Method 405 for the determination of Glyphosate and its metabolite AMPA in various plant materials
Test facility	Eurofins Dr. Specht GLP GmbH, Großmoorbogen 25 D-21079 Hamburg, Germany
Report No	FCS-0703V
Document No	Not applicable
Guidelines followed in study	EU 91/414/EEC amended by 96/46/EEC 4.2.1 SANCO/825/00 Rev. 7 BBA guideline: Residue Analytical Methods for Post-Registration Control Purposes OECD GLP
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Stability of analytes in sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Y and CA 6.1/003, 6.1/012 and 6.1/011 fit for purpose)
Category study in AIR 5 dossier (L docs)	Category 2a (with relevance for analytical methods)

Data point	CA 4.1.2/116
Report author	[redacted]
Report year	2008
Report title	1st Amendment to final report Validation of the analytical method DFG Method 405 for the determination of Glyphosate and its metabolite AMPA in various plant materials
Test facility	Eurofins Dr. Specht GLP GmbH, Großmoorbogen 25 D-21079 Hamburg, Germany
Report No	FCS-0703V
Document No	Not applicable
Guidelines followed in study	SANCO/825/00 Rev. 7 BBA guideline: Residue Analytical Methods for Post-Registration Control Purposes OECD GLP

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Stability of analytes in sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Y and CA 6.1/003, 6.1/012 and 6.1/011 fit for purpose)
Category study in AIR 5 dossier (L docs)	Category 2a (with relevance for analytical methods)

Data point	CA 4.1.2/117
Report author	[redacted]
Report year	1985
Report title	Validation of a new residue method for the analysis of glyphosate and aminomethylphosphonic acid (AMPA) – a round-robin study
Test facility	Analytical Biochemistry Corporation Labs (Columbia, MO.) and Craven Laboratories (Austin, TX)
Report No	MSL 4268
Document No	Not applicable
Guidelines followed in study	Not available
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Stability of analytes in sample extracts not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Y but CA 6.1/008 and 6.1/009 fit for purpose)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/131 (CA 6.3.1/015, CA 6.5.3/004)
Report author	[redacted]
Report year	1996
Report title	Residues of glyphosate and AMPA in olives and olive oil, following soil treatment with Roundup® herbicide. Spanish field trials, 1995
Test facility	Monsanto Europe SA, the agricultural Group, Parc Scientifique, Rue Laid Burniat, 1348 LOUVAIN-LA-NEUVE
Report No	MLL 30469
Document No	Not applicable
Guidelines followed in study	OECD GLP
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Stability of analytes in sample extracts not assessed • Linearity data is missing
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Y for grapes only
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/146 (CA 6.5.3/003)
Report author	[redacted]
Report year	1988
Report title	Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide
Test facility	Monsanto agricultural Company 800 N. Lindbergh Blvd, St Louis, Missouri 63167
Report No	MSL-7877
Document No	Not applicable
Guidelines followed in study	EPA Guideline 171-4: Magnitude of Residue-Crop Field Trials
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Not enough recoveries • Matrix effects not assessed • Stability of analytes in sample extracts not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes for potato whole tube only
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Principle of the method
An analytical method for the determination of glyphosate and aminomethylphosphonic acid (AMPA) using HPLC and post-column derivatisation with o-phthaldialdehyde (OPA) was developed in the 1980s and published as DFG method 405 by the Deutsche Forschungsgemeinschaft (DFG). This method was widely used and validated in different matrices of plant origin.

This method was used with minor modifications in the studies listed in the table below.

Table 5.1-11: Overview on residue studies

Data point	Report authors	Report year	Report number	Report title	Test facility
CA 6.1/003	[redacted]	2010	FCS-0707	Storage stability of residues of Glyphosate and AMPA in various plant materials	Eurofins Dr. Specht GLP GmbH Großmoorbogen 25 D-21079 Hamburg, Germany
CA 6.1/007	[redacted]	1997	IF-94/13882-00	Determination of the Storage Stability of Glyphosate in Beans, Oilseed Rape and Linseed	Institut Fresenius Chemische und Biologische Laboratorien GmbH Im Maisel 14 D-65232 Taunusstein Germany
CA 6.1/008	[redacted]	1993	91210	Determination of glyphosate in soybean raw agricultural commodities (RAC) stability report	Landis International, Inc. 3025 Madison Highway P.O. Box 5126 Valdosta, GA 31603-5126
CA 6.1/009	[redacted]	1993	91212	Determination of glyphosate in pasture grasses stability report	Landis International, Inc. 3025 Madison Highway P.O. Box 5126 Valdosta, GA 31603-5126

Table 5.1-11: Overview on residue studies

Data point	Report authors	Report year	Report number	Report title	Test facility
CA 6.1/011	[redacted]	1995	303614	Storage Stability of Glyphosate and AMPA in Wheat Grain and Straw and in Rye Grain and Straw	RCC UMWELTCHEMIE AG P.O. Box CH-4452 Itingen BL Switzerland
CA 6.1/012	[redacted]	1991	MSL10843	Storage stability of glyphosate residues in crop commodities	MONSANTO AGRICULTURAL COMPANY 700 Chesterfield Village Parkway St. Louis, Missouri 63198
CA 6.3.1/014	[redacted]	1989	MLL 30227	Glyphosate and AMPA residues in grapes following MON 8755 (Arcade) herbicide applications in vineyards. German field trials 1988	MONSANTO TECHNICAL CENTER Rue Laid Burniat, B-1348, Louvain-la-Neuve, Belgium
CA 6.3.1/015 CA 6.5.3/004	[redacted]	1996	MLL 30469	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with Roundup herbicide. Spanish field trials, 1995	MONSANTO TECHNICAL CENTER Rue Laid Burniat, B-1348, Louvain-la-Neuve, Belgium
CA 6.5.3/005	[redacted]	1993	MLL 30319	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with MON 65040 herbicide. Italian field trials, 1993	MONSANTO TECHNICAL CENTER Rue Laid Burniat, B-1348, Louvain-la-Neuve, Belgium
CA 6.5.3/006	[redacted]	1992	MLL 30297	Residues of glyphosate/AMPA in olives and olive oil following use of Sting SE - Spanish field trials 1990/1992.	MONSANTO TECHNICAL CENTER Rue Laid Burniat, B-1348, Louvain-la-Neuve, Belgium
CA 6.5.3/003	[redacted]	1988	MSL 7877	Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide	Monsanto agricultural Company 800 N. Lindbergh Blvd, St Louis, Missouri 63167

For the determination of glyphosate and the metabolite aminomethylphosphonic acid (AMPA) the samples were extracted with hydrochloric acid (130 or 150 mL 0.1 N HCl in water) and dichloromethane (40 mL or 50mL chloroform) for barley grain, maize green plant, sugar beet root and corn. Straw sample were extracted with water. After clean-up of the aqueous fraction by elution through Chelex 100 resin in the Fe(III) form glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron was removed using an anion exchange resin. After concentration to dryness to remove the hydrochloric acid and dissolving in water, glyphosate and AMPA were quantified by means of HPLC equipped with a post derivatisation unit and a fluorescence detector.
Determination involves post-column hypochlorite oxidation for glyphosate and reaction of the amine product with o-phthaldialdehyde (OPA) and mercaptoethanol to produce a fluorescent derivative.
Chromatographic conditions and equipment could vary slightly.

Following oxidative and derivatisation solutions were used:

Oxidative solution:	13.6 g KH_2PO_4, 11.6 g NaCl, 5.0 g NaOH dissolved in 0.9 L de-ionozed water, after addition of 1 mL $Na(ClO)_2$ solution was filled up to 1 L with de-ionozed water

Dervatisation solution:	25 g H_3BO_3 and 11 g NaOH dissolved in 0.9 L de-ionozed water, 1.6 g o-phthaldialdehyde were dissolved seperately in a solution of 4 mL mercapto-ethanol and 20 mL of methanol. Both solutions were combined and made up to 1 L with de-ionozed water

Validation data of studies that were performed in the same laboratory and using the analytical method DFG method 405 have been compiled by matrix.

Validation of method DFG Method 405 for glyphosate and AMPA in commodities of plant origin (CA 4.1.2/115 [redacted], 2007 and CA 4.1.2/116 [redacted] 2008)

Crop (study)	Commodity	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
Barley (FCS-0703V)	Grain	Glyphosate	0.05	80 – 91	85	4.2	4.9	5
			0.5	76 – 87	82	4.7	5.8	5
		AMPA	0.05	93 – 102	98	3.6	3.7	5
			0.5	91 – 102	98	4.6	4.7	5
	Straw	Glyphosate	0.05	72 – 103	84	14	16	5
			0.5	66 – 76	72	4.0	5.6	5
		AMPA	0.05	94 – 103	98	3.7	3.8	5
			0.5	86 – 103	98	7.0	7.2	5
Maize (FCS-0703V)	Green plant	Glyphosate	0.05	98 – 108	104	3.9	3.8	5
			0.5	103 – 106	105	1.3	1.2	5
		AMPA	0.05	67 – 73	71	2.4	3.4	5
			0.5	99 – 105	102	2.4	2.4	5
	Corn	Glyphosate	0.05	67 – 83	72	6.4	8.8	5
			0.5	73 – 85	79	4.4	5.5	5
		AMPA	0.05	87 – 96	91	4.0	4.4	5
			0.5	79 – 94	87	5.6	6.4	5
Sugar beet (FCS-0703V)	Root	Glyphosate	0.05	106 – 114	109	3.3	3.0	5
			0.5	87 – 105	99	7.4	7.5	5
		AMPA	0.05	95 – 104	100	3.6	3.6	5
			0.5	79 – 101	95	9.0	9.5	5

Validation of method DFG Method 405 for glyphosate and AMPA in commodities of plant origin (CA 4.1.2/115 [redacted] 2007 and CA 4.1.2/116 [redacted] 2008)

Crop (study)	Commodity	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
Oil seed rape (FCS-0703V)		Glyphosate	0.05	69 – 77	74	3.6	4.9	5
			0.5	70 – 80	75	3.7	5.0	5
		AMPA	0.05	67 – 85	75	8.0	11	5
			0.5	69 – 76	73	3.3	4.5	5
Citrus (FCS-0703V)	Fruit	Glyphosate	0.05	68 – 76	73	3.2	4.3	5
			0.5	64 – 83	77	7.4	9.7	5
		AMPA	0.05	74 – 81	78	3.3	4.3	5
			0.5	61 – 87	80	11	14	5

[1] Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.

Findings

Recoveries (accuracy)
Recoveries were obtained for the analysis of residues of glyphosate and AMPA in different matrices. The validation test included analysis of replicates fortified at the LOQ and higher fortification levels for each matrix. The recoveries for glyphosate and AMPA are summarized in the table above. All average recovery values were between 70 % and 110 %.

Specificity
Chromatograms from postcolumn derivatised standard solutions, samples and blank materials, as well as adequate recovery data and information on the precision of the method have been provided.
In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.

Linearity
In the study FCS-0703V standard solutions containing glyphosate and AMPA at concentrations from 0.016 to 3.27 µg/mL equivalent to 1.6 mg/kg – 0.327 mg/kg (n=10) were injected on the column and mean detector response plotted against standard concentration. Correlation coefficients (R^2) of 0.9996 for glyphosate and 1.0000 for AMPA were obtained.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20 %.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all crops investigated.

Matrix effects
Not assessed.

Stability of glyphosate and AMPA in sample extracts
Stability of the analytes in sample extracts was not assessed.

Conclusion
Method DFG 405 was successfully validated for the analysis of residues of glyphosate and AMPA in barley (grain and straw), maize (green plant and corn) sugar beet (roots), oil seed rape and citrus (fruit) at a LOQ of 0.05 mg/kg. The method validation meets criteria set in SANCO/3029/99 rev. 4, in most relevant points and is considered as fit-for-purpose for the determination of glyphosate and AMPA in different plant matrices.

Assessment and conclusion

Assessment and conclusion by applicant:
The method was previously evaluated at EU level. The presented studies were performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev. 4) in most aspects (deficits: efficiency of derivatisation and stability of the analytes in sample extract not assessed). Nevertheless, the method DFG 405 is a well-established and validated method and is therefore considered as fit-for-purpose to support the residue studies concerned.

Assessment and conclusion by RMS:
The method DFG 405 could be considered acceptable for the determination of glyphosate and AMPA in barley (grain and straw), maize (green plant and corn) sugar beet (roots), oil seed rape and citrus (fruit) at a LOQ of 0.05 mg/kg.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

The extraction solvent used for barley grain, maize green plant and corn (hydrochloric acid (130 mL 0.1 mol/L in water) and dichloromethane (40 mL)), consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

The others studies have not been performed in the same laboratory than the one of the initial method. Therefore, the data obtained in each laboratory have been reported by RMS in order to assess the method for each laboratory. (see summary below)

Residue study: Storage stability of residues of Glyphosate and AMPA in various plant materials Linseed (CA 6.1/003 FCS-0707, [redacted] 2010)

Findings:

At each time point after day 0 one control sarnple, three stored fortified samples for glyphosate and its metabolite AMPA were analysed together with one freshly fortified sample containing glyphosate and its metabolite AMPA. Fortification level: each 1.0 mg/kg.

The following recoveries were obtained for glyphosate in barley (grain and straw), maize (corn) and suger beet (root and leaves):

Storage Time Months	**Recovery in stored samples**			**Recovery in freshly fortified samples**
	Recoveries (%)	Mean (%)	Mean Corrected (%)*	Recovery (%)
Glyphosate - Barley (grain)				
0	77, 74, 71	74	100	-
6	80, 76,66	74	101	73
12	73,64, 75	71	101	70
18	69, 73,68	70	99	71
Glyphosate -Barley (straw)				

0	75, 72, 75	74	100	-
6	67,64,67	66	92	72
12	67, 70,68	68	87	78
18	77,63, 75	72	86	84
Glyphosate-Maize (corn)				
0	81, 77, 82	80	100	-
6	64,66,68	66	87	76
12	80, 79, 78	79	100	79
18	72, 74, 73	73	96	76
Glyphosate- Sugar Beet (root)				
0	82,85,86	84	100	-
6	91, 92, 84	89	95	94
12	79, 78,67	75	95	79
18	80, 86, 77	81	114	71
Glyphosate- Sugar beet (leaves)				
0	81, 91, 81	84	100	-
6	75, 70, 71	72	90	80
12	66,64, 70	67	96	70
18	64, 74,66	68	85	80

*) corrected for procedural recovery of freshly fortified sample in the same set

The following recoveries were obtained for AMPA in barley (grain and straw), maize (corn) and suger beet (root and leaves):

Storage Time Months	**Recovery in stored samples**			**Recovery in freshly fortified samples**
	Recoveries (%)	Mean (%)	Mean Corrected (%)*	Recovery (%)
AMPA - Barley (grain)				
0	95, 102,95	97	100	-
6	81,82,86	83	111	75
12	72,68, 77	72	88	82
18	74,66,64	68	96	71
AMPA-Barley (straw)				
0	79, 72, 75	75	100	-
6 **	51, 49, 59	53	75	71
12 **	33,40,36	36	42	85
18	77, 74,80	77	100	77
AMPA - Maize (corn)				
0	82,95, 104	94	100	-
6	83, 90, 73	82	106	77
12	72,84,80	79	93	85
18	90, 83,84	86	108	80
AMPA-Sugar Beet (root)				
0	94,87,90	90	100	-
6	88, 79,96	88	110	80
12	71, 67, 72	70	89	79
18	67,67,66	67	91	74
AMPA - Sugar beet (leaves)				
0	84, 89, 89	87	100	-
6	76,67,67	70	86	81
12 **	54,59,56	56	69	81
18	74,81,67	74	101	73

* Corrected for procedural recovery of freshly fortified sample in the same set.
** Low recoveries for the stored samples due to problems within the extraction of these samples.

Linearity:

The linearity of the detector response was confirmed by injecting seven standard solutions of 0.0160 µg/mL - 2.50 µg/mL for glyphosate and its metabolite AMPA covering the working range (r > 0.99).

Specificity:
Chromatograms of external standard, control specimen of barley (grain and straw), maize (corn), sugar beet (root and leaves) and freshly fortified specimen of barley (grain and straw), maize (corn), sugar beet (root and leaves), 18 months stored specimen of barley (grain and straw), maize (corn), sugar beet (root and leaves) for glyphosate and AMPA have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate and its metabolite AMPA in any of the control specimens.

Assessment and conclusion

Assessment and conclusion by RMS:
The validation data show several deficiencies. The linearity range is not available in mg/kg, the matric effect. However, the recoveries for glyphosate and AMPA are in the acceptable range (except for AMPA in barley straw where recoveries are below the acceptable limit). No repeatability has been performed.

However, as the objective of the residue study is to validate the stability of the sample at targeted concentrations, the data available for the method can be considered as acceptable to validate the content of glyphosate and AMPA at 1 mg/kg in tested matrices of the study.

Method validation: Validation of a new residue method for the analysis of glyphosate and aminomethylphosphonic acid (AMPA) – a round-robin study (CA 4.1.2/117 [redacted] 1985)
Test facility: Analytical Biochemistry Corporation Labs (Columbia, MO.) and Craven Laboratories (Austin, TX)

Findings

Validation of method DFG Method 405 for glyphosate and AMPA in commodities of plant origin ([redacted] 1985)

Crop (study)	Commodity	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
Alfalfa (MSL-4268)	Forage	Glyphosate	0.05	64 – 130	100	19	19	8
			0.10	76 – 121	92	16	17	8
			0.50	78 – 97	87	7.0	8.1	8
			1.0	80 – 100	88	7.4	8.4	8
			5.0	80 – 95	89	5.6	6.3	8
		AMPA	0.05	64 – 128	87	22	**25**	10
			0.10	57 – 105	87	17	20	10
			0.50	67 – 101	90	10	11	10
			1.0	75 – 96	89	6.5	7.2	10
			5.0	74 – 102	89	9.6	11	10
Cabbage, green (MSL-4268)	-	Glyphosate	0.05	74 – 106	86	10	12	10
			0.10	66 – 92	77	7.8	10	10
			0.50	67 – 84	76	4.7	6.2	10

Validation of method DFG Method 405 for glyphosate and AMPA in commodities of plant origin ([redacted] 1985)

Crop (study)	Commodity	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
			1.0	69 – 84	78	5.0	6.4	10
			5.0	48 – 80	72	8.7	12	10
		AMPA	0.05	62 – 96	77	9.3	12	10
			0.10	65 – 87	74	7.4	10	10
			0.50	63 – 83	76	6.1	8.0	10
			1.0	66 – 81	76	4.2	5.5	10
			5.0	51 – 76	71	7.5	11	10
Grapes, green (MSL-4268)	-	Glyphosate	0.05	62 – 104	83	14	17	10
			0.10	63 – 95	77	12	15	10
			0.50	71 – 97	80	7.4	9.4	10
			1.0	65 – 95	77	8.4	11	10
			5.0	60 – 106	76	13	17	10
		AMPA	0.05	62 – 102	74	13	17	10
			0.10	62 – 97	72	11	15	10
			0.50	69 – 90	77	6.5	8.5	10
			1.0	61 – 98	76	11	14	10
			5.0	57 – 108	77	14	18	10
Soybean (MSL-4268)	Grain	Glyphosate	0.05	46 – 140	85	27	**31**	10
			0.10	55 – 101	76	15	20	10
			0.50	73 – 114	87	14	16	10
			1.0	72 – 107	86	12	14	10
			5.0	76 – 108	90	8.7	9.7	10
		AMPA	0.05	80 – 144	102	21	20	10
			0.10	73 – 102	85	10	12	10
			0.50	69 – 96	84	7.4	8.8	10
			1.0	66 – 96	83	9.3	11	10
			5.0	77 – 105	89	9.5	11	10

[1] Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.

Recoveries (accuracy)

Recoveries were obtained for the analysis of residues of glyphosate and AMPA in different matrices. The validation test included analysis of replicates fortified at the LOQ and higher fortification levels for each matrix.

The recoveries for glyphosate and AMPA are summarized in the table above. All average recovery values were between 70 % and 110 %.

Specificity
Chromatograms of standard for glyphosate and AMPA, of control samples (alfalfa, cabbage, grapes and soybean grain) and fortified samples (samples (alfalfa, cabbage, grapes and soybean grain) have been provided.
In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.

Linearity
In the study, standard solutions containing glyphosate and AMPA at concentrations from 0.25 to 5 µg/mL (n=6) were injected on the column and mean detector response plotted against standard concentration. Correlation coefficients were not reported.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below or equal to 20 % (except for glyphosate in Soybean (grain) and for AMPA in alfalfa forage where RSD at 0.05 mg/kg is > 20%).

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all crops investigated except for AMPA if alfalfa forage and glyphosate in soybean grain (LOQ of 0.1 mg/kg)

Matrix effects
Not assessed.

Stability of glyphosate and AMPA in sample extracts
Stability of the analytes in sample extracts was not assessed.

Assessment and conclusion

Assessment and conclusion by RMS:
The validation data show several deficiencies. The linearity range is not available in mg/kg and the matrix effect was not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed and that the linearity range cover the fortification levels.

The accuracy of the method DFG 405 are acceptable for the determination of glyphosate in alfalfa forage cabbage green and grapes greenat a LOQ of 0.05 mg/kg and in soybean grain at a LOQ of 0.1 mg/kg. .
The accuracy of the method DFG 405 are acceptable for the determination of AMPA in cabbage green, grapes green a,n soybean grain at LOQ of 0.05 mg/kg and in alfalfa forageat the LOQ is 0.1 mg/kg.

The extraction solvent used (150 mL HCl 0.1 mol/L in water and chloroform (50 mL), consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Therefore, the analytical method can be considered as validated.

Residues of glyphosate and AMPA in olives, olive oil, and grapes

Residue studies:
Residues of glyphosate and AMPA in olives and olive oil, following soil treatment with Roundup® herbicide. Spanish field trials, 1995 (CA 6.3.1/015, CA 6.5.3/004 [redacted] 1996)
Glyphosate and AMPA residues in grapes following MON 8755 (Arcade) herbicide applications in vineyards. German field trials 1988 (CA 6.3.1/014 [redacted] 1989)
Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with MON 65040 herbicide. Italian field trials, 1993 **(**CA 6.5.3/005 [redacted] 1993)

Residues of glyphosate/AMPA in olives and olive oil following use of Sting SE - Spanish field trials 1990/1992 (CA 6.5.3/006 [redacted] 1992)
Test facility : Monsanto Europe SA, the agricultural Group, Parc Scientifique, Rue Laid Burniat, 1348 LOUVAIN-LA-NEUVE

Findings

Recovery/precision						
Matrix	*fortification level (mg/kg)*	*n*	*recovery range (%)*		*mean recovery (%)*	*RSD (%)*
glyphosate						
grapes (MLL 30227)	0.05	18	58	95	73,6	13,54
	0.1	5	68	80	74,2	6,90
	0.2	3	74	93	82,7	11,62
	0.5	2	73	89	81,0	13,97
olive fruit (MLL 30469, MLL 30319, MLL 30297)	0.05	12	63	110	79,3	**20,19**
	0.1	13	62	109	88,5	19,78
	0.2	1	71	71	71,0	-
	0.5	10	60	100	82,8	**20,11**
	1,00	11	61	108	93,2	15,39
olive oil (MLL 30469, MLL 30319, MLL 30297)	0.05	13	66	96	82,0	10,53
	0.1	13	66	99	82,8	15,31
AMPA						
grapes (MLL 30227)	0.05	4	52	70	**61,8**	14,75
	0.1	24	50	92	70,4	15,79
olive fruit (MLL 30469, MLL 30319, MLL 30297)	0.05	12	53	90	**67,8**	16,12
	0.1	12	57	96	72,3	14,02
	0.2	5	53	61	**57,2**	6,35
	0.5	5	68	80	76,4	6,45
	1,00	2	53	82	**67,5**	**30,38**
olive oil (MLL 30469, MLL 30319, MLL 30297)	0.05	13	57	101	76,9	16,78
	0.1	14	55	106	73,9	18,80

Recoveries (accuracy)
Recoveries were obtained for the analysis of residues of glyphosate and AMPA in different matrices. The validation test included analysis of replicates fortified at the LOQ and higher fortification levels for each matrix. The recoveries for glyphosate and AMPA are summarized in the table above. All average recovery values were between 70 % and 110 % (except for AMPA in grapes and olive fruit where the mean recovery is < 70).

Specificity
MLL 30469, MLL 30319 and MLL 30297: For both glyphosate and AMPA, chromatograms of a standard, of untreated samples and treated sample have been provided in olive fruit and olive oil.
In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.

MLL 30227: For both glyphosate and AMPA, chromatograms of a standard, of untreated samples and treated sample have been provided in grapes.
In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.

Linearity
MLL 30469: The glyphosate and AMPA standard solution linearity ranges from 0.05 to 5 µg/mL with a coefficient of correlation equal or greater than 0.995. The number of concentrations levels is missing.

MLL 30227, MLL 30319 and MLL 30297: The linearity is missing.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below or equal to 20 % (except for glyphosate in olive fruit where RSD at 0.05 mg/kg is > 20%).

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all crops investigated except for glyphosate in olive fruit, and AMPA is grapes and olive fruit (LOQ of 0.1 mg/kg).

Matrix effects
Not assessed.

Stability of glyphosate and AMPA in sample extracts
Stability of the analytes in sample extracts was not assessed.

Assessment and conclusion

Assessment and conclusion by RMS:
The validation data show several deficiencies. The linearity range is not not available in mg/kg and the matrix effect was not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed.

The accuracy of the method DFG 405 are acceptable for the determination of glyphosate and AMPA in grapes at LOQ of 0.05 mg/kg. The accuracy are not acceptable for the determination of glyphosate and AMPA for olive.

The extraction solvent used (150 mL HCl 0.1 mol/L in water and chloroform (50 mL), consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

The method can be considered as validated for the determination of glyphosate and AMPA in grapes but not in olive.

Residues of glyphosate and AMPA in potatoes and processed fractions

Residue study:
Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide (CA 4.1.2/146 [redacted] 1988)
Test facility : Monsanto agricultural Company 800 N. Lindbergh Blvd, St Louis, Missouri 63167

Findings

Crop (study)	Commodity	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
Potato (MSL 7877)	Whole tuber	Glyphosate	0.05	94 – 109	101	10.1	10.0	2
			0.1	99 – 101	100	1.6	1.6	2
			0.2	100	100	N/A	N/A	1
			0.5	97 – 104	100	3.8	3.8	3
			1.0	92 – 105	97	6.4	6.6	3
			2.0	103	103	N/A	N/A	1
		AMPA	0.05	82 – 110	96	20.2	**21.1**	2
			0.1	81 – 96	89	10.6	12.0	2
			0.2	80	80	N/A	N/A	1
			0.5	82 – 91	88	5.5	6.3	3
			1.0	91 – 98	95	3.5	3.7	3
			2.0	91	91	N/A	N/A	1
	Chips	Glyphosate	0.05	67	**67**	N/A	N/A	1
			0.2	100	100	N/A	N/A	1
			0.5	102	102	N/A	N/A	1
			2.0	96	96	N/A	N/A	1
		AMPA	0.05	135	135	N/A	N/A	1
			0.2	111	111	N/A	N/A	1
			0.5	102	102	N/A	N/A	1
			2.0	86	86	N/A	N/A	1
	Chips, stock feed	Glyphosate	0.05	96	96	N/A	N/A	1
			0.2	88	88	N/A	N/A	1
			0.5	92	92	N/A	N/A	1
			2.0	91	91	N/A	N/A	1
		AMPA	0.05	103	103	N/A	N/A	1
			0.2	85	85	N/A	N/A	1
			0.5	91	91	N/A	N/A	1
			2.0	85	85	N/A	N/A	1
	Flakes	Glyphosate	0.05	80 – 91	86	7.9	9.2	2
			0.1	78	78	N/A	N/A	1
			0.2	85	85	N/A	N/A	1

Findings

Crop (study)	Commodity	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
		AMPA	0.05	91 – 109	100	12.6	12.5	2
			0.1	74	74	N/A	N/A	1
			0.2	82	82	N/A	N/A	1
	Flakes, raw stock	Glyphosate	0.1	85 – 92	89	4.8	5.4	2
			1.0	92 – 95	93	2.5	2.6	2
		AMPA	0.1	94 – 92	88	5.3	6.0	2
			1.0	82 – 87	85	3.2	3.8	2
	Flakes, dry stock	Glyphosate	0.05	90 – 93	92	2.3	2.5	2
			0.1	64	64	N/A	N/A	1
			0.2	82 – 95	88	8.7	9.9	2
		AMPA	0.05	60 – 87	78	14.9	19.2	3
			0.1	91	91	N/A	N/A	1
			0.2	70 – 78	74	5.4	7.4	2
			0.5	69	69	N/A	N/A	1
	Granules	Glyphosate	0.05	84 – 92	88	3.8	4.3	3
			0.2	80	80	N/A	N/A	1
			0.5	80 – 85	83	3.7	4.5	2
		AMPA	0.05	72 – 94	83	10.8	13.0	3
			0.2	69	69	N/A	N/A	1
			0.5	68 – 71	69	2.1	3.1	2

[1] Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.

Recoveries (accuracy)
Recoveries were obtained for the analysis of residues of glyphosate and AMPA in different matrices. The validation test included analysis of replicates fortified at the LOQ and higher fortification levels for each matrix. The recoveries for glyphosate and AMPA are summarized in the table above. All average recovery values were between 70 % and 110 % (except for glyphosate in chips where the recovery is < 70).

Specificity
For both glyphosate and AMPA, chromatograms of a standard, of untreated samples and treated sample have been provided in all matrices.
In all control samples the intensity of signals at the retention time of glyphosate and AMPA was below 30 % LOQ.

Linearity
The linearity is missing.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below or equal to 20 % (except for AMPA in potato whole tuber where RSD at 0.05 mg/kg is > 20%). Moreover, the number of sample per fortification level is not sufficient for all processed fractions.

Limit of Quantification and Detection
The limit of quantification (LOQ) is 0.1 mg/kg for glyphosate in potato whole tuber and 0.5 mg/kg for AMPA in potato whole tuber. For processed fractions, the number of sample in the recovery is insufficient to determine a LOQ.

Matrix effects
Not assessed.

Stability of glyphosate and AMPA in sample extracts
Stability of the analytes in sample extracts was not assessed.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guidance document SANCO 3029/99 re.4.
The linearity data are missing. The matrix effect were not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed.

The accuracy of the method DFG 405 are acceptable only for the determination of glyphosate and AMPA in potato whole tuber with a LOQ of 0.1 mg/kg for glyphosate and 0.5 mg/kg for AMPA. The accuracy is not considered as validated for other processed matrices as the number of sample to demonstrate the recovery is too low.

The extraction solvent used (150 mL HCl 0.1 mol/L in water and chloroform (50 mL), consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 660). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Therefore, the analytical method cann be considered as validated only for the determination of glyphosate and AMPA in potato whole tuber

Residue study:
Determination of the Storage Stability of Glyphosate in Beans, Oilseed Rape and Linseed (CA 6.1/007 [redacted] 1997)
Test facility : Institut Fresenius, Chemische und Biologische Laboratorien GmbH, Im Maisel 14, D-65232 Taunusstein, Germany

Findings:

For the determination of the storage stability of glyphosate in beans, oilseed rape and in linseed, untreated sample material was fortified with certain amounts of glyphosate. The spike levels were:
beans: 2.6 mg/kg
oilseed rape: 0.6 mg/kg
linseed: 5.6 mg/kg

Recoveries (accuracy)

The following recoveries were obtained for glyphosate in beans, oilseed rape and linseed. Recovery values were between 70 % and 110 %:

Sample	Storage time period in months				
	0	6	12	15	18
beans	90 %	92 %	108%	105 %	98 %
oilseed rape	87 %	88 %	95 %	110 %	96 %
linseed	93 %	88%	97 %	106 %	89 %

Linearity:
The linearity of the detector response was confirmed by injecting six standard solutions of 0.051 µg/mL - 2.54 µg/mL for glyphosate (R > 0.99).

Specificity:
Chromatograms of glyphosate standard, of untreated samples (for beans, oilseed rape and linseed) and fortified samples (for beans, oilseed rape and linseed) have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate in any of the control specimens.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guida,nce document SANCO 3029/99 re.4.
The linearity is not available in mg/kg this does not allow to verify if the fortification levels are in linearity range. The matrix effect was not demonstrated.

The recoveries for glyphosate in beans, oilseed rape and linseed are in the acceptable range. No repeatability have been performed.

However, as the objective of the study is to validate the stability of the sample at targeted concentrations, the data available can be considered as sufficient for the stability study for glyphosate at 2.6 mg/kg in beans, 0.6 mg/kg in oilseed rape and 5.6 mg/kg in linseed.

Residue study:
Determination of glyphosate in soybean raw agricultural commodities (RAC) stability report (CA 6.1/008 [redacted] 1993)
Test facility : Landis International, Inc. 3025 Madison Highway P.O. Box 5126 Valdosta, GA 31603-5126

Findings:

For the determination of the storage stability of glyphosate and AMPA in soybean seed and straw, stability samples were fortified at 1 mg/kg.

Recoveries (accuracy):
The following recoveries were obtained for glyphosate and AMPA in soybean seed and straw. Recovery values were sometimes below 70%:

Number of days after treatment	**Recovery in stored samples**	
	Glyphosate Recoveries (%)	AMPA recoveries (%)
Soybean straw		
0	84.6 , 70.5	80.2 , 73.3
Soybean seed		
5	76.2 , 73.9	78.9 , 77.9

Linearity:
The linearity of the detector response was confirmed by injecting seven standard solutions of 0.25 µg/mL – 3 µg/mL for glyphosate and AMPA. Correlation coefficients were not reported.

Specificity:
Chromatograms of glyphosate and AMPA typical standard, typical control (untreated soybean forage) and fortified sample (fortified soybean forage) have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate and AMPA.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guida,nce document SANCO 3029/99 re.4.

The number of sample used to demonstrate the recovery is too low for glyphosate and AMPA in soybean straw and soybean seed. The linearity is not available in mg/kg this does not allow to verify if the fortification levels are in linearity range. The matrix effect was not demonstrated.

However, the recoveries of AMPA and glyphosate are in acceptable range. As the objective of the residue study is to validate the stability of the sample at targeted concentrations,, the data available for method can be considered as acceptable to validate the content of glyphosate and AMPA at 1 mg/kg in matrices..

Residue study:
Determination of glyphosate in pasture grasses stability report (CA 6.1/009 [redacted] 1993)
Test facility : Landis International, Inc. 3025 Madison Highway P.O. Box 5126 Valdosta, GA 31603-5126

Findings:

For the determination of the storage stability of glyphosate and AMPA in pasture grass, stability samples were fortified at 1 mg/kg.

Recoveries (accuracy)
The following recoveries were obtained for glyphosate in pasture grass. Recovery values were sometimes below 70%:

Number of days after treatment	Recovery in stored samples	
	Glyphosate Recoveries (%)	AMPA recoveries (%)
Pasture grass		
6	78.1 , 92.2	62.2 , 55.3

Linearity:
The linearity of the detector response was confirmed by injecting seven standard solutions of 0.25 µg/mL - 3 µg/mL for glyphosate and AMPA. Correlation coefficients were not reported.

Specificity:
Chromatograms of non treated pasture grass sample, of spike pasture grass sample, of aged glyphosate and AMPA pasture grass sample have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate and AMPA.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guida,nce document SANCO 3029/99 re.4.

The number of sample used to demonstrate the recovery for glyphosate and AMPA in pasture grass is low. The linearity is not available in mg/kg this does not allow to verify if the fortification levels are in linearity range. The matrix effect was not demonstrated.

The recoveries measured for glyphosate are in the acceptable range. The recoveries measured for AMPA is not in the acceptable range, however the results is obtained after 6 days, therefore cannot conclude if the the low value is linked to the stability or to the method.

As the objective of the residue study is to validate the stability of the sample at targeted concentrations,the data available for method can be considered sufficient to validate the content of glyphosate at 1 mg/kg and cannot be considered as acceptable to validate the content of AMPA at 1 mg/kg.

Residue study:
Storage Stability of Glyphosate and AMPA in Wheat Grain and Straw and in Rye Grain and Straw (CA 6.1/011 [redacted] 1995)
Test Facility: RCC UMWELTCHEMIE AG P.O. Box CH-4452 Itingen BL Switzerland

Findings:

For the determination of the storage stability of glyphosate and AMPA in wheat grain, wheat straw, rye grain and rye straw, stability samples were fortified at 1 mg/kg for glyphosate and 0.5 mg/kg for AMPA.

Recoveries (accuracy)

The following recoveries were obtained for glyphosate and AMPA in wheat grain, wheat straw, rye grain and rye straw. Recovery values were sometimes below 70%:

Number of days after treatment	**Recovery in stored samples**	
	Glyphosate Recoveries (%)	AMPA recoveries (%)
Wheat grain		
0	76.1	78.6
Wheat straw		
0	87.3	72.2
Rye grain		
0	71.2	79.8
Rye straw		
0	85	85.7

Linearity:
The linearity of the detector response was confirmed by injecting six standard solutions of 0.025 µg/mL - 1 µg/mL for AMPA and glyphosate. Correlation coefficients were not reported.

Specificity:
Chromatograms of both standards, of control sample and spiked storage stability sample in rye grain, wheat grain, rye straw and wheat straw for glyphosate and AMPA have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate and AMPA.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guida,nce document SANCO 3029/99 re.4.

The number of sample to demonstrate the fortification for glyphosate and AMPA is low for wheat grain, wheat straw, rye grain and rye straw. The results at T0 are in the acceptable range and no repeatability have been performed.
The linearity is not available in mg/kg this does not allow to verify if the fortification levels are in linearity range. The matrix effect were not demonstrated

As the objective of the residue study is to validate the stability of the sample at targeted concentrations , the data available for method can be considered as acceptable to validate the content of glyphosate at 1 mg/kg and ofr AMPA at 0.5 mg/kg in matrices.

Residue study:
Storage stability of glyphosate residues in crop commodities (CA 6.1/012 [redacted] 1991)
Test Facility : MONSANTO AGRICULTURAL COMPANY 700 Chesterfield Village Parkway St. Louis, Missouri 63198

Findings:
For the determination of the storage stability of glyphosate and AMPA in crop commodities, stability samples were fortified at 0.5 mg/kg for glyphosate and 0.4845 mg/kg for AMPA.

Recoveries (accuracy)
The following recoveries were obtained for glyphosate and AMPA in crop commodities. Almost all recovery values were between 70 % and 110 % (Only a few recovery values were below 70%):

Month after sampling	Recovery in stored samples	
	Glyphosate Recoveries (%)	AMPA recoveries (%)
corn grain		
13	83.1	88.2
Soybean forage		
32	112.1	102.2
Sorghum stover		
7	89.1	78.6
Clover		
17	96.1	87.5
Tomatoes		
4	83.3	82.1
Alfalfa seed		
2	87	77.2
Potatoes		
1	79.5	75.1

Linearity:
Linearity data were not reported.

Specificity:
Chromatograms of both standards, of control sample and spiked storage stability sample in all matrices for glyphosate and AMPA have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate and AMPA.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guida,nce document SANCO 3029/99 re.4.

The linearity is not available in mg/kg this does not allow to verify if the fortification levels are in linearity range. The matrix effect was not demonstrated.

The number of samples used to demonstrate the recovery for glyphosate and AMPA in crop commodities is low. However, the recoveries available are in acceptable range.

As the objective of the residue study is to validate the stability of the sample at targeted concentrations, the data available for method can be considered as acceptable to validate the content of glyphosate at 0.5 mg/kg and of AMPA at 0.4845 mg/kg in crop commodities tested.

Determination of glyphosate and AMPA in animal tissues by post-column derivatisation with OPA

Data point	CA 4.1.2/120
Report authors	[redacted]
Report year	1988
Report title	Validation of an analytical determination of glyphosate residues in animal tissues
Test facility	MONSANTO AGRICULTURAL COMPANY

	700 Chesterfield Village Parkway St. Louis, Missouri 63198
Report No	MSL-7358 (Data owner: Monsanto (MON))
Document No	Not applicable
Guidelines followed in study	Not reported
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No full validation set provided • Matrix effects not assessed • Stability of analyte in sample extract not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/140 (CA 6.4.1/003)
Report authors	[redacted]
Report year	1987
Report title	Residue Determination of Glyphosate and AMPA in Laying Hen Tissues and Eggs Following a 28 Day Feeding Study
Test facility	[redacted]
Report No	[redacted]-6676
Document No	Not applicable
Guidelines followed in study	Not applicable
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No full validation set provided • Not enough data on linearity • Matrix effects not assessed • Stability of analyte in sample extract not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, GLP statement included, no GLP certificate provided
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/141 (CA 6.4.2/002)
Report authors	[redacted]
Report year	1987
Report title	Residue Determination of Glyphosate and AMPA in Dairy Cow Tissues and Milk Following a 28-Day Feeding Study
Test facility	[redacted]

	[redacted]
Report No	[redacted]-6729
Document No	Not applicable
Guidelines followed in study	Not applicable
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No full validation set provided • Not enough data on linearity • Matrix effects not assessed • Stability of analyte in sample extract not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/142 (CA 6.4.3/001)
Report authors	[redacted]
Report year	1987
Report title	Residue Determination of Glyphosate and AMPA in Swine Tissues Following a 28 Day Feeding Study
Test facility	[redacted]
Report No	[redacted]-6627
Document No	Not applicable
Guidelines followed in study	Not applicable
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No full validation set provided • Not enough data on linearity • Matrix effects not assessed • Stability of analyte in sample extract not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, GLP statement included, no GLP certificate provided
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Full summary of the study according to OECD format
The analytical method DFG method 405 was used to determine the residues of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in different crop matrices by HPLC and post-column derivatisation for fluorescence detection. This method was adapted to the determination of residues of glyphosate and AMPA in various animal tissues. The method was also used to determine the levels of glyphosate and AMPA in feed diets used in feeding studies.

This method was used with minor modifications in the studies listed in the table below.

Table 5.1-12: Overview on residue studies

Data point	Report authors	Report year	Report number	Report title	Test facility
CA 6.1/014	[redacted]	1988	MSL-7515	Storage stability of Glyphosate and AMPA in swine tissues, dairy cow tissues and milk laying hen tissues and eggs	MONSANTO AGRICULTURAL COMPANY 700 Chesterfield Village Parkway St. Louis, Missouri 63198
CA 6.4.1/003	[redacted]	1987	[redacted]-6676	Residue determination of Glyphosate and AMPA in laying hen tissues and eggs following a 28 day feeding study	[redacted]
CA 6.4.2/002	[redacted]	1987	[redacted]-6729	Residue determination of Glyphosate and AMPA in dairy cow tissues and milk following a 28 day feeding study	[redacted]
CA 6.4.3/001	[redacted]	1987	[redacted]-6627	Residue determination of Glyphosate and AMPA in swine tissues following a 28-day feeding study	[redacted]

Principle of the method
Samples were blended with chloroform (50 mL) and water (100 mL). For milk, samples were blended with water and mixed with 0.1 N HCl (final pH 2.0 ± 0.4). After clean-up of the aqueous fraction by elution through Chelex 100 resin in the Fe(III) form, glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron ions removed using a anion exchange resin. After evaporation to dryness to remove the hydrochloric acid and reconstitution in distilled deionized water, glyphosate and AMPA were quantified by cation exchange HPLC equipped with a post-derivatisation unit and a fluorescence detector. Determination involves post-column calcium hypochlorite oxidation for glyphosate. Oxidized glyphosate and AMPA were coupled with Fluoraldehyde® or o-phthalaldehyde and mercaptoethanol to produce fluorescent derivatives.

Chromatographic conditions:

HPLC system:	Model 590 pump, WISP Model 710B autosampler, Post column derivatization amino acid analysis system, Model 420-AC fluorescence spectrometer (all Waters) or equivalent
HPLC Column:	Bio-Rad Labs analytics Aminex-A9, 100 mm × 4.6 mm or 300 mm x 4.6 mm Brownlee Labs RP-18 Spheri-10 guard column, 150 mm x 3.2 mm
Column temperature	50 °C
Mobile phase:	680 mg KH_2PO_4 dissolved in 4% methanol / de-ionozed water, pH 2.1 (H_3PO_4)
Oxidative solution:	1.36 g KH_2PO_4, 11.6 g NaCl, 0.4 g NaOH dissolved in 0.5 L de-ionozed water, after addition of 10 mg $Ca(ClO)_2$ (10 mL of 0.50 g $Ca(ClO)_2$ dissolved in 500 mL de-ionized water) solution was filled up to 1 L with de-ionozed water
Dervatisation solution:	Fluoraldehyde® or alternative OPA solution (25 g H_3BO_3 dissolved in 0.95 L de-ionozed water, adjust pH to 10.40 ± 0.2, add 3 mL 30% Brig 35 solution and 2 mL 2-mercaptoethanol, add 800 mg fluoropa dissolved in 10 mL methanol)
Flow rate:	Mobile phase: 0.50 mL/L Oxidation solution: 0.25 – 0.50 mL/min Derivatisation solution: 0.50 mL/min

Injection volume:	60 µL
Retention time:	Not reported
Detection:	Excitation wavelength 338 nm Emission wavelength 425 nm

Findings

Recoveries (accuracy)

The samples were fortified with glyphosate and AMPA at different fortification levels in range of 0.05 mg/kg to 15 mg/kg. All average recoveries were between 70 % and 110 % except for beef liver and pork liver at a fortification level of 0.05 mg/kg, where the mean recoveries were slightly below or above the limit of 70 – 110 %, respectively. The detailed results are given in the table below.

Results of method validation for the determination of glyphosate and AMPA in animal tissues

Matrix (Study)	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Beef muscle (MSL-7358)	Glyphosate	0.05	87 – 88	87	N/A	N/A	2
		0.10	86 – 87	87	N/A	N/A	2
		0.25	81 – 84	82	N/A	N/A	2
		0.50	89 – 90	90	N/A	N/A	2
		Overall	81 – 90	87	2.9	3.4	8
	AMPA	0.05	78 – 89	83	N/A	N/A	2
		0.10	83	83	N/A	N/A	2
		0.25	84 – 86	85	N/A	N/A	2
		0.50	86 – 88	87	N/A	N/A	2
		Overall	78 – 89	85	3.5	4.2	8
Beef milk (MSL-7358)	Glyphosate	0.025	95 – 99	97	N/A	N/A	2
		0.05	94 – 96	95	N/A	N/A	2
		0.125	89 – 89	80	N/A	N/A	2
		0.5	94 – 95	94	N/A	N/A	2
		1.25	94	94	N/A	N/A	2
		Overall	89 – 99	94	2.9	3.1	10
	AMPA	0.025	94 – 96.7	95.4	N/A	N/A	2
		0.05	95 – 98	97	N/A	N/A	2
		0.125	90 – 91	90	N/A	N/A	2
		0.5	92 – 95	93	N/A	N/A	2
		1.25	90 – 91	91	N/A	N/A	2
		Overall	90 – 98	93	2.8	3.1	10
Beef kidney	Glyphosate	0.05	93 – 97	95	N/A	N/A	2

Results of method validation for the determination of glyphosate and AMPA in animal tissues

Matrix (Study)	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
(MSL-7358)		0.25	91 – 91	91	N/A	N/A	2
		1.00	90 – 92	91	N/A	N/A	2
		5.00	94 – 95	95	N/A	N/A	2
		15.00	91 – 93	92	N/A	N/A	2
		Overall	90 – 97	93	2.3	2.5	10
	AMPA	0.05	98 – 103	100	N/A	N/A	2
		0.25	92 – 96	94	N/A	N/A	2
		1	90 – 93	92	N/A	N/A	2
		5	90	90	N/A	N/A	2
		15	90 – 92	91	N/A	N/A	2
		Overall	90 – 103	93	4.0	4.3	10
Beef fat (MSL-7358)	Glyphosate	0.05	102 – 112	107	N/A	N/A	2
		0.1	87 – 91	89	N/A	N/A	2
		0.25	94 – 98	96	N/A	N/A	2
		0.5	94 – 98	96	N/A	N/A	2
		1	87 – 87	87	N/A	N/A	2
		Overall	87 – 112	95	7.8	8.3	10
	AMPA	0.05	86 – 90	88	N/A	N/A	2
		0.1	87 – 91	89	N/A	N/A	2
		0.25	90 – 91	90	N/A	N/A	2
		0.5	86 – 92	89	N/A	N/A	2
		1	88 – 89	89	N/A	N/A	2
		Overall	86 – 92	89	2.1	2.4	10
Beef liver (MSL-7358)	Glyphosate	0.05	60 – 78	69	N/A	N/A	2
		0.1	69 – 71	97	N/A	N/A	2
		0.25	71 – 72	72	N/A	N/A	2
		1	78 – 85	81	N/A	N/A	2
		2.5	80 – 81	81	N/A	N/A	2
		Overall	60 – 85	74	7.4	10.0	10
	AMPA	0.05	85 – 94	89	N/A	N/A	2
		0.1	85 – 88	86	N/A	N/A	2
		0.25	81 – 84	82	N/A	N/A	2

Results of method validation for the determination of glyphosate and AMPA in animal tissues

Matrix (Study)	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		1	80 – 87	84	N/A	N/A	2
		2.5	75 – 77	76	N/A	N/A	2
		Overall	75 – 94	84	5.5	6.6	10
Chicken muscle (MSL-7358)	Glyphosate	0.05	89 – 90	90	N/A	N/A	2
		0.1	90 – 95	93	N/A	N/A	2
		0.25	85 – 87	86	N/A	N/A	2
		0.5	95 – 96	96	N/A	N/A	2
		Overall	85 – 96	91	4.1	4.5	8
	AMPA	0.05	82 – 86	84	N/A	N/A	2
		0.1	82 – 91	87	N/A	N/A	2
		0.25	82 – 89	86	N/A	N/A	2
		0.5	84 – 89	87	N/A	N/A	2
		Overall	82 – 91	86	3.8	4.4	8
Chicken liver (MSL-7358)	Glyphosate	0.05	68 – 73	70	N/A	N/A	2
		0.1	68 – 74	71	N/A	N/A	2
		0.5	77 – 78	78	N/A	N/A	2
		1	89	89	N/A	N/A	2
		5	78 – 80	80	N/A	N/A	2
		Overall	68 – 89	77	7.2	9.4	10
	AMPA	0.05	77 – 79	78	N/A	N/A	2
		0.1	72 – 78	75	N/A	N/A	2
		0.5	76 – 77	76	N/A	N/A	2
		1	84 – 86	85	N/A	N/A	2
		5	77	77	N/A	N/A	2
		Overall	72 – 86	78	4.1	5.2	10
Chicken kidney (MSL-7358)	Glyphosate	0.05	72 – 75	73	N/A	N/A	2
		0.25	83 – 88	86	N/A	N/A	2
		1	82 – 83	83	N/A	N/A	2
		5	92 – 94	93	N/A	N/A	2
		15	91 – 92	91	N/A	N/A	2
		Overall	71.8 – 93.5	87.0	7.8	9.0	10
	AMPA	0.05	94	94	N/A	N/A	2

Results of method validation for the determination of glyphosate and AMPA in animal tissues

Matrix (Study)	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.25	87 – 93	90	N/A	N/A	2
		1	86 – 87	86	N/A	N/A	2
		5	85 – 88	86	N/A	N/A	2
		15	90 – 91	91	N/A	N/A	2
		Overall	85 – 94	89	3.5	4.0	10
Chicken eggs (MSL-7358)	Glyphosate	0.025	91 – 92	92	N/A	N/A	2
		0.05	93 – 94	93	N/A	N/A	2
		0.125	89 – 92	90	N/A	N/A	2
		0.5	89 – 90	90	N/A	N/A	2
		Overall	89 – 94	91	1.8	2.0	8
	AMPA	0.025	89 – 91	90	N/A	N/A	2
		0.05	90 – 96	93	N/A	N/A	2
		0.125	89	89	N/A	N/A	2
		0.5	82 – 84	83	N/A	N/A	2
		Overall	82 – 96	89	4.4	5.0	8
Chicken fat (MSL-7358)	Glyphosate	0.05	86 – 88	87	N/A	N/A	2
		0.1	89 – 90	89	N/A	N/A	2
		0.25	83 – 85	84	N/A	N/A	2
		0.5	87 – 92	89	N/A	N/A	2
		Overall	83.1 – 91.5	87	2.8	3.2	8
	AMPA	0.05	88	88	N/A	N/A	2
		0.1	88	88	N/A	N/A	2
		0.25	84 – 85	85	N/A	N/A	2
		0.5	80 – 85	83	N/A	N/A	2
		Overall	80.1 – 88.4	86	3.0	3.5	8
Pork kidney (MSL-7358)	Glyphosate	0.05	86 – 87	87	N/A	N/A	2
		0.1	85	85	N/A	N/A	2
		0.5	98 – 99	98	N/A	N/A	2
		1	94 – 96	95	N/A	N/A	2
		5	96 – 97	97	N/A	N/A	2
		Overall	85 – 99	92	5.8	6.3	10
	AMPA	0.05	92 – 94	93.	N/A	N/A	2

Results of method validation for the determination of glyphosate and AMPA in animal tissues

Matrix (Study)	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.1	94 – 95	94	N/A	N/A	2
		0.5	93 – 94	93	N/A	N/A	2
		1	94 – 95	95	N/A	N/A	2
		5	93 – 94	93	N/A	N/A	2
		Overall	92 – 95	94	0.9	1.0	10
Pork muscle (MSL-7358)	Glyphosate	0.05	79 – 85	82	N/A	N/A	2
		0.1	85 – 87	86	N/A	N/A	2
		0.25	87 – 88	88	N/A	N/A	2
		0.5	89 – 91	90	N/A	N/A	2
		Overall	79 – 91	86	3.5	4.1	8
	AMPA	0.05	79 – 87	83	N/A	N/A	2
		0.1	86 – 87	86	N/A	N/A	2
		0.25	84 – 86	85	N/A	N/A	2
		0.5	89 – 91	90	N/A	N/A	2
		Overall	79 – 91	86	3.4	3.9	8
Pork fat (MSL-7358)	Glyphosate	0.05	95 – 101	99	N/A	N/A	2
		0.1	83 – 84	84	N/A	N/A	2
		0.25	89 – 94	92	N/A	N/A	2
		0.5	90 – 94	92	N/A	N/A	2
		Overall	83 – 101	91	6.0	6.6	8
	AMPA	0.05	98 – 101	99	N/A	N/A	2
		0.1	77 – 78	77	N/A	N/A	2
		0.25	75 – 81	78	N/A	N/A	2
		0.5	75 – 76	76	N/A	N/A	2
		Overall	75 – 101	83	10.5	12.8	8
Pork liver (MSL-7358)	Glyphosate	0.05	89 – 91	90	N/A	N/A	2
		0.1	83 – 91	87	N/A	N/A	2
		0.25	80 – 81	80	N/A	N/A	2
		0.5	86	86	N/A	N/A	2
		1	80 – 81	81	N/A	N/A	2
		Overall	80 – 91	85	4.4	5.1	10
	AMPA	0.05	109 – 114	112	N/A	N/A	2

Results of method validation for the determination of glyphosate and AMPA in animal tissues

Matrix (Study)	Analyte	Fortification level (mg/kg)	Recovery [1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.1	91 – 92	92	N/A	N/A	2
		0.25	82 – 85	83	N/A	N/A	2
		0.5	80	80	N/A	N/A	2
		1	78 – 81	80	N/A	N/A	2
		Overall	78 – 114	89	12.6	14.2	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.

Results of method validation for the determination of glyphosate and AMPA in feed diets for feeding studies

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Pig chow ([redacted]-6627)	Glyphosate	36	Not reported	95.6	Not reported	Not reported	12
		108	Not reported	94.4	Not reported	Not reported	8
		360	Not reported	98.1	Not reported	Not reported	12
	AMPA	4	Not reported	91.5	Not reported	Not reported	12
		12	Not reported	90	Not reported	Not reported	8
		40	Not reported	89.5	Not reported	Not reported	12
Chicken chow ([redacted]-6676)	Glyphosate	36	Not reported	94.7	Not reported	Not reported	10
		108	Not reported	93.5	Not reported	Not reported	4
		360	Not reported	94.4	Not reported	Not reported	8
	AMPA	4	Not reported	93.3	Not reported	Not reported	10
		12	Not reported	88.3	Not reported	Not reported	4
		40	Not reported	92	Not reported	Not reported	8
Cow chow ([redacted]-6729)	Glyphosate	144	Not reported	97.9	Not reported	Not reported	12
		432	Not reported	100	Not reported	Not reported	8
		1440	Not reported	94.9	Not reported	Not reported	10

Results of method validation for the determination of glyphosate and AMPA in feed diets for feeding studies

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	AMPA	16	Not reported	96.9	Not reported	Not reported	12
		48	Not reported	99	Not reported	Not reported	8
		160	Not reported	93.1	Not reported	Not reported	10

Specificity
MSL-7358: A confirmatory method is not considered necessary and is not specifically required for data generation methods. For glyphosate and AMPA no interferences from the specimen matrices were detected, for liver as matrix an additional clean up-step was necessary to eliminate an interfering peak. Sample quantification was based on comparison of peak height data to a calibration curve generated from concurrency run external standards.
Chromatograms of standards, of fortified samples and control samples of glyphosate and AMPA in all matrices tested have been repoted. No relevant interferences were detected at the retention time corresponding to glyphosate and AMPA

[redacted]-6627: Chromatograms of standards, of fortified samples and control samples of glyphosate and AMPA in pig chow have been repoted. No relevant interferences were detected at the retention time corresponding to glyphosate and AMPA.

[redacted]-6676: Chromatograms of standards, of fortified samples and control samples of glyphosate and AMPA in chicken chow have been repoted. No relevant interferences were detected at the retention time corresponding to glyphosate and AMPA.

[redacted]-6729: Chromatograms of standards, of fortified samples and control samples of glyphosate and AMPA in cow chow have been repoted. No relevant interferences were detected at the retention time corresponding to glyphosate and AMPA.

Linearity
MSL-7358: Eight standards were used to calibrate the HPLC instrument from 0.25 µg/mL to 50 µg/mL. These calibration curves were exponential for all the matrices except pork fat and pork liver, which had linear calibration curve. For the exponential curves the correlation coefficients (R^2) of >0.96 for glyphosate and >0.92 for AMPA were obtained. However, no calibration curve is available in the report.

[redacted]-6627, [redacted]-6676 and [redacted]-6729: Linearity data are not available.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20%. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The Limit of Quantification (LOQ), is 0.1 mg/kg for glyphosate and AMPA in beef muscle, chiken muscle and pork muscle and in beef fat, chicken fat and pork fat
The LOQ is 0.05 mg/kg for glyphosate and AMPA in beef milk and chicken eggs.
The LOQ is 0.25 mg/kg for glyphosate and AMPA in beef kidney and chicken kidney, and for glyphosate in beef liver.
The LOQ is 0.1 mg/kg for AMPA in beef liver, and for AMPA and glyphosate in chicken liver, pork liver and pork kidney.

Interference
The only tissue analysed in which an interference was encountered was liver. Therefore an additional clean up-step was necessary to eliminate the interfering peak.

Matrix effects
Not assessed.

Extraction efficiency
To confirm the method selected tissues of chicken egg yolk and liver, and goat kidney and liver from metabolism studies, containing ^{14}C-labeled endogenous residues, were analysed by the presented method. No information on extraction efficiency was reported in the report.

Stability of glyphosate and AMPA in sample extracts
Stability in sample extracts was not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate and AMPA in animal tissues. The method validation meets criteria set in SANCO/3029/99 rev. 4, in most relevant points and is considered as fit-for-purpose for the determination of glyphosate and AMPA in animal matrices.

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate and AMPA was not previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4) in most aspects (deficit: calibration curve and coefficient of determination not available and matrix effects not assessed). Nevertheless, the method is considered as fit-for-purpose to support the storage stability study concerned.

Assessment and conclusion by RMS:
As the validation have been performed in the same laboraotory, all data can be compiled by matrix.

The method has several deficiencies, the calibration curve, coefficient of determination are not available. Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

The other parameters meet the requirements.

The extraction solvent used is (100 mL) water and chloroform (50 mL), consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate and AMPA in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane/chloroform (see extraction efficiency part p 691). It is not expected that chloroform modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Therefore, the analytical method cannot be considered as validated

Residue study:
Storage stability of Glyphosate and AMPA in swine tissues, dairy cow tissues and milk laying hen tissues and eggs (CA 4.1.2/142 [redacted], 1988)

Findings:

For fat and muscle (swine, cows and chickens), the stability samples were fortified at 0.2 mg/kg of glyphosate and 0.05 mg/kg of AMPA.
For liver (swine), the stability samples were fortified at 0.8 mg/kg of glyphosate and 0.1 mg/kg of AMPA.
For liver (cows), the stability samples were fortified at 4 mg/kg of glyphosate and 0.5 mg/kg of AMPA.
For liver (chickes), the stability samples were fortified at 2 mg/kg of glyphosate and 0.25 mg/kg of AMPA.
For kidney (swine and chickens), the stability samples were fortified at 4 mg/kg of glyphosate and 0.5 mg/kg of AMPA.

For kidney (cows), the stability samples were fortified at 6 mg/kg of glyphosate and 1.5 mg/kg of AMPA.
For cow milk and chicken egg, the stability samples were fortified at 0.2 mg/kg of glyphosate and 0.05 mg/kg of AMPA.

Recoveries (accuracy)
The following recoveries were obtained for glyphosate. Recovery values were between 70 % and 110 % (except for egg in analysis point 3 and 4):

ANIMAL	MATRIX	0 DAY	ANALYSIS POINT 1	ANALYSIS POINT 2	ANALYSIS POINT 3	ANALYSIS POINT 4
Swine	Fat	94.3%	95.0%	83.5%	82.5%	103%
	Muscle	93.7%	91.0%	95.5%	91.0%	82.0%
	Liver	96.2%	90.0%	86.8%	89.4%	80.9%
	Kidney	101%	95.8%	98.5%	86.0%	101%
Cow	Fat	96.2%	89.2%	85.5%	85.2%	87.0%
	Muscle	92.5%	88.8%	93.0%	90.8%	102%
	Liver	98.0%	91.3%	89.9%	92.0%	99.4%
	Kidney	97.0%	93.5%	98.9%	94.4%	102%
	Milk	92.2%	84.2%	85.5%	84.5%	—
Chicken	Fat	96.0%	84.5%	85.2%	82.8%	87.8%
	Muscle	104%	95.8%	87.0%	90.5%	91.2%
	Liver	94.2%	69.9%	88.6%	78.3%	86.4%
	Kidney	97.7%	99.0%	97.0%	101%	—
	Egg	99.2%	87.2%	87.2%	42.5%	48.0%

The following recoveries were obtained for glyphosate. Recovery values were between 70 % and 110 % (except for egg in analysis point 3 and 4, and for chicken liver in analysis point 1):

ANIMAL	MATRIX	0 DAY	ANALYSIS POINT 1	ANALYSIS POINT 2	ANALYSIS POINT 3	ANALYSIS POINT 4
Swine	Fat	94.7%	91.7%	73.8%	73.5%	75.8%
	Muscle	101%	88.9%	84.5%	83.8%	75.0%
	Liver	95.2%	79.3%	82.8%	83.8%	80.2%
	Kidney	102%	93.0%	100%	85.3%	97.9%
Cow	Fat	98.0%	84.0%	82.7%	78.9%	80.6%
	Muscle	92.6%	85.0%	85.6%	71.0%	91.6%
	Liver	112%[1]	90.4%	88.1%	86.8%	92.6%
	Kidney	97.4%	91.1%	95.0%	89.8%	112%
	Milk	89.5%	82.1%	76.3%	86.5%	—
Chicken	Fat	98.7%	86.0%	86.1%	82.9%	81.5%
	Muscle	99.1%	93.9%	89.9%	87.3%	97.7%
	Liver	92.4%	65.4%	83.0%	78.6%	90.2%
	Kidney	97.5%	103%	92.0%	99.3%	—
	Egg	97.7%	89.5%	89.0%	44.5%	48.5%

Linearity:
Linearity data is missing.

Specificity:
Chromatograms of glyphosate and AMPA standards, of control samples and fortified samples in all tested matrices have been provided. No relevant interferences from the specimen matrix were detected at the retention time corresponding to glyphosate in any of the control specimens.

Assessment and conclusion

Assessment and conclusion by RMS:
The analytical method does not meet the requirement of the guida,nce document SANCO 3029/99 re.4.

The number of sample to demonstrate the recovery for glyphosate and AMPA is low. The linearity is not available in mg/kg this does not allow to verify if the fortification levels are in linearity range. The matrix effect was not demonstrated. However, the recoveries for glyphosate and AMPA are in the acceptable range.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

As the objective of the residue study is to validate the stability of the sample at targeted concentrations, the data available for method can be considered as acceptable to validate the content of glyphosate and AMPA at level tested in matrices.

Data point	CA 4.1.2/124 (CA 6.1/015, CA 6.4.1/002)
Report authors	[redacted]
Report year	1987
Report titles	Magnitude of SC-0224 Residues in Eggs and Poultry
Report No	[redacted] 87-43
Document No	Not applicable
Guidelines followed in study	Data Requirement Guideline §161-2
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No full validation set provided • Not enough data on linearity Stability of analyte in sample extract not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Data point	CA 4.1.2/125 (CA 6.1/016, CA 6.4.2/003)
Report authors	[redacted]
Report year	1987
Report titles	Magnitude of SC-0224 Residues in Meat and Milk
Report No	[redacted] 87-44
Document No	Not applicable
Guidelines followed in study	Guideline §161-2
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No full validation set provided • Not enough data on linearity
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, GLP statement included, no GLP certificate provided

Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

The analytical method describes the determination of SC-0224 (trimethylsulfonium carboxymethyl aminomethyl phosphonate) residues in biological samples. In this method, glyphosate (carboxymethyl aminomethyl phosphonic (CMP) anion) and its metabolite AMPA are quantified by LC-anion exchange-UV (200 nm) analysis. The method was validated in cow tissue (fat, muscle, liver, kidney), milk, chicken tissue and eggs.

An overview of the feeding studies, for which this method was used, is given in the table below.

Principle of the method
Glyphosate and AMPA were extracted from animal matrices using deionised water and clean-up by a cation exchange column. Milk was diluted with glacial acetic acid and for liver a 2-part clean-up system with methanol/water (1/10, v/v) was used. After separate collection of glyphosate and AMPA by use of an ion exchange column the analytes were converted to fluorescent derivatives with 9-fluorenylmethyl chloroformate (FMCL or FMOC) by adding borate buffer and FMOC-Cl derivatisation solution. Quantitation was achieved by HPLC-fluorescence analysis.

Chromatographic conditions:

HPLC system:	Beckman Model l00A or similar two-solvent HPLC system capable of pulse-free operation at 1500 psi, equipped with a sensitive fluorescence detector (Perkin-Elmer Model LC-10 or equivalent, with 10 µL sample loop)
HPLC column:	Ultrasil AX, 25 cm x 4.6 mm , 5 µm or 10 µm, Beckman No. 235347, or equivalent
Column temperature	50 °C
Dervatisation solution:	Prepare an acetone solution containing 1.0 mg/mL 9-fluorenylmethyl chloroformate, Aldrich No.16,051-2
Mobile phase glyphosate:	Buffer pH 2.5/acetonitrile/water (11/22/67, v/v/v)
Mobile phase AMPA:	Buffer pH 5/acetonitrile/water (10/22/68, v/v/v)
Dervatisation solution:	Prepare an acetone solution containing 1.0 mg/mL 9-fluorenylmethyl chloroformate, Aldrich No.16,051-2
Flow rate:	1.0 mL/min
Injection volume:	60 µL
Retention time:	Not reported
Detection:	Excitation wavelength 254 nm Emission wavelength 300 nm to 315 nm

Findings
Recoveries
Untreated samples were fortified with glyphosate and AMPA at different fortification levels in range of 0.01 mg/kg to 1.0 mg/kg. All average recoveries were between 70 % and 110 %.
The recoveries for glyphosate and AMPA are summarized in the table below.

Table 5.1-13: Recovery results of glyphosate and AMPA in samples of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Milk ([redacted] 87-44)	Glyphosate	0.02	99 – 107	103	N/A	N/A	2
		0.05	83 – 102	93	7.6	8.2	8
		0.10	83 – 91	87	5.7	6.5	2
		0.20	105	105	N/A	N/A	1
		0.5	107	107	N/A	N/A	1
	AMPA	0.02	73 – 89	81	N/A	N/A	2
		0.05	78 – 121	91	15	17	8
		0.10	96 – 98	97	N/A	N/A	2
		0.20	95	95	N/A	N/A	1
		0.5	95	95	N/A	N/A	1
Cow muscle ([redacted] 87-44)	Glyphosate	0.2	96	96	N/A	N/A	1
		0.5	69 – 73	71	N/A	N/A	2
	AMPA	0.2	86	86	N/A	N/A	1
		0.5	73 – 83	78	N/A	N/A	2
Cow fat ([redacted] 87-44)	Glyphosate	0.2	84	84	N/A	N/A	1
		0.5	65 – 84	75	9.5	13	3
	AMPA	0.2	100	100	N/A	N/A	1
		0.5	83 – 84	84	N/A	N/A	2
Cow liver ([redacted] 87-44)	Glyphosate	0.5	75	75	N/A	N/A	1
		1.0	67 – 73	70	N/A	N/A	2
	AMPA	0.5	46	46	N/A	N/A	1
		1.0	58 – 66	62	N/A	N/A	2
Cow kidney ([redacted] 87-44)	Glyphosate	0.5	84	84	N/A	N/A	1
		1.0	87	87	N/A	N/A	1
		2.0	81	81	N/A	N/A	1
	AMPA	0.5	69 – 91	80	N/A	N/A	2
		1.0	64	64	N/A	N/A	1
		2.0	69	69	N/A	N/A	1
Eggs ([redacted] 87-43)	Glyphosate	0.01	90	90	N/A	N/A	1
		0.02	100	100	N/A	N/A	1
		0.03	77	77	N/A	N/A	1
		0.05	79 – 107	93	11.7	12.6	6

Table 5.1-13: Recovery results of glyphosate and AMPA in samples of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.4	71 – 89	79	9.2	11.6	3
	AMPA	0.01	100	100	N/A	N/A	1
		0.02	75	75	N/A	N/A	1
		0.03	73	73	N/A	N/A	1
		0.05	67 – 70	69	N/A	N/A	2
		0.4	73 – 89	80	8.1	10.1	3
Chicken muscle ([redacted] 87-43)	Glyphosate	0.2	71	71	N/A	N/A	1
		0.5	73	73	N/A	N/A	1
	AMPA	0.2	65	65	N/A	N/A	1
		0.5	68 – 87	78	N/A	N/A	2
Chicken fat ([redacted] 87-43)	Glyphosate	0.2	114	114	N/A	N/A	1
		0.5	90	90	N/A	N/A	1
	AMPA	0.2	85	85	N/A	N/A	1
		0.5	86	86	N/A	N/A	1
Chicken liver ([redacted] 87-43)	Glyphosate	0.2	73	73	N/A	N/A	1
		0.5	65	65	N/A	N/A	1
		1.0	71	71	N/A	N/A	1
	AMPA	0.2	66	66	N/A	N/A	1
		0.5	82	82	N/A	N/A	1
		1.0	93	93	N/A	N/A	1
Chicken kidney ([redacted] 87-43)	Glyphosate	0.2	62 – 108	76	21	28	4
		0.4	64 – 68	66	2.1	3.1	3
		0.5	99	99	N/A	N/A	1
	AMPA	0.2	58	58	N/A	N/A	1
		0.4	71	71	N/A	N/A	1
		0.5	70	70	N/A	N/A	1

[1] Calculations of mean, SDs, RSDs and overall values were performed using Excel with recovery values as given in the report.

N/A Not applicable

Specificity

For study [redacted] 87-43: Chromatograms of standards solution, of control sample, of fortified sample at 0.025mg/kg (cow milk) and 0.05mg/kg (for other matrices) are provided. No interference is observed at the retention time at glyphosate and AMPA.

For study [redacted] *87-44*: Chromatograms of standards solution, of control sample, of fortified sample at 0.05mg/kg for milk, 0.5mg/kg for other matrices for glyphosate; 0.5mg/kg for muscle, 0.05mg/kg for other matrices for AMPA. No interference is observed at the retention time at glyphosate and AMPA.

Linearity
Glyphosate and AMPA were quantified with few calibration standards. The basic, mid-level calibration standard used is 0.05 ug each/mL water (acidified), while the high check standard is 0.2 ug each/mL, No calibration curve or degree of linearity is reported.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20%, except for glyphosate in chicken kidney, where the values very slightly above 20% (overall RSD 23%). Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for all the analysed matrices. The only exceptions were AMPA in cow liver (57%) and in chicken kidney (66%).

Limit of Quantification (LOQ)
The limit of quantitation (LOQ) of glyphosate and AMPA in animal tissue (kidney, liver, fat and muscle from cow and chicken) is 0.05 mg/kg (except cow liver, with LOQ of 0.2 mg/kg), while the LOQ of glyphosate and AMPA in milk and eggs is 0.02 mg/kg.

Note RMS: The LOQ is lowest fortification level (n≥5) with acceptable %RSD and % mean recovery. RMS considered for animal matrices, validation data are not compilable. In consequence, in the study [redacted] 87-47, the LOQ for milk is 0.05mg/kg for AMPA and glyphosate; for the other matrices, the number of sample is not sufficient
For the study [redacted] 87-43, the LOQ is 0.05mg/kg in Eggs for AMPA and glyphosate; For the others matrices, the number of sample is not sufficient at the fortification level.

Interference
No significant interferences from the specimen matrix were detected at the retention times, except in the case of cow liver, as noted below (matrix effects).

Matrix effects
Except in the case of cow liver, no significant interferences were noted for the analytes at the observed limits of detection. Liver contained compounds which interfered with the determination of the analytes. Detection limits for liver were 0.2 mg/kg for glyphosate and AMPA.

Stability of analyte
The stability of the analytes were evaluated as part of this study. All analytes were stable in milk, eggs and tissue for at least 671 days.

Conclusion
The analytical method was validated for the determination of glyphosate and AMPA in milk and eggs, at a limit of quantitation (LOQ) of 0.02 mg/kg and animal tissue (kidney, liver, fat and muscle from cow and chicken) with an LOQ of 0.05 mg/kg (except cow liver, with LOQ of 0.2 µg/g). The method validation meets criteria set in SANCO/3029/99 rev. 4, in most points and is considered as fit-for-purpose for the determination of glyphosate and AMPA in animal matrices.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was not previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation set, not enough information on linearity). Nevertheless, the method is fit for purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The method used for the determination of AMPA and glyphosate are not in agreement with SANCO 3029/99 rev 3.
The linearity range and calibration curve are missing. The number of sample by fortification level is low even if the matrices are compiled. However, for milk, the number of sample by fortification level are considered sufficient to demonstrate the validation of recovery.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

The extraction efficiency of the method can be considered as demonstrated according to the SANTE/2017/10632 as the solvent used in the method can be considered identical to the solvent used in the metabolism studies (see extraction efficiency part p691).

The method cannot be considered as fully validated for glyphosate and AMPA

Determination of glyphosate and degradate residues in various animal matrices using LC-MS/MS

Data point	CA 4.1.2/121
Report author	[redacted]
Report year	2007
Report title	Analytical method for the determination of *N*-acetyl-glyphosate and other analytes in various animal matrices using LC/MS/MS
Report No	DuPont-20009
Document No	Not applicable
Guidelines followed in study	EPA OPPTS 860.1340 SANCO/825/00 rev. 6
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 2a (with relevance for analytical methods)
Test facility	E.I du Pont de Nemours and Company Dupont Crop Protection Global Technology Division Stine-Haskell Research Center Newark, Delaware 19714-0030

[1] See Art.3 of Annex of Regulation No. 283/2013 and 284/2013

[2] 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).

Principle of method
The analytical method DuPont-20009 was validated for the determination of residues of glyphosate, *N*-acetylglyphosate, AMPA, and *N*-acetyl AMPA in matrices of animal origin

This method was used within several residue studies; an overview of the relevant studies is given in the table below.

Table 5.1-14: Overview on residue studies

Data point	Report authors	Report year	Report number	Test facility	Report title
CA 6.4.1/001	[redacted]	2007	[redacted]-20088	[redacted]	Magnitude of residues of *N*-Acetylglyphosate and degradates in laying hen tissues and eggs
CA 6.4.2/001	[redacted]	2007	[redacted]-20087	[redacted]	Magnitude of residues of *N*-Acetylglyphosate and degradates in dairy cow tissues and milk

For milk and egg commodities, matrix samples (2 g) were diluted in aqueous 0.1 % formic acid/ methanol (96:4, v/v) and shaken. The dilute sample was partitioned with hexane and the remaining aqueous fraction was partitioned with methylene chloride. The methylene chloride fraction was back extracted with additional 0.1 % formic acid/ methanol (96/4, v/v) for quantitative recovery of analytes. The aqueous fractions were combined and diluted to a final volume of 50 mL. An aliquot of the aqueous fraction was filtered through a C_{18} SPE cartridge. The C_{18} filtered extract was further purified by solid phase extraction using polymeric anion exchange (MAX) SPE cartridge and/ or polymeric cation exchange (MCX) SPE cartridge, depending on matrix and analytes to be examined.

For animal tissue commodities, samples (2 g) were blended with C_{18} sorbent material (4 g) prior to extraction in 0.1 N HCl solution (96 % water/4 % methanol) using vortexing and mechanical shaking followed by water for afinal extract volume of 50 mL. An aliquot of the extract was diluted in acetonitrile and methanol, then purified by solid phase extraction using polymeric anion exchange (MAX) SPE cartridge and/ or polymeric cation exchange (MCX) SPE cartridge, depending on matrix and analytes to be examined.

Final extract and calibration solutions were adjusted to 0.02 M phosphoric acid and all final extracts were filtered (0.2 µm) prior to LC-MS/MS analysis with reverse phase chromatography. The HPLC uses a phenyl-hexyl column and a triple quadrupole mass spectrometer with an electrospray ionisation in with MS/MS detection to acquire 2 molecular ion transitions (only 1 ion transition is monitored for AMPA in positive ion mode). Quantitative analysis was accomplished using a single molecular ion transition. The relative abundance of the 2 MS/MS fragment ions provides confirmatory evidence for glyphosate, *N*-acetylglyphosate, *N*-acetyl AMPA, and AMPA (negative mode). The reported results are based on calibration with an internal standard for glyphosate and AMPA.

Chromatographic conditions:

HPLC-system:	Agilent HP1100: G1322A vacuum degasser, G1311A quaternary pump, G1367A chilled autosampler, G1330A chiller, G1316A column compartment		
MS System:	Waters Quattro Premier triple quadrupole mass spectrometer, ESI interface, MassLynx version 4 SP4 software		
Column:	Phenomenex Luna® Phenyl-Hexyl (15.0 cm x 4.6 mm i.d. 3 µm diameter particle)		
Column temperature:	40 °C		
Injection volume:	25 µL (may be varied to correct for MS sensitivity)		
Mobile phase:	A: aqueous 0.2 M formic acid (positive ion) or 0.05 % formic acid (negative ion)B: methanol		
Flow rate:	0.35 – 0.5 mL/min		
Retention time:	**Analyte**	**0.2 M formic**	**0.05 % formic**
	AMPA	4.4 min	4.4 min
	Glyphosate	4.9 min	7.0 min
	N-acetyl AMPA	6.5 min	10.0 min
	N-acetyl-glyphosate	6.7 min	11.0 min

Scan type:	Positive or negative Ion MRM				
Ion source:	Electronspray (ESI)				
Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Dwell (secs)	Cone (volts)	Collision Energy (eV)
Glyphosate	170	88	0.10	14	9
	170	60	0.10	14	17
N-acetylglyphosate	212	88	0.10	17	17
	212	170	0.10	17	10
1,2-$^{13}C^{15}N$-glyphosate	173	91	0.10	14	9
AMPA	112	30	0.30	12	8
N-acetyl AMPA	154	30	0.10	14	15
	154	112	0.10	14	9
$^{13}C^{15}N$-AMPA	114	32	0.30	12	8

Quantitative analysis was accomplished using a single molecular ion transition. The relative abundance of the 2 MS/MS fragment ions provides confirmatory evidence for glyphosate, N-acetylglyphosate, N-acetyl AMPA, and AMPA (negative mode).

Findings

Recoveries

The method proved to be suitable to determine glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl AMPA in various animal matrices. Samples were spiked with the analytes at LOQ and higher levels. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-15: Recovery results for *N*-acetyl-glyphosate, glyphosate, *N*-acetyl AMPA and AMPA residues in commodities of animal origin

Matrix (study)	**Analyte**	**Fortification level (mg/kg)**	**Recovery[1]**				
			Range (%)	**Mean (%)**	**Standard deviation (%)**	**Relative standard deviation (%)**	**Number analyses (n)**
Whole milk (DuPont-20009)	*N*-acetyl-glyphosate	0.025	75 – 91	80	5	6	9
		0.05	75 – 83	78	3	3	7
		0.25	85 – 90	88	N/A	N/A	2
		0.5	75 – 83	79	3	4	7
		Overall	75 – 91	80	4	5	25
	Glyphosate	0.025	82 – 119	97	14	14	9
		0.05	87 – 126	100	13	13	7
		0.25	83 – 88	86	N/A	N/A	2
		0.5	71 – 94	80	7	9	7
		Overall	71 – 126	92	14	15	25
	N-acetyl	0.025	72 – 91	81	7	8	9

Table 5.1-15: Recovery results for *N*-acetyl-glyphosate, glyphosate, *N*-acetyl AMPA and AMPA residues in commodities of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	AMPA	0.05	74 – 85	79	4	4	7
		0.25	67 – 73	70	N/A	N/A	2
		0.5	76 – 86	82	3	4	7
		Overall	67 – 91	80	6	7	25
	AMPA	0.025	82 – 93	87	3	4	9
		0.05	73 – 96	85	7	9	7
		0.25	71 – 83	77	N/A	N/A	2
		0.5	71 – 87	80	6	7	7
		Overall	71 – 96	84	6	7	25
Skim milk (DuPont-20009)	*N*-acetyl-glyphosate	0.025	93 – 98	95	2	2	5
		0.05	81 – 103	93	8	9	5
		0.5	86 – 97	91	5	6	5
		Overall	81 – 103	93	6	6	15
	Glyphosate	0.025	81 – 111	93	12	13	5
		0.05	79 – 89	85	4	5	5
		0.5	78 – 95	85	7	8	5
		Overall	78 – 111	88	9	10	15
	N-acetyl AMPA	0.025	82 – 101	95	8	8	5
		0.05	91 – 107	99	7	7	5
		0.5	98 – 105	101	3	3	5
		Overall	82 – 107	98	6	6	15
	AMPA	0.025	93 – 96	94	N/A	N/A	2
		0.05	81 – 87	84	N/A	N/A	2
		0.5	N/A	76	N/A	N/A	2
		Overall	76 – 96	85	8	10	6
Cream (DuPont-20009)	*N*-acetyl-glyphosate	0.025	74 – 81	78	3	4	5
		0.05	75 – 86	82	4	5	5
		0.5	80 – 86	82	3	3	5
		Overall	74 – 86	81	4	5	15
	Glyphosate	0.025	79 – 113	99	13	13	5
		0.05	91 – 103	95	5	5	5

Table 5.1-15: Recovery results for *N*-acetyl-glyphosate, glyphosate, *N*-acetyl AMPA and AMPA residues in commodities of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.5	77 – 90	83	5	6	5
		Overall	77 – 113	92	11	12	15
	N-acetyl AMPA	0.025	68 – 108	87	14	17	5
		0.05	71 – 92	83	8	9	5
		0.5	86 – 98	93	5	5	5
		Overall	68 – 108	88	10	11	15
	AMPA	0.025	87 – 98	93	5	5	5
		0.05	78 – 96	88	8	9	5
		0.5	75 – 87	82	4	5	5
		Overall	75 – 98	88	7	8	15
Whole eggs (DuPont-20009)	*N*-acetyl-glyphosate	0.025	80 – 111	97	13	13	5
		0.05	82 – 97	87	6	7	5
		0.5	81 – 91	87	4	5	5
		Overall	80 – 111	90	9	10	15
	Glyphosate	0.025	79 – 93	88	6	7	5
		0.05	84 – 101	89	7	7	5
		0.5	82 – 87	85	2	3	5
		Overall	79 – 101	88	5	6	15
	N-acetyl AMPA	0.025	80 – 101	92	9	10	5
		0.05	90 – 109	99	8	8	5
		0.5	89 – 104	97	6	6	5
		Overall	80 – 109	96	8	8	15
	AMPA	0.025	92 – 110	105	7	7	5
		0.05	80 – 107	96	10	10	5
		0.5	79 – 86	84	3	4	5
		Overall	79 – 110	95	11	12	15
Egg whites (DuPont-20009)	*N*-acetyl-glyphosate	0.025	87 – 108	103	9	8	5
		0.05	88 – 107	96	10	10	5
		0.5	92 – 98	95	2	2	5
		Overall	87 – 108	98	8	8	15
	Glyphosate	0.025	74 – 94	83	7	9	5

Table 5.1-15: Recovery results for *N*-acetyl-glyphosate, glyphosate, *N*-acetyl AMPA and AMPA residues in commodities of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.05	84 – 91	88	3	4	5
		0.5	81 – 95	89	6	6	5
		Overall	74 – 95	87	6	7	15
	N-acetyl AMPA	0.025	86 – 96	90	4	4	5
		0.05	77 – 94	87	8	9	5
		0.5	85 – 100	92	5	6	5
		Overall	77 – 100	90	6	6	15
	AMPA	0.025	74 – 111	91	16	17	4
		0.05	90 – 103	94	5	5	5
		0.5	84 – 89	86	2	3	5
		Overall	74 – 111	91	9	10	14
Egg yolks (DuPont-20009)	*N*-acetyl-glyphosate	0.025	83 – 98	88	6	7	5
		0.05	85 – 97	90	5	5	5
		0.5	83 – 111	95	10	11	5
		Overall	83 – 111	91	8	8	15
	Glyphosate	0.025	89 – 115	98	10	11	5
		0.05	84 – 104	90	8	9	5
		0.5	83 – 99	89	7	7	5
		Overall	83 – 115	92	9	10	15
	N-acetyl AMPA	0.025	87 – 101	93	5	5	5
		0.05	93 – 99	96	2	3	5
		0.5	99 – 112	106	5	5	5
		Overall	87 – 112	99	7	7	15
	AMPA	0.025	106 – 114	110	N/A	N/A	2
		0.05	80 – 100	90	N/A	N/A	2
		0.5	91 – 97	94	N/A	N/A	2
		Overall	80 – 114	98	12	12	6
Liver[3] (DuPont-20009)	*N*-acetyl-glyphosate	0.05[2]	75 – 112	90	11	12	11
		0.5[2]	76 – 109	89	10	11	9
		Overall	75 – 112	90	10	12	20
		0.05[2]	74 – 105	90	10	11	11

Table 5.1-15: Recovery results for *N*-acetyl-glyphosate, glyphosate, *N*-acetyl AMPA and AMPA residues in commodities of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	Glyphosate	0.5[2]	71 – 88	82	5	7	9
		Overall	71 – 105	86	9	10	20
	N-acetyl AMPA	0.05[2]	58 – 107	83	16	19	10
		0.5[2]	63 – 95	81	12	13	9
		Overall	58 – 107	82	14	16	19
	AMPA	0.05[2]	77 – 118	97	12	12	10
		0.5[2]	81 – 110	94	10	10	9
		Overall	77 – 118	96	11	11	19
Beef kidney[3] (DuPont-20009)	*N*-acetyl-glyphosate	0.05[2]	80 – 112	99	11	11	6
		0.5[2]	73 – 88	83	5	6	7
		Overall	73 – 112	90	11	13	13
	Glyphosate	0.05[2]	78 – 116	98	15	15	6
		0.5[2]	81 – 92	87	4	5	7
		Overall	78 – 116	92	11	13	13
	N-acetyl AMPA	0.05[2]	69 – 94	82	10	12	6
		0.5[2]	71 – 93	79	9	11	6
		Overall	69 – 94	80	9	11	12
	AMPA	0.05[2]	76 – 113	92	15	17	6
		0.5[2]	71 – 108	89	13	14	7
		Overall	71 – 113	90	13	15	13
Fat (DuPont-20009)	*N*-acetyl-glyphosate	0.05[2]	91 – 107	100	6	6	6
		0.5[2]	83 – 97	90	6	6	6
		Overall	83 – 107	95	8	8	12
	Glyphosate	0.05[2]	86 – 113	98	11	12	6
		0.5[2]	86 – 98	94	4	5	6
		Overall	86 – 113	96	8	9	12
	N-acetyl AMPA	0.05[2]	82 – 95	88	5	6	6
		0.5[2]	71 – 93	87	8	9	6
		Overall	71 – 95	88	7	8	12
	AMPA	0.05[2]	95 – 109	103	6	6	5
		0.5[2]	89 – 97	93	3	4	5

Table 5.1-15: Recovery results for *N*-acetyl-glyphosate, glyphosate, *N*-acetyl AMPA and AMPA residues in commodities of animal origin

Matrix (study)	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	89 – 109	98	7	7	10
Muscle[3] (DuPont-20009)	*N*-acetyl-glyphosate	0.025[2]	76 – 113	92	13	14	7
		0.25[2]	70 – 92	81	8	10	7
		Overall	70 – 113	87	12	14	14
	Glyphosate	0.025[2]	77 – 103	92	10	11	7
		0.25[2]	78 – 91	84	4	5	7
		Overall	77 – 103	88	8	10	14
	N-acetyl AMPA	0.025[2]	69 – 96	83	10	13	6
		0.25[2]	64 – 88	80	9	12	5
		Overall	64 – 96	81	10	12	11
	AMPA	0.025[2]	84 – 103	94	9	10	6
		0.25[2]	85 – 101	94	6	7	5
		Overall	84 – 103	94	8	8	11

1 Recovery values are not corrected for interference with matrix compounds/respective control samples. Calculation of overall values for egg yolks was performed using excel with recovery values as given in the report
2 mg/kg glyphosate equivalents
3 Average of 2 analyses of same extract for some samples
N/A Not applicable
Liver, fat and muscle include samples from cow and chicken

Specificity
The analysis have been performed with HPLC-M/MS which is considered as specific analytical technique. However, only one ion was used for the quantification that is no sufficient to confirm the identy of the compound. However, e confirmatory method is not considered necessary for data generation methods. No interference was observed at the retention times of interest for all matrices tested.

Linearity
Calibration standards were prepared in aqueous 0.02 M phosphoric acid or 80 % control mix/ 20 % aqueous 0.02 M phosphoric acid. The use of the glyphosate and AMPA stable isotopes as internal standards in calibration standards and extract solutions is recommended to normalise recoveries for matrix effects and SPE purification performance for sample analysis. Generally, 5 calibration solutions were analysed for quantitative LC-MS/MS analysis. Calibration standards typically yielded a linear response (r^2>0.99) with %RSD < 20 % for calibration standard response factors (peak area/concentration) over the range of 0.25 to 50 ng/mL (corresponding to 50% LOQ and 120% highest expected final) for glyphosate, *N*-acetyl-glyphosate *N*-acetyl-AMPA or 0.5 to 50 ng/mL for AMPA. Representative calibration curves for each analyte were constructed using calibration standards from a validation set.

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

Accuracy
Mean recoveries obtained at each level of fortification and overall for each matrix were in the range 70-110% in the method validation for glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl AMPA. The accuracy of the method is within the limits specified by current EU guidance.
Limit of Quantification (LOQ) and Detection (LOD)
The LOQ validated in this method was 0.025 mg/kg in milk, egg, and muscle matrices and 0.050 mg/kg in kidney, liver, and fat matrices for the analysis of glyphosate, *N*-acetyl-glyphosate, *N*-acetyl AMPA, and AMPA. The LOQ is defined as the lowest fortification level at which average recoveries of 70-110 % and a RSD <20 % are achieved. Acceptable recoveries were achieved at the lowest fortification level. The LOD is defined by applicant as the analyte concentration in matrix with a response equivalent to a signal-to-noise ratio of approximately 3 to 1. The LOD estimates of this method are shown in the table below.

Estimated LODs (mg/kg glyphosate equivalents):

Matrix	**Glyphosate**	***N*-acetyl-glyphosate**	**AMPA**	***N*-acetyl AMPA**
Milk	0.008	0.005	0.008	0.008
Egg	0.003	0.008	0.006	0.007
Liver	0.009	0.018	0.019	0.008
Kidney	0.004	0.014	0.009	0.008
Fat	0.008	0.015	0.015	0.009
Muscle	0.004	0.006	0.008	0.006

Variation in the LOD was observed and each laboratory using this method should estimate an LOD value.

Interference
The chromatogram of a control sample did not reveal any significant interferences, which would interfere with the determination of the analytes.

Extraction Efficiency
Chicken liver, fat, and muscle samples treated with 14C N-acetylglyphosate in a poultry metabolism study ([redacted]-19795, [redacted]. 2007) were extracted using procedures in DuPont-20009 analytical method for radiochemical validation of the extraction efficiency.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does mainly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate other analytes in animal matrices.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was not performed under GLP and mainly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (stability of sample extracts and matrix effects not assessed). Nevertheless, the method is considered as fit for purpose to support the feeding study.

Assessment and conclusion by RMS:
The specificity, linearity, recovery and repeatability are in agreement with the SANCO 3029/99 rev.4 for milk, eggs, liver, kidney, fat and muscle. Only a minor deficiency has been identified, the matrix effect is not demonstrated. However, as the recoveries are in acceptable range, no further dat required.

In the study report, it is indicated that the metabolism study used DuPont-20009 analytical method. However, the extraction solvents used for each matrix tested in this study are not identical to the solvent used in the Dupont 2009 (more than 20% of deviation). However, we can consider, the solvent used in the method, identical to the solvent used in the metabolism studies (see extraction efficiency part p691)
Therefore, the analytical method can be considered as validated.

B.5.1.2.2 Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Overview Table for Analytical Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

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
CA 4.1.2/024 (CA 5.2.3/002)	[redacted] 2010 Report No. [redacted] 24603	Acute inhalation toxicity study of glyphosate TC in rats	N/A [redacted] 2010 Report No. [redacted] 24603	HPLC-FD (Fluorescence) *LOQ not reported in the studies report.*	No	Method fit-for-purpose at the targeted dose 5 mg/L	Y
CA 4.1.2/025 (CA 5.2.3/003)	[redacted] 2010 Report No. [redacted] 24875	Acute inhalation toxicity study of glyphosate TC in rats	N/A [redacted] 2010 Report No. [redacted] 24875				
CA 4.1.2/026 (CA 5.2.3/004)	[redacted] 2009 Report No. [redacted] 23911	Acute inhalation toxicity study of glyphosate TC in rats	N/A [redacted] 2009 Report No. [redacted] 23911				
CA 4.1.2/027 (CA 5.2.3/010)	[redacted] 2004 Report No. [redacted]-2003-116	An acute nose-only inhalation toxicity study in rats with MON 78623	N/A [redacted] 2004 Report No. [redacted]-2003-116	HPLC-UV *LOQ not reported* 2.038 – 7.206 mg/L	No	Method fit-for-purpose at the targeted doses for each trial	Y
CA 4.1.2/028 (CA 5.2.3/013)	[redacted] 1995 Report No. [redacted] 94-0155	HR-001: Acute inhalation toxicity study in rats	N/A [redacted] 1995 Report No. [redacted] 94-0155	HPLC-FD (Fluorescence) *LOQ not reported*	No	-	N

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**
CA 4.1.2/029 (CA 5.2.3/020)	[redacted], 1988 Report No. [redacted]-87147 / [redacted]-87-228	Acute inhalation study of MON-8750 technical	N/A [redacted] 1988 Report No. [redacted]87147 / [redacted]-87-228	HPLC-UV *LOQ not reported*	No	-	N
CA 4.1.2/030 (CA 5.2.3/021)	[redacted] 1987 Report No. [redacted]-6582	Acute toxicity of Rodeo herbicide administered by inhalation to male and female Sprague-Dawley rats	N/A [redacted], 1987 Report No. [redacted]-6582	HPLC-UV *LOQ not reported* *765 mg/L*	No	-	N
CA 4.1.2/031 CA 4.1.2/032 (CA 5.3.1/001)	[redacted] 1991 Report No. [redacted].881.28.DDR	28-day dietary study in Wistar rats. Test compound technical glyphosate (FSG 03090 H/05 March 1990)	RESI-953 [redacted] [redacted] 1991, Report No. ES.953.R.FST	Spectrophotometry *LOQ not reported* *2000 – 20000 mg/kg*	No	Method fit-for-purpose for the determination of glyphosate in diet at targeted doses	Y
CA 4.1.2/061 (CA 5.5/005) CA 4.1.2/031	[redacted] 1996 Report No. 886.C.C-R	Combined chronic toxicity and carcinogenicity study with glyphosate technical in Wistar rats	RESI-953 [redacted] [redacted] 1991, Report No. ES.953.R.FST				
CA 4.1.2/075 (CA 5.6.1/006) CA 4.1.2/031	[redacted], 1993 Report No. 885-RP-G2	Two generation reproduction study in Wistar rats	RESI-953 [redacted] [redacted] 1991, Report No. ES.953.R.FST				
CA 4.1.2/033	[redacted] 1990 Report No. 4823	Validation of analytical method no. 3750 for the analysis of glyphosate in rodent dietary formulations	3750 [redacted] 1990 Report No. 4823	HPLC-UV *LOQ not reported* 30 to 14200mg/kg	No	Method fit-for-purpose for the determination of	Y

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
CA 4.1.2/034 (CA 5.3.1/004) CA 4.1.2/033	[redacted] 1989 Report No. 5626	Glyphosate: 4 week dietary toxicity study in rats	3750 [redacted] 1990 Report No. 4823 [redacted] 1989 Report No. 5626	HPLC-UV *LOQ not reported*		glyphosate in rodent diet.	
CA 4.1.2/042 (CA 5.3.2/011) CA 4.1.2/033	[redacted] 1989 Report No. 7136	Glyphosate: 13 week dietary toxicity study in rats	3750 [redacted] 1990 Report No. 4823 [redacted]., 1989 Report No. 7136	HPLC-UV *LOQ not reported* 225 to 16781mg/kg			
CA 4.1.2/047 (CA 5.3.2/018) CA 4.1.2/033	[redacted] 1991 Report No. 7024	Glyphosate: 13 week dietary toxicity study in mice	3750 [redacted] 1990 Report No. 4823 [redacted] 1991 Report No. 7024	HPLC-UV *LOQ not reported* 970 to 27218mg/kg			
CA 4.1.2/063 (CA 5.5/007, CA 5.5/008, CA 5.5/009) CA 4.1.2/033	[redacted] 1993 Report No. 7867	Glyphosate – 104 week combined chronic feeding/ oncogenicity study in rats with 52 week interim kill (results after 104 weeks)	3750 [redacted] 1990 Report No. 4823 [redacted] 1993 Report No. 7867	HPLC-UV *LOQ not reported 10 – 14200 mg/kg* HPLC-UV *LOQ not reported 10 – 1000 mg/kg*			

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
CA 4.1.2/069 (CA 5.5/020) CA 4.1.2/033	[redacted] 1993 Report No. 7793	Glyphosate: 104 week dietary carcinogenicity study in mice	3750 [redacted] 1990 Report No. 4823 [redacted] 1993 Report No. 7793	HPLC-UV *LOQ not reported* 342 to 4636mg/kg			
CA 4.1.2/036 (CA 5.3.1/006) CA 4.1.2/035	[redacted] 1978 Report No. 77-2110	A four week pilot study with glyphosate in mice	N/A [redacted] 1978 Report No. MSL0000462 [redacted] 1978 Report No. 77-2110	HPLC-UV *LOQ not reported* *100 – 1000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/037 (CA 5.3.2/001, CA 5.3.2/002)	[redacted] 1996; Report No. [redacted]/P/1599	First revision to Glyphosate acid: 90 day feeding study in rats	N/A [redacted] 1996; Report No. [redacted]/P/1599	HPLC-UV *(LOQ 50 mg/kg)* *1000 – 20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/038 (CA 5.3.2/003)	[redacted] 1996 Report No. 434/016	Technical glyphosate: ninety day sub-chronic oral (dietary) toxicity study in the rat	N/A [redacted] 1996 Report No. 434/016	HPLC-FD (Fluorescence) *LOQ not reported* *1000 – 50000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/039 (CA 5.3.2/004)	[redacted] 1995 Report No. [redacted] 94-0138	HR-001: 13-week subchronic oral toxicity study in rats	N/A [redacted] 1995 Report No. [redacted] 94-0138	HPLC-FD (Fluorescence) *(LOD 2 mg/kg)* *3000 – 30000 mg/kg*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/040 (CA 5.3.2/005, CA 5.3.2/006, CA 5.3.2/007)	[redacted], 1993 Report No. 011-0001	90 day range finding study of glyphosate in rats	N/A [redacted] 1993 Report No. 011-0001	HPLC-FD (Fluorescence) *(LOQ 100 mg/kg) 2000 – 20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/041 (CA 5.3.2/008, CA 5.3.2/009, CA 5.3.2/010)	[redacted] 1992 Report No. TOXI: [redacted].882.90 OR	90-Day Oral Toxicity Study in Wistar Rats with Glyphosate Technical (FSG 03090 H/05 March 1990); Amendment to Final Report. 90-Day Oral Toxicity Study in Wistar Rats	N/A [redacted] 1992 Report No. TOXI: [redacted].882.90 OR	HPLC-FD (Fluorescence) *LOQ not reported 200 – 20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/043 (CA 5.3.2/012)	[redacted] 1990 Report No. [redacted]-900914	Glyphosate technical: 90 day oral toxicity study in the rat	N/A [redacted] 1990 Report No. [redacted]-900914	HPLC-UV *LOQ not reported 2000 – 7500 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/044 (CA 5.3.2/013)	[redacted]1989 Report No. [redacted]-891002	Glyphosate technical: 90 day oral toxicity study in the rat	N/A [redacted] 1989 Report No. [redacted]-891002	HPLC-UV *LOQ not reported 2000 – 7500 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/045 (CA 5.3.2/014)	[redacted] [redacted], 1987 Report No. [redacted]-7375	90-day study of glyphosate administered in feed to Sprague-Dawley rats	N/A [redacted] [redacted], 1987 Report No. [redacted]-7375	HPLC-FD (Fluorescence) *LOQ not reported 1000– 20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/046 (CA 5.3.2/017)	[redacted]1995 Report No. [redacted] 94-0136	HR-001: 13-week oral subchronic toxicity study in mice	N/A [redacted] 1995 Report No. [redacted] 94-0136	HPLC-FD (Fluorescence) *LOQ not reported 5000 – 50000 mg/kg*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/048 (CA 5.3.2/019)	[redacted], 1979 Report No. 77-2111	A three month feeding study of glyphosate (Roundup® technical) in mice	N/A [redacted] 1979 Report No. 77-2111	HPLC-UV *5000 – 5000 mg/kg*	No	Method fit-for-purpose as fit-for-purpose for the determination of glyphosate acid in rodent diet with an LOQ of 30mg/kg	Y
CA 4.1.2/078 (CA 5.6.1/014)	[redacted], 1981 Report No. 77-2063	A three generation reproduction study in rats with glyphosate	N/A [redacted], 1981 Report No. 77-2063	HPLC-UV *30 – 1000 mg/kg*			
CA 4.1.2/049 (CA 5.3.2/021, CA 5.3.2/022, CA 5.3.2/023, CA 5.3.2/024)	[redacted], 1999 Report No. 1816 and 1817-R.FST	Subchronic (90 day) oral toxicity study with glyphosate technical in Beagle dogs (Analytical phase: Test compound stability in experimental diet (dog feed))	N/A [redacted], 1999 Report No. 1816 and 1817-R.FST	Spectrophotometry *LOQ not reported* *200 – 10000 mg/kg*	No	Method fit-for-purpose as fit for purpose for the determination of glyphosate in the dog diets at 200mg/kg	Y
CA 4.1.2/050 (CA 5.3.2/025, CA 5.3.2/026)	[redacted] 1996 Report No. [redacted]/P/1802	First revision to glyphosate acid: 90-day oral toxicity study in dogs	N/A [redacted] 1996 Report No. [redacted]/P/1802	HPLC-UV *(LOD 5 mg/kg)* *2000 – 50000 mg/kg*	No	Method fit-for-purpose for the determination of glyphosate in the dog diets at the LOQ 2000mg/kg	Y
CA 4.1.2/051 (CA 5.3.2/027)	[redacted] 1996 Report No. [redacted] 94-0158	HR-001: 13-week oral subchronic toxicity study in dogs	N/A [redacted] 1996 Report No. [redacted] 94-0158	HPLC-FD *(LOD 2 mg/kg)* *1600 – 40000 mg/kg*	No	Method fit-for-purpose for the determination of glyphosate acid in dog diets at the LOQ 1600mg/kg	Y

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**
CA 4.1.2/052 (CA 5.3.2/032)	[redacted] 1997 Report No. [redacted] 94-0157	HR-001: 12-month oral chronic toxicity study in dogs	N/A [redacted], 1997 Report No. [redacted] 94-0157	HPLC-FD *(LOD 2 mg/kg)* *1600 – 50000 mg/kg*	No	Method fit-for-purpose for the determination of glyphosate acid in dog diets at the LOQ 1600mg/kg	Y
CA 4.1.2/053 (CA 5.3.2/033, CA 5.3.2/034)	[redacted] 1996 Report No. [redacted]/P/5079	Glyphosate acid: 1 year dietary toxicity study in dogs	N/A [redacted] 1996 Report No. [redacted]/P/5079	HPLC-UV *(LOD 30 mg/kg)* *3000 – 30000 mg/kg*	No	Method fit-for-purpose for the determination of glyphosate acid in dog diets at the LOQ 3000mg/kg	Y
CA 4.1.2/055 (CA 5.3.3/003) CA 4.1.2/054	[redacted] 1993 Report No. 7839	Glyphosate: 3 week toxicity study in rats with dermal administration	N/A [redacted] [redacted] 1993 Report No. 9112 [redacted] 1993 Report No. 7839	HPLC-FD *LOQ not reported* *5.34 – 333 mg/L*	No	Method fit-for-purpose	Y
CA 4.1.2/056 (CA 5.4.2/003) CA 4.1.2/057 (CA 5.4.2/004)	[redacted] 2008 Report No. 3996.402.395.07	Evaluation of the mutagenic potential of glyphosate technical by micronucleus assay in mice	N/A [redacted] 2008 Report No. 3996.402.395.07	HPLC-UV *LOQ not reported* *1.04 – 25 mg/L*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/058 (CA 5.5/001)	[redacted] 2009 Report No. 2060-0012	Glyphosate technical: Dietary combined chronic toxicity/carcinogenicity study in the rat	N/A [redacted] 2009 Report No. 2060-0012 [redacted] 2009 Report No. SPL2060-0011	HPLC-FD *LOQ not reported* *1500 – 24000 mg/kg* HPLC-FD *LOQ not reported* *500 – 5000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/065 (CA 5.5/012, CA 5.5/013, CA 5.5/014, CA 5.5/015)	[redacted] 2009 Report No. SPL2060-0011	Glyphosate technical: Dietary carcinogenicity study in mouse	N/A [redacted] 2009 Report No. 2060-0012 [redacted] 2009 Report No. SPL2060-0011	HPLC-FD *LOQ not reported* *1500 – 24000 mg/kg* HPLC-FD *LOQ not reported* *500 – 5000 mg/kg*			
CA 4.1.2/059 (CA 5.5/002)	[redacted] 2001 Report No. [redacted]/PR1111	Glyphosate acid: Two year dietary toxicity and oncogenicity study in rats	N/A [redacted] 2001 Report No. [redacted]/PR1111	HPLC-UV *(LOD 50 mg/kg)* *2000 – 20000 mg/kg*	No	Method fit-for-purpose for the determination of glyphosate acid in rat diets at the LOQ of 2000mg/kg	Y
CA 4.1.2/060 (CA 5.5/004)	[redacted] 1997 Report No. [redacted]-94-0150	HR-001: 24-month oral chronic toxicity and oncogenicity study in rats	N/A [redacted] 1997 Report No. [redacted]-94-0150	HPLC-FD *(LOD 2 mg/kg)* *3000 – 30000 mg/kg*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/062 (CA 5.5/006)	[redacted] 1996 Report No. [redacted]/P/5143	Glyphosate acid: One year dietary toxicity study in rats	N/A [redacted] 1996 Report No. [redacted]/P/5143	HPLC-UV *(LOD 25 mg/kg) 2000 – 20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/064 (CA 5.5/010)	[redacted] [redacted], 1990 Report No. [redacted]-10495	Chronic study of glyphosate administered in feed to albino rats	N/A [redacted] [redacted], 1990 Report No. [redacted]-10495	HPLC-UV *(LOD 25 mg/kg) 2000 – 21000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/066 (CA 5.5/016)	[redacted] 2001 Report No. 1559.CARCI-M	Carcinogenicity study with glyphosate technical in Swiss albino mice	N/A [redacted] 2001 Report No. 1559.CARCI-M	Spectrophoto-metrically *LOQ not reported 100 – 10000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/067 (CA 5.5/017)	[redacted]2017 Report No. 11921	Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice	[redacted] 2017 Report No. 11921		No		N
CA 4.1.2/068 (CA 5.5/018)	[redacted] 1997 Report No. [redacted] 94-0151	HR-001: 18-month oral oncogenicity study in mice	N/A [redacted] 1997 Report No. [redacted] 94-0151	HPLC-FD *(LOD 2 mg/kg) 1600 – 40000 mg/kg*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/071 (CA 5.5/023) CA 4.1.2/070	[redacted], 1983 Report No. 77-2061	A chronic feeding study of glyphosate (Roundup® Technical) in mice	N/A [redacted], 1982 Report No. MSL-2291 [redacted] 1983 Report No. 77-2061	HPLC-FD *(LOD 25 mg/kg)* *50 – 30000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/072 (CA 5.6.1/001, CA 5.6.1/002, CA 5.6.1/003)	[redacted] 2007 Report No. 2060/0013	Glyphosate technical: Dietary two generation reproduction study in the rat	N/A [redacted] 2007 Report No. 2060/0013	HPLC-FD *LOQ not reported* *1500 – 15000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/073 (CA 5.6.1/004)	[redacted] 2000 Report No. [redacted]/P/6332	Glyphosate acid: Multigeneration reproduction toxicity study in rats	N/A [redacted], 2000 Report No. [redacted]/P/6332	HPLC-UV *(LOD 76 mg/kg)* *1000 – 10000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/074 (CA 5.6.1/005)	[redacted], 1997 Report No. [redacted]-96-0031	HR-001: A two-generation reproduction study in rats	N/A [redacted], 1997 Report No. [redacted]-96-0031	HPLC-FD *(LOD 2 mg/kg)* *1200 – 30000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/076 (CA 5.6.1/007, CA 5.6.1/008)	[redacted] 1992 Report No. [redacted] 47/911129	The effect of dietary administration of glyphosate on reproductive function of two generations in the rat	N/A [redacted] 1992 Report No. [redacted]47/911129	HPLC-UV *(LOD 25 mg/kg)* *500 – 30000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/077 (CA 5.6.1/010)	[redacted] 1990 Report No. [redacted]-10387	Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats	N/A [redacted] 1990 Report No. [redacted]-10387	HPLC-UV *LOQ not reported* *2000 – 41000 mg/kg*	-	-	The part of the study report where the analytical data are reported is not available.

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**
CA 4.1.2/079 (CA 5.6.2/001) attention combinée avec 4.1.2/082 4.1.2/079	[redacted], 1996 Report No. [redacted]/P/4819 [redacted], 1996 Report No. [redacted] P 5009	Glyphosate acid: Developmental toxicity study in the rat	N/A [redacted], 1996 Report No. [redacted]/P/4819 [redacted] 1996 Report No. [redacted] P 5009	HPLC-UV *(LOD 0.4 mg/mL)* *25 – 150 mg/mL*	No	Method fit-for-purpose	Y
CA 4.1.2/080 (CA 5.6.2/002)	[redacted] 1995 Report No. [redacted]-94-0152	HR-001: Teratogenicity study in rats	N/A [redacted] 1995 Report No. [redacted]-94-0152	HPLC-FD *(LOD 2 mg/kg)* *3000 – 10000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/081 (CA 5.6.2/003)	[redacted] 1991 Report No. [redacted] 43 & 41-90716	The effect of glyphosate on pregnacy of the rat (incorporates preliminary investigations)	N/A [redacted] 1991 Report No. [redacted] 43 & 41-90716 [redacted] 1991 Report No. [redacted] 45 & 39 & 40/901303	HPLC-UV *LOQ not reported* *2 – 350 g/L* *(0.2 – 35 % w/v)*	No	Method fit-for-purpose for the determination of glyphosate solution at the targeted doses	Y

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
CA 4.1.2/085 (CA 5.6.2/014, CA 5.6.2/015)	[redacted] 1991 Report No. [redacted] 45 & 39 & 40/901303	The effect of glyphosate on pregnancy of the rabbit (incorporates preliminary investigations)	N/A [redacted] 1991 Report No. [redacted] 43 & 41-90716 [redacted] 1991 Report No [redacted] 45 & 39 & 40/901303				
CA 4.1.2/083 (CA 5.6.2/010)	[redacted], 1996 Report No. 434/020	Glyphosate technical: Oral gavage teratology study in the rabbit	N/A [redacted], 1996 Report No. 434/020	HPLC-UV *LOQ not reported* *10 – 80 mg/mL*	No	Method fit-for-purpose	Y
CA 4.1.2/084 (CA 5.6.2/011)	[redacted], 1995 Report No. [redacted]-94-0153	HR-001: Teratogenicity study in rabbits	N/A [redacted], 1995 Report No. [redacted]-94-0153	HPLC-FD *(LOD 100 mg/L)* *2000 – 60000 mg/L*	No	Method fit-for-purpose	Y
CA 4.1.2/086 (CA 5.7.1/001)	[redacted], 1996 Report No. [redacted]/P/4866	Glyphosate acid: Acute neurotoxicity study in rats	N/A [redacted] 1996 Report No. [redacted]/P/4866	HPLC-UV *(LOD 2.6 mg/mL)* *10 – 200 mg/mL*	No	Method fit-for-purpose	Y
CA 4.1.2/087 (CA 5.7.1/002)	[redacted] 2006 Report No. 2060-0010	Glyphosate technical: Ninety day repeated dose oral (dietary) neurotoxicity study in the rat	N/A [redacted] 2006 Report No. 2060-0010	HPLC-FD *LOQ of 1000mg/kg* *1000 – 20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/088 (CA 5.7.1/003)	[redacted] 1996 Report No. [redacted]/P/4867	Glyphosate acid: Subchronic neurotoxicity study in rats	N/A [redacted] 1996 Report No. [redacted]/P/4867	HPLC-UV *LOQ of 2000mg/kg* *2000 – 20000 mg/kg*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/089 (CA 5.7.2/001)	[redacted] 1996 Report No. [redacted]/C/3122	Glyphosate acid: Acute delayed neurotoxicity study in the domestic hen	N/A [redacted] 1996 Report No. [redacted]/C/3122	HPLC-UV *(LOD 0.15 mg/mL) – LOQ of 208mg/mL 208.8 mg/mL*	No	Method fit-for-purpose	Y
CA 4.1.2/090 (CA 5.8.1/005)	[redacted], 1988 Report No. [redacted]/P/2266	Aminomethyl phosphonic acid: Acute oral toxicity to the rat	N/A [redacted], 1988 Report No. [redacted]/P/2266	HPLC-FD *LOQ not reported 500 mg/mL*	No	-	N
CA 4.1.2/092 (CA 5.8.1/014) CA 4.1.2/091	[redacted] 1993 Report No. 7803	AMPA: 4 week dose range finding study in rats with administration	5391 [redacted] 1993 Report No. 8918 [redacted] 1993 Report No. 7803	HPLC-UV *LOQ not reported 10.2 – 509 µg/mL* HPLC-UV *LOQ not reported 1 – 100 mg/mL*	No	Method fit-for-purpose	Y
CA 4.1.2/093 (CA 5.8.1/016) CA 4.1.2/091	[redacted] 1993 Report No. 7866	AMPA: 13 week toxicity study in rats with administration by gavage	5391 [redacted] 1993 Report No. 8918 [redacted] 1993 Report No. 7866				
CA 4.1.2/098 (CA 5.8.1/028) CA 4.1.2/091	[redacted] 1992 Report No. 7891	AMPA: Teratogenicity study in rats	5391 [redacted], 1993 Report No. 8918 [redacted] 1992 Report No. 7891				

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
CA 4.1.2/094 (CA 5.8.1/017) CA 4.1.2/095	[redacted], 1979 Report No. 401-050	90-Day subacute rat toxicity study (IRD-78-174)	N/A [redacted] 1979 Report No. MSL-0682	HPLC-UV *(LOD 25 mg/kg) 100 – 50000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/096 (CA 5.8.1/018) CA 4.1.2/097	[redacted] 1991 Report No. [redacted]-50173	90-Day oral (capsule) toxicity study in dogs with AMPA	N/A [redacted] 1991 Report No. MSL-11291	HPLC-UV *LOQ not reported 400 mg/L*	No	Method fit-for-purpose	Y
CA 4.1.2/099 (CA 5.8.1/030) CA 4.1.2/100	[redacted], 1991 Report No. [redacted]-50159	A developmental toxicity study of AMPA in rats	N/A [redacted], 1990 Report No. MSL-10674	HPLC-UV *LOQ not reported 15 – 100 mg/mL*	No	Method fit-for-purpose	Y
CA 4.1.2/101 (CA 5.8.1/033)	[redacted], 2008 Report No. [redacted]-23316	IN-EY252 technical: Subchronic toxicity 90-day feeding study in rats	N/A [redacted] 2008 Report No. [redacted]-23316	LC-MS *LOQ not reported 900 – 18000 mg/kg*			
CA 4.1.2/102 (CA 5.8.1/037)	[redacted], 2007 Report No. [redacted]-22226	IN-EY252: Mouse bone marrow micronucleus test	N/A [redacted] 2007 Report No. [redacted]-22226	HPLC-UV *LOQ not reported 50 – 200 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/103 (CA 5.8.1/040)	[redacted], 2007 Report No. [redacted]-19008	IN-MCX20: Subchronic toxicity 90-day feeding study in rats	N/A [redacted], 2007 Report No. [redacted]-19008	LC-MS/MS *LOQ not reported 180 – 18000 mg/kg*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/104 (CA 5.8.1/044)	[redacted], 2006 Report No. [redacted]-20154	IN-MCX20: Mouse bone marrow micronucleus test	N/A [redacted], 2006 Report No. [redacted]-20154	LC-MS/MS *LOQ not reported* *50 – 200 mg/kg*	Yes	-	Y
CA 4.1.2/105 (CA 5.8.2/001)	[redacted], 2012 Report No. [redacted]-50393	Glyphosate – A 28-day oral (dietary) immunotoxicity study in female B6C3F1 mice	N/A [redacted] 2012 Report No. [redacted]-50393	HPLC-UV *LOQ not reported* *250 – 7500 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/106 (CA 5.8.2/002)	[redacted], 2010 Report No. [redacted]-50361	An eight week oral (diet and gavage) toxicity study of citric acid in male rats	N/A [redacted], 2010 Report No. [redacted]-50361	HPLC-UV *LOQ not reported* *50 – 200 mg/mL* *14000 mg/kg* *21400 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/107 (CA 5.8.2/003)	[redacted]1996 Report No. [redacted]/P/5160	Glyphosate acid: Comparison of salivary gland effect in three strains of rat	N/A [redacted] 1996 Report No. [redacted]/P/5160	HPLC-UV *LOQ not reported* *20000 mg/kg*	No	Method fit-for-purpose	Y
CA 4.1.2/109 (CA 5.8.3/005) CA 4.1.2/108	[redacted], 2012 Report No. [redacted]-843002	A uterotopic assay of glyphosate administered orally in ovariectomized rats	N/A [redacted], 2011 Report No. WIL-843004 [redacted] 2012 Report No. [redacted]-843002	HPLC-UV *LOQ not reported* *10 – 80 mg/mL* HPLC-UV *LOQ not reported* *20 – 200 mg/mL*	No	Method fit-for-purpose	Y

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**
CA 4.1.2/110 (CA 5.8.3/006) CA 4.1.2/108	[redacted], 2012 Report No. [redacted]-843003	A Hershberger Assay of glyphosate administered orally in peripubertal orchidoepididymectomized rats	N/A [redacted], 2011 Report No. WIL-843004 [redacted], 2012 Report No. [redacted]-843003	HPLC-UV *LOQ not reported 10 – 80 mg/mL* HPLC-UV *LOQ not reported 20 – 200 mg/mL*			
CA 4.1.2/111 (CA 5.8.3/007) CA 4.1.2/108	[redacted], 2012 Report No. [redacted]-843005	A pubertal development and thyroid function assay of glyphosate administered orally in intact juvenile/peripubertal male rats	N/A [redacted] 2011 Report No. WIL-843004 [redacted], 2012 Report No. [redacted]-843005	HPLC-UV *LOQ not reported 10 – 80 mg/mL* HPLC-UV *LOQ not reported 20 – 200 mg/mL*			

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**
CA 4.1.2/112 (CA 5.8.3/008) CA 4.1.2/108	[redacted], 2012 Report No. [redacted]-843007	A pubertal development and thyroid function assay of glyphosate administered orally in intact juvenile/peripubertal female rats	N/A [redacted], 2011 Report No. WIL-843004 [redacted], 2012 Report No. [redacted]-843007	HPLC-UV *LOQ not reported* *10 – 80 mg/mL* HPLC-UV *LOQ not reported* *20 – 200 mg/mL*			
KCA 4.1.2/225	[redacted] 2021 Report 8442132	Aminomethylphosphonic acid (AMPA): Method Validation with Stability and Homogeneity	[redacted] 2021 Report 8442132	HPLC-MS/MS LOQ:0.0501mg/mL 0.0001 – 0.0015mg/mL	Y	-	Y
KCA 5.8.1/045	[redacted] 2021 CV-2020-0209	Aminomethylphosphonic acid (AMPA): Reverse Mutation Assay 'Ames Test' using Salmonella typhimurium and Escherichia coli	[redacted] 2021 Report 8442132	HPLC-MS/MS LOQ:0.0501mg/mL 0.0001 – 0.0015mg/mL	Y	-	Y

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
5.8.1/046	[redacted] CV-2020-0233	Aminomethylphosphonic acid (AMPA): V79 HPRT Gene Mutation Assay	[redacted] 2021 Report 8442132	HPLC-MS/MS LOQ:0.0501mg/mL 0.0001 – 0.0015mg/mL	Y	-	Y
5.8.1/047	[redacted] CV-2020-0208	Aminomethylphosphonic acid (AMPA): Micronucleus Test in Human Lymphocytes in vitro	[redacted] 2021 Report 8442132	HPLC-MS/MS LOQ:0.0501mg/mL 0.0001 – 0.0015mg/mL	Y	-	Y
KCA 4.1.2/226	[redacted] 2021 8442134	Glyphosate: Method Validation with Stability and Homogeneity	[redacted] 2021 8442134	HPLC-RI LOQ:0.503mg/mL 0.2 – 1.0mg/mL	Y	-	Y
5.4.1/040	[redacted] 2021 CV-2020-0234	Glyphosate: V79 HPRT Gene Mutation Assay	[redacted] 2021 8442134	HPLC-RI LOQ:0.503mg/mL 0.2 – 1.0mg/mL	Y	-	Y
5.4.1/041	[redacted] 2021 CV-2020-0236	Glyphosate: Micronucleus Test in Human Lymphocytes in vitro	[redacted] 2021 8442134	HPLC-RI LOQ:0.503mg/mL 0.2 – 1.0mg/mL	Y	-	Y

Determination of glyphosate in acetonitrile sampling solutions from aerosol exposure compartments

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/024 (CA 5.2.3/002)
Report author(s)	[redacted]
Report year	2010
Report title	Acute inhalation toxicity study of glyphosate TC in rats
Report No	24603
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries available • Information to calibration function not available • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/025 (CA 5.2.3/003)
Report author(s)	[redacted]
Report year	2010
Report title	Acute inhalation toxicity study of glyphosate TC in rats
Report No	24875
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries available • Information to calibration function not available • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/026 (CA 5.2.3/004)
Report author(s)	[redacted]
Report year	2009
Report title	Acute inhalation toxicity study of glyphosate TC in rats
Report No	23911
Document No	
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries available • Information to calibration function not available • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed using HPLC-Fluorescence for the determination of glyphosate sampled from air exposure compartments by drawing air through acetonitrile in wash bottles, which were used for air sampling in the inhalation study.
Aliquots of these were directly analysed for glyphosate by high performance liquid chromatography (HPLC) with fluorescence detection at 258 nm excitation/305 nm emission using an external standard procedure.

Chromatographic conditions:

Column:	Nucleosil 100-5, C-8, 125 x 4.6 mm ID
Column oven temperature:	40 °C
Injection volume:	20 µL
Mobile phase:	55 % acetronitrile / 45 % pure water containing 0.025 % H_3P0_4 (pH 7)
Flow rate:	0.6 mL/min
Derivatisation agent:	Not derivatised
Detection:	Fluorescence at 258 nm excitation/305 nm emission
Retention time:	Glyphosate: not provided (no chromatograms provided)

This method was used within several toxicological studies; an overview of the relevant studies is given in the table below.

Table 5.1-16: Overview on toxicological studies which used the analytical method

Data point	Report authors	Report year	Report number	Report title
CA 5.2.3/002	[redacted]	2010	24603	Acute Inhalation Toxicity Study of Glyphosate TC In Rats
CA 5.2.3/003	[redacted]	2010	24875	Acute Inhalation Toxicity Study of Glyphosate TC In Rats
CA 5.2.3/004	[redacted]	2009	23911	Acute Inhalation Toxicity Study of Glyphosate TC In Rats

Findings

Recoveries

Method validation data from recoveries of spiked samples are not available.

However, glyphosate concentration (dust concentration) was measured gravimetrically, and alternatively by the HPLC Fluorescence method described above. None of the samples has been determined by the two methods. The recoveries determined by HPLC were calculated by comparison with average concentrations determined by gravimetry at other experiment times and are presented in the table below.

Table 5.1-17: Results of method performance for the determination of glyphosate in sampling solutions (acetonitrile)

Matrix	Analyte	Nominal concentration[1] (mg/L)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Sampling solution (sampled from aerosol exposure compartments through acetonitrile)	Glyphosate (Study 23911)	4.99	101 – 104	103	–	–	2
	Glyphosate (Study 24603)	5.05	99 – 106	102	–	–	2
	Glyphosate (Study 24875)	4.99	100 – 101	101	–	–	2
	Overall	4.99 – 5.05	99 – 106	102	2.3	2.3	6

1 Nominal concentration as determined by gravimeter.

2 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

Not assessed, no chromatograms provided in the report.

Linearity

No details to calibration reported.

Repeatability (Precision)

The overall relative standard deviation (RSD) of 2 determinations of glyphosate in acetonitrile sampling solutions was < 20 %. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values in each study and overall between 70 % and 110 % for glyphosate were found in acetonitrile sampling solutions compared to the mean concentration calculated by gravimetry. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not provided in the report.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points (minimal reporting of analytical method). Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in acetonitrile sampling solutions. Aerosol chamber concentrations were alternatively determined gravimetrically.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries given, details to calibration not provided, matrix effect and stability of sample extracts not assessed, repeatability/precision not reported, interference/specificity not addressed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned. Aerosol chamber concentrations were also determined gravimetrically.

Assessment and conclusion by RMS:
No validation data in agreement with SANCO/3029/99 rev. 4 is available in any of the three studies for the analytical method based on HPLC with fluorescence spectroscopy detection. No derivatisation step was described while the glyphosate cannot detected by fluorescence wihout derivatisation.
No typical chromatogram and no linearity data was provided. Matrix effects were not mentioned. Recoveries were estimated by comparing the mean result obtained by a gravimetric method over the time of the experiment (4 sampling at 30 min, 90 min, 150 min and 210 min) with results obtained by HPLC determination at other times of experiment (60 min and 180 min, single injections). As no sample has been analyzed more than one time, precision cannot be evaluated.
However both gravimetric method and HPLC method gave comparable results and no significant variations on glyphosate concentration in air are observed during the experiments. Therefore, the method can be considered as fit for purpose at the targeted dose 5 mg/L.

Determination of glyphosate in glass fibre filters used for determination of aerosol concentrations

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/027 (CA 5.2.3/010)
Report author(s)	[redacted]
Report year	2004
Report title	An acute nose-only inhalation toxicity study in rats with MON 78623

Report No.	[redacted]-2003-116
Document No.	3044.969
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Method validation recoveries not sufficiently reported • Details to calibration curve not given • Chromatograms not provided • 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
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of the glyphosate component of MON 78623 in glass fiber filters used for determination of aerosol concentrations by HPLC-UV.
Glass fiber filters which were used for gravimetric determination of aerosol concentrations in exposure chambers were weighted and placed in capped containers for extraction with 10 mL water. The solutions were agitated mechanically for 10 minutes and filtered through Whatman Puradisc 25PP 0.45 µm filters. The sample solutions were diluted with water (1:25) prior to pre-column derivatization of a 1.2 mL aliquot using 0.37 M tetraborate solution (0.8 mL) and 25 mM NBD-CL (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole; 2.4 mL) solution. Samples were heated at 80 °C for 30 minutes followed by addition of 0.9 mL of HCl solution after cooling. The sample was analysed by high performance liquid chromatography (HPLC) with UV detection at 500 nm using an external standard procedure.

Chromatographic conditions:

Column:	Synergi, Hydro-RP (Phenomenex), 4µ, 80Å, 250 x 4.6 mm ID
Pre-column:	SecurityGuard C18 (Phenomenex), 4.0 x 3.0 mm ID
Column oven temperature:	Ambient
Injection volume:	10 µL
Mobile phase:	(A) 0.05 M HCO_2NH_4, pH 3.6 / 5 % Acetonitrile (B) Acetonitrile 100 %
Gradient:	100 % A, hold for 6 minutes; linear change to 25 % A/75 % B over 1 minute; hold for 5 minutes; linear change to 100 % A over 1 minute; hold at 100 % A for 15 minutes
Flow rate:	1.0 µL/min
Derivatisation agent:	4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)
Detection:	UV at 500 nm
Retention time:	Glyphosate: not reported (no chromatograms provided)

Findings

Recoveries

System suitability injections were performed following the last sample injection. At least six consecutive injections of standard solutions were performed to demonstrate reproducibility. The percent relative standard deviations of the peak area response and peak retention time response were calculated for the consecutive injections. The first injection of each standard solution in the replicate injections was back calculated into the standard curve. Single injections of standard solution were also performed between samples such that no more than six consecutive sample injections were performed during the analysis. These single standard injections were also back calculated into the standard curve.

Individual recovery values are not provided in the report. The results of method validation are stated as follows:
For Trial Work, Standard 3 (nominal concentration 7.206 mg/L) was used to calculate the system suitability. The percent relative standard deviation for the peak areas was 1.45 %, and the percent relative standard deviation for the retention times was 0.67 %. Recovery values were within 2.0 % of the nominal concentration.
For Exposure #1 (analytical exposure 2.21 mg/L), Standard 2 (nominal concentration 2.038 mg/L) was used to calculate the system suitability. The percent relative standard deviation for the peak areas was 0.73 %, and the percent relative standard deviation for the retention times was 0.35 %. Recovery values were within 6.4 % of the nominal concentration.
For Exposure #2 (analytical exposure 5.27 mg/L), Standard 2 (nominal concentration 4.700 mg/L) was used to calculate the system suitability. The percent relative standard deviation for the peak areas was 0.69 %, and the percent relative standard deviation for the retention times was 0.41 %. Recovery values were within 2.4 % of the nominal concentration.

Specificity

Not assessed, sample chromatograms are not provided in the report.

Linearity

For Trial Work, linearity was assessed over the range of 2.402 to 12.01 mg/L (n=5). A correlation coefficient of 0.999 is reported.
For Exposure #1 (analytical exposure 2.21 mg/L), linearity was assessed over the range of 1.019 to 4.076 mg/L (n=4). A correlation coefficient of 0.9996 is reported.
For Exposure #2 (analytical exposure 5.27 mg/L), linearity was assessed over the range of 2.350 to 9.400 mg/L (n=4). A correlation coefficient of 0.9999 is reported.
No further details such as linearity plots or calibration functions are provided in the report.

Repeatability (Precision)

The relative standard deviations (RSDs) of repeated standard injections were < 20 %. In detail:
For Trial Work, Standard 3 (7.206 mg/L) was injected 6 times, , standard deviation for the peak area was 1.45%
For Exposure#1, Standard 2 (2.038 mg/L) was injected 6 times, , standard deviation for the peak area was 0.73%
For Exposure#2, Standard 2 (4.700 mg/L) was injected 6 times, , standard deviation for the peak area was 0.69%
Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Accuracy

Individual recovery values are not provided in the report. Acceptable mean recovery values were reported, please refer to recoveries above.

Limit of Quantification and Detection

Not reported.

Matrix effects

Not assessed. Standard and calibration samples were prepared in ultrapure water.

Stability of analytes in sample extracts

Not assessed.

Conclusion

The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method is considered as fit-for-purpose

for the determination of glyphosate in glass fibre filters used for determination of aerosol concentrations. Aerosol chamber concentrations were also determined gravimetrically.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (recovery data not reported, calibration curves/functions not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study, aerosol concentrations in exposure chambers were also determined gravimetrically.

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, no calibration curve was provided. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.
However, the method can be considered as fit for purpose at the targeted doses for each trial as aerosol concentrations in exposure chambers were also determined gravimetrically, and no particular differences between the two methods was observed.

Determination of glyphosate in glass fibre filters used for determination of aerosol concentrations

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/028 (CA 5.2.3/013)
Report author(s)	[redacted]
Report year	1995
Report title	HR-001: Acute inhalation toxicity study in rats
Report No.	[redacted] 94-0155
Document No.	-
Guidelines followed in study	None stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Very limited method validation recoveries provided • Details to calibration curve not given • Interference not assessed (no chromatograms provided) • 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
Test facility	[redacted]
Acceptability/Reliability	No

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 glass fiber filters used for determination of aerosol concentrations by HPLC-Fluorescence.
Samples of exposure chamber air (5 L volume) were drawn from the chamber sampling port at a flow rate of 10 L/min, and the test substance dust was trapped on glass fiber filters. Filters were then transferred into Erlenmeyer flasks and extracted with water (100 mL) by shaking for 20 minutes. An aliquot (1 mL) of this solution was evaporated to dryness, and the residue was re-dissolved in 0.05 M tetraborate solution (5 mL). The residue was then derivatised using 9-fluorenylmethyl chloroformate (FMCF; 5 mL; 1 mg/mL) for 20 minutes. Following addition of ethyl acetate (10 mL), the flask was shaken for 1 minute, and an aliquot of the separated aqueous phase was analysed by high performance liquid chromatography (HPLC) with fluorescence at 255 nm using an external standard procedure.

Chromatographic conditions:

HPLC system:	Shimadzu LC-6A with RF-535 detector and C-R4A integrator
Column:	TSK gel QAE-2SW, 250 x 4.6 mm ID
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/acedic acid/phosphoric acid (300/200/4/1, v/v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent:	FMCF (9-Fluorenylmethylchloroformate)
Detection:	Fluorescence: excitation 255 nm; emission 315 nm
Retention time:	Glyphosate: not provided (no chromatograms available)

Findings
Recoveries
Detailed recovery results for method validation are not provided in the report.
However, it is stated in the method description that the mean recovery of glyphosate from fortified filter papers was 95 – 96 %.
The mean actual atmospheric concentration of HR-001 (glyphosate technical) was 5.48 mg/L (RSD = 17.0%) as determined by HPLC analysis, and the ratio of the mean actual atmospheric concentration of HR-001 to the nominal concentration was 6.6 %.

Specificity
Chromatograms are not provided in the report.

Linearity
The calibration curve was prepared by injecting 10 µL aliquots of derivatised HR-001 standard solutions into the HPLC and plotting the peak heights against the original amounts of HR-001 injected. The number of plots is not reported. It is stated in the method description that the calibration curve was linear over the range from 0.32 to 3.2 ng (corresponding to 0.032 – 0.32 mg/L), and the coefficient of correlation (r) was higher than 0.999. Linearity plots and calibration functions are not provided in the report.

Repeatability (Precision)
Very limited data provided in the report, please refer to the recovery section above. The standard deviation (SD) of three analytical determinations in air was 0.93 mg/L, corresponding to a relative standard deviation (RSD) of 17.0 %. However this repeatability has been derived from a single analysis of 3 different samples (sampled at 60, 120 and 180 min of the experiment)

Accuracy
Detailed recovery results for method validation are not provided in the report. It is stated in the method description that the mean recovery of HR-001 (glyphosate technical) from fortified filter papers was 95 – 96 %, however it is not detailed how this was calculated.

Limit of Quantification and Detection
No details are reported, however it is stated in the report that the limit of detection of HR-001 (glyphosate technical) from the filter was 8 µg.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in many points, also due to insufficient reporting of method validation results. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in glass fibre filters used for the determination of aerosol concentrations. Aerosol chamber concentrations were also determined gravimetrically.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (very limited validation recoveries given, calibration curve not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned. Aerosol chamber concentrations were also determined gravimetrically.

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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferences, stability of sample extracts and matrix effects were not assessedwas not examined.
Aerosol concentrations in exposure chambers were also determined gravimetrically, but only the particle size distribution is reported. Therefore no comparison between results obtained from gravimetry and HPLC method could be evaluated. The data available are not sufficient to conclude. Therefore, the method cannot be considered as fit for purpose.

Determination of glyphosate in adsorption filters

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/029 (CA 5.2.3/020)
Report author(s)	[redacted]
Report year	1988

Report title	Acute inhalation study of MON-8750 technical
Report No	[redacted]-87-228[redacted]87147
Document No	Project No. [redacted]-87-228
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited true validation recoveries provided • Limited information to calibration curve provided • Interference not clearly assessed (limited chromatograms provided) • Matrix effect and stability of sample extracts not assessed • Repeatability (RSD) not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method using HPLC-UV was developed for the determination of glyphosate technical (ammonium salt) in Gelman type A/E glass fiber filters (25 mm), which were used for air sampling in the inhalation study. Filters were placed in sample jars in face-up position and extracted with deionized water by shaking and/or sonicating. An aliquot of the extract was then directly analysed for glyphosate ammonium by high performance liquid chromatography (HPLC) with UV detection at 195 nm using an external standard procedure.

Chromatographic conditions:

Column:	Brownlee AX-300, 220 x 4.6 mm ID, with 3 cm Brownlee RP-18 pre-column
Column oven temperature:	Ambient
Injection volume:	25 – 250 µL
Mobile phase:	4 % methanol in 0.0062 M KH_2PO_4 (adjusted to pH 2.1 with 85 % H_3PO_4)
Flow rate:	0.8 mL/min
Derivatisation agent:	Not derivatised
Detection:	UV at 195 nm
Retention time:	Glyphosate: ~ 7.6 min

Findings

Recoveries
For method validation, one filter (Gelman type A/E glass fiber, 25 mm) was fortified with glyphosate ammonium salt technical (MON 8750) at a nominal rate of 800 mg/L and analysed in duplicate using the analytical method. The recovery results are shown in table below. The average recovery of duplicate analyses was 98.9 %.

Table 5.1-18: Results of method validation (spike recovery) for the determination of glyphosate in sampling filters

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Filter	Glyphosate	800	Not provided	98.9	–	–	2

[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
One chromatogram of a calibration standard is provided. No major interfering peaks could be observed.

Linearity
Linearity was assessed by plotting peak heights of six standard solutions prepared in water covering the range of 150 to 1500 mg/L against concentration (duplicate determinations). No further details are provided to the calibration parameters.

Repeatability (Precision)
Not assessed, only the results of one quality control sample (fortified sample) are provided in the report.

Accuracy
An acceptable mean recovery value of 98.9% (n=2) was found for glyphosate technical at 800 g/L concentration (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
Not stated in the report. The lowest standard used for linearity testing/calibration was 150 mg/L, corresponding to ca. 0.75 mg/L air.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate technical in filter of aerosol sampling tubes. Aerosol concentrations in exposure chambers were also determined gravimetrically.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries given, limited information to calibration, matrix effect and stability of sample extracts not assessed, repeatability/precision not reported). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned. Aerosol concentrations in exposure chambers were also determined gravimetrically.

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: only one chromatogram and no calibration curve was provided. No information on precision and limited validation recoveries are reported. Stability of sample extracts and matrix effects were not assessed.
Moreover, it is noted that high variations in the concentration in air were observed during the experiment, from 8 measures in duplicate, leading to a mean concentration of 1.9 mg/L with a RSD of 47% (ie varying from 0.7 to 3.1 mg/L in air during the experiment). It is indicated that this loss from the exposure atmosphere of the test material would be due to the observed occurrence of impaction on animals, cages and/or the walls of the chamber. However, as no precision data were provided, it is not possible to conclude whether this variation is due to insufficient analytical precision or if the deviation was due to the experimental protocole itself.
Therefore, the method cannot be considered as fit for purpose.

Determination of glyphosate technical in distilled water

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/030 (CA 5.2.3/021)
Report author(s)	[redacted]
Report year	1987
Report title	Acute toxicity of Rodeo herbicide administered by inhalation to male and female Sprague-Dawley rats
Report No	[redacted]-6582
Document No	Project No. [redacted]-86-281
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited validation recoveries given • Limited information to calibration curve given • Interference not clearly assessed (limited chromatograms provided) • Matrix effect and stability of sample extracts not assessed • Repeatability (RSD) not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	No
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate sampled from exposure chambers by drawing air through distilled water in glass impingers by HPLC-UV, which were used for air sampling in the inhalation study.

Samples of water from impingers were directly analysed for glyphosate by high performance liquid chromatography (HPLC) with UV detection at 195 nm using an external standard procedure.

Chromatographic conditions:

Column:	Brownlee AX-300, 220 x 4.6 mm ID, with 1.5 cm Brownlee Amino pre-column
Column oven temperature:	Ambient
Injection volume:	250 µL
Mobile phase:	0.0062 M KH_2PO_4 (adjusted to pH 1.9 with 85 % H_3PO_4)
Gradient:	Isocratic (15 min runtime)
Flow rate:	0.75 mL/min
Derivatisation agent:	Not derivatised
Retention time:	Glyphosate: ~ 8.66 min
Detection:	UV at 195 nm

Findings

Recovery

For method validation, a quality control sample was prepared using EHL test substance No. T860049 (isopropylamine salt of glyphosate) at a nominal rate of 765 mg/L and analysed using the analytical method. The recovery result is shown in the table below.

Table 5.1-19: Results of method validation (spike recovery) for the determination of glyphosate in sampling filters

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Filter	Glyphosate	765	–	100.1	–	–	1

[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

One chromatogram of a calibration standard is provided. No major interfering peaks could be observed.

Linearity

Linearity was assessed by plotting peak heights of six standard solutions prepared in water covering the range of 150 to 1500 mg/L against concentration (duplicate determinations). No further details are provided to the calibration parameters (plot, equation or correlation coefficient).

Repeatability (Precision)

Not assessed, only the result of one quality control sample (fortified sample) is provided in the report (see table above).

Accuracy

An acceptable recovery value of 100.1 % was found for glyphosate technical (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
Not stated in the report. The lowest standard used for linearity testing/calibration was 150 mg/L, corresponding to ca. 0.75 mg/L air.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in chamber air (aerosol) sampling water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries given, limited information to calibration, matrix effect and stability of sample extracts not assessed, repeatability/precision not reported). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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: only one chromatogram and no calibration curve was provided. No information on precision and limited validation recoveries are reported. Matrix effects were not assessed.

Low variations in the concentration in air were observed during the experiment, leading to a mean concentration of 1.3 mg/L with a RSD of 8% nevertheless as recovery was performed at level higher than the tested concentration (765 mg/L), the targeted dose cannot be confirmed. Therefore, the method cannot be considered as fit for purpose

Determination of glyphosate technical in rat diet

Studies previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/031
Report authors	[redacted]
Report year	1991
Report title	Stability study in experimental diet, test compound: glyphosate technical
Report No	ES.953.R.FST
Document No	Method RESI-953
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given

	• Interference not assessed (absorbane values of blank samples not provided) • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the Monograph (2002)
GLP/Officially recognised testing facilities	Yes
Test facility	Residue Department, Rallis India Limited, Rallis Agrochemical Research Station, Plot No 21 & 22, Post Box No 5813, Peenya II Phase, Bangalore 560058, India
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/032 (CA 5.3.1/001)
Report authors	[redacted]
Report year	1991
Report title	28-day dietary study in Wistar rats. Test compound technical glyphosate (FSG 03090 H/05 March 1990)
Report No	[redacted].881.28.DDR
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Interference not assessed (absorbane values of blank samples not provided) • 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
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/061 (CA 5.5/005)
Report authors	[redacted]
Report year	1996
Report title	Combined chronic toxicity and carcinogenicity study with glyphosate technical in Wistar rats
Report No	886.C.C-R
Document No	-

Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Interference not assessed (absorbane values of blank samples not provided) • 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
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/075 (CA 5.6.1/006)
Report authors	[redacted]
Report year	1996
Report title	Two generation reproduction study in Wistar rats
Report No	885-RP-G2
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Interference not assessed (absorbane values of blank samples not provided) • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, evaluated in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
Method RESI-953 was developed for the determination of glyphosate technical in rat diet (Gold Mohur, M/S Lipton India Ltd, Bangalore-560 052, India) by spectrophotometrical absorbance at 243 nm. The method of

extraction and clean up for estimation of glyphosate in experimental diet is derived from Roy and Konar (1989)[1]. After extraction and clean up, glyphosate was converted to its nitroso-derivative as described by Bronstad and Friestad (1976)[2] and final estimation was made spectrophotometrically by measuring the absorbance of the complex at 243 nm.

An aliquot of rat diet sample (10 g) was extracted with 200 mL distilled water by shaking for 2 hours. After centrifugation the supernatant was washed with chloroform, followed by hexane and finally with ethyl acetate; organic layers were discarded. Aqueous extract was cleaned up with Darco (G-60) charcoal. After filtration an aliquot was derivatised under acidic conditions (sulfuric acid) with 25 % KBr solution and with 0.2 N sodium nitrite solution. After standing for 30 minutes an aliquot was diluted with distilled water and the absorbance was measured at 243 nm in a 1 cm quartz cell using corresponding control sample as blank. The concentration of glyphosate in the sample was determined from the standard curve.

Chromatographic conditions:
Not relevant, measurement using spectrophotometry.

This method was used within several toxicological studies; an overview of the relevant studies is given in the table below.

Table 5.1-20: Overview on toxicological studies which used method RESI-953

Data point	Report authors	Report year	Report number	Report title
CA 5.3.1/001	[redacted]	1991	[redacted].881.28 DDR	28 Day dietary study in wistar rats (test compound Glyphosate technical)
CA 5.5/005	[redacted]	1996	886.C.C-R	Combined chronic toxicity and carcinogenicity study with glyphosate technical in wistar rats
CA 5.6.1/006	[redacted]	1993	885-RP-G2	Two generation reproduction study in wistar rat (test compound glyphosate technical)

Findings
Recoveries
The method proved to be suitable to determine residues of glyphosate in matrix rat diet. Samples were spiked with glyphosate at two fortification levels, 2000 and 20000 mg/kg. All average recovery values (mean of 6 replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the table below. Blank samples were also analysed, however absorbance values were not reported.

Table 5.1-21a: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate technical	2000	90 – 107	96.4	5.8	6.0	6
		20000	91 – 98	94.5	2.6	2.8	6
		Overall	90 – 107	95	4.4	4.6	12

[1] Roy, D.N., Konar, S.K., 1989, Development of an analytical method for determination of glyphosate and (aminomethyl) phosphonic acid residues in soils by Nitrogen-selective gas chromatography. J.Agric.Food Chem; 37: 441-443.
[2] Bronstad, J.O., Friestad, H.O., 1976, Method for determination of glyphosate residues in natural water based on polarography of the N-nitroso derivative, Analyst; 101: 820-824.

Table 5.1-21a: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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 recovery values as given in the report.

Table 5.1-22b: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate technical	100	96-100	97.7	4.2	4.3	6
		1000	98-101	99.4	2.4	2.4	6
		10000	99-100	99.2	2.0	2.0	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 recovery values as given in the report.

Specificity
Not assessed. Absorbance values of blank samples were not reported.

Linearity
Linearity of detector response was tested using 7 calibration standard concentrations in the range of 0.14 to 0.98 mg/mL. The calibration standards were prepared in solvent (water) and derivatised as described above. A linear equation was calculated. Further details on calibration curve and function are missing.

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate technical were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed, but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical RESI-953 method was successfully validated for the determination of glyphosate in rat diet. The analytical method fulfils the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate technical in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate technical was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits *(*calibration curve and function not given, limit of quantification and detection missing, interference not assessed (absorbance values of blank samples not reported), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil all European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4: calibration curves, equations and correlation parameters are not given, interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in diet at tageteted doses. From additional data obtained in the study [redacted] 1993, the method was shown to be also fit for purpose.

Study previously submitted to the EU (Analytical part submitted to the EU for the first time)

1. Information on the study

Data point	CA 4.1.2/033
Report authors	[redacted]
Report year	1990
Report title	Validation of analytical method no. 3750 for the analysis of glyphosate in rodent dietary formulations
Report No	4823
Document No	IRI Project No. 337502
Facility test	Inveresk Research International Musselburgh, EH21 7UB Scotland
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes Inveresk Research Intrnational Musselburgh, EH21 7UB Scotland
Acceptability/Reliability	Fit for purpose

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/034 (CA 5.3.1/004)
Report authors	[redacted]
Report year	1989
Report title	Glyphosate: 4 week dietary toxicity study in rats
Report No	5626
Document No	[redacted] Project No. 437462
Facility test	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/042 (CA 5.3.2/011)
Report authors	[redacted]
Report year	1991
Report title	Glyphosate: 13 week dietary toxicity study in rats
Report No	7136
Document No	[redacted] Project No. 437876
Facility test	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/047 (CA 5.3.2/018)
Report authors	[redacted]
Report year	1991

Report title	Glyphosate: 13 week dietary toxicity study in mice
Report No	7024
Document No	[redacted] Project No. 437918
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Data point	CA 4.1.2/063 (CA 5.5/007, CA 5.5/008, CA 5.5/009)
Report authors	[redacted]
Report year	1993
Report titles	Glyphosate – 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks)
Report No	7867
Document No	[redacted] Project No. 438623
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/069 (CA 5.5/020)
Report authors	[redacted]
Report year	1993
Report title	Glyphosate: 104 week dietary carcinogenicity study in mice
Report No	7793
Document No	[redacted] Project No. 438618
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed

Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Fit for purpose
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

The method no. 3750 was developed for the determination of glyphosate in rodent diets by reversed phase ion-pair liquid chromatography.

Aliquots of the test material (10 g) were weighed accurately and transferred into a screw cap jar. After adding 2 mL of the appropriate internal standard solution (N-phosphomethyl-ß-alanine) 100 mL of 0.1 M triethylamine in water was added. The mixture was shaken for 1 h at 40 °C. Aliquots of the extracts were derivatised with 1-fluoro-2,4-dinitrobenzene. Sodium chloride and citrate buffer were added and the pH adjusted to 5.0-5.5. After addition of ethyl acetate, the samples were mixed, centrifuged and the ethyl acetate layer was discarded. This step was repeated once followed by addition of 25 % orthophosphoric acid solution and more ethyl acetate. The samples were mixed, centrifuged, and the ethyl acetate layer was transferred to a separate tube. The solvent was removed under a gentle stream of nitrogen. The residues were reconstituted in mobile phase and analysed by HLPC.

Analytical method no. 3750 was developed primarily for assays on rodent diets formulated to contain between 30 mg/kg and 300 mg/kg glyphosate. The actual dietary concentrations used in the toxicity studies (Report No. 5626, 7136 and 7024) exceed 300 mg/kg. A modification of method no. 3750 was required. Aliquots of test material (10 g) of formulated rodent diet were weighed accurately and transferred into a screw cap jar. After adding the appropriate internal standard solutions to the samples at lower ppm levels, 100 mL of 0.1 M trimethylamine was added and the mixture was shaken for 1 h at 40 °C. Aliquots were centrifuged and supernatants transferred in a separate tube. Aliquots of sample supernatants were transferred into scintillation vial, internal standard solution was added to the samples with higher ppm levels and the mixture was shaken. Trimethylamine (0.1 M) was added to an aliquot (1:10). After derivatisation with 1-fluoro-2,4-dinitrobenzene, sodium chloride, distilled water and ethyl acetate were added and mixed by vortexing. After a centrifugation step, the ethyl acetate layer was discarded. Orthophosphoric acid (25 %) and ethyl acetate were added and mixed by vortexing. The ethyl acetate layer was transferred to a separate tube after centrifugation and the solvent was removed under a gentle stream of nitrogen. The residues were reconstituted in mobile phase and analysed by HLPC.

This method was used within several toxicological studies; an overview of the relevant studies is given in the table below.

Table 5.1-23: Overview on toxicological studies which used the analytical method

Data point	Report authors	Report year	Report number	Report title
CA 4.1.2/033	[redacted]	1990	4823	Validation of analytical method no. 3750 for the analysis of glyphosate in rodent dietary formulations
CA 4.1.2/034 (CA 5.3.1/004)	[redacted]	1989	5626	4 week dietary toxicity study in rats
CA 4.1.2/042 (CA 5.3.2/011)	[redacted]	1991	7136	13 week dietary toxicity study in rats
CA 4.1.2/047 (CA 5.3.2/018)	[redacted]	1991	7024	13 week dietary toxicity study in mice
CA 4.1.2/063 (CA 5.5/007)	[redacted]	1993	7867	104 week combined chronic feeding/ oncogenicity study in rats with 52 week interim kill (results after 104 weeks)

Table 5.1-23: Overview on toxicological studies which used the analytical method

Data point	Report authors	Report year	Report number	Report title
CA 4.1.2/069 (CA 5.5/020)	[redacted]	1993	7793	104 week dietary carcinogenicity study in mice

Chromatographic conditions:

HPLC:	Pye Unicam Model 4700 autoinjector coupled to an Antex 110A pump; data handling was performed on a Trivector 2000 Data Station
Column:	Hichrom Limited, Nucleosil 120, 250 x 4.6 mm i.d., 5-C_{18} (Column No. 2794)
Column temperature:	Not stated.
Injection volume:	20 µL
Mobile phase:	0.02 M tetraethylammonium bromide, 0.05 M sodium dihydrogen orthophosphate adjusted to pH = 3.0 using orthophosphoric acid: acetonitrile (5/1, v/v)
Flow rate:	1.5 mL/min
Derivatisation agent:	l-fluoro-2,4-dinitrobenzene (FDNB)
Detector:	Pye Unicam 4020 UV spectrophotometric detector, UV at 383 nm
Retention time:	Glyphosate: ~ 3.8 min

Findings

Recoveries

In a first step, recoveries are provided from test diet analyses. Test diets were prepared in all studies in order to achieve the dosing levels expressed as mg/kg bw/day at several time points, adjusted for the body weights of the animals. These preparations were sampled and analysed in triplicate or more using the analytical method. Control blank diets were also analysed at each time point without detecting glyphosate above the limit of detection. Average recovery values between 70 % and 110 % were found at each dosing level and overall, with relative standard deviations (RSDs) of <20 %. The detailed results provided in the summary of applicant correspond to recalculated value based on different studies. However, the detail of the calcul performed was not provided an dthe results were expressed as mg/kg bw/day that is not correspond to unit used to validate the nalytical method, therefore the RMS revised to table to be in qagreement with the data reported in analytical part of the studies.
The recoveries were in acceptable range for the range concentration 300 ppm to 30000ppm in formulation diets. All recoveries are not reported in the table.

Table 5.1-24: Results of the analysis of test diets (revised by RMS)

Report No.	Matrix	Analyte	Nominal dosing level (ppm)	Recovery[1]		
				Mean (%)	Relative standard deviation (%)	Number analyses[2] (n)
7136	Rodent diet	Glyphosate	397	93	1.33	3
			521	87.5	21	3
			3386	98	4.6	3
			5142	95	4.8	3
			11623	101	16.5	3
			16781	96	3.7	10

Table 5.1-24: Results of the analysis of test diets (revised by RMS)

Report No.	Matrix	Analyte	Nominal dosing level (ppm)	Recovery[1]		
				Mean (%)	Relative standard deviation (%)	Number analyses[2] (n)
			225	101.8	2.4	3
			233	100	3.4	3
			2250	89	25.4	3
			2325	99.8	7.7	3
			7667	96.6	9.9	3
			7917	104.1	4.4	3
			2394	99.3	4.2	5
			325	96.2	2.6	4
			358	108.2	7.9	4
			2928	99.3	1.9	5
			3727	98.5	2.7	5
			10511	97	2.7	5
			11364	104.1	1.8	5
7024	Rodent diet	Glyphosate	4262	103	3.8	5
			5738	97.4	4.8	5
			970	90.3	4.1	3
			1188	91.4	0.6	3
			4508	85.8	7.1	3
			6678	85.2	9.8	3
			20631	102.8	4.8	3
			27218	103.6	4.3	3
7793	Rodent diet	Glyphosate	388	94.9	5.5	3
			351	91.7	2.2	3
			1196	92.9	2.1	3
			1020	95.8	2.4	3
			4063	90.9	3.3	3
			3486	95.8	0.9	3
			454	94.3	1.1	3
			342	101.8	0.6	3
			1327	94.6	1.1	3
			1032	94.2	0.4	3

Table 5.1-24: Results of the analysis of test diets (revised by RMS)

Report No.	Matrix	Analyte	Nominal dosing level (ppm)	Recovery[1]		
				Mean (%)	Relative standard deviation (%)	Number analyses[2] (n)
			4636	98.8	1.4	3
			3494	98.4	1.8	3

[1]

Additionally, ten samples of rat and mouse diets were prepared at the 30, 300, 120 and 14200 mg/kg level by direct addition of the test substance to untreated rat and mouse type 1 diet. The samples were analysed along with standard samples at each level of analysis. The overall assay accuracy was 101 %. The overall precision expressed as the RSD was 7.0 %. The results are shown in the table below.

Table 5.1-25: Results of the determination of assay accuracy and precision

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
4823	Rodent diet	Glyphosate	30	96 – 115	106	6.1	5.7	10
			300	89 – 114	106	7.2	6.8	9
			120	91 – 99	94	3.2	3.4	9
			14200	97 – 107	99	3.2	3.3	10
			Overall	89 – 115	101	7.1	7.0	38

[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 recovery values as given in the report.

Furthermore, the homogeneity of dietary preparations (SDS rat and mouse no. 1 or 3 diet) containing glyphosate at levels of 30 to 300 mg/kg or 50 to 5000 mg/kg, respectively, was analysed. The results are shown in the tables below and indicate satisfactory homogeneity and accuracy of mixing using both types of diet.

Table 5.1-26: Assessment of homogeneity and accuracy of SDS rat and mouse no. 1 diet

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
4823	Rodent diet	Glyphosate	30	91 – 104	96	4.2	4.4	9
			300	78 – 103	90	7.6	8.5	10

Table 5.1-26: Assessment of homogeneity and accuracy of SDS rat and mouse no. 1 diet

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			Overall	78 – 104	93	6.7	7.3	19

[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 recovery values as given in the report.

Table 5.1-27: Assessment of homogeneity and accuracy of SDS rat and mouse no. 3 diet

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
4823	Rodent diet	Glyphosate	50	96 – 102	99	2.7	2.7	8
			5000	95 – 105	101	3.1	3.1	10
			Overall	95 – 105	100	2.9	2.9	18

[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 recovery values as given in the report.

In addition, samples of type 1 diet containing both 30 mg/kg and 300 mg/kg or glyphosate were stored at ambient temperature in the dark. Subsamples were analysed after 7, 14 and 25 days. Samples of type 3 diet containing glyphosate at both 50 mg/kg and 5000 mg/kg were stored in the same manner and analysed after 21 days. The results are shown in the tables below.

Table 5.1-28: Assessment of stability of glyphosate on SDS rat and mouse no. 1 diet

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
4823	Rodent diet	Glyphosate	30	91 – 113	101	6.6	6.5	24
			300	78 – 108	93	7.2	7.7	25
			Overall	78 – 113	97	7.8	8.1	49

[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 recovery values as given in the report.

Table 5.1-29: Assessment of stability of glyphosate on SDS rat and mouse no. 3 diet

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
4823	Rodent diet	Glyphosate	50	96 – 102	100	2.4	2.4	13
			5000	93 – 105	100	3.4	3.4	15
			Overall	93 – 105	100	2.9	2.9	28

[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 recovery values as given in the report.

Specificity
Chromatogram of standards solution, of control sample, of fortified sample are not provided. Specificity is not demonstrated. Nevertheless, regarding data on the recoveries and %RSD, the specificity can be considered as acceptable. As the method will be no longer used for data generation, no additional confirmatory technique is necessary.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 3.072 to 12.288 µg/mL.The equivalency in mg/kg was not reporyed in the analytical report The correlation coefficient was determined during validation to be 0.9976 using a linear regression.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for rodent diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in rodent diets.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level. They were performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided.. Interferences and extracts and matrix effects were not assessed.
However, recovery and repeatability data are acceptable. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in rodent diet.

Determination of glyphosate in mouse diet

Study previously submitted to the EU (Analytical part submitted to the EU for the first time)

1. Information on the study

Data point	CA 4.1.2/035
Report authors	[redacted]
Report year	1978
Report title	Analysis of animal feed diets in the glyphosate 4-week mouse pilot study, performed at Bio/Dynamics Inc.
Report No	MSL0000462
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • 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	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Test facility	Monsanto, Agricultural Research Department, St. Louis, Missouri 63110, USA
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point:	CA 4.1.2/036 (CA 5.3.1/006)
Report authors	[redacted]
Report year	1978
Report title	A four week pilot study with glyphosate in mice
Report No	77-2110
Document No	[redacted] 77-418
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Analytical methods were reported separately (see [redacted] 1978)

Previous evaluation	No, not accepted in RAR 2015
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Test facility	[redacted]
Acceptability/Reliability:	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

The analyses for the above mentioned toxicological study ([redacted], Report No. 77-2110) were reported within a separate report ([redacted] Report No. MSL0000462).

Principle of the method

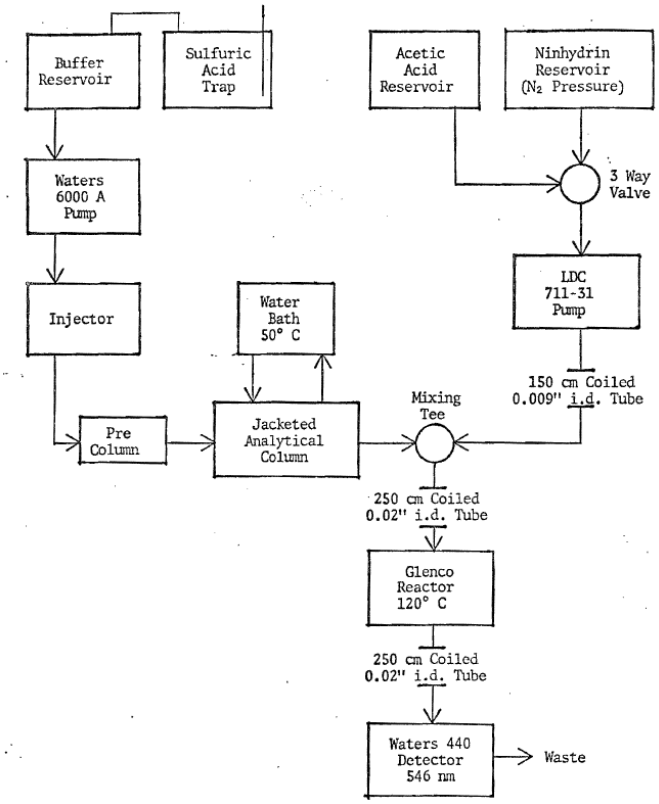
The method was developed for the determination of glyphosate in animal feed diets [redacted], Report No. 77-2110: Purina Laboratory Chow® and glyphosate) by HPLC-UV.

An aliquot (10 g) of mixed animal diet was extracted with deionised water and chloroform (2/1, v/v) by shaking for 30 minutes. The extract was centrifuged and an aliquot of the aqueous layer (2 mL) was withdrawn. The remaining extract was decanted off and the extraction step was repeated for a second time. A second aliquot (2 mL) was withdrawn and the remaining extracts combined. The solution was filtered, layers separated and the organic layer was discarded. The final volume of the aqueous layer was determined as this was necessary for calculation purposes. The two aliquots of the aqueous phase were combined and subjected to an ion exchange resin cleanup (AG 50W-X8, 200 – 400 mesh hydrogen form analytical grade cation exchange resin, Bio-Rad Laboratories Richmond, Calif.). The eluant is filtered, diluted appropriately and subjected to HPLC system fitted with a ninhydrin post column reactor and measuring the colour generated using a UV detector. A graphical illustration of the HPLC system used is given in the figure below. Sample quantitation is based on the relative sample peak height/area to standard peak heights/areas across the range of expected sample concentrations.

Chromatographic conditions:

HPLC-system:	Waters 6000A pump with a Waters U6K injector or a Varian 8500 autosampler LDC 711-31 pump, and column heater block (Waters - 84119) Glenco RC-1 reaction coil (120 °C)
HPLC column:	Aminex A-9, 30 cm x 4.6 mm i.d.
Guard column:	C_{18}/Corasil, 4.5 cm x 0.6 cm o.d. 0.3 cm i.d.
Column temperature:	50 °C
Injection volume:	Not given within the report
Mobile phase:	HPLC buffer solution: 0.005 M potassium dihydrogen phosaphate in 4 % methanol/deionisied water (adjusted to pH 1.9 by phosphoric acid) Ninhydrin-solution: Solution of 80 g ninhydrin and 2.5 g hydrindantin in a solvent-mixture of dimethyl-sulfoxide, deionisied water and 4.0 M sodium acetate solution (3/2/1, v/v/v; stored for a maximum of two weeks under N_2)
Flow rate:	0.5 mL/min (buffer flow rate) 0.5 mL/min (ninhydrin flow rate)
Pressure.	~2000 psi buffer ~600 psi ninhydrin
Derivatisation agent:	Ninhydrin post column reactor
Detection:	Waters Model 440 Absorbance detector with 546 nm filter
Retention time:	Glyphosate: not readable within the chromatograms

Figure 5.1-1: Graphical illustration of the HPLC system used for analysis.



Findings

Recoveries

The method proved to be suitable to determine glyphosate in animal feed diets. Samples were spiked with the analyte at 3 fortification levels at 100, 300 and 1000 mg/kg. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-30: Results of the method validation for the determination of glyphosate in animal feed diets

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
77-2110	Mouse diet	Glyphosate	100	79 – 95	87	8.3	9.5	4
			300	85 – 98	92	4.8	5.2	8
			1000	92 – 96	93	1.7	1.9	4
			Overall	79 – 98	91	5.5	6.1	16
MSL-0462	Feed diet	Glyphosate	100	79.2 – 94.6	87	8.3	9.5	4
			300	84.6 – 97.6	91.6	4.7	5.2	8
			1000	92.7 - 93	93.25	1.7	1.8	4

[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 recovery values as given in the report.

Specificity
The chromatogram of a control sample did not reveal any significant interferences, which would interfere with the determination of glyphosate.
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Linearity
The calibration standards were prepared in deionised water with concentrations in the range of 0.1 µg/mL to 50.0 µg/mL (n=9). The equivalency in mg/kg was not reported in the analytical part of the studies Information about calibration curves and functions is missing.

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found for rodent diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The sensitivity was stated to be 25 mg/kg for a 10 g sample. Acceptable recoveries were achieved at the lowest fortification level of 100 mg/kg.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in animal diets.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was not performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve and function not given, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil all European requirements for risk assessment methods as defined by SANCO/3029/99 rev. 4: calibration curves, equations and correlation parameters are not given, matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable. Therefore, the method can be considered as fit for purpose.

Determination of glyphosate acid in rat diet

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/037 (CA 5.3.2/001, CA 5.3.2/002)
Report author(s)	[redacted]
Report year	1996
Report title	First revision to Glyphosate acid: 90 day feeding study in rats
Report No	[redacted]/P/1599
Document No	PR0663 ([redacted] Study No)
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Validation with fortified samples not provided • Limited data on calibration provided • Chromatograms not provided • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes accepted in RAR 2015
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat CT1 diet by HPLC-UV. Accurately weighed samples of diet were extracted by mechanical shaking with distilled water. The extracts were centrifuged and diluted with water as required to give solutions containing theoretically between 50 and 400 µg/mL glyphosate acid. Aliquots were analysed by high performance liquid chromatography (HPLC) after pre-column derivatisation with UV detection at 265 nm using an external standard procedure.
For derivatisation, diet extracts were warmed to room temperature, 1 mL aliquots transferred to vials and 1 mL di-sodium tetraborate and 2 mL 9-fluorenylmethyl chloroformate added. After shaking the vials for about 10 minutes, 10 mL ethyl acetate were added, briefly shaken and left to stand for 5 minutes. Aliquots of the lower aqueous layer were subjected to analysis by HPLC-UV.

Chromatographic conditions:

HPLC system:	LDC Constametric III pump with LDC Spectromonitor III uv detector at 265nm; Trilab 2000 data system (Trivector Scientific)
Column:	Spherisorb S5NH, 125 x 4.9 mm ID (Hitchrom Ltd)
Column oven temperature:	Not provided
Injection volume:	10 µL
Mobile phase:	Acetonitrile/0.025 M KH_2PO_4 (50/15, v/v)

Flow rate:	2 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMOC-Cl)
Detection:	UV at 265 nm
Retention time:	Glyphosate acid: not provided (no chromatograms available)

Findings

Recoveries

Rat CT1 diets were prepared at three concentration levels, i.e. 1000, 5000 and 20000 mg glyphosate acid/kg diet. Aliquots were taken at two occasions and analysed for glyphosate acid content. The results are shown in table below. These are not true procedural recovery data, however they show the robustness of the analytical method.

Table 5.1-31: Results of test CT1 diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat CT1 diet	Glyphosate acid	1000	93– 98	96	–	–	2
		5000	100 – 102	101	–	–	2
		20000	96 – 98	97	–	–	2

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

Additionally, the homogeneity of dosing formulation was tested by analysis of samples from three different trays. The results are shown in table below. Acceptable recoveries were obtained using the analytical method.

Table 5.1-32: Results of rat CT1 diet analyses for dosing formulation homogeneity approval

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat CT1 diet	Glyphosate acid	1000	93 – 94	93	0.5	0.6	3
		20000	97 – 102	100	2.5	2.5	3

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

Specificity

Chromatograms are not provided in the report.

Linearity

Two linearity curves were produced to cover the wide range of concentration. The first function was tested using 4 calibration standard concentrations in the range of 500 – 5000 mg/kg diet, and the second one to cover the range of 5000 – 20000 mg/kg diet (duplicate determinations). Extracts were diluted to produce solutions in the range of 50 – 400 µg/mL. Details to the calibration functions are not provided in the report.

Repeatability (Precision)
The relative standard deviations (RSDs) of repeated diet analyses at each concentration level were < 20 % (required according to SANCO/3029/99 rev. 4).

Accuracy
Acceptable mean recovery values at all diet concentrations were between 70 % and 110 % for glyphosate acid (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
It is stated in the report that the detection limit was calculated to be equivalent to approximately 50 mg/kg for glyphosate acid in the diet.

Matrix effects
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. However, it is considered as fit-for-purpose for the determination of glyphosate acid in rat CT1 diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (validation with fortified samples not provided, limited data on calibration provided, chromatograms not provided, matrix effects not assessed, efficiency of derivatisation not assessed). However the analytical method is considered as fit-for-purpose in support of the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are accepatble, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat CT1 diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/038 (CA 5.3.2/003)
Report author(s)	[redacted]
Report year	1996
Report title	Technical glyphosate: ninety day sub-chronic oral (dietary) toxicity study in the rat
Report No.	434/016
Document No.	-
Guidelines followed in study	Not stated (with relevance to analytical methods)

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No details to calibration provided • Matrix effect not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet (Rat and Mouse SQC Ground Diet No. 1) by HPLC-Fluorescence.

The test material dietary admixtures were extracted with 0.1 M disodium tetraborate to give a final theoretical concentration of 50 mg/L. A 5 mL aliquot of the extract was derivatised using 9-fluoroenyl methyl chloroformate (1 % w/v in acetone). After approximately 30 minutes the solution was partitioned with toluene (10 mL) and the remaining aqueous phase was analysed by high performance liquid chromatography (HPLC) with Fluorescence detection at excitation 254 nm, emission 310 nm using an external standard procedure.

Chromatographic conditions:

Column:	Spherisorb SAX 55, 250 x 4.6 mm ID
Column oven temperature:	Not provided
Injection volume:	100 µL
Mobile phase:	Water/acetonitrile/glacial acedic acid/orthophoshoric acid (800/200/10/5, v/v/v/v)
Flow rate:	2 mL/min
Derivatisation agent:	9-fluorenylmethyl chloroformate (FMOC)
Detection:	Excitation 254 nm, emission 310 nm
Retention time:	Glyphosate: ~ 10.3 minutes

Findings
Recoveries
Rat diets (Rat and Mouse SQC Ground Diet No. 1) were prepared at three concentration levels, i.e. 1000, 10000 and 50000 mg glyphosate technical/kg diet. The mixtures were prepared at three occasions, i.e. prior to treatment and twice during the three month study period, using Hobard mixers. Aliquots were taken from these three prepared mixtures from different sampling locations in the mixer, i.e. from the middle and from opposite sides, and analysed for the content of glyphosate. The results are shown in table below. These are not true validation recovery data, however they show the robustness of the analytical method.

Table 5.1-33: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1000	104 – 116	109	4.0	3.7	9
		10000	95 – 110	102	4.9	4.8	9
		50000	87 – 101	93	5.1	5.4	9

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

Specificity
Chromatograms of standard solutions, control diet admixture and fortified (test material) admixtures are provided in the report. No interfering peaks were observed at the retention time of glyphosate technical.

Linearity
No details are provided to the calibration functions.

Repeatability (Precision)
The relative standard deviations (RSDs) of repeated diet analyses at each concentration level were <20 % (required according to SANCO/3029/99 rev. 4).

Accuracy
Acceptable mean recovery values at all diet concentrations were between 70 % and 110 % for glyphosate technical (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
Not reported. The method has shown acceptable performance at all concentration levels.

Matrix effects
Not assessed. Standard solutions for calibration were prepared in solvent (0.1 M disodium tetraborate).

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000). However, it is considered as fit-for-purpose for the determination of glyphosate technical in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no details to calibration provided, matrix effect not assessed, efficiency of derivatisation not assessed). The method is fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/039 (CA 5.3.2/004)
Report author(s)	[redacted]
Report year	1995
Report title	HR-001: 13-week subchronic oral toxicity study in rats
Report No.	[redacted] 94-0138
Document No.	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No chromatograms provided • Matrix effect not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet by HPLC-Fluorescence.
Aliquots of the test material dietary admixtures (5 g) were accurately weighted into an Erlenmeyer flask and extracted with water (100 mL). The extract was filtered and diluted with water to obtain a solution containing approximately 1 mg/L glyphosate. An aliquot (1 mL) of this solution was evaporated to dryness, and the residue is re-dissolved in 0.05 M tetraborate solution (5 mL). The residue was then derivatised using 9-fluorenylmethyl chloroformate (FMCF) for 20 minutes. Following addition of ethyl acetate, the flask was shaken for 1 minute, and an aliquot of the separated aqueous phase was analysed by high performance liquid chromatography (HPLC) with fluorescence detection at excitation 255 nm, emission 315 nm using an external standard procedure.

Chromatographic conditions:

HPLC:	L-4000W (Yanagimoto)
Column:	SAX-1253-P, 250 x 4.6 mm ID
Column oven temperature:	40 °C
Injection volume:	10 µL

Mobile phase:	Acetonitrile/water/phosphate buffer (pH 2.5) (35/54/11, v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMCF)
Detection:	650-lOS, fluorescence spectrophotometer (Hitachi), wavelength excitation: 255 nm, emission; 315 nm
Retention time:	Glyphosate: not stated (no chromatograms provided in the report)

Findings

Recoveries

For method validation, rat diets were fortified at relevant concentrations of 3000, 10000 and 30000 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD (<2 mg/kg). The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-34: Results of the method validation (spike recovery) for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	3000	95 – 100	98	–	–	2
		10000	92 – 93	93	–	–	2
		30000	94 – 95	95	–	–	2

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

Additionally duplicate samples of test diets prepared at five different time points (6 samples from initial preparation) 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 performance of the method.

Table 5.1-35: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	3000	90 – 107	96	5.0	5.2	14
		10000	88 – 105	95	5.0	5.3	14
		30000	90 – 109	95	6.3	6.6	14

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

Specificity

Chromatograms are not provided in the report.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL. the equivalency in mg/kg is not available. The calibration graph was linear in this range with correlation coefficients of > 0.999. The calibration standards were prepared in solvent (water) and derivatised as described above. All glyphosate determinations were chromatographed at concentrations within this linear range. Linearity plots and calibration functions are not provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection (LOD) of the method was stated to be 2 mg/kg.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. However, the method is considered as fit-for-purpose for the determination of glyphosate in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no chromatograms provided, matrix effect not assessed, efficiency of derivatisation not assessed. The method is fit for purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/040 (CA 5.3.2/005, CA 5.3.2/006, CA 5.3.2/007)
Report author(s)	[redacted]
Report year	1993
Report title	90 day range finding study of glyphosate in rats
Report No.	011-0001
Document No.	-

Guidelines followed in study	Not stated (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
Test facility	[redacted]
Acceptability/Reliability	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet by HPLC-FLD. The method is based on reference method US EPA Method 547 (July 1990) "Determination of glyphosate in drinking water by direct aqueous injection HPLC, post-column derivatisation, and fluorescence detection".

The test material dietary admixtures (1 g) were extracted with water. The extract was diluted with water to obtain a solution containing approximately 2 mg/L. The analyte was derivatised at post-column using hypochlorite (formation of glycine) followed by treatment with o-phthaldeyde and 2-mercaptoethanol (formation of 1-hydoxyethylthio-2-methylisoindole). The derivate was analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 330 nm excitation/465 nm emission using an external standard procedure.

Chromatographic conditions:

Column:	Hamilton RPR X-400, 250 x 4.1 mm ID
Column oven temperature:	38 °C
Injection volume:	50 µL
Mobile phase:	5 mM aqueous KH_2PO_4 (pH 2)
Flow rate:	0.5 mL/min
Derivatisation agent:	OPA (phthaldialdehyde) reagent
Detection:	Fluorescence at 330 nm excitation/465 nm emission
Retention time:	Glyphosate: ~ 6.1 minutes

Findings
Recoveries
For method validation, rat diets were fortified at relevant concentrations of 2000 and 20000 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD. The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-36: Results of the method validation (spike recovery) for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000	102 – 103	102	0.4	0.4	5
		20000	99 – 102	101	1.0	1.0	5

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

Specificity
Chromatograms of standard solutions, control diet admixture and fortified admixtures are provided in the report. No interfering peaks were observed at the retention time of glyphosate technical.

Linearity
Linearity of detector response was tested using 6 calibration standard concentrations in the range of 0.108 to 10.79 µg/mL with correlation coefficients of > 0.999. he equivalency in mg/kg is not available.The calibration standards were prepared in solvent (water). The calibration graph was linear in this range (y = 816.45 x + 19.89). All glyphosate determinations were chromatographed at concentrations within this linear range.

Repeatability (Precision)
The relative standard deviations (RSDs) of repeated diet analyses at each fortification level were < 20 %. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.
Additional samples at each of two concentration levels were analysed in quintuplet in order to validate the precision of the method. Acceptable RSDs were found in this analytical set, details are provided in the table below.

Table 5.1-37: Results of the method validation (precision test, analysis of test diets) for the determination of glyphosate in rat diet

Matrix	Analyte	Nominal concentra-tion (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate technical	2000	106 – 112	108	2.3	2.1	5
		20000	104 – 107	106	1.3	1.2	5

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was calculated from the lowest standard concentration in linearity tests. The LOQ of the method was stated to be 100 mg/kg.

The limit of detection (LOD) was calculated using the lowest standard concentration for the instrument detection limit (0.01 mg/kg). The LOD describes injections that show no detectable signal and are below the region of less-certain quantification. The LOD of the method was stated to be 10 mg/kg.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

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 is considered as fit-for-purpose for the determination of glyphosate in rat diet.

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 in most points (EU guideline SANCO/3029/99 rev. 4) with very minor deficits (matrix effects not assessed). The method is fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, linearitry, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/041 (CA 5.3.2/008, CA 5.3.2/009, CA 5.3.2/010)
Report authors	[redacted]
Report year	1992
Report title	90-Day Oral Toxicity Study in Wistar Rats with Glyphosate Technical (FSG 03090 H/05 March 1990); Amendment to Final Report. 90-Day Oral Toxicity Study in Wistar Rats
Report No	TOXI: [redacted].882.90 OR
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Insufficient data available to provide assessment • Only the results of test diet analyses are available, which are acceptable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
It is stated in the report that prepared test diets (Standard "Gold Mohur" brand powdered rat feed, M/S Lipton India Ltd) were sampled at five time points and analysed using a recommended method for extraction and clean-up and analysis by HPLC. The following reference is made to the method:
Jarczyk, van H.J. in Pflanzenschutz-Nachrichten Bayer (1986): 39(1): 73 – 92, with slight modifications. No further details are provided to the method.

Findings
Recoveries
Test diets were prepared and sampled at five time points and analysed using the analytical method. The average recovery values at each fortification level were between 70 % and 110 %. Control blank diets were also analysed without detecting glyphosate. The results are summarised in the table below.

Table 5.1-38: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	200	96– 99	97	1.2	1.2	5
		2000	98 – 100	99	1.0	1.0	5
		20000	98 – 99	99	0.4	0.4	5

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

Specificity
Insufficient data available to provide assessment.

Linearity
Insufficient data available to provide assessment.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were <20 %, therefore in compliance with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not reported. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Insufficient data available to provide assessment.

Conclusion
Sufficient data are not available to provide an assessment on the validity of the analytical phase of the study concerned. The results of the test diet analyses show good performance of the method and the nominal test concentrations are verified by analytical data.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. Sufficient data are not available to provide an assessment on the validity of the analytical phase of the study concerned. The results of the test diet analyses show good performance of the method and the nominal test concentrations are verified by analytical data.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/043 (CA 5.3.2/012)
Report author(s)	[redacted]
Report year	1990
Report title	Glyphosate technical: 90 day oral toxicity study in the rat
Report No	[redacted]-900914
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods) OECD 408
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited true validation recoveries given, recoveries calculated from analysis of rat diet • Limited information to calibration curve given • Interference not clearly assessed (limited chromatograms provided) • Matrix effect not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]

Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate technical in rat diet by HPLC-UV. Accurately weighted samples of diet (5 g) were extracted three times by mechanical shaking with distilled water and chloroform. Following centrifugation, the aqueous phases were combined and cleaned up by chromatography on a cation-exchange column (Dowex-50 W, hydrogen form, 8 % cross-linked, 200 – 400 dry mesh). The purified sample extracts were analysed for glyphosate by high performance liquid chromatography (HPLC) with UV detection at 195 nm using an external standard procedure.

Chromatographic conditions:

Column:	Partisil 10 SAX, 250 x 4.6 mm ID (HPLC Technology, Cheshire, UK)
Column oven temperature:	Not provided
Injection volume:	25 µL
Mobile phase:	4 % methanol in 0.004 M KH_2PO_4 (adjusted to pH 2.1 with 85 % H_3PO_4)
Flow rate:	2.3 mL/min
Derivatisation agent:	Not derivatised
Detection:	UV at 195 nm
Retention time:	Glyphosate: ~ 4.5 min

Findings
Recoveries
Rat diets (special quality control powdered diet; SDS Ltd, Witham, Essex, UK) were fortified at relevant concentrations of 2000, 5000 and 7500 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate. The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-39: Results of method validation (spike recovery) for the determination of glyphosate in rat diet

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000	94 – 104	98	4.3	4.3	4
		5000	77 – 86	81	4.2	5.2	3
		7500	81 – 92	85	5.9	6.9	3

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

Rat diets for the study were prepared at four occasions and samples were taken from the bottom, middle and top of the mixing device for analysis of glyphosate content. The results are shown in table below.

The glyphosate levels determined in the diet samples were generally lower than the declared levels of 2000, 5000 and 7500 mg/kg. Therefore, correction of the results for the recovery of added glyphosate from fortified samples was made. The peculiarly low and high results for some of the batch C samples (third test diet preparation) were confirmed by repeat analyses of these samples.

Table 5.1-40: Results of test diet analyses for dosing formulation test and homogeneity approval (results are corrected for recoveries detected from spiked samples)

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000	80.0 – 119.1	98	12.7	12.9	12
		5000	46.2 – 105.8	81	17.5	21.7	12
		7500	79.3 – 102.8	90	7.3	8.1	11[2]

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

[2] One very high recovery/glyphosate content was treated as an outlier.

Specificity

Chromatograms of standards, fortified diet samples and test diet samples are provided in the report. No major interfering peaks could be observed.

Linearity

Linearity was assessed by plotting peak heights of five standard solutions prepared in water covering the range of 100 to 500 µg/mL against concentration (duplicate determinations). The equivalency in mg/kf is not available. Linear relationships were found for each of 10 calibrations. Peak heights, linearity functions and plots are provided in the report, however no coefficients of correlation.

Repeatability (Precision)

The relative standard deviations (RSDs) during method validation using recoveries from spiked samples at each concentration level were < 20 % (required according to SANCO/3029/99 rev. 4).

Accuracy

During method validation with fortified samples (spike recovery), acceptable mean recovery values at all diet concentrations were between 70 % and 110 % for glyphosate technical (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection

Not stated in the report. The lowest standard used for linearity testing/calibration was 100 µg/mL.

Matrix effects

Not assessed.

Conclusion

The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate technical in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries given, limited information to calibration, matrix effect not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/044 (CA 5.3.2/013)
Report author(s)	[redacted]
Report year	1989
Report title	Glyphosate technical: 90 day oral toxicity study in the rat
Report No.	[redacted]-891002
Document No.	Study No [redacted]-401
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of rat diet • Missing information to calibration and linearity • LOQ not reported • Matrix effect not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet by HPLC-UV.
Samples of diet (10 g) were extracted twice by mechanical shaking with hot deionised water. The extracts were filtered, combined and evaporated to dryness under vacuum. The residue was redissolved in water and cleaned up on an equilibrated anion exchange resin (AG 1XB) column. The eluate was evaporated to dryness and redissolved in HPLC mobile phase for analysis by HPLC with UV detection at 195 nm using an external standard procedure.

Chromatographic conditions:

Column:	Partisil 10 SAX, 25 cm x 4.6 mm ID
Column oven temperature:	Not provided
Injection volume:	25 µL
Mobile phase:	KH_2PO_4 (0.005 M)/MeOH (pH 2.4 – 2.5) (96/4, v/v)
Flow rate:	2.3 mL/min
Derivatisation agent (pre-column):	No derivatisation
Detection:	UV at 195 nm
Retention time:	Glyphosate: ~7.9 min (it is stated that the retention time of glyphosate is pH dependent, i.e. increasing the pH of the mobile phase increases the retention time of glyphosate)

Findings

Recoveries

Rat test diets were prepared at two occasions at four concentration levels, i.e. 2000, 3000, 5000 and 7500 mg glyphosate/kg diet. Following preparation, aliquots were taken from three locations of the mixing vessel (bottom, middle, top) and analysed for glyphosate content. The results are shown in table below. These are not true procedural recovery data, however, they show the robustness of the analytical method and the achieved test concentrations.

Table 5.1-41: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000	94 – 111	102	9.2	9.0	4
		3000	96 – 109	101	5.2	5.2	5
		5000	96 – 108	102	5.8	5.7	5
		7500	88 – 109	96	6.8	7.1	11

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

Specificity

Chromatograms of standards and analytical samples are provided in the report. No interfering peaks are present at the retention time of glyphosate

Linearity

No information is reported on calibration and linearity.

Repeatability (Precision)

The relative standard deviations (RSDs) of repeated diet analyses at each concentration level were < 20 % (required according to SANCO/3029/99 rev. 4).

Accuracy

Acceptable mean recovery values at all diet concentrations were between 70 % and 110 % for glyphosate acid (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
No information provided in the report.

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Matrix effects
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no true validation recoveries given, missing information to calibration and linearity, LOQ not reported, matrix effect not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, matrix effects are not assessed.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/045 (CA 5.3.2/014)
Report author(s)	[redacted]
Report year	1987
Report title	90-day study of glyphosate administered in feed to Sprague-Dawley rats
Report No.	[redacted]-7375
Document No.	[redacted]-86-351/[redacted] 86128
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of rat diet • Details to calibration curve not given • LOQ/LOD not stated • Limited chromatograms provided • Matrix effect not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]

Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet by HPLC-UVD.
Aliquots of the test material dietary admixtures (10 g) were accurately weighted into an iodine flask and extracted with water (40 mL) and chloroform (15 mL) under agitation for 30 minutes. The extract was left for stand for 15-20 minutes and an aliquot (6-8 mL) was centrifuged for 10 minutes. An aliquot of the extract (1.2 mL) was treated with 0.37 M tetraborate solution (0.8 mL) and 25 mM NBD-CL (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole; 2.4 mL) solution for derivatisation. The mixture was heated to 80°C for 15 minutes and 1.2 N HCl solution (0.9 mL) was added. The sample was then filtered (0.2 micron) and analysed by high performance liquid chromatography (HPLC) with UV detection at 500 nm using an external standard procedure.

Chromatographic conditions:

HPLC system:	HP 1090 LC with autosampler and SP 4270 integrator
Column:	Spherex C-18 (Phenomenex), 250 x 4.6 mm ID
Pre-column:	Brownlee C-18, 30 x 4.6 mm ID
Column oven temperature:	Ambient
Injection volume:	15 µL
Mobile phase:	(A) 0.01 M phosphate buffer (KH_2PO_4; pH 3.6) (B) Acetonitrile
Gradient:	Time (min) / Eluent A (%) / Eluent B (%) 0-5 / 95 / 5 5-10 / 50 / 50 10-15 / 95 / 5
Flow rate:	Not reported
Derivatisation agent:	4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)
Detection:	UV at 500 nm
Retention time:	Glyphosate: ~ 3.35 min

Findings
Recoveries
For method validation, rat diets (Ralston Purina RODENT CHOW No. 5002) were fortified at relevant concentrations of about 1000, 5000 and 20000 mg/kg at 8 different time points (concurrent with test diet preparation) and analysed using the analytical method. The recovery results are shown in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-42: Results of the method validation (spike recovery) for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1000	91-100	96	4.3	4.5	8

Table 5.1-42: Results of the method validation (spike recovery) for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		5000	90-100	94	3.2	3.4	8
		20000	90-100	95	3.7	3.9	8

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

Additionally samples of test diets prepared at eight different time points 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 performance of the method and the acceptable concentrations achieved in the test diets.

Table 5.1-43: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	5000	88-110	95	7.1	7.5	8
		10000	84-100	93	4.8	5.1	9
		20000	85-100	94	5.0	5.3	9

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

Specificity
One sample chromatogram is provided in the report. No interferences are observed at the retention time of glyphosate.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 90 to 600 µg/mL. The equivalency in mg/kg is not available. No further details such as linearity plots and calibration functions are provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not reported.

Matrix effects
Not assessed. Standard and calibration samples were prepared in ultrapure water.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless the method is considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve not given, interference not assessed (only 1 chromatogram provided), matrix effect not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given,), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as it is considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/046 (CA 5.3.2/017)
Report author(s)	[redacted]
Report year	1995
Report title	HR-001: 13-week oral subchronic toxicity study in mice
Report No.	[redacted] 94-0136
Document No.	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No chromatograms provided • Matrix effect not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	[redacted]
Acceptability/Reliability	Valid (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 mouse diet by HPLC-Fluorescence. Aliquots of the test material dietary admixtures (5 g) were accurately weighted into an Erlenmeyer flask and extracted with water (100 mL). The extract was filtered diluted with water to obtain a solution containing approximately 1 mg/L. An aliquot (1 mL) of this solution was evaporated to dryness, and the residue is re-dissolved in 0.05 M tetraborate solution (5 mL). The residue was then derivatised using 9-fluorenylmethyl chloroformate (FMCF) for 20 minutes. Following addition of ethyl acetate, the flask was shaken for 1 minute, and an aliquot of the separated aqueous phase was analysed by high performance liquid chromatography (HPLC) with fluorescence detection at excitation 255 nm, emission 315 nm using an external standard procedure.

Chromatographic conditions:

HPLC:	L-4000W (Yanagimoto)
Column:	SAX-1253-P, 250 x 4.6 mm ID
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/phosphate buffer (pH 2.5) (35/54/11, v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent:	9-fluorenylmethyl chloroformate (FMCF)
Detection:	650-lOS, fluorescence spectrophotometer (Hitachi), wavelength excitation: 255 nm, emission; 315 nm
Retention time:	Glyphosate: not provided (no chromatograms available)

Findings
Recoveries
For method validation, mouse diets were fortified at relevant concentrations of 5000, 10000 and 50000 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD (< 2 mg/kg). The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-44: Results of the method validation (spike recovery) for the determination of glyphosate in mouse diet

Matrix	**Analyte**	**Fortification level (mg/kg)**	**Recovery[1]**				
			Range (%)	**Mean (%)**	**Standard deviation (%)**	**Relative standard deviation (%)**	**Number analyses (n)**
Mouse diet	Glyphosate technical	5000	95 – 96	96	~~0.7~~	~~0.7~~	2
		10000	92 – 93	93	~~0.7~~	~~0.8~~	2
		50000	92 – 94	93	~~1.4~~	~~1.5~~	2

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

Additionally duplicate samples of test diets prepared at five different time points (6 samples from each of 2 time points) were analysed using the analytical method. The results of these analyses are provided in table below. These are not true validation recovery data, however the results show the performance of the method.

Table 5.1-45: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Mouse diet	Glyphosate technical	5000	89 – 105	96	4.9	5.1	18
		10000	65 – 109	95	9.5	9.9	18
		50000	90 – 106	98	4.9	5.1	18

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

Specificity
Chromatograms are not provided in the report.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL. The equivalency in mg/kg is not available. The calibration graph was linear in this range with correlation coefficients of > 0.999. The calibration standards were prepared in solvent (water) and derivatised as described above. All glyphosate determinations were chromatographed at concentrations within this linear range. Linearity plots and calibration functions are not provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found for mouse diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection (LOD) of the method was stated to be 2 mg/kg.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. However, the method is considered as fit-for-purpose for the determination of glyphosate in mouse diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no chromatograms provided, matrix effect

not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:

The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points : interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in diet.

Determination of glyphosate in rodent feed diets

Data point	CA 4.1.2/048 (CA 5.3.2/019)
Report authors	[redacted]
Report year	1979
Report title	A three month feeding study of glyphosate (Roundup® technical) in mice
Report No	77-2111
Document No	Analytic report number MSL-1146, author [redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Supportive (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	CA 4.1.2/078 (CA 5.6.1/014)
Report authors	[redacted]
Report year	1981
Report title	A three generation reproduction study in rats with glyphosate
Report No	77-2063
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Test facility	[redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Principle of the method
The method was developed for the determination of glyphosate ([redacted], 77-2111: Purina brand rat/mouse chow and technical glyphosate or [redacted], 77-2063: Purina Lab Chow® 5001 and glyphosate) by HPLC-UV.
An aliquot (10 g) of mixed rodent diet was extracted with deionised water and chloroform (2/1, v/v) by shaking for 30 minutes. The extract was centrifuged and an aliquot of the aqueous layer (2 mL) was withdrawn. After filtration the extraction step was repeated for a second time. A second aliquot (2 mL) was withdrawn, the whole extraction solution was filtered, layers separated, and the organic layer was discarded. The final volume of the aqueous layer was determined as this was necessary for calculation purposes. The two aliquots of the aqueous phase were combined and were subjected to an ion exchange resin cleanup (AG 50W-X8, 200 – 400 mesh hydrogen form analytical grade cation exchange resin, Bio-Rad Laboratories Richmond, Calif.). The eluant is filtered, diluted appropriately and subjected to HPLC using a system fitted with a ninhydrin post column reactor and measuring the colour generated using a UV detector. A graphical illustration of the HPLC system used is given in the figure below. Sample quantitation is based on the relative sample peak height/area to standard peak heights/areas across the range of expected sample concentrations.

Chromatographic conditions:

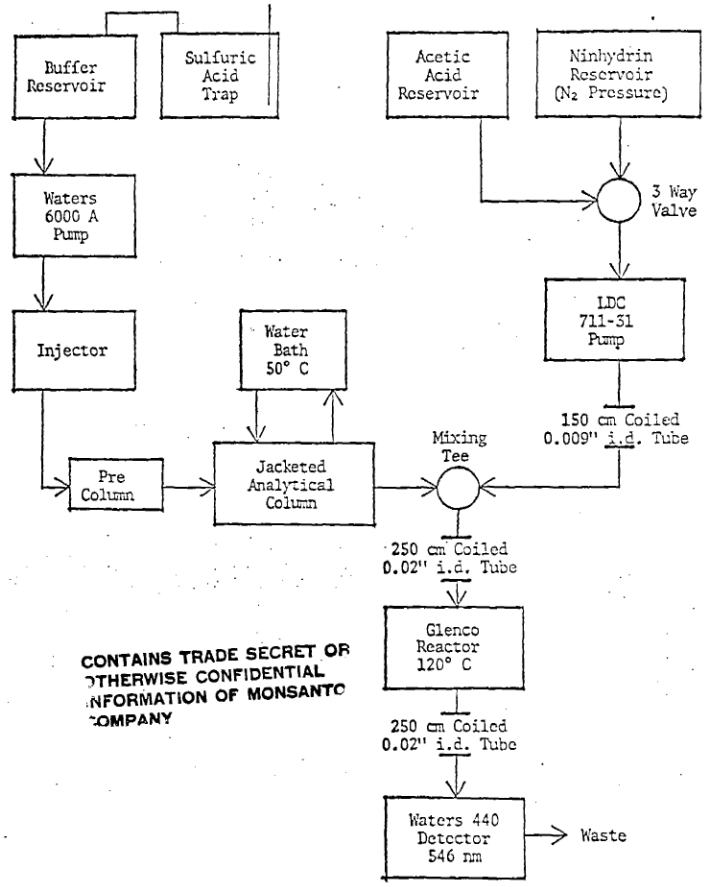
HPLC-sytem:	Waters 6000A pump with a Waters U6K injector or a Varian 8500 autosampler LDC 711-31 pump Glenco RC-1 reaction coil (120 °C)
Column:	Pre-column: C_{18}/Corasil, 4.5 cm x 0.6 cm o.d. 0.3 cm i.d. Column: Aminex A-9, 30 cm x 4.6 mm i.d.
Column temperature:	50 °C
Injection volume:	Not given within the report
Mobile phase:	HPLC buffer solution: 0.005 M potassium dihydrogen phosaphate in 4 % methanol/deionisied water (adjusted to pH 1.9 by phosphoric acid) Ninhydrin-solution: Solution of 80 g ninhydrin and 2.5 g hydrindantin in a solvent-mixture of dimethyl-sulfoxide, deionisied water and 4.0 M sodium acetate solution (3/2/1, v/v/v; stored for a maximum of two weeks under N_2)
Flow rate:	0.5 mL/min (buffer flow rate) 0.5 mL/min (ninhydrin flow rate)
Pressure:	~ 2000 psi buffer ~ 600 psi ninhydrin
Derivatisation agent:	Ninhydrin post column reactor
Detection:	Waters Model 440 Absorbance detector with 546 nm filter
Retention time:	Glyphosate: not readable within the chromatograms

This method was used within several toxicological studies; an overview of the relevant studies is given in the table below.

Table 5.1-46: Overview on toxicological studies which used above described method

Data point	Report authors	Report year	Report number	Report title
CA 4.1.2/048 (CA 5.3.2/019)	[redacted]	1979	77-2111	A three month feeding study of glyphosate (Roundup® technical) in mice.
CA 4.1.2/078 (CA 5.6.1/014)	[redacted]	1981	77-2063	A three generation reproduction study in rats with glyphosate

Figure 5.1-2: Graphical illustration of the HPLC system used for analysis.



Findings

Recoveries

In a first step, the mixing technique was verified (mixing efficiency; homogeneity) by sampling dietary admixtures from six different positions in the mixer (top, middle, bottom; left and right, respectively) in duplicate. These samples were analysed by applying the analytical method. During these analyses fortification experiments were done along with each set of samples and the results are presented in the table below. Samples were spiked with the analyte at 3 fortification levels at 5000, 10000 and 50000 mg/kg (Report No. 77-2111) or 30, 100 and 300 mg/kg (Report No. 77-2063). All average recovery values (mean of 2 to 5 replicates per fortification level) were between 70 % and 110 %.

Table 5.1-47: Results of diet preparation verification

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
77-2111 (table 6 in the study)	Rodent diet	Glyphosate	5000	86 – 104	95	12.7	13.5	2
			10000	99 – 104	101	3.5	3.4	2
			50000	93 – 99	96	4.2	4.4	2

Table 5.1-47: Results of diet preparation verification

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
77-2063	Rodent diet	Glyphosate	30	68 – 99	83	12.2	14.7	5
			100	84 – 94	89	4.9	5.5	3
			300	76 – 83	78	3.3	4.2	4
			Overall	68 – 99	83	8.9	10.8	12

In a second step, the feed diet was analysed at seven time points during study duration in duplicate (13 weeks, Report No. 77-2111) or at 15 time points during the study duration (95 weeks, Report No. 77-2063). These samples were analysed by applying the analytical method. During these analyses fortification experiments were done along with each set of samples and the results are presented in the table below. Samples were spiked with the analyte at 3 fortification levels at 5000, 10000 and 50000 mg/kg (Report No. 77-2111) or at 11 fortification levels in the range of 30 to 1000 mg/kg (Report No. 77-2063). All average recovery values (mean of 2 to 16 replicates per fortification level) were between 70 % and 110 %.

Table 5.1-48: Results of test diet analyses

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
77-2111	Rodent diet	Glyphosate	5000	93 – 109	101	4.7	4.7	10
			10000	93 – 109	102	5.0	4.9	8
			50000	93 – 108	102	5.3	5.2	6
77-2063	Rodent diet	Glyphosate	30	93 – 110	105	5.8	5.6	6
			40	85 – 105	96	7.4	7.7	7
			50	89 – 111	103	6.0	5.8	12
			100	93 – 104	99	3.4	3.4	16
			150	85 – 105	95	7.6	8.0	8
			200	93 – 93	93	0.1	0.2	2
			300	94 – 96	95	0.8	0.9	5
			400	95 – 98	96	1.3	1.4	5
			450	85 – 90	87	2.5	2.9	4
			500	88 – 108	98	8.0	8.2	7
			1000	102 – 110	106	5.7	5.3	2
			Overall	85 – 111	98	6.8	6.9	74

Table 5.1-48: Results of test diet analyses

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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 recovery values as given in the report.

Specificity
Report 77-2111: chromatograms of fortified samples at 50000ppm, of treated samples, of standards solution and of control sample are provided. No interference is observed at the retention time of glyphosate.

Linearity
The calibration standards (n>5) were prepared in deionised water with concentrations in the range of 0.1 µg/mL to 50.0 µg/mL. The equivalency in mg/kf is nt available. Information about calibration curves and functions is missing.

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found for rodent diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The sensitivity was stated to be 25 mg/kg for a 10 g sample. Acceptable recoveries were achieved at the lowest fortification level of 30 mg/kg.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in rodent diets.

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level. They were not performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve and function not given, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, linearity, interference, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rodent diet..

Determination of glyphosate technical in dog diet

Data point	CA 4.1.2/049 (CA 5.3.2/021, CA 5.3.2/022, CA 5.3.2/023, CA 5.3.2/024)
Report author(s)	[redacted] (Analytical phase: [redacted].)
Report year	1999 (Analytical phase: 1997)
Report title	Subchronic (90 day) oral toxicity study with glyphosate technical in Beagle dogs (Analytical phase: Test compound stability in experimental diet (dog feed)) CA 4.1.2/049 - TEST COMPOUND STABILITY IN EXPERIMENTAL DIET (DOG FEED) – Report 1817
Report No.	TOXI-1816 (Analytical phase: Report No. 1817-R.FST)
Document No.	002/1-GPT-90-OD
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Spectrophotometry is not considered highly specific • Calibration functions and correlation coefficients not provided • LOQ not reported • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Principle of the method
The objective of this study was to determine the stability of Glyphosate Technical in the dog feed in order to decide the frequency of preparing the dietary admixtures for oral toxicity studies

An analytical method was developed for the determination of glyphosate in feed.
Samples of dietary admixtures (25 g for low level, 10 g for high level diets) were accurately weighted and extracted with 150 mL methanol/chloroform (2/1, v/v) using a mechanical shaker for 30 min. The extract was filtered and the filter cake was extracted again with 150 mL methanol/chloroform (1/2, v/v) and filtered. Both filtrates were discarded and the filter cake was finally extracted with 150 mL distilled water for 1 hour on a shaker. Following centrifugation at 15000 rpm for 15 min, the supernatant was transferred to a flask and 1 g Darco (G-60) charcoal was added. The mixture was then filtered and the volume was made up to 250 mL with distilled water.
Aliquots of these extracts (20 mL for low level, 1 mL for high level diets) were transferred to volumetric flasks, and 1 mL of 1/1 aqueous sulfuric acid solution followed by 0.5 mL of 25 % aqueous potassium bromide and 1 mL 0.2 N aqueous sodium nitrite solution were added and the contents were mixed thoroughly. The volume was finally

made up to 100 mL with distilled water and allowed to stand for 30 minutes before the nitroso derivative of glyphosate in the solutions were measured using a spectrophotometer at 243 nm, and the concentration was determined using external standard calibration.

Chromatographic conditions:
Not relevant, measurement using spectrophotometry.

Findings
Recoveries
For method validation, dog diets were fortified at relevant concentrations of 200 and 10000 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD. The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-49: Results of the method validation (spike recovery) for the determination of glyphosate in dog diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate	200	83 – 87	86	1.4	1.7	6
		10000	88 – 92	90	1.4	1.6	6

Specificity
The specificity is not demonstrated. Nevertheless, regarding data on the recoveries and %RSD, the specificity can be considered as acceptable.

Linearity
The method is based on determination of glyphosate using spectrophotometry with external calibration. Linearity of detector response was tested using 8 calibration standard concentrations in the range of 0.976 to 7.806 µg/mL. The equivalency in mg/kg is not available. It is stated in the report that linear response of the spectrophotometer was detected, however calibration functions and correlation coefficients are not provided.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for dog diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not provided in the report.
Regarding the data of the accuracy, the LOQ could be set at the lowest fortification level which is 200mg/kg.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. However, the method is considered as fit-for-purpose for the determination of glyphosate in dog diet.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (spectrophotometry is not considered highly specific, calibration functions and correlation coefficients not provided, LOQ not reported, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, interferences and matrix effects are not assessed, the efficiency of derivatisation was not examined.

Nevertheless, recovery and repeatbilty data are acceptable in the study, therefore the method can be considered as fit for purpose for the detertermination of glyphosate in the dog diets.

Data point	CA 4.1.2/050 (CA 5.3.2/025, CA 5.3.2/026 Appendix)
Report author(s)	[redacted]
Report year	1996
Report title	First revision to glyphosate acid: 90-day oral toxicity study in dogs
Report No.	[redacted]/P/1802
Document No.	PD0674 ([redacted] Study No)
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No chromatograms provided • Missing information to calibration and linearity • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Not available

Principle of the method
An analytical method was developed for the determination of glyphosate acid in dog diet by HPLC-UV detection. Accurately weighted samples of diet were extracted by mechanical shaking with distilled water. The extracts were centrifuged and diluted with water as required to give solutions containing theoretically between 50 and 400 µg/mL

glyphosate acid. Aliquots were analysed by high performance liquid chromatography (HPLC) after pre-column derivatisation with UV detection at 265 nm using an external standard procedure.
For derivatisation, diet extracts were warmed to room temperature, 1 mL aliquots transferred to vials and 1 mL di-sodium tetraborate (0.025 M) and 2 mL 9-fluorenylmethyl chloroformate (0.01 M) added. After shaking the vials for about 10 minutes, 10 mL ethyl acetate were added, briefly shaken and left to stand for 5 minutes. Aliquots of the lower aqueous layer were subjected to analysis by HPLC-UV.

Chromatographic conditions:

HPLC:	Constametric III (LDC), WISP 71OB (Waters Associates)
Column:	Spherisorb S5NH, 125 x 4.9 mm ID (Hitchrom Ltd)
Column oven temperature:	Not provided
Injection volume:	10 µL
Mobile phase:	Acetonitrile/0.025 M KH_2PO_4 (pH 8.5) (50/50, v/v)
Flow rate:	2 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMOC)
Detection:	UV at 265 nm
Retention time:	Glyphosate acid: not provided (no chromatograms available)

Findings
Recoveries
Dog test diets were prepared at three concentration levels, i.e. 2000, 10000 and 50000 mg glyphosate acid/kg diet. Aliquots were taken at three occasions (duplicate samples from initial test diet preparation) and analysed for glyphosate acid content. The results are shown in table below. These are not true procedural recovery data, however they show the robustness of the analytical method and the achieved test concentrations.

Table 5.1-50: Results of dog diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate acid	2000	91 – 105	98	5.7	5.8	4
		10000	95 – 105	101	4.2	4.1	4
		50000	93 – 99	96	3.1	3.3	4

Additionally, the homogeneity of glyphosate acid in the test diets was tested by analysis of samples from three different sampling points in the mixing trays (top, middle, bottom). The results are shown in table below. Acceptable recoveries were obtained using the analytical method.

Table 5.1-51: Results of dog diet analyses for dosing formulation homogeneity approval

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate	2000	95 – 101	98	3.2	3.2	3

Table 5.1-51: Results of dog diet analyses for dosing formulation homogeneity approval

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	acid	50000	88 – 97	93	2.8	3.0	6

Specificity
No chromatograms is provided for the demonstration of the specification. Nevertheless, regarding data on the repeatability (nominal concentration), the specificity of the method can be considered as acceptable.

Linearity
Standards were prepared by fortifying control diet at levels of 2000, 10000 and 50000 mg/kg in duplicate. These standards were extracted and derivatised alongside the test diet samples. Further details to the linearity of the detector response are not provided in the report.

Repeatability (Precision)
The relative standard deviations (RSDs) of repeated diet analyses at each concentration level and overall were <20 % (required according to SANCO/3029/99 rev. 4).

Accuracy
Acceptable mean recovery values at all diet concentrations were between 70 % and 110 % for glyphosate acid (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
It is stated in the report that the detection limit was calculated to be equivalent to approximately 5 mg/kg for glyphosate acid in the diet. The LOQ could be set at 2000mg/kg regarding the results of the lowest level of nominal concentration.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000). However, it is considered as fit-for-purpose for the determination of glyphosate acid in dog diet.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no true validation data provided, no chromatograms provided, missing information to calibration and linearity, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, interferences are not assessed (values obtained from

blank samples are not reported), matrix effects are not assessed, the efficiency of derivatisation was not examined. However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid dog diets.

Data point	CA 4.1.2/051 (CA 5.3.2/027)
Report author(s)	[redacted]
Report year	1996
Report title	HR-001: 13-week oral subchronic toxicity study in dogs
Report No.	[redacted] 94-0158
Document No.	
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited validation recoveries given • Limited information to calibration curve given • Interference not clearly assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Principle of the method
An analytical method was developed for the determination of glyphosate in dog diet by HPLC with fluorescence detection.
Aliquots of the test material dietary admixtures (5 g) were accurately weighted into an Erlenmeyer flask and extracted with water (100 mL). The extract was filtered and diluted with water to obtain a solution containing approximately 1 mg/L. An aliquot (1 mL) of this solution was evaporated to dryness, and the residue was re-dissolved in 0.05 M tetraborate solution (5 mL). The residue was then derivatised using 9-fluorenylmethyl chloroformate (FMCF) for 20 minutes. Following addition of ethyl acetate, the flask was shaken for 1 minute, and an aliquot of the separated aqueous phase was analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 255 nm excitation/315 nm emission using an external standard procedure.

Chromatographic conditions:

Column:	SAX-1253-P, 250 x 4.6 mm ID
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/phosphate buffer (pH 2.5) (35/54/11, v/v/v)
Flow rate:	1.5 mL/min

Derivatisation agent:	9-fluorenylmethyl chloroformate (FMCF)
Detection:	Fluorescence at 255 nm excitation/315 nm emission
Retention time:	Glyphosate: not provided (no chromatograms available)

Findings

Recoveries

For method validation, dog diets were fortified at relevant concentrations of 1600, 8000 and 40000 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD (<2 mg/kg). The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-52: Results of the method validation (spike recovery) for the determination of glyphosate in dog diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate	1600	94 – 95	95	0.7	0.7	2
		8000	95 – 96	96	0.7	0.7	2
		40000	92 – 94	93	1.4	1.5	2

Additionally duplicate samples of test diets prepared at four different timepoints (6 samples from initial preparation) were analysed using the analytical method. The results of these analyses are provided in table below. These are not true validation recovery data, however the results show the performance of the method and the correct concentrations in the diets.

Table 5.1-53: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate	1600	89 – 108	98	7.0	7.1	12
		8000	89 – 101	94	4.4	4.6	12
		40000	90 – 100	93	2.6	2.8	12

Specificity

No chromatograms is provided for the demonstration of the specification. Nevertheless, regarding data on the repeatability (nominal concentration), the specificity of the method can be considered as acceptable

Linearity

Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL. the equivalency in mg/kg is not available. The calibration graph was linear in this range with

correlation coefficients of > 0.999. The calibration standards were prepared in solvent (water) and derivatised as described above. All glyphosate determinations were chromatographed at concentrations within this linear range. Linearity plots and calibration functions are not provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for dog diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection (LOD) of the method was stated to be 2 mg/kg. The LOQ could be set at 1600mg/kg regarding the results of the lowest level of nominal concentration.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. However, the method is considered as fit-for-purpose for the determination of glyphosate in dog diet.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited true validation data provided, no chromatograms provided, missing information to calibration and linearity, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves and equations are not given, interferences anf matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in dog diets.

Data point	CA 4.1.2/052 (CA 5.3.2/032)
Report author(s)	[redacted]
Report year	1997
Report title	HR-001: 12-month oral chronic toxicity study in dogs
Report No.	[redacted] 94-0157
Document No.	
Guidelines followed in study	Not stated (with relevance to analytical methods)

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited information to calibration curve given • Interference not clearly assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

Principle of the method
An analytical method was developed for the determination of glyphosate in dog diet by HPLC with fluorescence detection.
Aliquots of the test material dietary admixtures (5 g) were accurately weighted into an Erlenmeyer flask and extracted with water (100 mL). The extract was filtered and diluted with water to obtain a solution containing approximately 1 mg/L. An aliquot (1 mL) of this solution was evaporated to dryness, and the residue was re-dissolved in 0.05 M tetraborate solution (5 mL). The residue was then derivatised using 9-fluorenylmethyl chloroformate (FMCF; 5 mL; 1 mg/mL) for 20 minutes. Following addition of ethyl acetate, the flask was shaken for 1 minute, and an aliquot of the separated aqueous phase was analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 255 nm excitation/315 nm emission using an external standard procedure.

Chromatographic conditions:

Column:	SAX-1253-P, 250 x 4.6 mm ID
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/phosphate buffer (pH 2.5) (35/54/11, v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent:	9-fluorenylmethyl chloroformate (FMCF)
Detection:	Fluorescence at 255 nm excitation/315 nm emission
Retention time:	Glyphosate: not provided (no chromatograms available)

Findings
Recoveries
For method validation, dog diets were fortified at relevant concentrations of 1600, 8000 and 50000 mg/kg and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD (< 2 mg/kg). The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-54: Results of the method validation (spike recovery) for the determination of glyphosate in dog diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate	1600	94 – 101	97	3.1	3.2	4
		8000	95 – 105	100	5.2	5.2	4
		50000	96 – 104	101	3.6	3.5	4

Additionally duplicate samples of test diets prepared at 14 different time points (6 samples from initial preparation for the highest dose level) were analysed using the analytical method. The results of these analyses are provided in table below. These are not true validation recovery data, however the results show the performance of the method and the correct concentrations in the diets.

Table 5.1-55: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate	1600	88 – 106	96	4.6	4.8	28
		8000	88 – 111	97	6.2	6.4	28
		50000	90 – 111	98	5.4	5.5	32

Specificity
No chromatograms are provided for the demonstration of the specification. Nevertheless, regarding data on the repeatability (nominal concentration), the specificity of the method can be considered as acceptable.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL. The equivalency in mg/kg is not available. The calibration graph was linear in this range with correlation coefficients of >0.999. The calibration standards were prepared in solvent (water) and derivatised as described above. All glyphosate determinations were chromatographed at concentrations within this linear range. Linearity plots and calibration functions are not provided in the report. The number of sample is not available.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for dog diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection (LOD) of the method was stated to be 2 mg/kg. The LOQ could be set at 1600mg/kg regarding the results of the lowest level of nominal concentration.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. However, the method is considered as fit-for-purpose for the determination of glyphosate in dog diet.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no chromatograms provided, missing information to calibration and linearity, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: : The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points., interferences and matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in dog diets.

Data point	CA 4.1.2/053 (CA 5.3.2/033, CA 5.3.2/034)
Report author(s)	[redacted]
Report year	1996
Report title	Glyphosate acid: 1 year dietary toxicity study in dogs
Report No.	[redacted]/P/5079
Document No.	[redacted] Study No. PD1006
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries available • Information to calibration function not available • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Not available

Principle of the method
An analytical method was developed for the determination of glyphosate acid in dog diet by HPLC-UV.
Accurately weighted samples of test diet (10 g) were extracted by mechanical shaking with distilled water for 30 minutes. The extracts were centrifuged and diluted with water as required to a known nominal concentration within the appropriate range of calibration standards. Aliquots were analysed by high performance liquid chromatography (HPLC) after pre-column derivatisation with UV detection at 265 nm using an external standard procedure.
For derivatisation, diet extracts were warmed to room temperature, 1 mL aliquots transferred to vials and 1 mL di-sodium tetraborate (0.025 M) and 2 mL 9-fluorenylmethyl chloroformate (0.01 M) added. After shaking the vials for about 20 minutes, 10 mL ethyl acetate were added, briefly shaken and left to stand for 5 minutes. Aliquots of the lower aqueous layer were subjected to analysis by HPLC-UV.

Chromatographic conditions:

Column:	S5NH, 250 x 4.6 mm ID (Hitchrom Ltd)
Column oven temperature:	Ambient
Injection volume:	25 µL
Mobile phase:	Acetonitrile/0.025 M Na_2HPO_4 (pH 8.5) (60/40, v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMOC-Cl)
Detection:	UV at 265 nm
Retention time:	Glyphosate acid: not provided (no chromatograms available)

Findings
Recoveries
Dog test diets were prepared at three concentration levels, i.e. 3000, 15000 and 30000 mg glyphosate acid/kg diet. Aliquots were taken at six occasions (duplicate samples from initial test diet preparation) and analysed for glyphosate acid content. The results are shown in table below. These are not true procedural recovery data, however they show the robustness of the analytical method and the achieved test concentrations. Control diet samples were also analysed, where no residues above the limit of detection (30 mg/kg) were found.

Table 5.1-56: Results of dog test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate acid	3000	81 – 102	92	7.1	7.8	7
		15000	88 – 105	98	5.7	5.8	7
		30000	92 – 105	98	4.9	5.0	7

Additionally, the homogeneity of glyphosate in the test diets was tested by analysis of samples from three different sampling points in the mixing trays (top, middle, bottom). The results are shown in table below. Acceptable recoveries were obtained using the analytical method.

Table 5.1-57: Results of dog diet analyses for dosing formulation homogeneity approval

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dog diet	Glyphosate acid	3000	83 – 100	92	8.9	9.6	3
		30000	96 – 99	98	1.3	1.3	3

Specificity
No chromatograms is provided for the demonstration of the specification. Nevertheless, regarding data on the repeatability (nominal concentration), the specificity of the method can be considered as acceptable.

Linearity
The analysis system was calibrated using a range of standards to determine the linearity of response. An appropriate standard of known concentration was interspersed at intervals throughout the analysis. Further details to the linearity of the detector response are not provided in the report.

Repeatability (Precision)
The relative standard deviations (RSDs) of repeated diet analyses at each concentration level and overall were < 20 % (required according to SANCO/3029/99 rev. 4).

Accuracy
Acceptable mean recovery values at all diet concentrations were between 70 % and 110 % for glyphosate acid (required according to SANCO/3029/99 rev. 4).

Limit of Quantification and Detection
It is stated in the report that the detection limit was assessed to be approximately 0.30 µg/mL test substance in the analysed solution, corresponding to a dietary concentration of 30 mg/kg for glyphosate acid in the diet. The LOQ could be set at 3000mg/kg regarding the results of the lowest level of nominal concentration.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000). However, it is considered as fit-for-purpose for the determination of glyphosate acid in dog diet.

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no true validation recoveries available no chromatograms provided, missing information to calibration and linearity, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not given, interferences and matrix effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in dog diets.

Determination of glyphosate in dermal solutions (diethylphthalate)

Data point	CA 4.1.2/054
Report authors	[redacted]
Report year	1993
Report titles	Adaption of existing methodology to the analysis of glyphosate in dermal solutions; assessment of formulation homogeneity, accuracy and stability, including support for IRI Project No. 450881
Report No	9112
Document No	IRI Project No. 353922
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient number of validation recoveries given • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Inveresk Research International, Tranet Scotland.

Data point	CA 4.1.2/055 (CA 5.3.3/003)
Report authors	[redacted]
Report year	1993
Report titles	Glyphosate: 3 week toxicity study in rats with dermal administration
Report No	7839
Document No	[redacted] Project No. 450881
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Analytical methods were reported separately (see [redacted], 1993)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted].

To enable topical administration of the test material at the concentrations necessary for the toxicology study (Report No. 7839), the only practical vehicle usable was diethylphthalate. Extensive experimental investigation into the adaption of existing methods to analyse glyphosate was carried out without finding an appropriate method for analysing suspensions of glyphosate in diethylphthalate.
First, only assessment of the use of method AM280 was required, since the understanding was that the vehicle for the dermal study would be aqueous. Then, the vehicle was chosen to be diethylphthalate. This required a different analytical method as diethylphthalate absorbs strongly over the absorbance range of glyphosate. An adaption of method M3750 was used, but revealed poor recoveries due to incomplete derivatisation of glyphosate. A further HPLC method M3936 was tried. This method was found to be unsuitable for the dermal formulations as diethylphthalate masked the glyphosate peak during refractive index detection. **Method M5392 was found to be suitable for confirmation of homogeneity and stability of formulations and was validated for linearity with quality control samples prepared at the midpoint of the curve.**

Principle of the method
The method M5392 was developed for the determination of glyphosate in diethylphthalate suspensions by HPLC with fluorescence detection.
Aliquots of the test material (5 mL) were mixed with 6 M NaOH and distilled water. The samples were placed in a sonic bath for approximately 0.5 h before centrifugation. The aqueous layer was pipetted into a clean tube and distilled water was added. After mixing, an aliquot was transferred to a separate tube and mixed. The samples were derivatised by first adding borate buffer and mixing. FMOC solution (FMOC chloride in acetone) was added, mixed and after 30 seconds ADAM solution (ADAM in acetonitrile) was added. After mixing, the samples were left for at least 10 min before injection and analysis by HPLC.

Chromatographic conditions:

HPLC:	Hewlett Packard HP1050 liquid chromatograph
Column:	Lichrosorb RP18 25 cm x 4.6 mm i.d.
Column temperature:	50°C
Injection volume:	10 µL
Mobile phase:	A: Milli Q water:phosphate buffer (5.82 g Na_2HPO_4 + 3.81 g KH_2PO_4, made up to 1 L with milli Q water) 19:1 (v/v) B: Acetonitrile
Gradient:	Time (min) / Eluent A (%) / Eluent B (%) / Flow rate (mL/min) 0 / 95 / 5 / 1.5 4 / 15 / 85 / 1.5 9 / 15 / 85 / 1.5 9.5 / 5 / 95 / 1.5 12 / 5 / 95 / 1.5
Derivatisation agent:	9-fluorenylmethyl chloroformate (FMOC-Cl)
Detector:	Shimadzu Fluorescence HPLC Monitor RF-535
Detector settings:	Excitation: 266 nm Emission: 315 nm Sensitivity: low Response: slow Range: 8
Retention time:	Glyphosate: ~ 5.0 min

Findings
Recoveries
This methodology was validated for linearity with quality control samples prepared at the midpoint of the curve. The average recovery value was between 70 % and 110 % and RSD was below 20 %. The detailed results are given in the table below.

Table 5.1-58: Results of the validation for linearity with quality control samples

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
9112	Water	Glyphosate	5.34	102-103	102	0.7	0.7	3

Additionally, a representative formulation was prepared containing 333 mg glyphosate/mL and its homogeneity and stability was assessed at two time points (0 h and 24 h). The results are shown in the table below and indicate satisfactory homogeneity and stability of glyphosate in diethylphthalate.

Table 5.1-59: Assessment of homogeneity and stability of glyphosate in diethylphthalate

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
9112	Diethyl-phthalate	Glyphosate	333	94-121	104	7.9	7.5	8

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Specificity
Only chromatograms of standards solution and sample are provided. Data provided are not sufficient to demonstrate the absence of interference. Nevertheless, regarding data on the repeatability (nominal concentration), the specificity of the method can be considered as acceptable

Linearity
Linearity of detector response was tested using calibration standard concentrations (n>5) in the range of 2.17 to 21.72 µg/mL. the equivalency in mg/kg is not available. All standards were prepared in water. The correlation coefficient was determined to be 0.9960 and a linear regression was calculated.

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found in diethylphthalate. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed. The LOQ could be set at 333mg/L regarding the results of nominal concentration.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in dermal solutions.

Assessment and conclusion by applicant:
The toxicological study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (not sufficient number of validation recoveries given, limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. interferences and effects are not assessed, the efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in diethylphthalate suspensions at the targeted dose.

Determination of the glyphosate technical concentration in corn oil dosing solution

Data point	CA 4.1.2/056 (CA 5.4.2/003)
Report authors	[redacted]
Report year	2008
Report title	Evaluation of the mutagenic potential of glyphosate technical by micronucleus assay in mice
Report No	[redacted]-3996.402.395.07
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No details on sample workup given • No true validation recoveries given, recoveries calculated from analysis of the dosing solution • Matrix effect and stability of sample extracts not assessed • Only limited information about linearity • Limit of quantification and detection not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (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	CA 4.1.2/057 (CA 5.4.2/004)
Report authors	[redacted]
Report year	2010
Report title	Amendment n°1 to final report: Evaluation of the mutagenic potential of glyphosate technical by micronucleus assay in mice

Report No	[redacted]-3996.402.395.07 Amendment
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No details on sample workup provided • No true validation recoveries given, recoveries calculated from analysis of the dosing solution • Matrix effect and stability of sample extracts not assessed • Limit of quantification and detection not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

The aim of the study is to evaluate the mutagenic potential of the test substance GLYPHOSATE TECHNICAL in mice when administered intraperitoneally

Principle of the method
An analytical method was developed for the determination of glyphosate technical in samples of the dosing solutions (vehicle: sterilised corn oil).
The test substance concentration in the test solutions were determined by analyzing the solution for the active ingredient with a validated analytical method following VM-040/08 and SOP-M0456-Rev.02. No further information is provided by the report. The analyses were carried out using a high performance liquid chromatograph (HPLC) with an ultra violet (UV) absorption detector.

Within the amendment N°1 to final report further analytical results were described, concerning intermediary dose results. A different retention time for glyphosate was mentioned (2.6 min instead of 4.8 min), presumable different chromatographic conditions were used, but no further information were provided.

Chromatographic conditions:

HPLC:	HP 1050 (CL#01) POP-E 0018 Rev.04
Column:	SAX 250 x 4.6 mm, 5 µm
Column oven temperature:	Not stated
Injection volume:	20 µL
Mobile phase:	H_2O/Met/KH_2PO_4 (960 mL + 40 mL + 0.8435 g) pH 2.0 phosphoric acid
Flow rate:	1.5 mL/min
Detection:	UV, 195 nm
Retention time:	Glyphosate technical: ~ 4.8 min or 2.6 min

Findings
Recoveries
No validation recoveries were presented within the report and its amendments.
However, the achieved concentration of glyphosate technical in the dosing solutions was tested. These are not true validation recovery data and the results are very limited; however the results show the performance of the method. The results are shown in the table below.

Table 5.1-60: Results of vehicle analyses

Matrix	Analyte	Nominal concentration (mg/mL)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solutions	Glyphosate technical	1.04	92	92	–	–	1
		2.08	100	100	–	–	1
		4.16	96	96	–	–	1
		8.33	106	106	–	–	1
		16.66	106	106	–	–	1
		25.00	88	88	–	–	1

Specificity
Chromatograms of standards solution and of test sample are provided. No interference is observed. Control sample and fortified sample are missing. Regarding the recoveries data, the specificity of the method is acceptable.

Linearity
Two different calibration functions were provided within the report. Linearity of detector response was tested using different calibration standard concentrations with correlation coefficients of > 0.99. Details to the calibration are provided below.

Table 5.1-61: Details on linearity

Calibration function	Calibration concentrations (ng/µL)	Number of determinations	Equation	Coefficient of correlation (r)
Linear	No information[1]	No information[2]	y = 45.2 x – 1800	1.0
Linear	1024 – 4099	3	y = 0.562662 x + 8.09020	0.99997

[1] Concentrations were between approximately 0-4000 ng/µL (further details not possible due to the bad quality of the linearity plot).
[2] Approximately 4-5 different concentrations were analysed (further details not possible due to the bad quality of the linearity plot).

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values of the dosing solutions analyses were < 20 %. Therefore these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate technical were found for the dosing solutions analyses. Therefore these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The LOQ was not addressed in the report but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) in most points. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate technical in the dosing solutions (vehicle: corn oil solution).

Assessment and conclusion by applicant:
The study was previously evaluated at EU level (the amendment is submitted to the EU for the first time). It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no details on sample workup given, no true validation recoveries given, recoveries calculated from analysis of the dosing solution, matrix effect and stability of sample extracts not assessed, only limited information about linearity, limit of quantification and detection not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not complete, interferences are not assessed (values obtained from blank samples are not reported), matrix effects are not assessed, the number of sample used to demonstrate the repeatability and the recovery is low,the efficiency of derivatisation was not examined.

However, recovery and repeatability data available are in acceptable range, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in vehicle sterilised corn oil at the targeted doses.

Determination of glyphosate technical in rat and mice diet

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/058 (CA 5.5/001)
Report authors	[redacted]
Report year	2009
Report titles	Glyphosate technical: Dietary combined chronic toxicity/carcinogenicity study in the rat
Test facility	[redacted]
Report No	2060-0012
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient validation recoveries given, additional recoveries calculated from analysis of diet preparations • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed

Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/065 (CA 5.5/012, CA 5.5/013, CA 5.5/014, CA 5.5/015)
Report authors	[redacted]
Report year	2009
Report titles	Glyphosate technical: Dietary carcinogenicity study in mouse
Test facility	[redacted]
Report No	2060-0011
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient validation recoveries given, additional recoveries calculated from analysis of diet preparations • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Both toxicological studies from [redacted] (Report No. 2060-0012 and 2060-0011) used the same analytical method. A summary of this method is given in the following.

Principle of the method
The concentration of glyphosate technical in the rodent diet (Rat and Mouse SQC certified ground diet No.1, Special Diet Services Limited, Witham, Essex, UK) was determined by high performance liquid chromatography (HPLC) with fluorescence detection using an external standard technique.
Samples of rodent diet were extracted with 0.05 M di-sodium tetraborate to give a final, theoretical test material concentration of approximately 25 mg/kg and then derivatised using 0.25 % 9-fluoroenyl methyl chloroformate (FMOC-Cl) in acetone. The sample solutions were analysed by HPLC using an external standard technique.

Chromatographic conditions:

HPLC:	Agilent Technologies I 050, incorporating autosampler and workstation
Column:	Hypersil SAX 5µ (100 x 4.6 mm id)
Injection volume:	5 µL

Mobile phase:	Acetonitrile/0.1 % orthophosphoric acid (60/40 v/v)
Flow rate:	1 ml/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Fluorescence detector wavelengths	Excitation 254 nm, emission 315 nm
Retention time:	Glyphosate: ~ 3.9 min

Findings

Recoveries

The method proved to be suitable to determine residues of glyphosate in rat diet. Samples were spiked with the analyte at nominal fortification levels of 1100, 5000 and 21000 mg/kg. All average recovery values (mean of two replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-62: Results of the method validation for the determination of glyphosate in rodent diet

Matrix	Analyte	Nominal fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1100	97 – 106	102	6.4	6.3	2
		5000	104 – 105	105	0.7	0.7	2
		21000	100 – 101	101	0.7	0.7	2

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

Additionally samples of test diets prepared during study duration (13 different time points) 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. All average recovery values (mean of 20 – 42 replicates per fortification level) were between 70 % and 110 %.

Table 5.1-63: Results of test diet analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
SPL2060-0012	Rat diet	Glyphosate	1500	70 – 105	96	6.2	6.4	42
			5000	79 – 118	99	6.4	6.4	42
			15000 – 19000	94 – 102	97	2.0	2.1	20
			21000 – 24000	75 – 103	97	5.6	5.8	21

Table 5.1-63: Results of test diet analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
SPL2060-0011	Mouse diet	Glyphosate	500	94 – 117	102	4.1	4.0	33
			1500	94 – 110	100	3.3	3.3	33
			5000	93 – 105	101	2.5	2.5	33

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

Specificity
Analysis of the solvent and a blank rat diet produced no signal that interfered with the signal of the test material glyphosate technical.

Linearity
Linearity of detector response was tested using six calibration standard concentrations in the range of 0 to 38.175 mg/kg with correlation coefficients of > 0.999. The calibration standards were prepared in water. Details to the calibration are provided below.

Table 5.1-64: Details on linearity

Calibration function	Calibration concentrations (mg/kg)	Number of determinations	Equation	Coefficient of correlation (r)
Linear	0 – 38.175	6 levels	y = 6.7557 x – 2.0798	0.9996

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification
Not assessed but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. The method is considered as fit-for-purpose for the determination of glyphosate technical in rat diet.

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) with deficits (not sufficient validation recoveries given, limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. matrix effects are not assessed, the number of sample used to demonstrate the repeatability and the recovery is low,the efficiency of derivatisation was not examined.

However, recovery and repeatability data available are in acceptable range, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rodent diet.

Data point:	CA 4.1.2/059 (CA 5.5/002)
Report authors	[redacted]
Report year	2001
Report titles	Glyphosate acid: Two year dietary toxicity and oncogenicity study in rats
Report No	[redacted]/PR1111
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No details on validation recoveries given, additionally recoveries calculated from analysis of diet • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Not available

Principle of the method
An analytical method was developed for the determination of glyphosate acid in rat diet (CT 1 diet supplied by Special Diet Services Limited, Stepfield, Witham, Essex, UK) by HPLC-UV.
An aliquot of the diet (10 g) was extracted with water. The extract filtered and the supernatant was diluted with water to obtain a solution to a known nominal concentration within the range of the calibration standards. An aliquot of the extract was taken, a di-sodium tetraborate solution (945 mg in 100 mL water), and a 9-

Fluorenylmethyl chloroformate solution (FMOC-Cl, 259 mg in 100 mL acetone) were added for derivatisation. After shaking for at least 20 min, ethyl acetate was added, shaken and the layers were allowed to separate. An aliquot of the lower aqueous layer was removed for subsequent analysis by high performance liquid chromatography (HPLC) with UV detection at 265 nm using an external standard procedure.

Chromatographic conditions:

HPLC:	600 Series (Waters)
Column:	S5NH, 25 cm x 4.6 mm (Hichrom)
Column oven temperature:	Ambient
Injection volume:	20 µL
Mobile phase:	Acetonitrile (60 % v/v), 0.025 M KH_2PO_4, pH 6.0 (40 % v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Detector:	486 Series (Waters), SA6504 UV detector (Severn Analytical) or 2487 Dual wavelength UV detector (Waters), Detector wavelength 265 nm
Retention time:	Not available (no chromatograms provided)

Findings

Recoveries

For each analysis, recovery was determined in triplicate at each level (2000, 6000 and 20000 mg/kg). No further information was provided within the report.

Additionally samples of test diets prepared at twelve time points during the study duration 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 performance of the method. Mean recovery values at each fortification level and overall were acceptable, between 70 % and 110 %.

Table 5.1-65: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate acid	2000	88 – 108	99	5.0	5.1	49
		6000	91 – 108	101	3.9	3.9	29
		20000	94 – 113	101	4.8	4.7	44

Specificity

No chromatograms are provided for the demonstration of the specification. Nevertheless, regarding data on the repeatability (nominal concentration), the specificity of the method can be considered as acceptable.

Linearity

Nominally 100 mg of glyphosate acid was accurately weighed into a 100 ml volumetric flask, diluted to volume with water and sonicated for approximately 5 minutes (nominally 1.0 mg/mL). Further appropriate dilutions were made with water in volumetric flasks to give a range of solutions, nominally within l0 µg/mL to 60 µg/mL. the equivalency in mg/kg is not available.

The analysis system was calibrated using a range of standards to determine the linearity of response. An appropriate standard of known concentration was interspersed at intervals throughout the analysis. Linearity plots and calibration functions are not provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate acid were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection was calculated to be approximately 1.0 µg/mL test substance in the analysed solution, corresponding to a dietary concentration of 50 mg/kg. Acceptable recoveries were obtained at lowest fortification level. The LOQ could be set at 2000mg/kg regarding the results of the lowest level of nominal concentration.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of glyphosate acid in extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate acid in rat diet within the toxicological study concerned.

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate acid was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no details on validation recoveries given, calibration curve and function not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. calibration curves, equations and correlation parameters are not complete, interferences and matrix effects are not assessed, ,the efficiency of derivatisation was not examined.

However, recovery and repeatability data available are in acceptable range, therefore the method can be considered as fit-for-purpose for the determination of glyphosate acid in rat diets.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/060 (CA 5.5/004)
Report authors	[redacted]
Report year	1997
Report titles	HR-001: 24-month oral chronic toxicity and oncogenicity study in rats
Test Facility	[redacted]
Report No	[redacted] 94-0150

Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient validation recoveries given, additionally recoveries calculated from analysis of diet • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes [redacted]
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate (HR-001) in rat diet (MF Mash, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo) by HPLC with fluorescence spectrophotometer.
An aliquot of the diet (5 g) was extracted with water. The extract was filtered and the supernatant was diluted with water to obtain a solution to a known nominal concentration within the range of the calibration standards. An aliquot of the extract was evaporated to dryness and a sodium tetraborate solution (0.05 M in water) was added. A 9-Fluorenylmethyl Chloroformate (FMCF) solution (FMCF dissolved in acetone, 1 mg/mL) was added and the mixture and allowed to stand for 20 minutes at room temperature for derivatisation. Following the addition of ethyl acetate, the mixture was shaken for 1 min and allowed to stand for a moment. An aliquot of the lower aqueous layer was removed for subsequent analysis by high performance liquid chromatography (HPLC) with fluorescence spectrophotometer using an external standard procedure.

Chromatographic conditions:

HPLC:	L-4000W (Yanagimoto) or PU-980 (Jasco)
Column:	SA.X-1253-P, 250 mm x 4.6 mm
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/ phosphate buffer, pH 2.5 (35:54:11, v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-Fluorenylmethylchloroformate (FMCF)
Detector:	650- lOS, fluorescence spectrophotometer (Hitachi), FP-920, fluorescence spectrophotometer (Jasco) Wavelength: excitation; 255 nm, emission; 315 nm, slit width: excitation; 15 or 18 nm, emission; 10, 15 or 18 nm, lamp: Xe lamp
Retention time:	Not available (no chromatograms provided)

Findings
Recoveries
The method proved to be suitable to determine residues of glyphosate in rat diet. Samples were spiked with the analyte at 3000, 10000 and 30000 mg/kg fortification levels. All average recovery values (mean of 4 replicates per fortification level and analyte) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-66: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	3000[2]	93 – 100	96	3.1	3.3	4
		10000[2]	92 – 93	93	0.6	0.6	4
		30000[2]	90 – 95	93	2.2	2.4	4

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

[2] Two of four recoveries were conducted within study [redacted] 94-0138.

Additionally duplicate samples of test diets prepared at each month during study duration (25 different timepoints; 6 samples from initial preparation) 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 5.1-67: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	3000	86 – 111	98	5.0	5.1	54
		10000	90 – 107	98	4.5	4.6	54
		30000	86 – 109	98	5.6	5.7	54

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

Specificity

Chromatograms are not provided in the report.

Linearity

Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL with correlation coefficients of > 0.999. the equivalency in mg/kg is not available. The calibration standards were prepared in solvent (water) and derivatised as described above. All glyphosate determinations were chromatographed at concentrations within this linear range. Linearity plots and calibration functions are not provided in the report.

Repeatability (Precision)

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification
Not assessed, but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in most points. However, the method is considered as fit-for-purpose for the determination of glyphosate in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (not sufficient validation recoveries given, calibration curve and function not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferencesand matrix effects were not assessed, efficiency of derivatisation was not examined.

However, sufficient precision data are available to consider that the method is fit for purpose, for the determination of glyphosate in rat diet

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/062 (CA 5.5/006)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate acid: One year dietary toxicity study in rats
Test facility	Test facility not available
Report No	[redacted]/P/5143
Document No	PR1012
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Details on validation recoveries missing, additionally recoveries calculated from analysis of diet

	• Calibration curve and equation not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet (CTI diet supplied by Special Diet Services Limited, Stepfield, Witham, Essex, UK) by HPLC-UV.
An aliquot of the diet (10 g) was extracted with water. The extract was filtered or centrifuged and the supernatant was diluted with water to obtain a solution to a known nominal concentration within the range of the calibration standards. An aliquot of the extract was taken, a di-sodium tetraborate solution (945 mg in 100 mL water), and a 9-Fluorenylmethyl chloroformate solution (FMOC-Cl, 260 mg in 100 mL acetone) were added for derivatisation. After shaking for at least 10 min, ethyl acetate was added, shaken and the layers were allowed to separate. An aliquot of the lower aqueous layer was removed for subsequent analysis by high performance liquid chromatography (HPLC) with UV detection at 265 nm using an external standard procedure.

Chromatographic conditions:

HPLC:	600 Series (Waters) or SA6410B (Severn Analytical)
Column:	25 cm x 4.6 mm ID S5NH (Hichrom)
Column oven temperature:	Ambient
Injection volume:	15 µL or 25 µL
Mobile phase:	Acetonitrile (60 % v/v) 0.025 M KH_2PO_4, pH 8.5 or 6 (40 % v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethylchloroformate)
Detector:	486 Series (Waters), SA6500 (Severn Analytical) or LC235 Diode Array (Perkin Elmer), Detector wavelength 265 nm
Retention time:	Not available (no chromatograms provided)

Findings
Recoveries
For each analysis, recovery was determined in triplicate at each level. No further information is given within the report.
Additionally samples of test diets prepared at five to seven time points during the study duration 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. Acceptable recovery values at each fortification level and overall between were between 70 % and 110 %

Table 5.1-68: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate acid	2000	90 – 104	97	3.4	3.5	18
		8000	94 – 103	99	2.9	3.0	11
		20000	90 – 106	98	5.1	5.3	15

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

Specificity
Not assessed. Chromatograms were not provided in the report.

Linearity
The analysis system was calibrated using a range of standards to determine the linearity of response. Standard and calibration samples were prepared in water to give ranges of solutions, nominally within 40 to 150 µg/mL, or 10 to 60 µg/mL. The equivalency in mg/kg is not available. An appropriate standard of known concentration was interspersed at intervals throughout the analysis. Further details are missing; linearity plots and calibration functions were not provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate acid were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification
Not assessed, but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of glyphosate acid in extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate acid in rat diet within the toxicological study concerned.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (details on validation recoveries missing, calibration curve and function not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferencesand matrix effects were not assessed, efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable. The method can be considered as fit for purpose for the determination of glyphosate acid in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/064 (CA 5.5/010)
Report authors	[redacted]
Report year	1990
Report titles	Chronic study of glyphosate administered in feed to albino rats
Test facility	[redacted]
Report No	[redacted]-10495
Document No	87122 (Study Number); [redacted]-87-148 (Project Number)
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet (Purina Mills Certified rodent chow #5002) by HPLC with UV detection.
An aliquot of the diet (10 g) was extracted with a mixture of water/chloroform (approx. 3/1). After shaking, the supernatant was centrifuged and then diluted with water. For derivatisation a borate solution (potassium tetraborate in water, 0.37 M) and a NBD-Cl solution (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in water, 25 mM) were added, and the solution was heated for 30 min (80 °C). Afterwards an HCl solution (1.2 N) was added, filtered and the extract was analysed by liquid chromatography (LC) with a variable wavelength UV detector using an external standard procedure.

Chromatographic conditions:

HPLC:	HP 1090 LC with autosampler and SP 4270 integrator
Column:	3 cm x 4.6 mm Brownlee C-18 Pre-column with 25 cm x 4.6 cm C-18 Spherex (Phenomenex)
Column oven temperature:	Ambient
Injection volume:	15 µL
Mobile phase:	0-5 minutes 95 % 0.01 M KH_2P0_4 at pH 3.6; 5 % acetonitrile (ACN). 5-10 minutes: 50/50 buffer/ACN. 10 to 15 minutes: same as 0-5 minutes
Derivatisation agent (pre-column):	4-chloro-7-nitrtobenzo-2-oxa-1, 3-diazole (NBD-Cl)
Detector:	Variable Wavelength UV; Sample @ 500 nm; Reference @ 580 nm
Retention time:	Glyphosate: ~ 7 min

Findings

Recoveries

The method proved to be suitable to determine residues of glyphosate in rat diet. Samples were spiked with the analyte at approx. 2000, 8000 and 20000 mg/kg fortification levels. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-69: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000	90 – 95	93	3.5	3.8	2
		2100	91 – 110	96	6.5	6.8	10
		2200	91 – 109	95	6.4	6.7	8
		2300	87 – 96	91	6.2	6.7	2
		2400	92 – 100	94	4.8	5.1	3
		2500	92	92	-	-	1
		8000	91 – 95	94	1.4	1.5	6
		8100	90 – 100	97	3.1	3.2	11
		8200	94 – 100	96	3.2	3.4	3
		8300	95	95	-	-	1
		8400	92 – 96	94	2.4	2.5	3
		8500	89 – 91	90	0.8	0.9	2
		20000	90 – 120	97	6.7	6.9	22
		21000	90 – 105	96	6.0	6.2	4

Table 5.1-69: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[1] Recovery values are not corrected for interference with matrix compounds/respective control samples. All values are means of duplicate determinations. Calculations of RSDs and overall values were performed using Excel with individual recovery values as given in the report.

Specificity
No chromatograms of control matrix or fortifications are provided. In one chromatogram of a standard no interference peaks at the retention time of the analyte are visible.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 90 to 600 mg/L. The equivalenvy in mg/kg is not available. The calibration standards were prepared in water. Further details are missing; linearity plots and calibration functions were not provided in the report.

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found in rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification
Not assessed, but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. However, the method is considered as fit-for-purpose for the determination of glyphosate in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limit of quantification and detection not assessed, calibration curve and function not given, interference not assessed (no chromatograms of control matrix or fortifications provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.

However, recovery and repeatability data are acceptable. The method can be considered as fit for purpose for the determination of glyphosate in rat diet.

Determination of glyphosate technical in mice diet

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/066 (CA 5.5/016)
Report authors	[redacted]
Report year	2001
Report titles	Carcinogenicity study with glyphosate technical in Swiss albino mice
Test facility	[redacted]
Report No	1559.CARCI-M
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of diet • Details on chromatographic conditions are missing • Limit of quantification and detection not given • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

1. Information on the study

Data point	CA 4.1.2/067 (CA 5.5/017)
Report authors	[redacted]
Report year	2017

Report titles	Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
Test facility	[redacted]
Report No	11921 (Data owner: AMADA)
Document No	90017583
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of diet • Details on chromatographic conditions are missing • Limit of quantification and detection not given • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • 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	No, not conducted under GLP/Officially recognised testing facilities (Statistical evaluation)
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

The method was developed for the determination of glyphosate technical in mice diet (Ssniff rat/mouse powder food maintenance meal - low in germs manufactured by M/s Ssniff Spezialdiaten GmbH., Ferdinand - Gabriel - Weg 16, D-59494 Säest, Germany) spectrophotometrically.

The test compound glyphosate technical was pre-extracted with a mixture of methanol and chloroform (2/1, v/v) and a mixture of methanol and chloroform (1/2, v/v). The pre-extracts were discarded and the compound glyphosate technical was extracted from experimental diet with water. It was then decolourised by shaking with charcoal (Darco, G-60). Quantification was achieved by converting the glyphosate to its nitroso-derivative which was measured spectrophotometrically at 243 nm. Further details on derivatisation are missing within the report.

Chromatographic conditions:

Not relevant, measurement using spectrophotometry.

Findings

Recoveries

No validation recoveries were presented within the report. However, samples of test diets prepared at five different time points during the conduct of the study were analysed using the analytical method. These are not true validation recovery data; however the results show the good performance of the method. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-70: Results of test diet analyses

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Mice diet (1559.CARCI-M)	Glyphosate	100	90 – 97	94	1.7	1.8	15
		1000	93 – 98	95	1.6	1.7	15
		10000	93 – 99	95	1.4	1.5	15

[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 information given within the report. No chromatograms were provided.

Linearity
No information given within the report.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values were < 20 %.

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found for mice diet.

Limit of Quantification
Not assessed, but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
No information given within the report.

Stability of analytes in sample extracts
No information given within the report.

Conclusion
The analytical method was used for the determination of glyphosate in mice diet. The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in some points. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in mice diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, calibration curve and function not given, limit of quantification and detection not given, interference not assessed, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different

samples single analyses. Interferencesand matrix effects were not assessed, efficiency of derivatisation was not examined.

Recovery and repeatability datas are provided from two differents laboratory and cannot be compiled. The method can be considered as fit-for-purpose to support the toxicological study . [redacted] 2001, 4.1.2/066. However, as neither analytical data are available and reference of the analytical method used in the study [redacted], 2017, 4.1.2/067, the method used for the determination of glyphosate in diet is not considered fit for purpose in this one.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/068 (CA 5.5/018)
Report authors	[redacted]
Report year	1997
Report title	HR-001: 18-month oral oncogenicity study in mice
Test facility	[redacted]
Report No	[redacted] 94-0151
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate (HR-001) in mice diet (certified diet MF Mash, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo) by HPLC with fluorescence spectrophotometer.
An aliquot of the diet (5 g) was extracted with water. The extract was filtered and the supernatant was diluted with water to obtain a solution to a known nominal concentration within the range of the calibration standards. An aliquot of the extract was evaporated to dryness and dissolved in a sodium tetraborate solution (0.05 M in water). A 9-fluorenylmethyl chloroformate (FMCF) solution (FMCF dissolved in acetone, 1 mg/mL) was added and the mixture was allowed to stand for 20 minutes at room temperature for derivatisation. Following the addition of ethyl acetate, the mixture shaken for 1 min and was allowed to stand for a moment. An aliquot of the lower aqueous layer was removed for subsequent analysis by high performance liquid chromatography (HPLC) with fluorescence spectrophotometer using an external standard procedure.

Chromatographic conditions:

HPLC:	L-4000W (Yanagimoto) or PU-980 (Jasco)
Column:	SA.X-1253-P, 4.6 mm x 250 mm
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/ phosphate buffer, pH 2.5 (35/54/11, v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMCF (9-Fluorenylmethylchloroformate)
Detector:	650- lOS, fluorescence spectrophotometer (Hitachi), FP-920, fluorescence spectrophotometer (Jasco) Wavelength: excitation; 255 nm, emission; 315 nm, slit width: excitation; 15 or 18 nm, emission; 10, 15 or 18 nm, lamp: Xe lamp
Retention time:	Glyphosate: not provided (no chromatograms available)

Findings

Recoveries

The method proved to be suitable to determine residues of glyphosate in mice diet. Samples were spiked with the analyte at 1600, 8000 and 40000 mg/kg fortification levels. All average recovery values (mean of four replicates per fortification level and analyte) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-71: Results of the method validation for the determination of glyphosate in mice diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Mice diet	Glyphosate	1600	93 – 98	96	2.4	2.5	4
		8000	89 – 96	94	3.1	3.3	4
		40000[2]	92 – 95	94	1.3	1.4	4

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

[2] Two of four recoveries were conducted within study [redacted] 94-0137.

Additionally duplicate samples of test diets prepared at each month during study duration (20 different time points; 6 samples from initial preparation) 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 performance of the method.

Table 5.1-72: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Mice diet	Glyphosate	1600	85 – 107	97	5.8	5.9	44
		8000	87 – 107	97	5.2	5.4	44
		40000	90 – 110	97	4.8	4.9	44

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

Specificity
Not assessed. Chromatograms were not provided in the report.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL with correlation coefficients of > 0.999. The equivalency in mg/kg is not available. The calibration standards were prepared in solvent (water) and derivatised as described above. All glyphosate determinations were chromatographed at concentrations within this linear range. Linearity plots and calibration functions are not provided in the report.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for mice diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification
Not assessed, but acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. However, the method is considered as fit-for-purpose for the determination of glyphosate in mice diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve and function not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit for purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.

However, recovery and repeatbility data are acceptable. The method can be considered as fit for purpose for the determination of glyphosate in mice diet.

Data point	CA 4.1.2/070
Report authors	[redacted]
Report year	1982
Report title	Analysis of animal feed diets in lifetime feeding study of glyphosate in mice performed by Bio/Dynamics, Inc.
Report No	MSL-2291
Document No	7163
Guidelines followed in study	Not stated (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, analytical phase submitted for the first time
GLP/Officially recognised testing facilities	No (pre-GLP; no certificate)
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	Not available

Data point	CA 4.1.2/071 (CA 5.5/023)
Report authors	[redacted]
Report year	1983
Report title	A chronic feeding study of glyphosate (Roundup® Technical) in mice
Report No	[redacted]-77-420
Test facility	[redacted]
Document No	77-2061
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Analytical methods were reported separately (see [redacted] 1982)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

The analyses for the above mentioned toxicological study ([redacted] Report No. 77-2061) were reported within a separate report ([redacted] Report No. MSL-2291). In the following a summary of the analytical report is given.

Principle of the method

The method was developed for the determination of glyphosate in animal feed diets by HPLC-UV.
An aliquot (10 g) of mixed animal diet was extracted with deionised water and chloroform (2/1, v/v) by shaking for 30 minutes. The extract was centrifuged and an aliquot of the aqueous layer (2 mL) was withdrawn. The remaining extract was decanted off and the extraction step was repeated for a second time. A second aliquot (2 mL) was withdrawn and the remaining extracts combined. The solution was filtered, layers separated, and the organic layer was discarded. The final volume of the aqueous layer was determined as this was necessary for calculation purposes. The two aliquots of the aqueous phase were combined and subjected to an ion exchange resin clean-up using a D-50 cation exchange resin mini-column and eluted with deionised water. After mixing, an aliquot was filtered and quantified for glyphosate by liquid chromatography fitted with a ninhydrin post-column reactor and measuring the colour generated using a UV detector.

Chromatographic conditions:

HPLC:	Waters 6000A pump, Waters U6K injector (for manual injection) or Varian 8500 autosampler, and column heater block (Waters – 84119)
Pre-column:	C_{18}/Corasil, 4.5 cm x 0.6 cm od. X 0.3 cm i.d.
Column:	Aminex A-9, 30 cm x 4.6 mm i.d.
Column temperature:	50°C
Injection volume:	Not stated
Derivatisation agent:	Ninhydrin
Flow rate:	Buffer: 0.5 mL/min Ninhydrin: 0.5 mL/min
Pressure:	~ 2000 psi buffer ~ 600 psi ninhydrin
Detector:	Waters Model 440 Absorbance Detector with 546 nm filter
Retention time:	Not stated

Findings

Recoveries

Along with each set of stability study feed samples analysed, checks and fortifications were run to verify the performance of the method. Samples were fortified at three levels (50 mg/kg, 300 mg/kg and 1000 mg/kg). Average recovery values were between 70 % and 110 % for each fortification level and the overall RSD was below 20 %. The detailed results are given in the table below.

Table 5.1-73: Results of the method performance verification

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Dog chow	Glyphosate	50	81 – 93	87	–	–	2
			300	95 – 99	97	–	–	2
			1000	94 – 98	96	–	–	2
			Overall	81 – 99	93	6.7	7.1	6

Table 5.1-73: Results of the method performance verification

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Rat chow	Glyphosate	50	75 – 93	84	–	–	2
			300	83 – 88	85	–	–	2
			1000	92 – 94	93	–	–	2
			Overall	75 – 94	87	7.5	8.6	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 recovery values as given in the report.

N/A Not applicable

Additionally, a laboratory stability study with glyphosate on dog and rat chow was performed. The analysis shows that glyphosate is stable for at least a one week period in both feed diets. The detailed results are shown in the table below.

Table 5.1-74: Recoveries from the laboratory stability study

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Dog chow	Glyphosate	50	71 – 96	84	9.6	11	12
			300	82 – 100	89	6.1	6.9	12
			1000	81 – 105	91	6.9	7.6	12
			Overall	71 – 105	88	8.0	9.1	36
MSL-2291	Rat chow	Glyphosate	50	88 – 133	102	12.9	12.7	12
			300	79 – 112	93	10.0	10.7	12
			1000	79 – 95	87	5.1	5.9	12
			Overall	79 – 133	94	11.4	12.2	36

[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 recovery values as given in the report.

Furthermore, the method was tested to establish mixing efficiency. The results demonstrate that an individual animal will ingest a dosed diet that is within ± 10 % of the planned amount. Along with each set of mixing efficiency samples analysed, checks and fortifications were run to verify the performance of the analytical method. The analytical results and method performance data for the mixing efficiency study are presented in the tables below.

Table 5.1-75: Results from mixing efficiency – analytical results

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Mouse feed	Glyphosate	1000	80 – 94	86	3.4	3.9	18
			5000	83 – 100	90	3.6	4.0	18
			30000	89 – 110	97	5.2	5.5	18
			Overall	80 – 110	91	5.9	6.5	54

[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 recovery values as given in the report.

Table 5.1-76: Results from mixing efficiency – method performance verification

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Mouse feed	Glyphosate	1000	88 – 98	93	4.0	4.3	6
			5000	90 – 94	92	1.4	1.5	6
			30000	95 – 109	103	6.2	6.0	6
			Overall	88 – 109	96	6.6	6.9	18

[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 recovery values as given in the report.

Weekly feed analyses were performed in a feed diet monitoring program to establish that test animals were fed as planned throughout the course of the study. The monitoring program consisted of analysis in duplicate of all samples from weeks 1, 2, 3, 4, 6, 9, 12, 16, 24, 36, 48, 72, 84, 96 and 102. Furthermore, a weekly quality control based on in-house fortified samples was performed. The detailed results are presented in the tables below.

Table 5.1-77: Results of the monitoring program

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Feed diet	Glyphosate	1000	79 – 105	92	6.0	6.5	32
			5000	79 – 115	95	8.9	9.4	32

Table 5.1-77: Results of the monitoring program

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			30000	88 – 112	96	6.5	6.8	32
			Overall	79 – 115	95	7.4	7.8	96

[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 recovery values as given in the report.

Table 5.1-78: Results of the monitoring program – quality control with in-house fortified samples

Report No.	Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-2291	Feed diet	Glyphosate	1000	83 – 99	92	4.1	4.5	14
			5000	78 – 111	94	7.7	8.2	12
			30000	90 – 100	95	3.7	3.9	6
			Overall	78 – 111	93	5.7	6.1	32

[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 recovery values as given in the report.

Specificity
No interferences were observed in control samples at the retention time of interest.

Linearity
All standards were prepared in water, further information is missing. A calibration curve and function is not given within the report.

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found in feed diets. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The sensitivity was stated to be 25 mg/kg for a 10 g sample. Acceptable recoveries were achieved at the lowest fortification level of 50 mg/kg.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in animal feed diets.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological 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 calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.

However, recovery and repeatbility data are acceptable. The method can be considered as fit for purpose for the determination of glyphosate in diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/072 (CA 5.6.1/001, CA 5.6.1/002, CA 5.6.1/003)
Report authors	[redacted]
Report year	2007
Report title	Glyphosate technical: Dietary two generation reproduction study in the rat
Report No	2060/0013
Document No	-
Test facility	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient validation recoveries, additional recoveries calculated from analysis of diet • Limit of quantification and detection not assessed • 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)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate technical in rat diet by HPLC.
The dietary admixtures were extracted with 0.05 M di-sodium tetraborate to give a final theoretical test material concentration of approximately 25 mg/kg, then derivatised using 0.25 % 9-fluoroenyl methyl chloroformate in acetone and analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 254 nm (extinction) and 315 nm (emission) using an external standard procedure.

Chromatographic conditions:

HPLC:	Agilent Technologies 1050, incorporating autosampler and workstation
Column:	Hypersil SAX, 5µm, 100 x 4.6 mm id
Column oven temperature:	Not stated
Injection volume:	5 µL
Mobile phase:	Acetonitrile: 0.1 % orthophosphoric acid (60:40 v/v)
Flow rate:	1 mL/min
Derivatisation agent (pre-column):	9-Fluorenylmethylchloroformate (FMCF)
Detection:	Fluorescence (extinction 254 nm, emission 315 nm)
Retention time:	Glyphosate technical: ~ 3.9 min

Findings
Recoveries
For method validation, rat diets were fortified at relevant concentrations of 1500, 5000 and 15000 mg/kg and analysed using the analytical method. Control samples were also analysed without detecting glyphosate technical. The recovery results are shown in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-79: Results of the method validation for the determination of glyphosate technical in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1500	96 – 98	97	–	–	2
		5000	101 – 105	103	–	–	2
		15000	101 – 102	101	–	–	2
		Overall	96 – 105	101	3.1	3.1	6

Table 5.1-79: Results of the method validation for the determination of glyphosate technical in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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 triplicate samples of test diets prepared at 10 different time points during study duration were analysed using the analytical method. The results of these analyses are provided in table below. These are not true validation recovery data; however the results show the performance of the method.

Table 5.1-80: Results of test diet analyses

Matrix	Analyte	Nominal concentra-tion (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1500	80 – 101	93	4.5	4.8	30
		5000	82 – 103	97	5.6	5.8	30
		15000	92 – 103	97	2.5	2.6	30
		Overall	80 – 103	96	4.7	4.9	90

[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

Analysis of the solvent and a blank basal laboratory diet (control) produced no signal that interfered with the signal due to the test material.

Linearity

Linearity of detector response was tested using six calibration standard concentrations in the range of 0 to 40.5 ppm with correlation coefficients of > 0.9998. The calibration standards were prepared in solvent (0.05 M Di-sodium tetraborate solution) and derivatised as described above. The calibration graph was linear in this range ($y = 6.6292 x - 1.2633$). All determinations of glyphosate technical were chromatographed at concentrations within this linear range. Details on the calibration are provided below.

Table 5.1-81: Details on linearity of the method

Calibration function	Calibration concentrations (ppm)	Number of determinations	Equation	Coefficient of correlation (r)
Linear	0 – 40.5	6 levels	y = 6.6292 x – 1.2633	0.9998

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.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate technical were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not reported. The method has shown acceptable performance at all concentration levels - acceptable recoveries were obtained at lowest fortification level.

I

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

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 is considered as fit-for-purpose for the determination of glyphosate technical in rat diet.

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) with minor deficits (not sufficient validation recoveries, additional recoveries calculated from analysis of diet, limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit for purpose to support the toxicological 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: precision data were derived from different samples single analyses,matrix effects were not assessed, efficiency of derivatisation was not examined.

However, acceptable linearity, recovery and repeatbility data are available to consider that the method is fit for purpose, for the determination of glyphosate in diet.

Determination of glyphosate acid in rat diet and deionised water

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/073 (CA 5.6.1/004)
Report authors	[redacted]
Report year	2000
Report title	Glyphosate acid: Multigeneration reproduction toxicity study in rats
Report No	[redacted]/P/6332
Document No	RR0784
Test facility	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of diet preparations and dosing solutions, respectively • Correlation coefficients and calibration functions not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point:	CA 4.1.2/079 (CA 5.6.2/001)
Report authors	[redacted]
Report year	1996 (year of the amendment 2002)
Report title	Glyphosate acid: Developmental toxicity study in the rat
Report No	[redacted]/P/4819/AMEND-001
Document No	RR0690
Test facility	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of diet preparations and dosing solutions, respectively

	• Correlation coefficients and calibration functions not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point:	CA 4.1.2/082 (CA 5.6.2/009)
Report authors	[redacted]
Report year	1996
Report titles	Glyphosate acid: Developmental toxicity study in the rabbit
Report No	[redacted]/P/5009
Document No	RB0709
Facility test	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of diet preparations and dosing solutions, respectively • Correlation coefficients and calibration functions not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet (CTL diet, supplied by Special Diet Services Limited, Witham, Essex, UK) and in dosing formulations (solutions of glyphosate acid in deionized water) for oral administration to rats and rabbits by HPLC-UV.

The samples of rat diet were extracted with water and portions of supernatant solutions were diluted with water to give sample solution concentrations within the range of the calibration standards. The samples of the dosing solutions were diluted with water to give sample solution concentrations within the range of the calibration standards. Portions of the different diluted extracts were combined with di-sodium tetraborate (Borax) and 9-fluorenylmethyl chloroformate (FMOC-Cl), shaken and rotated. Following the addition of ethyl acetate the sample

extracts were shaken then allowed to settle. Portions of the lower aqueous layer were taken and analysed by high performance liquid chromatography (HPLC) with UV detection at 265 nm.

Sample preparation:
Accurately weighed portions of diet samples, 10g, were added to tared conical tlasks, 100ml of water added, and the tlasks stoppered. The samples were extracted by mechanical shaking for 30 minutes at an appropriate speed. The samples were allowed to stand and a portion of the supematant removed, filtered through a 0.45µm disc filter and diluted as required, to a known nominal concentration within the range of the calibration standards. A 1 ml portion of diluted extract was taken (using a Gilson pipette), 1ml di-sodium tetraborate and 2 ml of 10 mM (SmM for standards) FMOCCL added. The samples were shaken for 30 seconds and rotated for 20 minutes prior to adding 10ml of ethyl acetate followed by shaking for 30 seconds. After standing to allow the layers to separate, the lower aqueous layer was transferred to autosampler vials for analysing.

Chromatographic conditions:

HPLC:	600 Series (Waters) or SA6410B (Severn analytical)
Column:	25 cm x 4.6 mm ID S5NH (Hichrom) 25 cm x 4.6 mm ID S10NH (Hichrom)
Column oven temperature:	Ambient
Injection volume:	20 µL
Mobile phase:	Acetonitrile/0.025M KH_2PO_4 (60/40, v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethylchloroformate (FMOC-Cl)
Detection:	UV at 265 nm
Retention time:	Glyphosate acid: not stated (no chromatograms available)

Findings
Recoveries
Within the study [redacted]/P/6332 for each analysis, validation recoveries were determined in triplicate at each level (1000, 3000, 10000 mg/kg) for each analysis; further information is missing. For the other studies [redacted]/P/4819 and [redacted]/P/5009 there is no information about validation recoveries presented within the reports.
Nevertheless, in each study samples of rat diet or dosing formulations prepared during each study (1-5 different time points) 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 performance of the method.

Table 5.1-82: Results of test diet analyses and dosing formulation analyses

Report No.	Matrix	Analyte	Nominal concen-tration (mg/kg or mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
[redacted]/P/6332	Rat diet	Glyphosate acid	1000	79 – 109	100	6.1	6.1	22
			3000	90 – 110	101	5.1	5.0	14
			10000	81 – 105	99	6.0	6.0	20
			Overall	79 – 110	100	5.8	5.8	56
[redacted]/P/4819	Dosing formulation	Glyphosate acid	25	101 – 104	102	2.0	1.9	2
			50	99 – 101	100	1.6	1.6	2

Table 5.1-82: Results of test diet analyses and dosing formulation analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/kg or mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			100	105 – 105	105	0.1	0.1	2
			Overall	99 – 105	102	2.5	2.4	6
[redacted]/P/5009	Dosing formulation	Glyphosate acid	50	87 – 106	95	8.6	9.1	4
			87.5	88 – 112	99	10.4	10.5	4
			150	95 – 107	101	5.9	5.8	4
			Overall	87 – 112	98	8.2	8.3	12

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

Specificity
Not assessed. Chromatograms are not provided in the reports.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 5 µg/mL to 60 µg/mL. The equivalency in mg/kg is not available. Correlation coefficients and calibration functions are not provided in the reports.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were < 20 %. Therefore these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate acid were found. Therefore these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection was calculated to be approximately 1.3 µg/mL test substance in the analysed solution, corresponding to a dietary concentration of 76 mg/kg ([redacted]/P/6332). The limit of detection was calculated to be approximately 0.4 mg/mL in the formulation ([redacted]/P/4819). The limit of detection was calculated to be approximately 0.04 µg/mL test substance in the analysed solution, corresponding to a formulation concentration of 0.08 mg/mL ([redacted]/P/5009). Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless the method is considered as fit-for-purpose for the determination of glyphosate acid in rat diet and in dosing formulations.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, calibration curve not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.

However, recovery and repeatbility data are acceptable. The method can be considered as fit for purpose for the determination of glyphosate in diet.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/074 (CA 5.6.1/005)
Report authors	[redacted]
Report year	1997
Report title	HR-001: A two-generation reproduction study in rats
Report No	[redacted] 96-0031
Document No	-
Test facility	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Insufficient quantity of validation recoveries given, additional recoveries calculated from analysis of dosing solutions • Calibration curve not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of glyphosate (HR-001) in rat diet (certified pulverized feed, MF Mash, Oriental Yeast Co., Ltd.) by HPLC using fluorescence detection.

An aliquot of the diet was extracted with water by mechanical shaking for 30 min and portions of supernatant solutions were filtered and diluted with water, as appropriate, to give sample solution concentrations within the range of the calibration standards. An aliquot of the diluted extract was combined with 0.05 M di-sodium tetraborate (solution in deionised water) and 9-fluorenylmethyl chloroformate (solution in acetone) and allowed to stand at room temperature for 20 minutes for derivatisation. Following the addition of ethyl acetate the mixture was shaken and then allowed to settle. An aliquot of the lower aqueous layer was subjected to high performance liquid chromatography (HPLC) with fluorescence detection (extinction 255 nm and emission 315 nm).

Chromatographic conditions:

HPLC:	Gulliver PU-980 (Jasco)
Column:	TSK-GEL QAE-2SW (Tosoh), 250 mm x 4.6 mm i.d.
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile/water/acetic acid/phosphoric acid (300/200/4/1, v/v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMOC-Cl)
Detection:	Fluorescence (excitation 255 nm, emission 315 nm)
Retention time:	Glyphosate: not stated (no chromatograms available)

Findings

Recoveries

For method validation, rat diets were fortified at relevant concentrations of 1200, 6000 and 30000 mg/kg and analysed using the analytical method. Control samples were also analysed without detecting glyphosate. The recovery results are shown in table below. All average recovery values were between 70 % and 110 %.

Table 5.1-83: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1200	99 – 101	100	–	–	2
		6000	100 – 101	101	–	–	2
		30000	97 – 98	98	–	–	2
		Overall	97 – 101	100	1.7	1.7	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 test diets prepared at each month during study duration (12 different time points; 6 samples from initial preparation) 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 performance of the method.

Table 5.1-84: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1200	90 – 104	96	3.7	3.9	28
		6000	89 – 109	96	5.2	5.5	28
		30000	90 – 107	99	4.2	4.3	28
		Overall	89 – 109	97	4.6	4.8	84

[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
Chromatograms are not provided in the report.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL with a correlation coefficient of >0.999. The equivalency in mg/kg is not available. The calibration standards were prepared in solvent (di-sodium tetraborate) and derivatised as described above. Further details on the calibration such as calibration functions are not provided by the report.

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.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection of glyphosate in rat diet was calculated to be 2 mg/kg. Acceptable recoveries were obtained at lowest fortification level

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate (HR-001) in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (insufficient quantity of validation recoveries given, calibration curve not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological 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, no calibration curve was provided. Recoveries were reported without details on how they were calculated, and precision data were derived from different samples single analyses. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.

However, recovery and repeatbility data are acceptable. The method can be considered as fit for purpose for the determination of glyphosate in diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/076 (CA 5.6.1/007, CA 5.6.1/008)
Report authors	[redacted]
Report year	1992
Report titles	The effect of dietary administration of glyphosate on reproductive function of two generations in the rat
Report No	[redacted] 47/911129
Document No	-
Facility test	[redacted]
Guidelines followed in study	Not stated (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	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)

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 rat diet (Biosure Laboratory Animal Diet No. 2) by HPLC-UV.

An aliquot (10 g) of rat diet was extracted with aqueous triethylamine by mechanical shaking for 1 hour at 40 °C. After centrifugation and filtration the extract was diluted, as appropriate, using aqueous triethylamine. An aliquot of the diluted extract was mixed with saturated borax solution and derivatising reagent (solution of 1-fluoro-2,4-dititrobenzene in ethanol). The mixture was incubated in darkness for 1.5 hours. The derivatised extract was transferred to a separating funnel, citrate buffer was added and the mixture partitioned with ethyl acetate by shaking vigorously for 15 seconds. The aqueous layer was acidified by adding 25 % aqueous orthophosphoric acid and partitioned with ethyl acetate by shaking vigorously for 15 seconds. The ethyl acetate layer was evaporated to dryness. The residue was re-dissolved in mobile phase to provide a solution containing glyphosate at an expected concentration in the range 4-8 µg/mL. The final solution was filtered and the concentration of glyphosate quantified by high performance liquid chromatography using UV detection using an external standard procedure.

Chromatographic conditions:

HPLC:	Waters model 510
Column:	Merck Ltd., LiChrospher 100 RP-18e, 5 µm, 250 mm x 4 mm ID
Column oven temperature:	Not stated
Injection volume:	40 µL
Mobile phase:	Acetonitrile/solvent A (1/5, v/v) Solvent A: Tetraethylammonium bromide (8.4 g) and sodium dihydrogen orthophosphate (15.6 g) were dissolved in water. The solution was adjusted to pH 3.0 using orthophosphoric acid, diluted to 2 L with water and filtered
Flow rate:	1.0 mL/min
Derivatisation agent (pre-column):	1-fluoro-2,4-dititrobenzene
Detector:	Spectra-Physics LC 481 UV-Vis variable wavelength ultra violet spectrophotometer; 383 nm
Retention time:	Glyphosate: ~ 7 min

Findings

Recoveries

For method validation, rat diets were fortified at relevant concentrations of 500 and 30000 mg/kg and analysed using the analytical method. The recovery results are shown in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-85: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	500	86-101	95	6.9	7.2	6
		30000	91-111	100	7.4	7.4	6
		Overall	86-111	98	7.3	7.4	12

Table 5.1-85: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

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

Additionally samples of rat diet prepared during the study (13 different time points) 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 5.1-86: Results of test diet analyses

Matrix	Analyte	Nominal concen-tration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1000	83 – 115	102	9.2	9.0	29
		3000	79 – 119	96	10.9	11.3	32
		10000	80 – 115	97	9.0	9.2	26
		Overall	79 – 119	99	10.0	10.1	87

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

Specificity
Analysis of the control sample produced no signal that interfered with the signal due to the test material.

Linearity
Linearity of detector response was tested using five calibration standard concentrations in the range of 2 to 10 µg/mL with a correlation coefficient of > 0.99. the equivalency in mg/kg is not available. At each analytical occasion, two extracted calibration standards were determined for each inclusion level by fortifying control rodent diet with glyphosate and analysing as described above. The test substance, glyphosate, was added either as a solution in aqueous trimethylamine or as the neat material. The calibration graph was linear and the details on the calibration are provided below.

Table 5.1-87: Details on linearity of the method

Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
Linear	2 – 10	5 levels	Not stated	0.993

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.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection of glyphosate was calculated to be 25 mg/kg. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

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 is considered as fit-for-purpose for the determination of glyphosate in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate was previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). The method is considered as fit-for-purpose to support the toxicological 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: matrix effects were not assessed, efficiency of derivatisation was not examined.

However, linearity, recovery and repeatbility data are acceptable. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in diet.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/077 (CA 5.6.1/010)
Report authors	[redacted]
Report year	1990
Report titles	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats
Report No	[redacted]-10387
Document No	-
Test facility	-
Guidelines followed in study	Not stated (with relevance to analytical methods)

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and equation not given • Interference not assessed (no chromatograms of control matrix are provided) • Matrix effect and stability of sample extracts not assessed • Limit of quantification and detection not assessed. • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet (Purina Mills Certified Rodent Chow No. 5002) by HPLC-UV.
An aliquot of the diet sample was extracted with a mixture of water/chloroform (approximately 10/3, v/v) by mechanical shaking for 60 min. An aliquot of the supernatant was centrifuged and diluted before derivatisation. For derivatisation a 0.37 M borate solution (potassium tetraborate in distilled water) and a 25 mM NBD-Cl solution (4-Chlor-7-nitrobenzo-2-oxa-1,3-diazol in methanol) were added and solution was heated for 15-30 min at 80°C. Solution was acidified with 1.2 N HCl; the supernatant was filtered and subjected to high performance liquid chromatograph utilising a variable wavelength UV/VIS detector using external standard procedure.

Chromatographic conditions:

HPLC:	HP 1090 LC with autosampler and SP 4270 integrator
Column:	Spherex C-18 (Phenomenex), 25 cm x 4.6 mm, with pre-column Brownlee C-18, 3 cm x 4.6 mm
Column oven temperature:	Ambient
Injection volume:	15 µL
Mobile phase:	0-5 min: 95% 0.01 M KH_2PO_4 at pH 3.6; 5% acetonitrile (ACN) 5-10 mins: 50/50 buffer/ACN 10-15 min: 95% 0.01 M KH_2PO_4 at pH 3.6; 5% acetonitrile (ACN)
Flow rate:	Not stated
Derivatisation agent (pre-column):	4-Chlor-7-nitrobenzo-2-oxa-1,3-diazol (NBD-Cl)
Detection:	UV, 500 nm
Retention time:	Glyphosate: 3.96 min

Findings
Recoveries
The method proved to be suitable to determine residues of glyphosate in rat diet. Samples were spiked with the analyte at approximately 2000, 10000 and 30000 mg/kg fortification levels. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-88: Results of the method validation for the determination of glyphosate in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000 – 2400	86 – 114	97	8.3	8.6	14
		9800 – 11000	89 – 110	96	5.4	5.6	14
		30000 – 41000	93 – 107	99	3.9	4.0	14
		Overall	86 – 114	97	6.1	6.3	42

[1] Recovery values are not corrected for interference with matrix compounds/respective control samples. All values are means of duplicate determinations. 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 test diets prepared at during study duration (50 different time points; 6 samples from initial preparation) 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 5.1-89: Results of test diet analyses

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	2000	85 – 110	97	6.0	6.2	23
		10000	90 – 110	96	5.3	5.5	22
		30000	87 – 107	98	5.2	5.3	21
		Overall	85 – 110	97	5.5	5.7	66

[1] Recovery values were corrected for interference with matrix compounds/respective control samples. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentrations values as given in the report.

Specificity

No chromatograms of control matrix are provided. In chromatograms of a standard no interference peaks at the retention time of the analyte are visible.

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 200 to 600 mg/L. A linear regression was used for calculations. The calibration standards were prepared in water. No further information is given within the report.

Repeatability (Precision)

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found in rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. The method is considered as fit-for-purpose for the determination of glyphosate in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve and equation not given, interference not assessed (no chromatograms of control matrix are provided), matrix effect and stability of sample extracts not assessed, limit of quantification and detection not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:

The part of the study report where the analytical data are reported is not available.
Comment to AGG : if the this toxicological study is used in the assessment, the analytical part of the study report will be requested to applicant.

Determination of glyphosate (HR-001) in dosing solution

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/080 (CA 5.6.2/002)
Report authors	[redacted]
Report year	1995
Report titles	HR-001: Teratogenicity Study in Rats
Report No	[redacted] 94-0152
Document No	-
Facility test	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4)

	• Insufficient amount of validation recoveries given, additional recoveries calculated from analysis of dosing solutions • Calibration curve and equation not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate (HR-001) in dosing solution (glyphosate in purified water with the aid of 0.5 % sodium carboxymethylcellulose (CMC, Lot no. CTJ0627, Wako Pure Chemical Industries, Ltd.)) by HPLC using fluorescence detection.
An aliquot of the dosing solution was diluted with water. An aliquot of this solution was diluted with water to provide a suitable concentration (0.75-1.0 mg/kg). An aliquot of this diluted solution was taken and evaporated to dryness in a water bath below 40 °C under reduced pressure. The residue was dissolved in 0.05 M sodium tetraborate solution. An aliquot of FMCF solution (9-Fluorenylmethyl chloroformate in acetone, concentration: 1 mg/mL) was added and the mixture was allowed to stand for 20 minutes at room temperature for fluorescence label derivatisation. Following the addition of ethyl acetate, the flask was shaken for 1 minute using a reciprocal shaker. The mixture was allowed to stand for a moment. The aqueous phase thus obtained was analysed by high performance liquid chromatography using fluorescence detection with external standardisation.

Chromatographic conditions:

HPLC:	L-4000W (Yanagimoto)
Column:	SAX-1253-P, 250 mm x 4.6 mm i.d.
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile-water-pH 2.5 phosphate buffer (35/54/11, v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (prior to chromatographic analysis):	9-Fluorenylmethyl chloroformate (FMCF)
Detection:	650-lOS, fluorescence spectrophotometer (Hitachi): 255 nm excitation, 315 nm emission
Retention time:	Glyphosate: not stated (no chromatograms available)

Findings
Recoveries
For method validation, dosing solutions were fortified at relevant concentrations of 3000, 30000 and 100000 mg/kg and analysed using the analytical method. Control samples were also analysed without detecting glyphosate. The recovery results are shown in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-90: Results of the method validation for the determination of glyphosate in dosing solutions

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	Glyphosate	3000	100 – 102	101	–	–	2
		30000	97 – 102	100	–	–	2
		100000	93 – 94	94	–	–	2
		Overall	93 – 102	98	3.9	4.0	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 test diets prepared during the study (2 different time points; 6 samples from initial preparation) 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 performance of the method.

Table 5.1-91: Results of dosing solutions

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	Glyphosate	3000	96 – 107	103	4.2	4.1	8
		30000	94 – 98	97	2.0	2.1	4
		100000	95 – 109	104	4.9	4.7	8
		Overall	94 – 109	102	4.7	4.7	20

[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
Not assessed. No chromatograms are provided by the report.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.008 to 0.32 µg/mL with a correlation coefficient of > 0.999. The equivalency in mg/kg is not available. The calibration curve was prepared by injecting aliquots of derivatised glyphosate standard solutions into the HPLC and plotting the peak heights against the original amounts of glyphosate injected. The calibration curve was linear. Further details on the calibration such as calibration functions are not provided by the report.

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.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate were found for the dosing solution. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection of glyphosate in the dosing solution was 2 mg/kg. Acceptable recoveries were obtained at lowest fortification level.

Interference

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in dosing solutions for oral administration to rats.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (insufficient amount of validation recoveries given, additional recoveries calculated from analysis of dosing solutions, calibration curve and equation not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit for purpose to support the toxicological 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, no calibration curve was provided. Recoveries and precision data were derived from different samples single analyses. Interferences and matrix effects were not assessed, efficiency of derivatisation was not examined.

However, linearity, recovery and repeatbility data are acceptable. Therefore the method can be considered as fit for purpose for the determination of glyphosate in solution ate the targeted doses.

Determination of glyphosate in methylcellulose formulations

Study previously submitted to the EU

1. Information on the studies

Data point	CA 4.1.2/081 (CA 5.6.2/003)
Report authors	[redacted]
Report year	1991a
Report title	The effect of glyphosate on pregnacy of the rat (incorporates preliminary investigations),
Laboratory	[redacted]
Report No	[redacted]43 & 41/90716

Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) 1. Calibration curve and function not given 2. Matrix effect and stability of sample extracts not assessed 3. Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/085 (CA 5.6.2/014, CA 5.6.2/015)
Report authors	[redacted]
Report year	1991b
Report title	The effect of glyphosate on pregnancy of the rabbit (incorporates preliminary investigations)
Laboratory	[redacted]
Report No	[redacted] 45 & 39 & 40/901303
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) 1. Calibration curve and function not given 2. Matrix effect and stability of sample extracts not assessed 3. Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the analytical method used in both studies according to OECD format

Principle of the method

An analytical method was developed for the determination of glyphosate technical in 1 % aqueous methylcellulose by HPLC.

An aliquot of the test suspension was dissolved in an appropriate volume of aqueous triethylamine (0.1 M) to provide suitable concentration range for derivatisation (containing glyphosate in the concentration range 2 to 4 µg/mL). A suitable volume of diluted extract was mixed with saturated borax solution. For derivatisation an aliquot of the diluted extract was mixed with saturated borax solution and derivatising reagent (3.75 g 1-Fluoro-2,4-dinitrobenzene in 1 L ethanol). The mixture was incubated in darkness for 1 hour. A citrate buffer (pH 3.0) was added and the mixture was partitioned two times with ethyl acetate. The ethyl acetate layers were discarded. The aqueous layer was acidified by adding 25 % aqueous orthophosphoric acid and partitioned with ethyl acetate. The ethyl acetate layer was collected and evaporated to dryness. The residue was re-dissolved in mobile phase and the concentration of glyphosate was quantified by high performance liquid chromatography using ultra-violet detection and an external standard procedure.

Chromatographic conditions:

HPLC:	Waters model 510, autosampler: Waters WISP model 710B

Analytical column:	Nucleosil ODS, 5 µm, 250 x 4.6 mm ID, Jones Chromatography Ltd.
Guard column:	Aquapore ODS cartridge, 7 µm, 15 x 3.2 mm ID, Brownlee Labs Inc.
Injection volume:	20 µL or 25 µL
Mobile phase:	Acetonitrile/Solvent A (l/5, v/v) Solvent A: solution of Tetraethylammonium bromide (8.4 g) and sodium dihydrogen orthophosphate (15.6 g) dissolved in 2 L water (pH 3.0)
Flow rate:	1.0 mL/min
Derivatisation agent (pre-column):	1-Fluoro-2,4-dinitrobenzene
Detector:	Waters Lambda-Max 481 variable wavelength LC spectrophotometer, wavelength UV 383 nm
Retention time:	Glyphosate technical: ~7.1 – 7.6 min

Findings

Recoveries

. Samples were spiked with the analyte at 2 fortification levels, the concentration of glyphosate in the control was 0.2% w/v and 35% w/v. All average recovery values (mean of 9 replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-92: Results of the method validation for the determination of glyphosate in methylcellulose formulations

Matrix	Analyte	Fortification level (% glyphosate in control (w/v))	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous methyl-cellulose	Glyphosate	0.2	92 – 102	98	3.0	3.1	9
		35	91 – 104	96	4.1	4.2	9
		Overall	91 – 104	97	3.6	3.7	18

[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

Analysis of the calibration, sample and control solutions (blank MC and MC fortified (3, 10 and 35 %)) did not reveal any peaks in the chromatogram, which would interfere with the determination of glyphosate.

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Linearity

Linearity of detector response was tested using 5 calibration standard concentrations in the range of 2 to 10 µg/mL . The equivalency in mg/kg is not available. The calibration standards were prepared in 0.1 M triethylamine. No more details (r^2 and equation) are given in the report.

Repeatability (Precision)

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

Accuracy

Acceptable mean recovery values at 0.2 % glyphosate in control (w/v) and 35 % glyphosate in control (w/v) between 70 % and 110 % for glyphosate were found for aqueous methylcellulose.

Limit of Quantification and Detection
The limit of detection, defined as the concentration of glyphosate in matrix producing a peak response equivalent to 3 x baseline noise, was stated to be as 0.015 % (w/v). Acceptable recoveries were obtained at lowest fortification level (0.2 % (w/v).

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method was successfully validated for the determination of glyphosate technical in 1 % aqueous methylcellulose. The analytical method fulfils the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. The method is considered as fit-for-purpose for the determination of glyphosate technical in 1 % aqueous methylcellulose.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (calibration curve and function not given, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

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 calibration curve was provided. Matrix effects were not assessed, efficiency of derivatisation was not examined.

However, linearity, recovery and repeatbility data are acceptable. Therefore, the method can be considered as fit for purpose for the determination of glyphosate solution at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/083 (CA 5.6.2/010)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate technical: Oral gavage teratology study in the rabbit
Report No	434/020
Document No	-
Laboratory	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, additional recoveries calculated from analysis of dosing solutions • Calibration curves and functions not given • Limit of quantification and detection not given • Matrix effect and stability of sample extracts not assessed

	• Confirmation of the absence of interferences
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate technical in dosing solution (1 % carboxymethyl cellulose) by HPLC-UV.
The test material formulations were diluted with mobile phase to give a theoretical test material concentration of 1 mg/mL. The samples were analysed by high performance liquid chromatography (HPLC) using an external standard technique and UV detection.

Chromatographic conditions:

HPLC:	Not stated
Column:	C_{18} Novapak (300 x 3.9 mm id)
Column oven temperature:	Not stated
Injection volume:	25 µL
Mobile phase:	Water:Tetrabutylammonium hydrogen sulphate (0.1% w/v), Orthophosphoric acid (0.01% v/v) in Methanol (99:1)
Flow rate:	1 mL/min
Retention time:	Glyphosate technical: 2.9 min
Detection:	UV, 200 nm

Findings
Recoveries
There were no validation data presented within the report. However triplicate samples of dosing solutions prepared during the study (three different time points) 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. All average recovery values were between 70 % and 110 %.

Table 5.1-93: Results of analysis of the dosing solutions

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	Glyphosate technical	10	94 – 104	100	3.6	3.6	9
		40	77 – 98	91	6.9	7.6	9
		80	69 – 102	89	10.6	12.0	9
		Overall	69 – 104	93	8.7	9.3	27

Table 5.1-93: Results of analysis of the dosing solutions

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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
Analysis of a standard solution and the test material formulation produced seems indicate no interference peaks at the retention time of glyphosate technical. Nevertheless, as blank chromatograms is missing the absence of interferences cannot be confirmed.

Linearity
Standard solutions were prepared in mobile phase at a nominal concentration of 1 mg/mL. No details (number of samples, r^2, and equation) were provided on the calibration curves and functions.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values of the dosing formulation analyses at each level and overall were < 20 %. Therefore, these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate technical were found for the dosing solution analyses. Therefore, these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed, but acceptable recoveries were obtained at lowest fortification level 10 mg/mL.

Matrix effects
Not assessed.
Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate technical in dosing solutions for orally administration to rabbits.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, recoveries calculated from analysis of dosing solutions, calibration curve and functions not given, matrix effect and stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:

The analytical method for the determination of glyphosate technical in 1 % carboxymethyl cellulose by HPLC/UV was provided. The method is not in agreement with the SANCO 3029/99. Some validation data on the linearity (number of samples, r^2, and equation) and on the specificity (interference / blank chromatogram) are missing, the matrix effect and the efficiency of the deritivatisation are not examined. However, recovery and repeatability data are in the acceptable range. Therefore, the method is considered as fit-for-purpose for the determination of glyphosate in solution at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/084 (CA 5.6.2/011)
Report authors	[redacted]
Report year	1995
Report title	HR-001: Teratogenicity study in rabbits
Report No	[redacted] 94-0153
Document No	-
Laboratory	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient validation recoveries given, additional recoveries calculated from analysis of dosing solutions • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of glyphosate (HR-001) in dosing solution (suspension of glyphosate in purified water with the aid of 0.5 % sodium carboxyrnethylcellulose (CMC, Kanto Chemical Co., Inc., Lot no. 502El706)) by HPLC using fluorescence detection.

An aliquot of the dosing solution was diluted with water to provide a suitable concentration. An aliquot of this diluted solution was taken and evaporated to dryness in a water bath below 40 °C under reduced pressure. The residue was dissolved in 0.05 M sodium tetraborate solution. An aliquot of 9-fluorenylmethyl chloroformate (FMCF) solution (dissolved in acetone, 1 mg/mL) was added and the mixture was allowed to stand for 20 minutes at room temperature for fluorescence label derivatisation. Following the addition of ethyl acetate, the flask was shaken for 1 minute using a reciprocal shaker. The mixture was allowed to stand for a moment. The aqueous phase thus obtained was analysed by high performance liquid chromatography using fluorescence detection with external standardisation.

Chromatographic conditions:

HPLC:	LC-6A (Shimadzu)

Column:	TSKgel QAE-2SW, 4.6 mm i.d. x 250 mm
Column oven temperature:	40 °C
Injection volume:	10 µL
Mobile phase:	Acetonitrile-water-phosphoric acid-acetic acid (300:200:4:1, v/v/v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMCF)
Detection:	Fluorescence RF-535 (Shimadzu), 255 nm excitation, 315 nm emission
Retention time:	Glyphosate: not stated (no chromatograms available)

Findings

Recoveries

For method validation, dosing solutions were fortified at concentrations of 2000, 20000 and 60000 mg/kg and analysed using the analytical method. Control samples were also analysed without detecting glyphosate (HR-001). The recovery results are shown in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-94: Results of the method validation for the determination of glyphosate (HR-001) in dosing solution

Matrix	Analyte	Fortification level (mg/L)	Recovery[1,2]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	Glyphosate (HR-001)	2000	100 – 105	103	–	–	2
		20000	92 – 94	93	–	–	2
		60000	93 – 95	94	–	–	2
		Overall	92 – 105	97	5.0	5.2	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.

2 Recovery values at 20000 and 60000 mg/L were generated in Report No. [redacted] 94-140.

Additionally duplicate samples of dosing solutions prepared at two different time points (6 samples from initial preparation for 2000 mg/kg) 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. Mean recoveries and %RSD are in the acceptable range for each concentration level.

Table 5.1-95: Results of dosing solution analyses

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing	Glyphosate	2000	95 – 105	98	3.7	3.8	8

Table 5.1-95: Results of dosing solution analyses

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
solution		20000	92 – 97	95	2.5	2.6	4
		60000	96 – 103	99	3.3	3.3	4
		Overall	92 – 105	98	3.5	3.6	16

[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
Not assessed. No chromatograms are provided by the report.

Linearity
Linearity of detector response was tested using calibration standard concentrations in the range of 0.032 to 0.32 µg/mL with a correlation coefficient of > 0.999. The calibration standards were prepared in solvent (water) and derivatised as described above**.** Linearity plots, number of samples and calibration functions are not provided in the report.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level and overall were < 20 %.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate (HR-001) were found for the dosing solution. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection of glyphosate in the dosing solution was stated to be 100 mg/kg. Acceptable recoveries were obtained at lowest fortification level 2000 mg/L.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate (HR-001) in dosing solutions for orally administration to rabbits.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (not sufficient validation recoveries given, additional recoveries calculated from analysis of dosing solutions, calibration curve and equation not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not

assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit for purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
An analytical method for the determination of glyphosate (HR-001) in sodium carboxyrnethylcellulose (CMC) solution by HPLC-Flu was provided. The method is not in agreement with the SANCO 3029/99. Some validation data on the linearity and specificity (interference) are missing, the matrix effect and the efficiency of the deritivatisation are not examined. However, recoveriy and repeatability data are in the acceptable range. Therefore, the method can be considered as fit-for-purpose for the determination of glyphosate in solution at targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/086 (CA 5.7.1/001)
Report authors	[redacted]
Report year	1996
Report titles	Glyphosate acid: Acute neurotoxicity study in rats
Laboratory	[redacted]
Report No	[redacted]/P/4866
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of dosing solutions • Calibration curve not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 dosing formulations (deionised water) by HPLC.
An aliquot of the dosing formulation was diluted with water to a known concentration within the range of calibration standards. An aliquot of the diluted sample was added to a borax-solution (disodium tetraborate in water) and an FMOC-Cl solution (9-Fluorenylmethylchloroformate in acetone), shaken for 30 seconds and rotated for 10 minutes. Ethyl acetate was added and shaken for a further 30 seconds; when the two layers had separated the lower aqueous layer was subjected to high performance liquid chromatography (HPLC) with UV detection (265 nm) using external standardisation.

Chromatographic conditions:

HPLC:	SA6410B (Severn Analytical) or similar 600 series (Waters)
Column:	S5NH 25 cm x 4.6 mm id (Hichrom)
Column oven temperature:	Ambient
Injection volume:	20 µL
Mobile phase:	Acetonitrile/0.025M Na_2HPO_4, pH 8.5 (60/40, v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethylchloroformate)
Detection:	SA6504 (Severn Analytical) or similar 486 (Waters): UV, 265 nm
Retention time:	Glyphosate acid: not provided (no chromatograms available)

Findings

Recoveries

No validation recoveries were presented within the report.

However the achieved concentration of glyphosate acid in the dosing solution was tested. These are not true validation recovery data; however the results show the good performance of the method. All average recovery values are in the acceptable range. The results are shown in the table below.

Table 5.1-96: Results of dosing solution analyses

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	Glyphosate acid	50	99 – 100	99	–	–	2
		100	101 – 104	103	–	–	2
		200	97 – 99	98	–	–	2
		Overall	97 – 104	100	2.4	2.4	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, the homogeneity of glyphosate acid in the dosing solution was tested by analysis of samples from three different sampling points (start, middle and end of the study duration). The results are shown in the table below. Acceptable recoveries were obtained using the analytical method.

Table 5.1-97: Results of homogeneity analyses of dosing solutions

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	Glyphosate acid	50	105 – 114	109	3.3	3.0	6
		200	98 – 111	106	5.6	5.3	6
		Overall	98 – 114	107	4.7	4.3	12

[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
Not assessed. No chromatograms are provided by the report.

Linearity
Standard solutions were prepared in water within a range of 10 to 150 µg/mL. The equivalency in mg/kg is not available. Standard solutions were placed at the start of the sample run to determine the linearity of response. The diluted sample solutions were injected so that they were bracketed at regular intervals with a standard solution of known nominal concentration. No details were provided to the calibration functions (r^2, number of samples and equation), no calibration curve is provided.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values of the dosing formulation and homogeneity analyses at each level and overall were < 20 %. Therefore, these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate acid were found for the dosing solution and during homogeneity analyses. Therefore, these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The detection limit was set in the study at 5.1 µg/mL in the analysed solution and calculated to be 2.6 mg/mL in the formulation. Acceptable recoveries were obtained at lowest fortification level 50 mg/mL..

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate acid in dosing formulations.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, recoveries calculated from analysis of dosing solutions, calibration curve not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
An analytical method for the determination of glyphosate acid in deionised water by HPLC/UV was provided. The method is not in agreement with the SANCO 3029/99. Validation data on the linearity, specificity are missing, the matrix effect and the efficiency of the deritivatisation are not examined. However, recovery and repeatability data are in the acceptable range. Therefore, the method can be considered as fit-for-purpose for the determination of glyphosate in solution at targeted doses.

Determination of glyphosate technical in rat diet

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/087 (CA 5.7.1/002)
Report authors	[redacted]
Report year	2006
Report title	Glyphosate technical: Ninety day repeated dose oral (dietary) neurotoxicity study in the rat
Laboratory	[redacted]
Report No	2060-0010
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not assessed • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate technical in rat diet by HPLC/Flu.
The dietary admixtures were extracted with 0.05 M di-sodium tetraborate to give a final theoretical test material concentration of approximately 25 mg/kg, then derivatised using 0.25 % (w/v) 9-fluoroenyl methyl chloroformate in acetone and left for 30 min to stand at room temperature. After dilution with water, the extract was analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 254 nm (excitation) and 315 nm (emission) using an external standard procedure.

Chromatographic conditions:

HPLC:	Agilent Technologies 1050, incorporating autosampler and workstation
Column:	Hypersil SAX 5µm (100 x 4.6 mm id)
Column oven temperature:	Not stated
Injection volume:	5 µL
Mobile phase:	Acetonitrile/0.1 % orthophosphoric acid (60/40, v/v)
Flow rate:	1 mL/min
Derivatisation agent (pre-column):	9-Fluorenylmethylchloroformate
Detection:	Fluorescence (excitation 254 nm, emission 315 nm)
Retention time:	Glyphosate technical: ~ 3.9 min

Findings

Recoveries

For method validation, rat diets were fortified at relevant concentrations of approximately 1000, 5000 and 20000 mg/kg and analysed using the analytical method. Control samples were also analysed without detecting glyphosate technical. The recovery results are shown in the table below. All average recovery values were between 70 % and 110 %.

Table 5.1-98: Results of the method validation for the determination of glyphosate technical in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1000	97 – 106	102	–	–	2
		5000	104 – 105	105	–	–	2
		20000	100 – 101	101	–	–	2
		Overall	97 – 106	102	3.4	3.4	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 triplicate samples of test diets prepared at three different time points during study duration were analysed using the analytical method. The results of these analyses are provided in table below. These are not true validation recovery data; however the results show the good performance of the method. Mean recoveries and %RSD are in the acceptable limits.

Table 5.1-99: Results of rat diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate	1000	81 – 100	92	7.8	8.6	9

Table 5.1-99: Results of rat diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		5000	80 – 106	94	9.4	10.0	9
		20000	79 – 98	91	8.0	8.9	9
		Overall	79 – 106	92	8.3	9.0	27

[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 interference was observed at the retention time of interest.

Linearity
Linearity of detector response was tested using six calibration standard concentrations in the range of 0 to 38.175 mg/L with correlation coefficients of > 0.99. The equivalency in mg/kg is not available. The calibration standards were prepared in solvent (0.05 M Di-sodium tetraborate solution) and derivatised as described above. The calibration graph was linear in this range. All determinations of glyphosate were chromatographed at concentrations within this linear range. Details on the calibration are provided below.

Table 5.1-100: Details on linearity of the method

Calibration function	Calibration concentrations (mg/L)	Number of determinations	Equation	Coefficient of correlation (r)
Linear	0 – 38.175	6 levels	y = 6.756 x – 2.080	1.000

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.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate technical were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not reported. Nevertheless, the limit of quantification can be set at the acceptable minimum concentration used in the recovery test i.e 1000 mg/kg.

Interference
Chromatograms of standard solutions, of control (blank basal laboratory diet) and of matrix fortified (1000, 5000 and 20000 ppm) were provided. No interference was observed at the retention time of the analyte.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

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 is considered as fit-for-purpose for the determination of glyphosate technical in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is submitted to the EU for the first time. It was performed under GLP and meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method isconsidered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
An analytical method for the determination of glyphosate in in rat diet by HPLC-Flu was provided. The method is not in agreement with the SANCO 3029/99 as some data on matrix effect and derivatisation efficiency are missing.
However, linearity and specificity were provided and validated. Recovery and repeatability are in the acceptable range . Therefore, the method can be considered as fit for purpose for the determination of glyphosate in rat diet.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/088 (CA 5.7.1/003)
Report authors	[redacted]
Report year	1996
Report titles	Glyphosate acid: Subchronic neurotoxicity study in rats
Laboratory	Not available
Report No	[redacted]/P/4867
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of rat diet • Calibration curve not given • Interference not assessed (no chromatograms provided) • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 rat diet (CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK) by HPLC/UV.

An aliquot of the rat diet was extracted with water and shaken for 30 minutes. An aliquot of the supernatant was removed, filtered and diluted with water to a known concentration within the range of calibration standards. An aliquot of the diluted sample was added to a borax-solution (disodium tetraborate in water) and an FMOC-Cl solution (9-Fluorenylmethylchloroformate in acetone) for the derivatisation, shaken for 30 seconds and rotated for 10 minutes. Ethyl acetate was added and shaken for a further 30 seconds, when the two layers had separated the lower aqueous layer was subjected to high performance liquid chromatography (HPLC) with UV detection (265 nm) using external standardisation.

Chromatographic conditions:

HPLC:	SA6410B (Severn Analytical) or similar 600 series (Waters)
Column:	S5NH, 25 cm x 4.6 mm id (Hichrom)
Column oven temperature:	Ambient
Injection volume:	15 or 25 µL
Mobile phase:	Acetonitrile/0.025M Na_2HPO_4, pH 8.5 (60/40, v/v) or Acetonitrile/0.025M KH_2PO_4, pH 8.5 (60/40, v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethylchloroformate)
Detection:	SA6500 (Severn Analytical) or similar 486 (Waters): UV, 265 nm
Retention time:	Glyphosate acid: not provided (no chromatograms available)

Findings

Recoveries

Samples of rat diet were spiked with glyphosate acid at three fortification levels 2000, 8000 and 20000 mg/kg. For each analysis, recovery was determined in triplicate at each level. These validation recoveries were not presented in detail within the report.

Additionally, the achieved concentration of the diet was tested at three time points during the study. These are not true validation recovery data; however the results show the good performance of the method. Mean recoveries and %RSD are in the acceptable limits. The results are shown in the table below.

Table 5.1-101: Results of test diet analyses

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Diet	Glyphosate acid	2000	83 – 114	92	10.8	11.7	10
		8000	98 – 105	101	3.0	3.0	6
		20000	98 – 104	100	2.1	2.1	6
		Overall	83 – 114	97	8.6	8.9	22

[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
Not assessed. No chromatograms are provided by the report.

Linearity
Standard solutions were prepared in water within a range of 10 to 150 µg/mL. The equivalency in mg/kg is not available. Standard solutions were placed at the start of the sample run to determine the linearity of response. The diluted sample solutions were injected so that they were bracketed at regular intervals with a standard solution of known nominal concentration. No details (r^2 and n) were provided to the calibration functions, no calibration curve is provided.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values of the diet analyses at each level and overall were < 20 %. Therefore, these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate acid were found for the diet analysis. Therefore, these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The detection limit in the study was calculated to be approximately 0.17 µg/mL in the analysed solution and calculated to be 10 mg/kg in rat diet. Acceptable recoveries were obtained at lowest fortification level with a validated recovery and repeatability 2000 mg/kg.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, recoveries calculated from analysis of rat diet, calibration curve not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method for the determination of glyphosate acid in rat diet (CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK) by HPLC/UV is not in agreement with the SANCO 3029/99. Some data on the linearity (r^2, n and equation), specificity (interference), matrix effect and derivatisation efficiency are missing. However, mean recoveries and repeatability are in the acceptable range.. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in diet at the targeted doses.

Determination of glyphosate acid in aqueous formulations

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/089 (CA 5.7.2/001)
Report authors	[redacted]
Report year	1996
Report titles	Glyphosate acid: Acute delayed neurotoxicity study in the domestic hen
Laboratory	[redacted]
Report No	[redacted]/C/3122
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) 1. Not sufficient validation recoveries given, additional recoveries calculated from analysis of dosing solutions 2. Matrix effect and stability of sample extracts not assessed 3. Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
Method was developed for the determination of glyphosate acid in aqueous formulations by HPLC-UV.
An aliquot of the test suspension was dissolved in an appropriate volume of aqueous trimethylamine (0.1 M) to provide a solution containing glyphosate acid in the expected concentration range 2 – 4 µg/mL. For derivatisation an aliquot of the diluted extract was mixed with saturated borax solution and derivatising reagent (3.75 g 1-fluoro-2,4-dinitrobenzene in 1 L ethanol). The mixture was allowed to stand in the dark for 1 hour. A citrate buffer (pH 3.0) was added and the mixture was partitioned two times with ethyl acetate. The ethyl acetate layers were discarded. The aqueous layer was acidified by adding 25 % aqueous orthophosphoric acid and partitioned with ethyl acetate. The ethyl acetate layer was collected and evaporated to dryness. The residue was re-dissolved in mobile phase to provide a solution containing glyphosate acid in the expected concentration range 4 – 8 µg/mL. The solution was filtered and the concentration of glyphosate acid was quantified by high performance liquid chromatography using ultra-violet detection and an external standard procedure.

Chromatographic conditions:

HPLC:	Pump: Perkin Elmer LC 200, Autosampler: Perkin Elmer ISS200, Detector: Spectra Physics Spectra 100, Data handling: Perkin Elmer Nelson Access*Chrom.
Analytical Column:	Nucleosil ODS, 5 µm, 250 x 4.6 mm id, Phase Sep.
Guard column:	Aquapore ODS, 7 µm, 15 x 3.2 mm id, Applied Biosystems.
Column temperature:	Ambient, nominally 21 °C ± l °C
Injection volume:	20 µL
Mobile phase:	Acetonitrile/Solvent A (l/5, v/v); (solvent A: solution of Tetraethyl-ammonium bromide and sodium dihydrogen orthophosphate in water (pH 3.0))
Flow rate:	1.0 ml/minute
Derivatisation agent (pre-column):	1-Fluoro-2,4-dinitrobenzene
Detector wavelength:	UV, 383 nm.
Retention time:	Glyphosate: ~ 7.5 min

Findings

Recoveries

Samples were spiked with the analyte at one fortification level at 208.8 mg/mL and diluted appropriately. All average recovery values (mean of 8 replicates) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-102: Results of the method validation for the determination of glyphosate acid in aqueous formulation

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous formulation	Glyphosate acid	208.8	92 – 102	98	3.0	3.1	7

[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 a formulation of glyphosate acid prepared for investigation of the stability of glyphosate acid in formulation was analysed at three different time points and at three different depth of formulation (representing the top, middle and bottom). These are not true validation recovery data; however the results show the good performance of the method. The results of these analyses are provided in the table below.

Table 5.1-103: Results of stability of glyphosate acid in formulation

Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous formulation	Glyphosate acid	208.8 (top)	93 – 98	96	2.7	2.8	3
		208.8 (middle)	95 – 97	96	1.4	1.4	3
		208.8 (bottom)	96 – 99	97	1.5	1.5	3
		Overall	93 – 99	96	1.9	1.9	9

[1] Results in the report were calculated using unrounded figures and corrected for the appropriate mean procedural recovery value. Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity
Chromatograms of control of standard solution and of sample fortified at 208.8 mg/mL were provided. No interference was observed at the retention time of the analyte.

Linearity
Linearity of detector response was tested using 5 calibration standard concentrations in the range of 2 – 10 µg/mL with correlation coefficients of >0.99. The equivale,ncy in mg/kg is not available. The calibration standards were prepared in 0.1 M aqueous triethylamine. Details to the calibration are provided below.

Table 5.1-104: Details on linearity of the method

Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
Linear	2 – 10	5 levels	y = 24700 x + 663.06	0.9985

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate acid were found for aqueous formulations. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection, defined as the concentration of glyphosate acid in control matrix producing a peak response equivalent to 3 x baseline noise, was calculated to be 0.15 mg/mL. Acceptable recoveries were obtained at lowest fortification level with a validated recovery and repeatability 208 mg/mL.

Matrix effects
Not assessed.

Stability of analytes in formulation
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate acid in dosing formulations.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (not sufficient validation recoveries given, additional recoveries calculated from analysis of dosing solutions, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study.

Assessment and conclusion by RMS:
An analytical method for the determination of glyphosate acid in rat diet (CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK) by HPLC/UV is not in agreement with the SANCO 3029/99. Some data on matrix effect and derivatisation efficiency are missing. However, linearity and specificity (interference) are acceptable. Recovery and repeatability data are in the acceptable range with a minimum validated level at 208 mg/mL. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in aqueous formulation at targeted doses.

Determination of aminomethyl phosphonic acid (AMPA) in dosing solution

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/090 (CA 5.8.1/005)
Report authors	[redacted]
Report year	1988
Report title	Aminomethyl phosphonic acid: Acute oral toxicity to the rat
Laboratory	[redacted]
Report No	[redacted]/P/2266
Document No	AR4690
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) 1. Details on chromatographic conditions missing 2. No validation recoveries given 3. Calibration curve and function not given 4. Limit of quantification and detection not assessed 5. Interference not assessed (no chromatograms provided) 6. Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
A method was developed for the determination of aminomethyl phosphonic acid in dosing solution (0.5 % (w/v) aqueous polysorbate 80) by HPLC using a fluorescence detector
The dosing preparation was shaken thoroughly and duplicate portions taken. These portions were diluted with distilled water to give solutions of known theoretical concentrations of aminomethyl phosphonic acid. The diluted dosing preparation was analysed by HPLC using a fluorescence detector by a single point calibration standard after demonstrating linearity of the detector.
Chromatographic conditions:
Detailed information missing.

Findings
Recoveries
No validation recoveries from fortified samples were presented within the report.
However, the achieved concentration of aminomethyl phosphonic acid in the dosing solution was tested. These are not true validation recovery data; however, the results are very limited, but show the good performance of the method. One concentration (500 mg/mL) was tested with a recovery in acceptable range.The results are shown in the table below.

Table 5.1-105: Results of dosing solution analyses

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	AMPA	500	103	103	–	–	1

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

Specificity
Not assessed, no chromatograms given within the report.

Linearity
Using the test formulation as the reference material, a range of standard solutions was prepared comparable in concentration with that of the diluted dosing preparation. A calibration graph was constructed to demonstrate detector linearity and the sample concentration calculated using a single point calibration standard. Number of samples, calibration function and curve, and correlation coefficient are missing.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values were not calculated due to limited data set.

Accuracy
Acceptable mean recovery values were not calculated due to a limited data set.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) as information given within the report is very limited. Nevertheless, the method is considered as fit-for-purpose for the determination of aminomethyl phosphonic acid in dosing formulations as analytic was not absolutely necessary. The dosing solution (correct gravimetrically produced) was administered by gavage, but no information about the time between preparation and administration was given within the report.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (details on chromatographic conditions missing, no validation recoveries given, calibration curve and function not given, limit of quantification and detection not assessed, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
An analytical method for the determination of aminomethyl phosphonic acid in dosing solution (0.5 % (w/v) aqueous polysorbate 80) by HPLC using a fluorescence detector was provided. The method is not in agreement with the SANCO 3029/99. Validation data on the specificity, repeatability and some data on linearity are missing. Furthermore, only one recovery is available at one fortification level. The recovery is in acceptable range, nevertheless the concentration tested cannot be linked to the targeted dose (5000mg/kg) as the unit used is not the same. Therefore, the method cannot be considered as fit for purpose.

Study previously submitted to the EU (Validation of analytical method report submitted to the EU for the first time)

1. Information on the study

Data point	CA 4.1.2/091
Report authors	[redacted]
Report year	1993
Report title	Establishment and validation of method no. 5391 for the analysis of aminomethylphosphonic acid in dosing suspension. Assessment of homogeneity, accuracy and stability of formulations
Report No	8918
Document No	IRI Project No. 353917
Test facility (analytical part)	Inveresk Research International Limited, Tranent, EH33 2NE, Scotland
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	No, submitted for the first time
GLP/Officially recognised testing facilities	No official GLP statement included, no GLP certificate provided, but the purpose for GLP mentioned on p.4 of the report.
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/092 (CA 5.8.1/014)
Report authors	[redacted]
Report year	1993
Report title	AMPA: 4 week dose range finding study in rats with administration by gavage
Report No	7803
Document No	[redacted] Project No. 450860
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/093 (CA 5.8.1/016)
Report authors	[redacted]
Report year	1993
Report title	AMPA: 13 week toxicity study in rats with administration by gavage
Report No	7866
Document No	[redacted] Project No. 450876
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/098 (CA 5.8.1/028)
Report authors	[redacted]
Report year	1992
Report title	AMPA: Teratogenicity study in rats
Report No	7891
Document No	[redacted] Project No. 490421
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

The method no. 5391 was developed for the determination of AMPA in 0.5 % carboxymethylcellulose (CMC) by HPLC with UV detection. The same method has been used in each report.

Samples (1 g) of formulated gavage were weighed accurately. After adding distilled water and internal standard (glyphosate) solutions, samples were derivatized. Aliquots were transferred into separate vials and saturated sodium tetraborate and 2,4-dinitrofluorobenzene (used for derivatisation) were added. The samples were sonicated for 2-3 min and left at room temperature. After half an hour, sodium chloride, distilled water and ethyl acetate were added, the samples were sonicated again, left until layers were separated and the ethyl acetate layer was removed. Aqueous orthophosphoric acid (25 %) and ethyl acetate were added to the aqueous layer. The ethyl acetate layer was extracted into a clean scintillation vial, blown down under N_2 and reconstituted in mobile phase. The samples were analysed for AMPA by HLPC with UV detection.

Chromatographic conditions:

HPLC:	Waters 510 pump, Waters Wisp 712 autosampler
Column:	Nucleosil 120 C18 (5 µm), 25 cm x 4.6 mm i.d.
Column temperature:	Not stated
Injection volume:	20 µL
Mobile phase:	0.05 M NaH_2PO_4 in 0.02 M trimethylammonium chloride (pH 3)/ Acetonitrile (5/1, v/v)
Flow rate:	1.0 mL/min
Derivatisation agent:	2,4-dinitrofluorobenzene
Detector:	Waters 484 UV Vis spectrometric detector, detection at 383 nm, data handling was performed on a Trivector 3000 data station
Retention time:	Glyphosate (IS): ~ 4.6 min AMPA: ~ 7.5 min

Findings

Recoveries

Analysis of dosing formulations was performed to determine the concentration accuracy and stability. The data show that the formulations used were prepared to an acceptable level of accuracy, were homogenous, and were stable for at least 24 hours. Average recovery values were between 70 % and 110 % for each fortification level and the RSD values were below 20 %. The detailed results are given in the tables below.

Full validation was performed in study 8918 (table 4.1-108 to 4.1-110). Other tables refer to additional recovery data only. Since the same method is used in each report, these additional recoveries are confirmatory data.

Table 5.1-106: Analysis of dosing formulations – initial stability analysis

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
7891 [redacted]	Dosing formulation	AMPA	1 (initial time point)	98 – 100	98.8	1.1	1.1	3
			1 (after 24h)	99-106	102	3.8	3.8	3
			10 (initial time point)	99-102	100	1.9	1.9	3
			10 (after 24h)	97-101	99	2.1	2.1	3
			100 (initial time point)	102 – 106	102	2.3	2.3	3
			100 (after 24h)	100-102	101	1.5	1.5	3

[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 recovery values as given in the report.

Table 5.1-107: Analysis of dosing formulations – concentration accuracy

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
7891 [redacted]	Dosing formulation	AMPA	1	93 – 103	98	3.1	3.2	12
			10	91 – 100	96	2.8	2.9	12
			100	87 – 103	96	5.4	5.6	12

[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 recovery values as given in the report.

Additionally, analyses of dosing suspensions were undertaken on samples prepared during weeks 1 and 4 (Report No. 7803) or weeks 1, 6, and 13 (Report No. 7866) of dosing. Analyses showed dosing suspensions were generally within acceptable limits (± 10 %) for accuracy of preparation and homogeneity. The detailed results are given in the table below.

Table 5.1-108: Analysis of dosing suspensions

Reference	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
7803 [redacted]	Dosing suspension	AMPA	1	104 – 115	107	4.2	3.9	6
			10	97 – 103	99	2.3	2.3	6
			35	90 – 93	91	1.2	1.3	6
			100	93 – 96	95	1.5	1.5	6
7866 [redacted]	Dosing suspension	AMPA	1	102 – 120	112	5.8	5.1	12
			10	104 – 126	116	7.6	6.5	15
			100	94 – 116	106	5.7	5.4	12

[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 recovery values as given in the report.

In Report No. 8918, 5 quality control samples were prepared at 10.2 µg AMPA/mL and 5 at 509 µg AMPA/mL for analysis of assay accuracy and precision according to method no. 5391 along with calibration standard samples. The detailed results are presented in the table below.

Table 5.1-109: Assay accuracy and precision for the determination of AMPA in dosing suspensions

Report No.	Matrix	Analyte	Fortification concentration (µg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
8918 [redacted]	Dosing suspension	AMPA	10.2	92 – 114	101	8.3	8.2	5
			509	101 – 105	103	1.4	1.3	5

[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 recovery values as given in the report.

Furthermore, 1.0 and 100 mg AMPA/mL dosing suspensions were prepared in the dispensary and 5 aliquots of each analysed. The gavage formulations were stored in the dark at ambient temperature for 25 hours and 32 days and the analysis repeated at each time point. No significant change in concentration was observed after either storage at ambient temperature for 24 hours or for 32 days. Both formulations showed satisfactory homogeneity. Before each analysis the test material was mixed to ensure resuspension. The detailed results are shown in the tables below.

Table 5.1-110: Determination of homogeneity of dosing suspensions

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
8918 [redacted]	Dosing suspension	AMPA	1	89 – 108	98	6.8	7.0	5
			100	93 – 101	98	3.1	3.2	5

[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 recovery values as given in the report.

Table 5.1-111: Determination of stability of dosing suspensions

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
8918 [redacted]	Dosing suspension	AMPA	1 (initial time point)	98-100	99	1.1	1.1	3
			1 (after 24h)	99-106	102	3.9	3.8	3
			1 (after 32 days)	98-101	99	2.1	2.2	3
			100 (initial time point)	100-104	102	2.4	2.3	3
			100 (after 24h)	100-102	101	1.5	1.5	3
			100 (after 32 days)	97-101	100	2.1	2.1	3

[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 recovery values as given in the report.

Specificity (interference)

Chomatograms have been submitted for calibration standards, control and fortified gavage solution. No interferences were noticed at the retention time of AMPA.

Linearity

All standards were prepared in water. Samples were prepared containing 0, 9.9, 49.5, 99, 248 and 495 µg AMPA/mL (eq to 0 – 10mg/mL in gavage solution) and all containing the internal standard, glyphosate, at a level of 100 µg/mL. Samples at higher level are further diluted (200 times for the gavage solution at 10mg/mL, 1000 times for the gavage solutionat 35mg/mL and 2000 times for the gavage solution at 100mg/mL) and fall within the linearity range.

A plot of the resultant peak area ratios versus the relevant concentration of AMPA showed good linearity over the range examined with a correlation coefficient of 0.9998 (linear regression).

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were <20 %. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.
Furthermore, the chromatographic system precision was determined by injecting one standard at 49.5 µg AMPA/mL ten times. The relative standard deviation (RSD) of recovery values was 2.1%.

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for AMPA were generally found in gavage suspensions, except for fortification levels 1 and 10 mg/mL (Report No. 7866) with minor exceedance (112 % and 116 %), which is acceptable. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method no. 5391 does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of AMPA in dosing suspensions (0.5 % carboxymethylcellulose).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level. They meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (LOQ and LOD not stated, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological studies.

Assessment and conclusion by RMS:
The method is not v validated according to guidance SANCO/3029/99/rev.4. Matrix effects and derivatisation efficiency were not investigated in this study.
However, specificity, linearity, accuracy/precision (2 fortified levels with n=5/level) were acceptable. A LOQ is not necessary since the method is used to confirm the content of the analyte in the gavage solution.
The method can be considered as fit for purpose for the determination of AMPA in solution.

Determination of aminomethyl phosphonic acid (AMPA) in rat diet

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/094 (CA 5.8.1/017)
Report authors	[redacted]
Report year	1979

Report title	90-Day subacute rat toxicity study ([redacted]-78-174)
Report No	401-050
Document No	-
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/095
Report authors	[redacted]
Report year	1979
Report title	Analysis of animal feed diets in the aminomethyl phosphonic acid (AMPA) 90-day subacute rat toxicity study, performed at International Research and Development Corporation
Report No	MSL-0682
Document No	1763
Test facility (analytical part)	International research and development corporation, Mattawan Michigan
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the Monograph (2002)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

The analyses for the above mentioned toxicological study ([redacted], 401-050) were reported within a separate report ([redacted], MSL-0682) in the following a summary of the analytical report is given.

Principle of the method

The analytical method was developed for the determination of AMPA in rat diet (Purina@ Laboratory Chow@) by liquid chromatography using a variable wavelength UV/Visible detector after post column derivatisation with ninhydrin.

An aliquot (10 g) of mixed rodent diet was extracted with deionised water and chloroform (2/1, v/v) by shaking for 30 minutes. The extract was centrifuged and an aliquot of the aqueous layer (2 mL) was withdrawn. After filtration the extraction step was repeated for a second time. A second aliquot (2 mL) was withdrawn, the whole extraction solution was filtered, layers separated and the organic layer was discarded. The final volume of the aqueous layer was determined as this was necessary for calculation purposes. The two aliquots of the aqueous phase were combined and were subjected to an ion exchange resin cleanup (AG 50W-X8, 200-400 mesh hydrogen

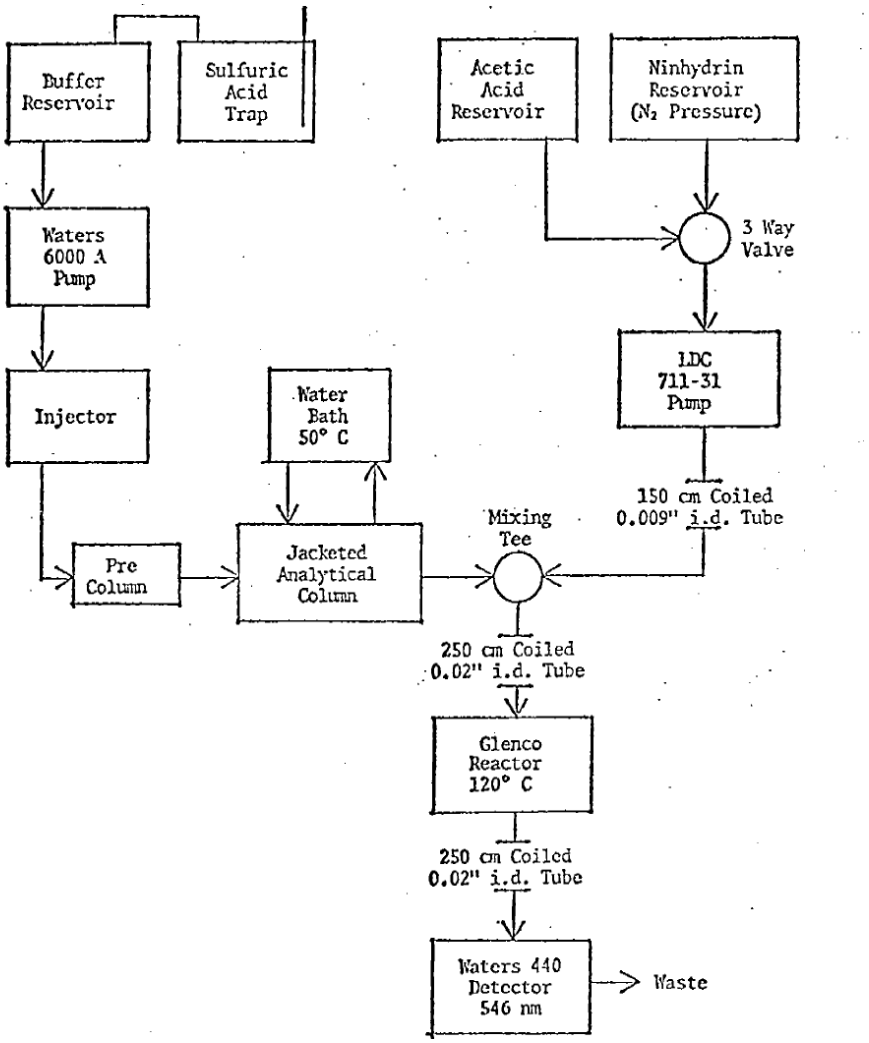
form analytical grade cation exchange resin, Bio-Rad Laboratories Richmond, Calif.). The eluant is filtered, diluted appropriately and subjected to HPLC system fitted with a ninhydrin post-column reactor and measuring the color generated using a UV detector. A graphical illustration of the used HPLC system is given in the figure below.

Chromatographic conditions:

HPLC:	Waters 6000A pump with a Waters U6K injector or a Varian 8500 autosampler LDC 711-31 pump Glenco RC-1 reaction coil (120 °C)
Column[(a)]:	Pre-column: C_{18}/Corasil, 4.5 cm x 0.6 cm o.d. 0.3 cm i.d. Column: Aminex A-9, 30 cm x 4.6 mm i.d.
Column temperature:	50 °C
Injection volume:	Not given within the report
Mobile phase:	HPLC buffer solution: 0.005 M potassium dihydrogen phosaphate in 4% methanol/deionisied water (adjusted to pH 1.9 by phosphoric acid) Ninhydrin-solution: Solution of 80 g ninhydrin and 2.5 g hydrindantin in a solvent-mixture of dimethyl-sulfoxide, deionisied water and 4.0 M sodium acetate solution (3/2/1, v/v/v; stored for a maximum of two weeks under N_2)
Flow rate[(a)]:	0.5 mL/min (buffer flow rate) 0.5 mL/min (ninhydrin flow rate)
Pressure[(a)].	~ 2000 psi buffer ~ 600 psi ninhydrin
Derivatisation agent:	Ninhydrin post column reactor
Detection:	Waters Model 440 Absorbance detector with 546 nm filter
Retention time:	AMPA: not readable within the chromatograms

(a): As page 16 is missing in pdf of final report of [redacted], 1979, MSL-0682, information was taken out of [redacted], 1981, 77-2063, p.338.

Figure 5.1-3: Graphical illustration of the HPLC system used for analysis.



Findings

Recoveries

The method proved to be suitable to determine residues of AMPA in rat diet. Samples were spiked with the analyte at five fortification levels. All average recovery values (mean of 2-14 replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-112: Results of the method validation for the determination of AMPA in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	AMPA	100	90 – 107	99	9.5	9.6	4

Table 5.1-112: Results of the method validation for the determination of AMPA in rat diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		200	110 – 112	111	1.5	1.3	2
		3000	82 – 106	93	6.4	6.9	12
		10000	85 – 99	90	4.1	4.6	10
		50000	92 – 100	96	3.0	3.1	14

[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 recovery values as given in the report.

Samples of test diets prepared at several weeks during study duration were analysed using the analytical method (week 1 – 4, 6, 8, 12 of study duration, samples of diet taken on day 1 and day 7 of each week). 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 5.1-113: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	AMPA	400	76 – 102	89	6.0	6.7	28
		1200	83 – 103	93	5.0	5.3	28
		4800	78 – 111	96	7.1	7.5	28

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

Specificity (interference)
Chromatograms were provided for fortified and treated diet. Chromatograms of control diet and standard are missing.

Linearity
Linearity of detector response was tested using 8 calibration standard concentrations in the range of 1.0 – 50.0 µg/mL (eq to approx. 44 – 2240 mg/kg). Standards were prepared in deionised water. Further information on calibration functions and curves are missing.

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

Accuracy
Acceptable mean recovery values at LOQ and 10x LOQ between 70 % and 110 % for AMPA were found for rat diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection (LOD) was 25 mg/kg for a sample of 10 g.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method was successfully validated for the determination of AMPA in rat diet. The analytical method fulfils the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. However, the method is considered as fit-for-purpose for the determination of AMPA in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve and function not given, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The method cannot be considered sufficiently validated according to guidance SANCO/3029/99 rev.4. Specificity cannot be checked due to absence of chromatogram for control diet. Matrix effects and derivatisation efficiency were also not investigated.
However, results obtained are acceptable for accuracy/precision As the aim of this study is to confirm the content of AMPA in the diets, the method can be considered as fit for purpose.
A LOQ is not necessary since the method is used to confirm the content of the analyte in the diet.

It should be noticed that chloroform has been used. This solvent should be avoided for future pre registration studies.

Determination of aminomethyl phosphonic acid (AMPA) in aqueous solutions

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/096 (CA 5.8.1/018)
Report authors	[redacted]
Report year	1991
Report title	90-Day oral (capsule) toxicity study in dogs with AMPA
Report No	[redacted]-50173
Document No	[redacted]-90-354

Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No validation recoveries given • Calibration curve and function not given • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/097
Report authors	[redacted]
Report year	1991
Report title	Results of the stability analyses of AMPA (aminomethyl phosphonic acid) test material used in a 90-day dog study at WIL Laboratories (WI-90-354)
Report No	MSL-11291
Test facility (analytical part)	Wil Reseach Laboratories, Inc, Ashland
Document No	ML-90-369, EHL 90163
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No validation recoveries given • Calibration curve and function not given • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the Monograph (2002)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

The analyses for the above mentioned toxicological study ([redacted], [redacted]-50173) were reported within a separate report ([redacted], MSL-11291). In the following a summary of the analytical report is given.

The aim of study [redacted]-50173 is to investigate the toxicologic potential of AMPA when administered to dogs. AMPA is added in the gelatin capsule used for dog feeding. The analytical method used in this study is only to demonstrate that the test material (AMPA) was stable throughout the course of the experiment. The analysis of

the test material was performed at the beginning and at the conclusion of the study. Results were provided in another report (ML 90-369).
Stability of the lot of AMPA used as test material in study [redacted]-50173 was determined by purity assays. To demonstrate it, several dilutions in deionized water of neat AMPA test material were analysed by HPLC-UV. The material was assayed by comparison to peak areas obtained using analytical grade (99.1% pure) AMPA for preparation of standard. Each assay analysis was repeated ten times to obtain a mean assay value. These mean assay values were used as a measure of the stability of the neat test material (lot PIT-9008-2407-T). The pre-test assay result was 91.8% (n=10; 14/09/1990) and the post test assay results was 91.2% (n=10; 04/04/1991). Results were in accordance with the purity obtained by supplier (88.3-90.6%).

The method used for the determination of the purity is described below.

Principle of the method
Method was developed for the determination of test material AMPA purity by liquid chromatography using a variable wavelength UV/Visible detector after post column derivatisation with ortho-phthalaldehyde.
Appropriate dilutions of AMPA in deionized water were analysed. The post-column reagent solution was prepared by dissolving 7.5 g boric acid and 4.5 g NaOH in water, followed by stirring until a total dissolved solution was established. After degassing and filtering 0.1 g o-phthalaldehyde in 15 ml MeOH and 0.25 mL of 2-mercaptoethanol were added, following by stirring until a total dissolved solution was received. Concentration of AMPA was assayed by external standard procedure.

Chromatographic conditions:

HPLC:	Varian 5500 LC with autosampler; integration by Spectra-Physics 4270 integrator
Column:	Dupont Zorbax 300 SCX. 4.6 mm x 25 cm
Column oven temperature:	No information
Injection volume:	20 µL
Mobile phase:	0.01 M KH_2P0_4/4 % MeOH, pH 2.3 with H_3P0_4.
Post column reagent:	Boric acid and NaOH with 1.5 % MeOH, 2-mercaptoethanol, o-phthalaldehyd
Flow rate:	0.6 mL/min for both eluent and post-column reagent solutions
Derivatisation (post-column):	ortho-Phthalaldehyde
Detection:	Variable wavelength; 340 nm
Retention time:	AMPA: ~ 5.86 min

Findings
Recoveries
No validation recoveries with fortified samples were presented within the analytical method.
As the aim of this study is to assess the stability of the test material AMPA used for the 90-Day oral (capsule) toxicity study in dogs, only analyses to determine the purity of the neat AMPA at the beginning and at the end of the toxicity was performed. The test material was analysed ten times in both case and results are given in the table below:

Table 5.1-114: Results of aqueous solution analyses

Matrix	Analyte	concentration in test solution analysed (mg/L)	Mean purity of test material (%)	Relative standard deviation (%)	Number analyses (n)
neat test material AMPA	AMPA	400 (begin)	92	0.3	10
		400 (end)	91	0.9	10

Specificity (interference)
No chromatogram was provided.

Linearity
Linearity of detector response was tested using 5 calibration standard concentrations in the range of 100 to 500 mg/L prepared in deionised water. A linear regression line was calculated. No details to the calibration functions are provided.

Repeatability (Precision)
Ten replicates were analysed at the beginning and at the end of the study. RSD were below 1%.

Accuracy
Accuracy was not assessed.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. However, the method is considered as fit-for-purpose for the determination of test material AMPA purity used in the toxicological study.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed not under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, calibration curve and function not given, limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:

The method is not validated according to guidance SANCO/3029/99/rev.4. Several data are missing : linearity, specificity (interference), matrix effects and derivatisation efficiency. Recovery and repeatability data are in acceptable range at the targeted dose. Therefore, the method can be considered fit for pupose for the determination of AMPA in gelatine capsule at the targeted dose

Determination of aminomethyl phosphonic acid (AMPA) in corn oil dosing solutions

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/099 (CA 5.8.1/030)
Report authors	[redacted]
Report year	1991
Report title	A developmental toxicity study of AMPA in rats
Report No	[redacted]-50159
Document No	[redacted]-90-266
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient number of validation recoveries given, further recoveries calculated from analysis of dosing solutions • Calibration curve and function not given • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in the Monograph (2002)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/100
Report authors	[redacted]
Report year	1990
Report title	Results of the analyses of corn oil samples from WIL Research Laboratories for AMPA (aminomethyl phosphonic acid)
Report No	MSL-10674
Document No	ML-90-290 / EHL 90164
Test facility (analytical part of study Wil-50159)	Not reported
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Not sufficient number of validation recoveries given, further recoveries calculated from analysis of dosing solutions

	• Calibration curve and function not given • Limit of quantification and detection not assessed • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in the Monograph (2002)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

The analyses for the above mentioned toxicological study ([redacted], [redacted]-50159) were reported within a separate report ([redacted], MSL-10674). In the following a summary of the analytical report is given.

Principle of the method
Method was developed for the determination of AMPA in corn oil dosing solutions by liquid chromatography (LC) using a variable wavelength UV/VIS detector.
An aliquot of corn oil samples was extracted with deionized water by shaking vigorously for at least 20 minutes. An aliquot was centrifuged at 1800 rpm for 20 minutes. The bottom aqueous layer was diluted and then subjected to a liquid chromatography (LC) with a variable wavelength UV/VIS detector using an external standard procedure. The analyte AMPA was quantified relative to bracketing standard solutions.

Chromatographic conditions:

HPLC:	Varian 5500 HPLC
Column:	Bio-Rad A-9; 300 x 4.6 mm with a Brownlee 30 x 4.6 mm PRP-1 guard column
Column oven temperature:	No information
Injection volume:	20 µL
Mobile phase:	5 mM KH_2PO_4 solution in water/methanol (96/4, v/v), adjusted to pH 2.1 with 85 % phosphoric acid; isocratic for 10 minutes
Flow rate:	1.0 mL/min
Detector:	Variable wavelength detector, wavelength 200 nm
Retention time:	AMPA: ~ 5.9 min

Findings
Recoveries
The method proved to be suitable to determine residues of AMPA in corn oil dosing solutions. Samples were spiked with the analyte at three fortification levels at 15, 40 and 100 mg/mL. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below; given values are means of duplicate analyses.

Table 5.1-115: Results of the method validation for the determination of AMPA in corn oil dosing solutions

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Corn oil	AMPA	15	87 – 111	99	12.0	12.1	3

Table 5.1-115: Results of the method validation for the determination of AMPA in corn oil dosing solutions

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
dosing solutions		40	108	108	–	–	1
		100	91 – 92	92	–	–	2

[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 recovery values as given in the report.

Additionally, the achieved concentration of the corn oil dosing solution was tested at two time points during the study (day 0 and last day); samples were taken from the top, middle and bottom of the well stirred dosing solution, respectively. These are not true validation recovery data; however the results show the good performance of the method. The results are shown in the table below; given values are means of duplicate analyses.

Table 5.1-116: Results of corn oil dosing solution analyses

Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Corn oil dosing solutions	AMPA	15	100 – 120	112	6.8	6.1	8
		40	105 – 108	107	–	–	2
		100	110 – 120	114	5.5	4.8	5

[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 recovery values as given in the report.

Specificity (interference)
No chromatograms of control corn oil have been included in the report. However, it is stated that blank corn oil samples at each time point containing no test material were also analysed together with samples and indicated no significant interferering materials were present.

Linearity
Linearity of detector response was tested using up to 6 calibration standard concentrations in the range of 1000 – 6000 mg/L (eq in mg/kg is not reported). Standard solutions were prepared in distilled water. A linear regression was calculated, further information is missing. A calibration curve and function is not given within the report.

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

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for AMPA were found for corn oil dosing solutions with minor exceedance of mean recovery values of 112 – 114 % for dosing solution analyses. These exceedances were accepted as they are only 2 – 4 % over the limit of 110 % and as the RSDs were well below 20 % (2.0 – 6.1 %). Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed. Acceptable recoveries were obtained at lowest fortification level.

Interference
Control samples did not reveal any peaks in the chromatogram, which would interfere with the determination of AMPA.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of AMPA in corn oil dosing solutions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was not performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (not sufficient number of validation recoveries given, further recoveries calculated from analysis of dosing solutions, calibration curve not given, limit of quantification and detection not assessed, matrix effect and stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The method does not fulfil criteria set in guidance SANCO/3029/99 rev.4. Some data are lackaging for linearity, matrix effect, accuracy, precision.

However, some recovery data (3 levels, with 1 to 3 replicates) were performed and are in the acceptable range. As this method is used to confirm the content of AMPA in corn oil dosing solution administered orally by gavage to animals, it can be considered sufficiently validated for its purpose.

Determination of N-Acetyl AMPA in rat diet

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/101 (CA 5.8.1/033)
Report authors	[redacted]
Report year	2008
Report title	IN-EY252 technical: Subchronic toxicity 90-day feeding study in rats
Report No	[redacted]-23316
Document No	-
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limit of quantification and detection not reported • Stability of sample extracts 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)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of N-Acetyl AMPA in rat diet (PMI Nutrition International, LLC Certified Rodent LabDiet® 5002, meal) by liquid chromatography (LC) with a mass spectrometric (MS) detection.

Aliquots (5 ± 0.05 g) of diet samples were mixed with methanol (100 mL), and the mixtures were placed in an ultrasonic bath and sonicated for approximately 60 minutes, with swirling every 15 minutes. The extracts were then filtered (Gelman type, 0.45 µm PFTE HPLC certified) and aliquots of the filtrates were further diluted using control diet (blank) extracts and methanol prior to analysis by LC-MS.

Chromatographic conditions:

HPLC:	Agilent (Hewlett-Packard) Model 1100			
Column:	Agilent Zorbax 300SB-CN, 5 µm, 150 x 4.6 mm			
Column temperature:	30 °C			
Injection volume:	2.0 µL			
Mobile phase:	A: Methanol/water (50/50, v/v) B: Acetonitrile			
Gradient Method 1:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.00	95	5	0.5
	2.50	95	5	0.5
	2.60	20	80	0.9
	5.00	20	80	0.9
	5.10	95	5	0.5
	10.00	95	5	0.5
Gradient Method 2:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.00	5	95	0.5
	2.80	5	95	0.5
	2.90	95	5	0.9
	7.00	95	5	0.9
	7.10	5	95	0.5
	12.00	5	95	0.5
Retention time:	N-Acetyl AMPA, Method 1: ~ 2.3 min N-Acetyl AMPA, Method 2: ~ 5.8 min			
Detection:	MS (Waters (Micromass) ZQ			
Ionization mode:	Electrospray negative (ES-)			
Monitored mass:	152 *m/z*			
MS parameters:	Capillary voltage:	3.0 kV	Source temperature:	120 °C

	Cone voltage:	30.0 V	Desolvation temperature:	300 °C

Findings

Recoveries

For method validation, blank control diet samples were fortified at relevant concentrations of nominally 900 and 18000 mg/kg with the analyte and analysed concurrently with the test diet samples. The average recovery values at each fortification level and overall were between 70 % and 110 %. The results are shown in the table below.

Table 5.1-117: Results of method validation for the determination of N-Acetyl AMPA in rat diet

Matrix	Analyte	Fortification concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	N-Acetyl AMPA	900	87– 105	99	6.7	6.8	5
		18000	102 – 110	106	3.3	3.1	5

[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 recovery values as given in the report.

Test diets prepared at three time points were sampled and analysed using the analytical method. Blank control samples were also analysed without detecting N-Acetyl AMPA. The average recovery values at each fortification level and overall were between 70 % and 110 %, with relative standard deviations (RSDs) of <20 %. The results are shown in the table below.

Table 5.1-118: Results of concentration verification of test diets

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	N-Acetyl AMPA	900	96 – 103	99	3.9	4.0	3
		6000	98 – 108	102	5.5	5.5	3
		18000	99 – 109	106	5.5	5.2	3

[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 recovery values as given in the report.

Additionally the homogeneity of the diet mixtures was verified by sampling test diet at the bottom, middle and top of the mixing vessels and analysed using the analytical method. The average recovery values at each fortification

level and overall were between 70 % and 110 %, with relative standard deviations (RSDs) of < 20 %. The results are shown in the table below.

Table 5.1-119: Results of homogeneity verification of test diet

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	N-Acetyl AMPA	900	90 – 97	94	3.7	3.9	3
		6000	100 – 104	102	1.7	1.7	3
		18000	91 – 106	100	8.4	8.4	3

[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 recovery values as given in the report.

Specificity (interference)
Chromatograms have been provided for standards, control and fortified diet. No significant interferences were noticed in blank diet.

Linearity
Linearity of detector response was tested using 5 calibration standard concentrations in the range of 1.06 to 5.30 µg/mL (replicate injections, eq to approx. 20 – 106 mg/kg) prepared in control diet extracts and methanol (matrix-matched standards). Linear correlations were found with coefficients of determination (r^2) of >0.99. Details of a representative LC-MS calibration curve are presented below. Note that samples are further diluted to be in the linearity range.

Table 5.1-120: Details of representative calibration function

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
N-Acetyl AMPA	Linear (1/x weighting)	1.06 – 5.30	5 levels	y = 1145.83 x – 422.438	>0.99

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values from all tests at each fortification level and overall were < 20 %, therefore in compliance with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for N-Acetyl AMPA were found in all tests. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not reported. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed and not required, analytical standards were prepared in matrix-matched solutions (blank diet extracts and methanol).

Stability of analytes in sample extracts
Not assessed. However stability of N-Acetyl AMPA was confirmed for neat test substance purity for the duration of the study and in the test diets for 15 days.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. The method is considered as fit-for-purpose for the determination of N-Acetyl AMPA in rat diet.

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) with minor deficits (limit of quantification and detection not assessed, stability in sample extracts not assessed). The method is considered as fit-for-purpose to support the toxicological study.

Assessment and conclusion by RMS:
The method is validated according to guidance SANCO 3029/99/rev.4. Specificity, linearity (with matrix matched calibration), accuracy and precision were assessed and found acceptable.
A LOQ is not necessary since the method is used to confirm the content of the analyte in the diet.

Determination of N-Acetyl AMPA in dosing solutions (DI water)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/102 (CA 5.8.1/037)
Report authors	[redacted]
Report year	2007
Report title	IN-EY252: Mouse bone marrow micronucleus test
Report No	[redacted]-22226
Document No	-
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation data (validation done by analysis of test samples) • Limit of quantification and detection not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of N-Acetyl AMPA (IN-EY252) in dosing solutions (distilled water) by high-performance liquid chromatography (HPLC) with a UV/Vis detector.
An aliquot (0.50 mL) of each dosing solution sample was quantitatively transferred to a 100 mL volumetric flask and diluted to volume with HPLC water. All samples except the control sample were further diluted with HPLC water to give a nominal concentration of 25.0 µg/mL, active ingredient. Samples were analysed by high-performance liquid chromatography (HPLC) with a UV/Vis detector at 210 nm.

Chromatographic conditions:

HPLC:	Agilent Model 1100
Column:	Zorbax 300SB-CN, 5 µm, 150 x 4.6 mm
Column temperature:	40 °C
Injection volume:	5.0 µL
Mobile phase:	A: 60 % H_3PO_4 3.1 mM B: 40 % Acetonitrile
Flow rate:	0.5 mL/min
Derivatisation agent:	Not applicable (not derivatised)
Detection:	UV absorbance at 210 nm
Retention time:	N-Acetyl AMPA: ~ 3.48 min

Findings
Recoveries
No validation recoveries from fortified samples were presented within the report.
However, the achieved concentrations of N-Acetyl AMPA in the dosing solutions was tested by duplicate analyses. These are not true validation recovery data. Despite the available data are limited, they show the good performance of the method with average recovery values between 70 % and 110 %. A control sample was also analysed without detecting N-Acetyl AMPA. The results are shown in the table below.

Table 5.1-121: Results of the analysis of dosing solutions

Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	N-Acetyl AMPA	50	99 – 108	104	/		2
		100	102 – 104	103	/		2
		200	101 – 110	105	/	/	2

[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 recovery values as given in the report.

Specificity (interference)
Chromatograms have been provided for calibration standard, control and dosing solution at 100 mg/mL. No significant interferences were noticed.

Linearity
Linearity of detector response was tested using four calibration standard concentrations in the range of 10 to 50 µg/mL (single injections, eq to 2 – 10 mg/mL in dosing solution) prepared in HPLC water. A linear correlation was found with a coefficient of correlation (r) of 0.99193 (y = 1.30750945 x – 0.6911716).
Note that all samples except the control sample were further diluted with HPLC water to give a nominal concentration of 25.0 µg/mL.

Repeatability (Precision)
Only 2 recoveries per level were performed. A RSD on two samples is not representative.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for N-Acetyl AMPA were found for dosing solutions. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed and not required, dosing solutions were prepared in distilled water.

Stability of analytes in sample extracts
Stability of N-Acetyl AMPA in dosing solutions was assessed and targeted concentrations were shown to be stable for 5 hours at room temperature.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of N-Acetyl AMPA in dosing solutions.

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) with deficits (no true validation data (validation done by analysis of test samples), limit of quantification and detection not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The analytical method is not completely validated according to guidance SANCO 3029/99/rev.4. Only 2 recoveries per level were analysed and no true RSD can be determined.
However, three different levels were tested (50, 100 and 200 mg/mL) and acceptable recoveries are obtained in all cases (70-110%), . The method can be considered as fit for purpose. A LOQ is not required in this case since the aim of the study is to confirm the dose of the analyte in the dosing solution which is further given to animals by oral intubation.

Determination of N-Acetyl glyphosate in rat diet

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/103 (CA 5.8.1/040)
Report authors	[redacted]
Report year	2007

Report title	IN-MCX20: Subchronic toxicity 90-day feeding study in rats
Report No	[redacted]-19008
Document No	-
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Insufficient validation data with fortified samples • Limit of quantification and detection not reported • Stability of sample extracts not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of N-Acetyl glyphosate (IN-MCX20) in rat diet (PMI Nutrition International, LLC Certified Rodent LabDiet® 5002) by liquid chromatography (LC) with tandem mass spectrometric (MS/MS) detection.

Aliquots (2.5 g) of diet samples were mixed with methanol (50 mL for 180 and 900 mg/kg samples; 100 mL for 4500 and 18000 mg/kg samples), followed by the addition of Nanopure® water in the same amounts as the aliquots of the stock solution used to make calibration standards. These mixtures were placed in an ultrasonic bath and sonicated for approximately 60 minutes, with swirling every 15 minutes. The extracts were then filtered (Gelman type, 0.45 µm PFTE HPLC certified) and aliquots of the filtrates were either diluted with 0.2 M formic acid or control diet (blank) extract together with 0.2 M formic acid prior to analysis by LC-MS/MS.

Chromatographic conditions:

HPLC:	Agilent (Hewlett-Packard) Model 1100			
Column:	Phenomenex Luna® Phenyl-Hexyl, 5 µm, 150 x 2.0 mm			
Column temperature:	30 °C			
Injection volume:	50 µL			
Mobile phase:	A: 0.2 M Formic acid in Nanopure® water B: Acetonitrile			
Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.00	95	5	0.1
	0.50	80	20	0.1
	2.00	80	20	0.1
	2.10	95	5	0.1
	6.00	95	5	0.3
	9.50	95	5	0.1
	10.00	95	5	0.1
Retention time:	N-Acetyl glyphosate: ~ 4.7 min			
Detection:	MS/MS (Waters (Micromass) Quattro Micro)			
Ionization mode:	Electrospray (ESI) positive			
Monitored transition:	*m/z* 212 → 170			

MS parameters:	Capillary voltage:	3.75 kV	Source temperature:	150 °C
	Cone voltage:	13.0 V	Desolvation temperature:	350 °C

Findings

Recoveries

For method validation, blank control diet samples were fortified at relevant concentrations of nominally 180, 883, 4486 and 18013 mg/kg with the analyte and analysed concurrently with the test diet samples. The average recovery values at each fortification level and overall were between 70 % and 110 %. The results are shown in the table below.

Table 5.1-122: Results of method validation for the determination of N-Acetyl glyphosate in rat diet

Matrix	Analyte	Fortification concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	N-Acetyl glyphosate	180	83 – 101	92	–	–	2
		883	100 – 106	103	–	–	2
		4486	100	100	–	–	1
		18013	97 – 101	99	–	–	2

[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 recovery values as given in the report.

Test diets prepared at two time points were sampled and analysed using the analytical method. Blank control samples were also analysed without detecting N-Acetyl glyphosate. The average recovery values at each fortification level and overall were between 70 % and 110 %, with an overall relative standard deviation (RSD) of < 20 %. The results are shown in the table below.

Table 5.1-123: Results of concentration verification of test diets

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	N-Acetyl glyphosate	180	65 – 82	74	–	–	2
		900	84 – 97	91	–	–	2
		4500	85 – 92	89	–	–	2
		18000	89 – 105	97	–	–	2

[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 recovery values as given in the report.

Additionally the homogeneity of the diet mixtures was verified by sampling test diet at the bottom, middle and top of the mixing vessels (in duplicate each time) and analysed using the analytical method. The average recovery

values at each fortification level and overall were between 70 % and 110 %, with relative standard deviations (RSDs) of < 20 %. The results are shown in the table below.

Table 5.1-124: Results of homogeneity verification of test diet

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	N-Acetyl glyphosate	180	84 – 96	89	6.1	6.9	3
		900	80 – 96	86	8.8	10.2	3
		4500	9 – 108	101	5.9	5.8	3
		18000	93 – 100	98	3.7	3.8	3

[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 recovery values as given in the report.

Specificity (interference)
Chromatograms have been provided for solvent, control diet and fortified diet. No interference were noticed in the control diet.

Linearity
Linearity of detector response was tested using four calibration standard concentrations in the range of 0.252 to 1.28 µg/mL (triplicate injections, eq of the linearity range in mg/kg was not reported) prepared in control diet extracts and 0.2 M formic acid in water (matrix-matched standards). Linear correlations were found with coefficients of determination (r^2) of > 0.99. Details of a representative LC-MS/MS calibration curve are presented below.
According to the report, calibration standards were prepared to bracket the target concentrations of the diluted diet extracts.

Table 5.1-125: Details of representative calibration function

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
N-Acetyl glyphosate	Linear (1/x weighting)	0.252 – 1.28	5 levels	y = 9359.89 x + 344.322	>0.99

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values from all tests at each fortification level and/or overall were < 20 %, therefore in compliance with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for N-Acetyl glyphosate were found in all tests.

Limit of Quantification and Detection
Not reported. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed and not required, analytical standards were prepared in matrix-matched solutions (blank diet extracts and 0.2 M formic acid in water).

Stability of analytes in sample extracts
Not assessed. However stability of N-Acetyl glyphosate in the test diets was confirmed for 21 days.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. The method is considered as fit-for-purpose for the determination of N-Acetyl glyphosate in rat diet.

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) with minor deficits (insufficient validation data with fortified samples, limit of quantification and detection not assessed, stability in sample extracts not assessed). The method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The method is not validated according to guidance SANCO/3029/99 rev.4. Accuracy/precision were not assessed with sufficient number of samples per level (only n=3).

However, several recovery determinations were performed for homogeneity verification, concentration and validation.the results are in acceptable range. Moreover, specificity (interference) and linearity are acceptable. Therefore, the method can be considered as fit for purpose for the determination of N-Acetyl glyphosate in rat diet.

Determination of N-Acetyl glyphosate in dosing solutions (Nanopure® water)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/104 (CA 5.8.1/044)
Report authors	[redacted]
Report year	2006
Report title	IN-MCX20: Mouse bone marrow micronucleus test
Report No	[redacted]-20154
Document No	-
Test facility (analytical part)	[redacted]
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation data (validation done by analysis of test samples) • Limit of quantification and detection not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of N-Acetyl glyphosate (IN-MCX20) in dosing solutions (Nanopure® water) by liquid chromatography (LC) with tandem mass spectrometric (MS/MS) detection. Each dosing sample (0.5 mL) was initially diluted with 100 mL of Nanopure® water. The samples were further diluted to a final expected concentration of 0.0005 mg/mL with Nanopure® water for analysis and 100 and 200 mg/mL levels were matrix corrected. The 0 mg/mL sample followed the 50 mg/mL sample dilutions. Samples were analysed by LC-MS/MS.

Chromatographic conditions:

HPLC:	Agilent (Hewlett-Packard) Model 1100			
Column:	Phenomenex Luna® Phenyl-Hexyl, 5 µm, 150 x 2.0 mm			
Column temperature:	30 °C			
Injection volume:	50 µL			
Mobile phase:	A: 0.2 M Formic acid in Nanopure® water B: Acetonitrile			
Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.00	95	5	0.1
	0.50	80	20	0.1
	2.00	80	20	0.1
	2.10	95	5	0.1
	6.00	95	5	0.3
	9.50	95	5	0.1
	10.00	95	5	0.1
Retention time:	N-Acetyl glyphosate: ~ 4.8 min			
Detection:	MS/MS (Waters (Micromass) Quattro Micro)			
Ionization mode:	Electrospray (ESI) positive			
Monitored transition:	*m/z* 212 → 170			
MS parameters:	Capillary voltage:	3.75 kV	Source temperature:	120 °C
	Cone voltage:	18.0 V	Desolvation temperature:	350 °C

Findings

Recoveries

No validation recoveries from fortified samples were presented within the report.

However, the achieved concentrations of N-Acetyl glyphosate in the dosing solutions was tested by duplicate analyses. These are not true validation recovery data. Despite the available data are limited, the data show the performance of the method with average recovery values between 108 % and 114 %. A control sample was also analysed without detecting N-Acetyl glyphosate. The results are shown in the table below.

Table 5.1-126: Results of the analysis of dosing solutions

Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dosing solution	N-Acetyl glyphosate	50	109 – 118	114			2
		100	105 – 120	113			2
		200	106 – 110	108			2

[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 recovery values as given in the report.

Specificity (interference)
Chromatograms have been provided for control and fortified Nanopure water. No interferences were noticed in nanopure water.

Linearity
Linearity of detector response was tested using four calibration standard concentrations in the range of 0.204 to 0.816 µg/mL (triple injections, eq in mg/mL in Nanopure solutions is not reported) prepared in Nanopure® (matrix matched). A linear correlation was found with a coefficient of determination (r) >0.99 (y = 1.5105e7 x – 217.897). Note that samples were further diluted to a final expected concentration of 0.5 µg/mL with Nanopure® water for analysis, falling in the linearity range.

Repeatability (Precision)
Only two samples per level were analysed. No representative RSD can be determined with only 2 determinations.

Accuracy
The mean recovery values at each fortification level and overall were above 110 % for N-Acetyl glyphosate in most cases and therefore not in compliance with EU guideline document SANCO/3029/99 rev. 4. However these recoveries were not determined with fortified samples, and the analyses show that the dosing solutions were even higher concentrated than nominally intended.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed and not required, analytical standards were prepared in matrix-matched solutions.

Stability of analytes in sample extracts
Stability of N-Acetyl glyphosate in dosing solutions was assessed and targeted concentrations were shown to be stable for 5 hours at room temperature.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of N-Acetyl glyphosate in dosing solutions.

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) with deficits (no true validation data (validation done by

analysis of test samples), limit of quantification and detection not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study.

Assessment and conclusion by RMS:
The analytical method is validated according to guidance SANCO 3029/99/rev.4. Only 2 recoveries per level were analysed and no true RSD can be determined.

However, three different levels have been tested (50, 100 and 200 mg/mL). Recoveries were slightly higher than 110% (up to 114%) but can be considered as acceptableThe method can be considered as fit for purpose for the determination of N-Acetyl glyphosate in dosing solution.

Determination of glyphosate in mice diet

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/105 (CA 5.8.2/001)
Report authors	[redacted]
Report year	2012
Report title	Glyphosate – A 28-day oral (dietary) immunotoxicity study in female B6C3F1 mice
Report No	[redacted]-50393
Document No	[redacted]-10-460
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Limit of quantitation not given • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

2. Full summary of the study according to OECD format

Principle of the method
This method was developed for the determination of glyphosate concentrations in diet formulations (PMI Nutrition International, LLC, Certified Rodent LabDiet® #5002 (meal)) containing test substance in concentration ranging from 400 to 6000 mg/kg using a high performance liquid chromatography (HPLC) method with ultraviolet (UV) absorbance detection at a wavelength of 265 nm.
An aliquot of the diet was extracted with water by shaking for approximately 30 minutes on a horizontal shaker at high speed and sonicated for approximately 10 minutes followed by centrifugation for approximately five minutes. A portion of the sample extract was filtered through a 0.45-µm pore-size syringe-end filter and further diluted with water as necessary. An aliquot of each diluted sample was combined with 25 mM di-sodium tetraborate solution and with 10 mM FMOCCl (9-fluoroenylmethyl chloroformate) solution in polypropylene tubes. The tubes were mixed with vortex action and placed on a sample rotator for approximately 20 minutes. An aliquot of each

processed formulation sample was diluted as necessary and was subjected to HPLC-UV analysis. Quantitation was done using external standard procedure both in water and in matrix.

Chromatographic conditions:

HPLC:	Agilent 1100 liquid chromatograph equipped with a variable wavelength detector, autosampler, and Dionex Chromeleon® software, or equivalent system
Column:	Waters Spherisorb®, 5µm particle-size NH2, 250 × 4.6 mm
Column temperature:	25 °C
Injection volume:	20.0 µL
Mobile phase:	Acetonitrile (ACN)/25 mM potassium phosphate monobasic (pH 6.0) (60/40, v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Detector:	UV at 265 nm
Retention time:	Glyphosate: approximately 9.9 to 10.8 minutes

Findings

Recoveries

The method proved to be suitable to determine residues of glyphosate in diet. Samples were spiked with the analyte at three fortification levels. All average recovery values (mean of nine replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the following table.

Table 5.1-127: Results of the method validation for the determination of glyphosate in diet (standards in water)

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Diet	Glyphosate	400	81 – 88	85	2.0	2.4	9
		2500	94 – 100	97	2.1	2.1	9
		6000	94 – 99	97	2.0	2.1	9
		Overall	81 – 100	93	6.2	6.7	27

[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.

In addition, the method was cross-validated for the use of matrix matched calibration standards. Matrix samples were spiked with the analyte at five fortification levels. All average recovery values (mean of 3 to 6 replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-128: Results of the cross validation for the determination of glyphosate in diet (matrix matched standards)

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Diet	Glyphosate	250	104 – 105	104	0.7	0.7	3
		400	100 – 104	101	1.5	1.5	6
		2500	98 – 100	99	0.7	0.7	6
		6000	100 – 101	101	0.4	0.4	6
		7500	99 – 100	100	0.9	0.9	3
		Overall	98 – 105	101	1.9	1.9	24

[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
Control samples did not reveal any peaks in the chromatogram, which would interfere with the determination of glyphosate.

Linearity
Linearity of detector response was tested using 5 calibration standard concentrations in triplicate in the range of 10.0 to 60.0 µg/mL. The equivalency in mg/kg is not available.. The glyphosate peak areas (y) and the theoretical concentrations (x) of the calibration standards were fit with least-squares regression analysis to the quadratic function: $y = ax^2 + bx + c$. The calibration standards were prepared in water or matrix. Linearity plots and calibration functions are not provided in the report.

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found for in diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not defined within the study. Acceptable recoveries were obtained at lowest fortification level for wich the repeatability and the recovery is acceptable (400 ppm).

Matrix effects
Matrix-matched standards as well as standards in solvent were used for establishment of calibration data.

Stability of analytes in sample extracts
Test substance stability was assessed in calibration and in processed samples for recovery experiments stored at room temperature for four days.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in diet formulations.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (calibration curve and function not given, limit of quantitation not given, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99. The linearity plots and calibration function were not provided.
However, the recoveries have been performed in water and in diet. The range of recoveries are in acceptable range and in agreement with the targeted dose. The method can be considered as fit for purpose for the determination of glyphosate in diet

Determination of citric acid and trisodium citrate dihydrate in water or/and diet

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/106 (CA 5.8.2/002)
Report authors	[redacted]
Report year	2010
Report titles	An eight week oral (diet and gavage) toxicity study of citric acid in male rats
Report No	[redacted]-50361
Document No	[redacted]-09-015
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve and function not given • Limit of Quantification and Detection not defined • Matrix effect not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

2. Full summary of the study according to OECD format

Principle of the method
Two different methods were developed within this current toxicological study.

The first method was developed for the determination of citric acid in water by HPLC-UV at concentrations of 50 – 200 mg/mL. An aliquot of the citric acid formulation was extracted with water under vortex assistance. Samples were further diluted with water to achieve a theoretical final citric acid concentration within the calibration range and subjected to HPLC-UV analyses.
The second method was developed for the determination of citric acid and trisodium citrate concentration by HPLC-UV in rodent diet admix formulations at concentrations of 14000 and 21400 mg/kg. An aliquot of the formulated diets was extracted with water by shaking at high speed for 30 minutes. After centrifugation and filtration, samples were further diluted with water to achieve a theoretical final citric acid concentration within the calibration range and subjected to HPLC-UV analyses.
Quantitation was done using external standard procedure.

Chromatographic conditions:

HPLC:	Agilent 1100 liquid chromatograph equipped with a variable wavelength detector, autosampler, and Dionex Chromeleon® software, or equivalent system
Column:	Phenomenex Rezex ROA-Organic Acid H+, 300 × 7.8 mm, 8 µm particle-size
Column temperature:	55 °C
Injection volume:	25.0 µL
Mobile phase:	0.005 N sulfuric acid (H_2SO_4) in deionised water
Flow rate:	0.500 mL/min
Detector:	UV at 230 nm
Retention time:	Citrate ion from citric acid and trisodium citrate dihydrate: approximately 10.3 to 10.6 minutes for the

Findings
Recoveries
Samples were spiked with the analyte at 4 fortification levels. All average recovery values (mean of 3 replicates per fortification level and analyte) were between 70 % and 110 %. The detailed results are given in the table below. Calculation of the trisodium citrate concentrations was achieved by applying a correction factor of 1.54 due to the difference in molar mass between citric acid and trisodium citrate dihydrate. Additionally, the concentration of citric acid inherent in blank diet formulations was subtracted from the overall measured concentrations of citric acid and trisodium citrate dihydrate in diet samples.

Table 5.1-129: Results of the method validation for the determination of citric acid and trisodium citrate dihydrate in water or/and diet

Matrix	Analyte	Fortification level (mg/mL or mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Water	Citric acid	50	99 – 99	99	0.3	0.3	3
		200	98 – 99	99	0.5	0.5	3
		Overall	98 – 99	99	0.4	0.4	6
Rat diet	Citric acid	14000	95 – 96	95	0.4	0.4	3

Table 5.1-129: Results of the method validation for the determination of citric acid and trisodium citrate dihydrate in water or/and diet

Matrix	Analyte	Fortification level (mg/mL or mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Trisodium citrate dihydrate	21400	97 – 99	98	1.0	1.0	3

[1] Calculations of RSDs and overall values were performed using Excel with individual recovery values as given in the report.

Specificity
Control samples did not reveal any peaks in the chromatogram, which would interfere with the determination of analytes. Citrate was detected in the analyzed basal diet administered to the control group at a similar concentration to that found in basal diet during method qualification.

Linearity
Linearity of detector response was tested using five calibration standard concentrations in triplicate in the range of 50.0 to 1000 µg/mL. The equivalency in mg/kg is not available. The resulting citric acid peak area versus theoretical citric acid concentration data were fit to the linear function using least-squares regression analysis. The calibration standards were prepared in water. Linearity plots and calibration functions are not provided in the report.
Concerning the trisodium citrate, calculation of concentrations was achieved by applying a correction factor of 1.54 due to the difference in molar mass between citric acid and trisodium citrate dihydrate. Additionally, the concentration of citric acid inherent in blank diet formulations was subtracted from the overall measured concentrations of citric acid and trisodium citrate dihydrate QC diet samples and formulation diet samples. The concentrations of the QC and formulation samples were further calculated by applying any necessary multiplication factors to correct for dilution and/or unit conversions.

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for citric acid and trisodium citrate dihydrate were found for water or/and diet. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not defined within the study. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Test substance stability was assessed in calibration standards prepared with citric acid and stored at room temperature for two days.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of citric acid and trisodium citrate dihydrate in water or/and diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve and function not given, limit of quantification and detection not defined, matrix effect not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99. The linearity plots and calibration function were not provided.
However, the recoveries have been performed in water and in diet. The recoveries are in acceptable range and in agreement with the targeted dose. The method can be considered as fit for purpose for the determination of citric acid and trisodium citrate in water and rat diet.

Determination of glyphosate acid in rat diet

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/107 (CA 5.8.2/003)
Report authors	[redacted]
Report year	1996
Report titles	Glyphosate acid: Comparison of salivary gland effect in three strains of rat
Report No	[redacted]/P/5160
Document No	PR1029
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries given, recoveries calculated from analysis of rat diet • Calibration curve and function not given • Interference not assessed (no chromatograms provided) • Matrix effect and stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Not available

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 rat diet (CTl, supplied by Special Diet Services Limited, Witham, Essex, UK) by HPLC-UV.

An aliquot of the diet (10 g) was extracted with water using mechanical shaking for 30 minutes. The extract was filtered or centrifuged and the supernatant was diluted with water to obtain a solution to a known nominal concentration within the range of the calibration standards. An aliquot of the extract was taken, to which a di-sodium tetraborate solution (945 mg in 100 mL water) and a 9-Fluorenylmethyl chloroformate solution (FMOC-Cl, 260 mg in 100 mL acetone) were added for derivatisation. After shaking for 20 min, ethyl acetate was added, shaken and the layers were allowed to separate. An aliquot of the lower aqueous layer was removed for subsequent analysis by high performance liquid chromatography (HPLC) with UV detection at 265 nm using an external standard procedure.

Chromatographic conditions:

HPLC:	600 Series (Waters)
Column:	S5NH (Hichrom), 25 cm x 4.6 mm ID
Column temperature:	Ambient
Injection volume:	20 µL
Mobile phase:	Acetonitrile (60 % v/v) 0.025 M KH_2PO_4, pH 6 (40 % v/v)
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Detector:	LC235 Diode Array (Perkin Elmer), Detector wavelength 265 nm
Retention time:	Glyphosate acid: not provided (no chromatograms available)

Findings
Recoveries
For each analysis, recovery was determined in triplicate. No further information is given within the report.
Additionally samples of test diets (two batches) prepared at one time point during the study were analysed using the analytical method. The limited 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 5.1-130: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Rat diet	Glyphosate acid	20000	100 – 103	101	1.3	1.3	4

[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
Not assessed. No chromatograms are provided by the report.

Linearity
The analysis system was calibrated using a range of standards to determine the linearity of response. An appropriate standard of known concentration was interspersed at intervals throughout the analysis. Linearity plots and calibration functions are not provided in the report.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values of the diet analyses at each level and overall were limited but < 20 %. Therefore these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at each fortification level and overall between 70 % and 110 % for glyphosate acid were found for the diet analysis. Therefore these recoveries are in line with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of detection was calculated to be approximately 0.1 µg/mL test substance in the analysed solution, corresponding to a dietary concentration of 50 mg/kg. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not assessed. Standard and calibration samples were prepared in water.

Stability of glyphosate acid in extracts
Not assessed.

Conclusion
The analytical method does partly fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate acid in rat diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries given, recoveries calculated from analysis of rat diet, calibration curve and function not given, interference not assessed (no chromatograms provided), matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99. The linearity plots and calibration function were not provided.. The quantification is based on derivatisation of the substance and no data have been provided to demonstrated that derivatisation reaction is complete.

However, recoveries available are based on analysis of rat diet and are in acceptable range.As the method is only used to check that the dose in diet corresponds to the targeted dose, the method can be considered as fit or purpose for the determination of glyphosate in rat diet at the targeted dose.

Determination of glyphosate in aqueous formulations containing 0.5 % methylcellulose

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/108
Report authors	[redacted]
Report year	2011
Report title	Analytical validation and stability study of glyphosate in aqueous formulations
Report No	WIL-843004
Document No	-
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Joint Glyphosate Task Force, LLC c/o Data Group Management 8325 Old Deer Trail Raleigh, NC 27615

Data point	CA 4.1.2/109 (CA 5.8.3/005)
Report authors	[redacted]
Report year	2012
Report title	A uterotopic assay of glyphosate administered orally in ovariectomized rats
Report No	[redacted]-843002
Document No	[redacted]-2011-0272
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (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	CA 4.1.2/110 (CA 5.8.3/006)
Report authors	[redacted]
Report year	2012
Report title	A Hershberger Assay of glyphosate administered orally in peripubertal orchidoepididymectomized rats
Report No	[redacted]-843003
Document No	[redacted]-2011-0271
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (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	CA 4.1.2/111 (CA 5.8.3/007)
Report authors	[redacted]
Report year	2012
Report title	A pubertal development and thyroid function assay of glyphosate administered orally in intact juvenile/peripubertal male rats
Report No	[redacted]-843005
Document No	[redacted]-2011-0302
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (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:	CA 4.1.2/112 (CA 5.8.3/008)
Report authors	[redacted]
Report year	2012

Report title	A pubertal development and thyroid function assay of glyphosate administered orally in intact juvenile/peripubertal female rats
Report No	[redacted]-843007
Document No	[redacted]-2011-0303
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • LOQ and LOD not stated • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

2. Full summary of the study according to OECD format

Principle of the method

The method was developed for the determination of glyphosate in aqueous formulations containing 0.5 % methylcellulose by HPLC with UV detection. Analyses to demonstrate homogeneity, resuspension homogeneity for stored suspensions, and stability of the test substance formulations for up to 15 days of room temperature storage were conducted prior to the start of dose administration (Report No. [redacted]-843002, [redacted]-843003, [redacted]-843005, [redacted]-843007) in a previous study (Report No. [redacted]-843004).

Formulation samples were processed by adding deionised water and mixing by vortexing. Samples were further diluted with deionised water. The samples were mixed and derivatised by combining an aliquot (1 mL) with 1 mL of 25 mM disodium tetraborate solution and 2 mL of the FMOC-Cl (10 mM 9-fluorenylmethyl chloroformate in acetone) solution. The preparations were mixed by vortexing and rotated for approximately 20 minutes. An aliquot of each processed formulation sample was transferred to an autosampler vial for analysis via HPLC with UV detection.

Chromatographic conditions:

HPLC:	Agilent 1100 liquid chromatograph equipped with a variable wavelength detector, autosampler, and Dionex Chromeleon® software, or equivalent system
Column:	Waters Spherisorb® NH2, 250 x 4.6 mm, 5 µm particle size
Column temperature:	25 °C
Mobile phase:	25 mM potassium phosphate monobasic (pH 6.0)/acetonitrile (ACN) (40/60, v/v)
Injection volume:	20 µL
Flow rate:	1.5 mL/min
Derivatisation agent (pre-column):	9-fluorenylmethyl chloroformate (FMOC-Cl)
Detection:	UV at 265 nm
Retention time:	Glyphosate: approximately 7.2 – 7.8 minutes

Findings

Recoveries

The formulations used for oral administration were analysed to assess the test substance concentration acceptability. The analysed formulations showed concentrations within 70 % and 110 % of the target concentration with RSDs < 20 %. No test substance was detected in the analysed vehicle administered to the control group. The detailed results are given in the table below.

Table 5.1-131: Results of the concentration assessment

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
[redacted]L-843002 [redacted]	Dosing formulation	Glyphosate	20	105 – 105	105	–	–	2
			60	105 – 105	105	–	–	2
			200	103 – 104	104	–	–	2
			Overall	103 – 105	105	0.8	0.8	6
[redacted]-843003 [redacted]	Dosing formulation	Glyphosate	20	104 – 105	105	0.5	0.5	4
			60	104 – 105	105	0.5	0.5	4
			200	104 – 114	109	5.5	5.1	4
			Overall	104 – 114	106	3.5	3.3	12
[redacted]-843005 [redacted]	Dosing formulation	Glyphosate	20	97 – 102	100	1.7	1.7	10
			60	100 – 105	102	1.9	1.9	10
			200	104 – 114	108	3.2	3.0	10
			Overall	97 – 114	103	4.3	4.2	30
[redacted]-843007 [redacted]	Dosing formulation	Glyphosate	20	99 – 101	99	0.8	0.8	6
			60	101 – 107	103	2.1	2.1	6
			200	102 – 112	108	3.9	3.7	6
			Overall	99 – 112	103	4.2	4.1	18

[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.

In addition, the calibration acceptability was determined. The mean back-calculated concentrations at each calibration level were within ±10 % of the theoretical values. Therefore the reproducibility of the calibration data was acceptable. The detailed results are shown in the table below.

Table 5.1-132: Results of calibration acceptability

Report No.	Matrix	Analyte	Nominal concentration (µg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
WIL-843004 [redacted]	Dosing formulation	Glyphosate	10	98 – 101	100	0.7	0.7	9
			25	99 – 100	100	0.4	0.4	9
			40	100 – 101	100	0.4	0.4	9
			50	100 – 101	100	0.4	0.4	9
			80	100 – 100	100	0.3	0.3	9
			Overall	98 – 101	100	0.5	0.5	45

[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.

During each of three validation sessions, triplicate QC samples at 3 concentrations were prepared by combining glyphosate stock solution with formulation vehicle (0.5 % methylcellulose (w/v) and derivatized and analysed as described above. Single injections were made of each processed QC sample. The results of the regression analyses were used to calculate the corresponding concentrations from the QC peak area data. The variability (RSD) of the calculated QC concentration data was used as a measure of assay precision, and the difference between the theoretical and calculated mean QC concentrations was used as a measure of assay accuracy. The RSD of the calculated concentrations at each level was ≤15 % and the mean concentration at each level was within ±15 %. Therefore, the precision and accuracy was acceptable. The detailed results are presented in the table below.

Table 5.1-133: Analysis of precision and accuracy

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
WIL-843004 [redacted]	Dosing formulation	Glyphosate	1	91 – 94	93	1.0	1.1	9
			100	91 – 96	93	1.6	1.7	9
			200	93 – 96	94	1.0	1.1	9
			Overall	91 – 96	93	1.3	1.4	27

[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.

Furthermore, the test substance stability in primary dilution QC samples was assessed. The detailed results shown in the table below confirm stability of the test substance at the primary dilution stage for 3 days at room temperature.

Table 5.1-134: Three day room temperature stability analysis of the primary dilution of the QC samples

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
WIL-843004 [redacted]	Dosing formulation	Glyphosate	1	98 – 100	99	–	–	2
			200	99 – 101	100	–	–	2
			Overall	98 – 101	99	1.4	1.4	4

[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.

Calibration standards prepared at 10 and 80 µg/mL and QC samples prepared at 1 and 200 mg/mL were stored for 3 days at room temperature before re-analysis to assess test substance stability. The acceptance criteria for stability were met. The detailed results are shown in the tables below.

Table 5.1-135: Stability analysis of calibration standards and processed QC samples

Report No.	Matrix	Analyte	Nominal concentration	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
WIL-843004 [redacted]	DI water	Glyphosate	10 µg/mL	93 – 94	94	0.3	0.3	3
			80 µg/mL	100 – 102	101	1.2	1.1	3
			Overall	93 – 102	97	3.9	4.0	6
WIL-843004 [redacted]	Dosing formulation	Glyphosate	1 mg/mL	98 – 99	98	0.5	0.5	3
			200 mg/mL	99 – 101	100	0.9	0.9	3
			Overall	98 – 101	99	1.1	1.1	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.

In addition, duplicate samples from the top, middle, and bottom strata of the formulations prepared at target test substance concentrations of 1 and 200 mg/mL were analysed to assess test substance homogeneity. The formulations that remained after sampling, were divided into aliquots as would be used for daily dispensation. Representative aliquots were stored at room temperature for 2, 5, and 15 days at which time the test substance was resuspended by stirring. Duplicate samples were collected from the top and bottom strata of the aliquots and analysed to assess 2-, 5-, and 15-day resuspension homogeneity and stability. The results are summarised in the table below.

Table 5.1-136: Results of test substance homogeneity and resuspension homogeneity of formulations

Report No.	Matrix	Analyte	Nominal concentration (mg/mL)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
WIL-843004 [redacted]	Dosing formulation	Glyphosate	1	92-102	95	3.4	3.5	18
			200	97-111	101	3.9	3.9	18
			Overall	92-111	98	4.5	4.6	36

[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 interferences were observed in control samples at the retention time of interest.

Linearity
All standards were prepared in deionised water. During each of three validation sessions, a minimum of triplicate calibration standards at 5 concentrations (10, 25, 40, 50 and 80 µg/mL) were prepared and analysed. Single injections were made of each calibration standard. The resulting glyphosate peak area versus theoretical glyphosate concentration data were fit to the linear function using least-squares regression analysis. The results of the regression analyses were used to back-calculate the corresponding concentrations from the peak area data. The reproducibility of the calibration curve data was acceptable. The linearityplots and calibration functions are not available.

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

Accuracy
Acceptable mean recovery values at each fortification level between 70 % and 110 % for glyphosate were found in aqueous formulations. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
Not assessed. Acceptable recoveries were obtained at the lowest fortification level with a validated recovery and repeatability (1 mg/mL).

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Stability of analyte in samples was assessed for 3 days. Processed samples were stored for 3 days at room temperature and re-analysed again. Post-storage concentrations ranged from 98.5% to 100%.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits. Nevertheless, the method is considered as fit-for-purpose for the determination of glyphosate in aqueous formulations containing 0.5 % methylcellulose.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (LOQ and LOD not stated, matrix effect and stability of sample extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the toxicological studies.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99 rev 4. The linearity plots and calibration fucntions are not available. The matrix effect and derivatisation efficiency are examined.

However, the specificity/interference and the recoveries are in acceptable range and can be compiled as all analysis have been performed in the same laboratory. Therefore, the method can be considered as fit for purpose for the determination of glyohosate in the dosing formulation.

Data point	KCA 4.1.2/225
Report authors	[redacted]
Report year	2021
Report titles	Aminomethylphosphonic acid (AMPA): Method Validation with Stability and Homogeneity
Report No	8442132 (Monsanto Ref. No CV-2020-0206)
Document No	
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	No (with relevance to SANCO/3029/99 rev. 4)
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	Covance Laboratories Limited Shardlow Business Park Shardlow Derbyshire DE72 2GD UK

Principle of the method
The method was developed for the determination of aminomethylphosphonic acid (AMPA; purity 99.2% w/w) in water as test vehicle. The formulations were prepared by accurately weighting test item and dissolving in water by shaking, followed by direct analysis by high performance liquid chromatography with Tandem Mass Spectrometric Detection (HPLC-MS/MS) using an external standard technique.

Chromatographic conditions**:**

HPLC system:	Agilent Technologies 1100, Agilent G1311A pump and CTC PAL autosampler
HPLC column:	Kintex C8 (100 × 4.6 mm ID, 5 µm)
Column temperature:	40 °C
Mobile phase:	A: 0.05% (w/v) ammonium formate in water B: 0.05% (w/v) ammonium formate in methanol

Gradient:	Time (min)	% A	% B	Flow rate (mL/min)
	0.0	95	5	0.6
	3.0	5	95	0.6
	5.0	5	95	0.6
	5.01	95	5	0.6
	10.0	95	5	0.6
Injection volume:	50 µL			
Retention time:	AMPA: approx. 2 min			
Detection mode:	MS/MS (Applied Biosystems, API 3000; triple stage quadrupole mass spectrometer)			
Scan type:	MRM (Multiple Reaction Monitoring)			
Ionisation mode:	ESI negative			
Gas temperature:	450 °C			
ESI turboflow:	7 L/min			
Mass transition for evaluation:	AMPA: *m/z* 110.1→63.0 (dwell time 80 ms) AMPA: *m/z* 110.1→78.8 (dwell time 80 ms) (All results were processed using Total Ion Chromatogram mode and therefore both transitions were monitored combined)			

Findings

Recoveries

Samples of water were accurately fortified with known amounts of test item at nominal concentrations of 0.05 mg/mL and 50 mg/mL and directly analysed. The results of the analyses of accuracy and precision samples are given in the table below.

Table 5.1-137: Results of test item analyses

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Water	AMPA	0.0501	90 – 94	93	2.0	2.2	4[2]
		50.1	91 – 102	96	4.0	4.1	5
		Overall	90 – 102	95	3.4	3.6	9

[1] Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

[2] One measurement with a recovery of 52 % was considered as an outlier and not considered for mean and RSD calculation (consideration of this outlier would yield a mean of 85 % and a RSD of 21.7 %).

Specificity

The control samples (vehicle without test item) and an analysed solvent blank showed no significant interfering response at the retention time of the test item. Chromatograms of control sample, calibration standard and test samples (low and high level) are provided in the report.

Linearity

Linearity of detector response was tested using six calibration standard concentrations (single injections) in the range of 0.0001 to 0.0015 mg/mL with coefficient of determination (r^2) of 0.9987. The calibration

standards were prepared in water. An example quadratic calibration fit is presented in the report, details are presented in the table below.

Table 5.1-138: Details on linearity

Calibration function	Calibration concentrations (mg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Quadratic	0.0001 – 0.0015	7 levels	$y = 10389207084.4463\ x^2 + 235376200.0025\ x + 2329.2694$	0.9987

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for AMPA were found for test formulations. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
No information given within the report. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not relevant, test formulations in water.

Stability of analytes in sample extracts
The test item formulations in water were shown to be stable at room temperature in the light for 24 hours. Moreover the homogeneity of the formulations was shown by applying the analytical method.

Conclusion
The analytical method was used for the determination of AMPA in test formulations prepared in water. The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) without relevant deficiencies. Stability and homogeneity of test formulations was shown by applying the analytical method.

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) without relevant deficits. The method is considered valid and acceptable to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000). The method is considered validated.

Data point	KCA 5.8.1/045
Report author	[redacted]
Report year	2021
Report title	Aminomethylphosphonic acid (AMPA): Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*
Report No	8442150
Document No	CV-2020-0209
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) no. 440/2008 Method B13/14 (2008), U.S. EPA OCSPP 870.5100 (1998), Japanese MAFF (2011), ICH S2 (R1, 2012)
Deviations from current test guideline OECD 471 (1997)	None
Previous evaluation	No, not previously submitted
GLP	Yes
Acceptability/Reliability	Yes/yes
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Covance Laboratories Ltd., Shardlow, Derbyshire, United Kingdom

Data point	5.8.1/046
Report author	[redacted]
Report year	2021
Report title	Aminomethylphosphonic acid (AMPA): V79 HPRT Gene Mutation Assay
Report No	8441963 (Covance Laboratories Ltd.)
Document No	CV-2020-0233
Guidelines followed in study	OECD 476 (2016), Commission Regulation (EC) No. 440/2008 method B17 (2008), US EPA OPPTS 870.5300 (1998)
Deviations from current test guideline OECD 476 (2016)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised	Yes/Yes
Acceptability/Reliability	Yes/Yes
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Covance Laboratories Limited, Shardlow, UK

Data point	5.8.1/047
Report author	[redacted]
Report year	2021
Report title	Aminomethylphosphonic acid (AMPA): Micronucleus Test in Human Lymphocytes *in vitro*
Report No	8442149
Document No	CV-2020-0208
Guidelines followed in study	OECD 487 (2016)
Deviations from current test guideline OECD 487 (2016)	None
Previous evaluation	No, not previously submitted.

GLP/Officially recognised	Yes/yes
Acceptability/Reliability	Yes/yes
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Covance Laboratories Ltd., Shardlow, Derbyshire, United Kingdom

The analytical method used is the method KCA 4.1.2/225 [redacted].report study 8442132.
The purpose of the study is the analysis of the concentration of AMPA in the test samples water with HPLC using an exyternal standard technique.

Validation data

Principle of method
See KCA 4.1.2/225 [redacted] report study 8442132

Specificity
Chromatograms of standards solution, of control sample, of fortified samples are provided. No interference is observed at the retention time of the analyte

Linearity
See KCA 4.1.2/225 [redacted] report study 8442132

Accuracy
See KCA 4.1.2/225 [redacted] report study 8442132
Some data of recoveries are performed in these studies:

KCA 5.8.1/045

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	Expressed as % of nominal
1	0	N/D	-
	0.0150	0.0165	110
	0.050	0.0498	100
	0.150	0.158	105
	0.500	0.457	91
	1.50	1.57	105
	5.00	5.17	103
	15.0	15.4	103
	50.0	48.8	98

Study 5.8.1/046

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	[expressed as % of nominal]
1	0	N/D	-
	0.350	0.318	91
	0.690	0.670	97
	1.39	1.38	99
	2.78	2.62	94
	5.55	5.12	92
	11.1	11.9	107

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	[expressed as % of nominal]
2	0	N/D	-
	0.350	0.315	90
	0.690	0.690	100
	1.39	1.29	93
	2.78	2.86	103
	5.55	4.84	87
	11.1	10.1	91

Study 5.8.1/047

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	[expressed as % of nominal]
1	0	N/D	-
	0.350	0.358	102
	0.690	0.722	105
	1.39	1.43	103
	2.78	2.70	97
	5.55	5.59	101
	11.1	11.1	100

Conclusion
The analytical method used for the determination of AMPA in the test sample is validated. The method is considered valid and acceptable to support the toxicological study concerned.

Data point	KCA 4.1.2/226
Report authors	[redacted]
Report year	2021

Report titles	Glyphosate: Method Validation with Stability and Homogeneity
Report No	8442134 (Monsanto Ref. No CV-2020-0237)
Document No	
Guidelines followed in study	Not stated (with relevance to analytical methods)
Deviations from current test guideline	No (with relevance to SANCO/3029/99 rev. 4)
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	Covance Laboratories Limited Shardlow Business Park Shardlow Derbyshire DE72 2GD UK

2. Full summary of the study according to OECD format

Principle of the method
The method was developed for the determination of glyphosate (purity 91% w/w) in water as test vehicle. The formulations prepared were analysed direct (low level, 0.5 mg/mL) or diluted with mobile phase (high level, 20 mg/mL) by high-performance liquid chromatography with refractive index detection (HPLC-RI).

Chromatographic conditions**:**

HPLC system:	Agilent Technologies 1200, incorporating workstation and autosampler
HPLC column:	Nucleosil 100-5SB (250 × 4.6 mm ID)
Column temperature:	40 °C
Mobile phase:	Methanol/6mM potassium dihydrogen orthophosphate (adjusted to pH 2 with orthophosphoric acid) (12/88, v/v)
Flow rate:	0.8 mL/min
Injection volume:	100 µL
Run time:	15 min
Detection:	Refractive index (RI) (temperature: 40 °C; polarity: positive)
Retention time:	Glyphosate: approx. 6.3 min

Findings
Recoveries
Samples of water were accurately fortified with known amounts of test item at concentrations of 0.5 mg/mL and 20 mg/mL. The formulations prepared were analysed direct (low level) or diluted with mobile phase (high level). The results of the analyses of accuracy and precision samples are given in the table below.

Table 5.1-139: Results of test item analyses

Matrix	Analyte	Fortification level (mg/mL)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Water	Glyphosate	0.503	95 – 110	106	6.4	6.0	5
		20.1	98 – 105	102	2.6	2.5	5
		Overall	95 – 110	104	5.2	5.0	10

[1] Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity
The control samples (vehicle without test item) and an analysed solvent blank showed no significant interfering response at the retention time of the test item. Chromatograms of control sample, calibration standard and test samples (low and high level) are provided in the report.

Linearity
Linearity of detector response was tested using six calibration standard concentrations (single injections) in the range of 0.2 to 1.0 mg/mL with coefficient of determination (r^2) of 0.9999. The calibration standards were prepared in mobile phase. An example linear fit is presented in the report, details are presented in the table below.

Table 5.1-140: Details on linearity

Calibration function	Calibration concentrations (mg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Linear	0.2 – 1.0	6 levels	y = 59399457.6062 x – 320207.1077	0.9999

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

Accuracy
Acceptable mean recovery values between 70 % and 110 % for glyphosate were found for test formulations. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
No information given within the report. Acceptable recoveries were obtained at lowest fortification level.

Matrix effects
Not relevant, test formulations in water.

Stability of analytes in sample extracts
The test item formulations in water were shown to be stable at room temperature in the light for 24 hours. Moreover the homogeneity of the formulations was shown by applying the analytical method.

Conclusion
The analytical method was used for the determination of glyphosate in test formulations prepared in water. The analytical method does fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000). Stability and homogeneity of test formulations was shown by applying the analytical method.

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) without deficits. The method is considered valid and acceptable to support the toxicological study concerned.

Assessment and conclusion by RMS: The analytical method fulfills the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000). The method is considered validated

Data point	5.4.1/040
Report author	[redacted]
Report year	2021
Report title	Glyphosate: V79 HPRT Gene Mutation Assay
Report No	8441968 (Covance Laboratories Ltd.)
Document No	CV-2020-0234
Guidelines followed in study	OECD 476 (2016), Council Regulation (EC) No. 440/2008 method B17 (2008), US EPA OPPTS 870.5300 (1998)
Deviations from current test guideline OECD 476 (2016)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised	Yes/Yes
Acceptability/Reliability	Yes/yes
Category study in AIR 5 dossier (L docs)	Category 1
Test Facility	Covance Laboratories Limited, Shardlow, UK.

Data point	5.4.1/041
Report author	[redacted]
Report year	2021
Report title	Glyphosate: Micronucleus Test in Human Lymphocytes *in vitro*
Report No	8441969 (Monsanto)
Document No	CV-2020-0236
Guidelines followed in study	OECD 487 (2016)
Deviations from current test guideline OECD 487 (2016)	None
Previous evaluation	No, not previously submitted.
GLP/Officially recognised	Yes/yes
Acceptability/Reliability	Yes/yes
Category study in AIR 5 dossier (L docs)	Category 1
Test Facility	Covance Laboratories Ltd., Shardlow, Derbyshire, United Kingdom

The analytical method used is the method KCA 4.1.2/226 [redacted] 8442134.
The purpose of the study is the analysis of the concentration of glyphosate in the test samples water with HPLC-RI using an external standard technique.

Validation data

Principle of method
See KCA 4.1.2/226 [redacted] 8442134

Specificity
Chromatograms of standards solution, of control sample, of fortified samples are provided. No interference is observed at the retention time of the analyte

Linearity
See KCA 4.1.2/226 [redacted] 8442134

Accuracy
See KCA 4.1.2/226 [redacted] 8442134
Some data of recoveries are performed in theses studies:

Study 5.4.1/040

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	[expressed as % of nominal]
1	0	N/D	-
	1.16	1.10	94
	2.32	2.10	90
	4.65	4.36	94
	9.30	8.93	96
	13.9	13.57	98
	18.58	17.42	94

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	[expressed as % of nominal]
2	0	N/D	-
	1.16	1.12	96
	2.32	2.26	97
	4.65	4.53	97
	9.30	8.99	97
	13.9	13.61	98
	18.58	17.89	96

study5.4.1/041

Analysis Number	Nominal Concentration [mg/mL]	Concentration Found [mg/mL]	[expressed as % of nominal]
1	0	N/D	-
	1.16	1.05	91
	2.32	2.16	93
	4.65	4.32	93
	9.30	9.20	99
	13.9	12.1	87
	18.58	19.3	104

Conclusion

The analytical method used for the determination of glyphosate in the test sample is validated. The method is considered valid and acceptable to support the toxicological study concerned.

B.5.1.2.3 Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Overview Table for Anlytical Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

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**
CA 4.1.2/147 (CA 8.1.1.1/003)	[redacted] 1991 Report No. [redacted] 48/91266	Glyphosate technical: Acute oral toxicity (LD_{50}) to the bobwhite quail	N/A [redacted] 1991 Report No. [redacted] 48/91266 [redacted] 1992 Report No. [redacted] 48/91843	HPLC-UV LOQ 2 g/L 2-400 g/L	No	Method fit-for-purpose	Y
CA 4.1.2/148 (CA 8.1.1.1/007)	[redacted] 1992 Report No. [redacted] 48/91843	Glyphosate technical: Acute oral toxicity (LD_{50}) to the mallard duck (*Anas platyrhynchos*)	N/A [redacted] 1992 Report No. [redacted] 48/91843				

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**
CA 4.1.2/149 (CA 8.1.1.3/001)	[redacted] 1999 Report No. 123-186	Glyphosate acid: A reproduction study with Northern bobwhite (*Colinus virginianus*)	N/A [redacted] 1999 Report No. 123-186 [redacted] 1999 Report No. 123-187	HPLC-DAD LOQ 50 mg/kg 50-3500 mg/kg	No	Method fit-for-purpose	Y
CA 4.1.2/150 (CA 8.1.1.3/004)	[redacted] 1999 Report No. 123-187	Glyphosate acid: A reproduction study with the mallard (*Anas platyrhynchos*)	N/A [redacted] 1999 Report No. 123-187	HPLC-DAD LOQ 50 mg/kg 50-3500 mg/kg			
CA 4.1.2/151 (CA 8.2.1/001)	[redacted] 2003 Report No. 139A-310C	MON 78623: A 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)	N/A [redacted] 2003 Report No. 139A-310C	HPLC-UV LOQ 10.5 mg/L 10.5-2500 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/167 (CA 8.2.4.1/001)	[redacted] 2003 Report No. 139A-309	MON 78623: A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)	N/A [redacted] 2003 Report No. 139A-309	HPLC-UV LOQ 10.5 mg/L 10.5-2500 mg/L			

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
CA 4.1.2/152 (CA 8.2.1/002)	[redacted] 1995 Report No. [redacted]5552/B	Glyphosate acid: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)	N/A [redacted] 1995 Report No. [redacted]5552/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L	Yes, with deficits	Method fit-for-purpose	Y
CA 4.1.2/155 (CA 8.2.1/009)	[redacted] 1995 Report No. [redacted]5553/B	Glyphosate acid: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*)	N/A [redacted] 1995 Report No. [redacted]5553/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L			
CA 4.1.2/168 (CA 8.2.4.1/004)	[redacted] 1996 Report No. BL5551/B	Glyphosate acid: Acute toxicity to *Daphnia magna*	N/A [redacted] 1996 Report No. BL5551/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L			
CA 4.1.2/189 (CA 8.2.6.1/005)	[redacted] 1995 Report No. BL5550/B	Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*	N/A [redacted] 1995 Report No. BL5550/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L			

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
CA 4.1.2/197 (CA 8.2.6.2/001)	[redacted] Report No. BL5698/B	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae*	N/A [redacted] 1996 Report No. BL5698/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L			
CA 4.1.2/199 (CA 8.2.6.2/004)	[redacted] 1996 Report No. BL5673/B	Glyphosate acid: Toxicity to the freshwater diatom *Navicula pelliculosa*	N/A [redacted] 1996 Report No. BL5673/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L			
CA 4.1.2/201 (CA 8.2.6.2/006)	[redacted] 1996 Report No. BL5684/B	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*	N/A [redacted] 1996 Report No. BL5684/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L - 560 mg/L			
CA 4.1.2/206 (CA 8.2.7/005)	[redacted] 1996 Report No. BL5662/B	Glyphosate acid: Toxicity to duckweed (*Lemna gibba*)	N/A [redacted] 1996 Report No. BL5662/B	HPLC-FD LOQ not assessed (LOD 0.0021 mg/L) 0.75 mg/L-560 mg/L			

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**
CA 4.1.2/153 (CA 8.2.1/004)	[redacted] 1993 Report No. 80-91-2328-03-93	Acute Toxicity Testing in Fish – Test article: "Glyphosate isopropylamine salt"	N/A [redacted] 1993 Report No. 80-91-2328-03-93	HPLC-UV LOQ 50 mg/L 50-2282 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/158 (CA 8.2.1/016)	[redacted] 1993 Report No. 80-91-2328-02-93	Acute Toxicity Testing in Fish – Test article: "Glyphosate isopropylamine salt"	N/A [redacted] 1993 Report No. 80-91-2328-02-93	HPLC-UV LOQ 50 mg/L 50-2282 mg/L			
CA 4.1.2/170 (CA 8.2.4.1/007)	[redacted] 1994 Report No. 83-91-0737-00-93	Acute toxicity study in *Daphnia magna* - Test article: Glyphosate isopropylamine salt	N/A [redacted] 1994 Report No. 83-91-0737-00-93	HPLC-UV LOQ 50 mg/L 50-2282 mg/L			
CA 4.1.2/180 (CA 8.2.5.1/003)	[redacted] 1993 Report No. 80-91-2328-05-93	21 d Reproduction test in Daphnia – Test article: "Glyphosate isopropylamine salt"	N/A [redacted] 1993 Report No. 80-91-2328-05-93	HPLC-UV LOQ 50 mg/L 50-2282 mg/L			
CA 4.1.2/192 (CA 8.2.6.1/013)	[redacted] 1993 80-91-2328-01-93	Algae growth inhibition test – Test article: "Glyphosate isopropylamine salt"	N/A [redacted] 1993 Report No. 80-91-2328-01-93	HPLC-UV LOQ 50 mg/L 50-2282 mg/L			

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**
CA 4.1.2/154 (CA 8.2.1/005)	[redacted] 1990 Report No. 271631	Glyphosate technical: 96-hour acute toxicity study (LC50) in the rainbow trout	N/A [redacted] 1990 Report No. 271631	HPLC-FD LOQ not assessed (lowest test concentration 95 mg/L with n=2) 95-1000 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/171 (CA 8.2.4.1/009)	[redacted] 1990 Report No. 272968	48-hour acute toxicity of glyphosate technical to *Daphnia magna* (OECD-Immobilization test)	N/A [redacted] 1990 Report No. 272968	HPLC-FD LOQ not assessed (lowest test concentration 95 mg/L with n=2) 95-1000 mg/L			
CA 4.1.2/156 (CA 8.2.1/010)	[redacted] 1991 Report No. 271642	Glyphosate technical: 96-hour acute toxicity study (LC50) in the bluegill sunfish	N/A [redacted] 1991 Report No. 271642	HPLC-FD LOQ not assessed (lowest test concentration 1.6 mg/L with n=2) 1.6-1000 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/181 (CA 8.2.5.1/004)	[redacted] 1990 Report No. 250795	Influence of glyphosate on the reproduction of *Daphnia magna*	N/A [redacted] 1990 Report No. 250795	HPLC-FD LOQ not assessed (lowest test concentration 1.6 mg/L with n=2) 1.6-1000 mg/L			

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
CA 4.1.2/193 (CA 8.2.6.1/015)	[redacted] 1990 Report No. 250773	Acute toxicity of glyphosate to *Scenedesmus subspicatus* (OECD – algae growth inhibition test	N/A [redacted] 1990 Report No. 250773	HPLC-FD LOQ not assessed (lowest test concentration 1.6 mg/L with n=2) 1.6 - 1000 mg/L			
CA 4.1.2/157 (CA 8.2.1/013)	[redacted] [redacted] 2006 Report No. 2060/015	Glyphosate technical: Acute toxicity to common carp (*Cyprinus carpio*)	N/A [redacted] 2006 Report No. 2060/015	HPLC-UV LOQ 5.3 mg/L 210mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/159 (CA 8.2.1/017)	[redacted] 1998 Report No. 232469	96-Hour acute toxicity study in rainbow trout with (aminomethyl)phosphonic acid (static)	N/A [redacted] 1998 Report No. 232469	HPLC-FD LOQ not assessed (lowest test concentration 10 mg/L with n=3) 10 - 220 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/172 (CA 8.2.4.1/012)	[redacted] 1998 Report No. 232471	Acute toxicity study in Daphnia magna with (aminomethyl)phosphonic acid (Static).	N/A [redacted] 1998 Report No. 232471	HPLC-FD LOQ not assessed (lowest test concentration 10 mg/L with n=3) 10-220 mg/L			
CA 4.1.2/194 (CA 8.2.6.1/016)	[redacted] 1998 Report No. 232458	Fresh water algal growth inhibition test with (aminomethyl)phosphonic acid	N/A [redacted] 1998 Report No. 232458	HPLC-FD LOQ not assessed (lowest test concentration 10 mg/L with n=3) 10-220 mg/L			

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**
CA 4.1.2/160	[redacted] 1990 Report No. [redacted]-90-403	Results of the analyses of AMPA in a 96-hour acute study with rainbow trout	N/A [redacted] 1990 Report No. [redacted] 90-403	HPLC-FD LOQ not assessed (lowest fortification level 32 mg/L with n = 1) 32-1000 mg/L	No	No validation data are available in the study reports provided by applicant.	N
CA 4.1.2/161 (CA 8.2.1/019)	[redacted] 1991 Report No. [redacted]-90-402	Acute toxicity of AMPA to rainbow trout (*Oncorhynchus mykiss*)	N/A [redacted] 1991 Report No. [redacted]-90-402	HPLC-FD LOQ not assessed (lowest fortification level 32 mg/L with n = 1) 32-1000 mg/L			
CA 4.1.2/174 (CA 8.2.4.1/014)	[redacted] [redacted] 1991 Report No. AB-90-401	Acute toxicity of AMPA to *Daphnia magna*	N/A [redacted] 1991 Report No. AB-90-401	HPLC-FD LOQ not assessed (lowest fortification level 32 mg/L with n=1) 32-1000 mg/L			
CA 4.1.2/162 (CA 8.2.1/020)	[redacted] 1991 Report No. [redacted]5070/B	AMPA: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)	N/A [redacted] [redacted] [redacted] 1994 Report No. [redacted]5070/B	HPLC-UV LOQ not assessed (LOD 6.9 mg/L) 18 mg/L-180 mg/L	No	Method fit-for-purpose	Y

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
CA 4.1.2/173 (CA 8.2.4.1/013)	[redacted] 1994 Report No. BL5061/B	AMPA: Acute toxicity to *Daphnia magna*	N/A [redacted] 1994 Report No. BL5061/B	HPLC-UV LOQ not assessed (LOD 6.9 mg/L) 18 mg/L-180 mg/L			
CA 4.1.2/163 (CA 8.2.2.1/001)	[redacted] 2010 Report No. 1005.029.321	Glyphosate acid: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions	N/A [redacted] 2010 Report No. 1005.029.321	HPLC-UV LOQ 0.064 mg/L 0.064 mg/L-10.7 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/164 (CA 8.2.2.1/004)	[redacted] 2011 Report No. 139A-394 ([redacted]-2010-328)	AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*)	N/A [redacted] 2011 Report No. 139A-394 ([redacted]-2010-328)	HPLC-UV LOQ 0.4 mg/L 0.75-12 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/185 (CA 8.2.5.1/007)	[redacted] 2011 Report No. 139A-393	AMPA (Aminomethylphosphonic acid): A semi-static life-cycle toxicity test with the Cladoceran (*Daphnia magna*)	N/A [redacted] 2011 Report No. 139A-393	HPLC-UV LOQ 1.0 mg/L 7.5-120 mg/L			
CA 4.1.2/165 (CA 8.2.3/001)	[redacted] 2012 Report No. 707A-102A	Glyphosate: Fish short-term reproduction assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*)	N/A [redacted] 2012 Report No. 707A-102A	HPLC-FD LOQ 0.30 mg/L 0.045-30 mg/L	No	Method fit-for-purpose	Y

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
CA 4.1.2/166 (CA 8.2.3/002)	[redacted] 2012 Report No. 707A-103	Glyphosate: Amphibian metamorphosis assay for the detection of thyroid active substances	N/A [redacted] 2012 Report No. 707A-103	HPLC-FD LOQ 0.10 mg/L 0.16-100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/169 (CA 8.2.4.1/006)	[redacted] 1995 Report No. 141863	Acute toxicity study in *Daphnia magna* with glyfosaat	N/A [redacted] 1995 Report No. 141863	HPLC-UV LOQ not available 100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/179 (CA 8.2.5.1/002)	[redacted] 1995 Report No. 141874	*Daphnia magna*, reproduction test with glyfosaat	N/A [redacted] 1995 Report No. 141874	HPLC-UV LOQ not available 5-100 mg/L			
CA 4.1.2/190 (CA 8.2.6.1/007)	[redacted] 1995 Report No. 141896	Fresh water algal growth inhibition test with glyfosaat	N/A [redacted] 1995 Report No. 141896	HPLC-UV LOQ not available 10-100 mg/L			
CA 4.1.2/175 (CA 8.2.4.1/015)	[redacted] 2011 Report No. 139A-395	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)	N/A [redacted] 2011 Report No. 139A-395	LC-MS LOQ 1.0 mg/L 100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/176 (CA 8.2.4.2/001)	[redacted] 1996 Report No. BL5713/B	Glyphosate acid: Acute toxicity to mysid shrimp (*Mysidopsis bahia*)	N/A [redacted] 1996 Report No. BL5713/B	HPLC-FD LOQ not assessed (lowest limit of determination 0.001 mg/L) 3.2-1000 mg/L	No	Method fit-for-purpose	Y

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
CA 4.1.2/177 (CA 8.2.4.2/003)	[redacted] 1996 Report No. BL5714/B	Glyphosate acid: Acute toxicity to larvae of the Pacific oyster (*Crassostrea gigas*)	N/A [redacted] 1996 Report No. BL5714/B	HPLC-FD LOQ not assessed (lowest limit of determination 0.001 mg/L) 3.2-1000 mg/L			
CA 4.1.2/178 (CA 8.2.5.1/001)	[redacted] 1999 Report No. BL6535/B	Glyphosate acid: Chronic toxicity to *Daphnia magna*	N/A [redacted] 1999 Report No. BL6535/B	HPLC-FD LOQ not assessed (lowest limit of determination 0.0044 mg/L) 12.5-100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/182	[redacted], 1989 Report No. ML 89-62 (Analytical phase for Report No. AB 89-58)	Results of the analyses of glyphosate in a 21-day chronic *Daphnia* exposure study	N/A [redacted], 1989 Report No. ML 89-62 (Analytical phase for Report No. AB 89-58)	HPLC-UV LOQ reported (lowest valid fortification level 6.5 mg/L) 6.5-100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/183 (CA 8.2.5.1/005)	[redacted] 1989 Report No. AB 89-58	21-day prolonged static renewal toxicity of glyphosate technical to *Daphnia magna*	N/A [redacted] 1989 Report No. ML 89-62	HPLC-UV LOQ reported (lowest valid fortification level 6.5 mg/L) 6.5-100 mg/L			
CA 4.1.2/184 (CA 8.2.5.1/006)	[redacted] [redacted] 1982 Report No. AB 82-036	Chronic toxicity of glyphosate to *Daphnia magna* under flow-through test conditions	N/A [redacted] [redacted] 1982 Report No. AB 82-036	GC-FPD LOQ not reported 25-400 mg/L	No	Method fit-for-purpose	Y

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
CA 4.1.2/186 (CA 8.2.5.3/001)	[redacted], 2020 Report No. 20FV2ME (Interim report)	MON 77973: A study on the toxicity to the sediment dweller *Chironomus riparius* using spiked water	The report was not available to the RMS at the time of submission.				
CA 4.1.2/187 (CA 8.2.6.1/001)	[redacted], 2002 Report No. A-99-02-04	A study on the toxicity of glyphosate isopropylamine salt 62.5% to algae (*Pseudokirchneriella subcapitata*)	N/A [redacted], 2002 Report No. A-99-02-04	HPLC-UV LOQ 4 mg/L 4-100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/188 (CA 8.2.6.1/002)	[redacted] 2002 Report No. 139A-311	MON 78623: A 72-hour toxicity test with the freshwater alga (*Selenastrum capricornutum*)	N/A [redacted]., 2002 Report No. 139A-311	HPLC-UV LOQ 2.0 mg/L 3.3-57 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/191 (CA 8.2.6.1/009)	[redacted] 1987 Report No. 1092-02-1100-1	Volume I: The toxicity of glyphosate technical to *Selenastrum capricornutum*	N/A [redacted] 1987 Report No. 1092-02-1100-1	HPLC-UV LOQ not assessed, LOD <0.05 mg/L 10-100 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/198 (CA 8.2.6.2/002)	[redacted], 1987 Report No. 1092-02-1100-4	Volume IV: The toxicity of glyphosate technical to *Anabaena flos-aquae*	N/A [redacted] 1987 Report No. 1092-02-1100-4	HPLC-UV LOQ not assessed, LOD <0.05 mg/L 10-100 mg/L			
CA 4.1.2/200 (CA 8.2.6.2/005)	[redacted] 1987 Report No. 1092-02-1100-2	Volume II: The toxicity of glyphosate technical to *Navicula pelliculosa*	N/A [redacted] 1987 Report No. 1092-02-1100-2	HPLC-UV LOQ not assessed, LOD < 0.05 mg/L 10-100 mg/L			

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
CA 4.1.2/202 (CA 8.2.6.2/008)	[redacted] 1987 Report No. 1092-02-1100-3	Volume III: The toxicity of glyphosate technical to *Skeletonema costatum*	N/A [redacted] 1987 Report No. 1092-02-1100-3	HPLC-UV LOQ not assessed, LOD < 0.05 mg/L 0.1-3.2 mg/L			
CA 4.1.2/207 (CA 8.2.7/007)	[redacted] 1987 Report No. 1092-02-1100-5	Volume V: The toxicity of glyphosate technical to *Lemna gibba*	N/A [redacted] 1987 Report No. 1092-02-1100-5	HPLC-UV LOQ not assessed, LOD < 0.05 mg/L 5-50 mg/L			
CA 4.1.2/195 (CA 8.2.6.1/018)	[redacted], 1994 Report No. IFU93006/01-Ss	Testing of toxic effects of Aminomethyl phosphonic acid (AMPA) on the single cell green alga *Scenedesmus subspicatus*	N/A [redacted], 1994 Report No. IFU93006/01-Ss	HPLC-UV LOQ not assessed 0.96 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/196 (CA 8.2.6.1/019)	[redacted] 2011 Report No. 139A-396A (Monsanto Study No. WL-2010-330)	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)	N/A [redacted], 2011 Report No. 139A-396A (Monsanto Study No. WL-2010-330)	LC-MS LOQ 1.0 mg/L 7.5-120 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/210 (CA 8.2.7/012)	[redacted] 2011 Report No. 139A-397	HMPA (hydroxymethylphosphonic acid): A 7-day static-renewal toxicity test with Duckweed (*Lemna gibba G3*)	N/A [redacted] 2011 Report No. 139A-397	LC-MS LOQ 1.0 mg/L 7.5-120 mg/L			

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**
CA 4.1.2/203 (CA 8.2.6.2/010)	[redacted] 1996 Report No. 960606FH	Glyphosate tec. – Alga, growth inhibition test to *Nitzschia palea*	N/A [redacted] 1996 Report No. 960606FH	HPLC-UV LOQ 0.28 mg/L 0.31 - 310 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/204 (CA 8.2.7/001)	[redacted], 2002 Report No. CEMR-1873	IPA salt of glyphosate: Effects on *Lemna minor*	N/A [redacted], 2002 Report No. CEMR-1873	HPLC-FD LOQ not assessed, LOD < 0.26 mg/L 2.16 - 72 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/205 (CA 8.2.7/003)	[redacted], 1999 Report No. TLA60871 (980909FH)	Glyphosate 62 % IPA-Salt – Aquatic plant toxicity test using *Lemna gibba*	N/A [redacted] 1999 Report No. TLA60871 (980909FH)	HPLC-UV LOQ 0.90 mg/L 3.9-62.4 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/208 (CA 8.2.7/010)	[redacted], 2012 Report No. CHE-015/4-80/A	Effect of MON77973 (glyphosate acid) on the growth of *Myriophyllum aquaticum* in the presence of sediment. Test with a subsequent recovery period	N/A [redacted], 2012 Report No. CHE-015/4-80/A	LC-MS/MS LOQ 0.25 mg/L 0.25 - 2.5 mg/L	Yes	-	Y
CA 4.1.2/209 (CA 8.2.7/011)	[redacted] 2012 Report No. CHE-022/4-80/A	Effect of AMPA (aminomethyl phosphonic acid) on the growth of *Myriophyllum aquaticum* in the presence of sediment, with a subsequent recovery period	N/A [redacted] 2012 Report No. CHE-022/4-80/A	LC-MS/MS LOQ 0.5 mg/L 0.5 - 5.0 mg/L	Yes	-	Y

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**
CA 4.1.2/211 (CA 8.3.1.2/001)	[redacted], 2017 Report No. IO-2016-0508	MON 0139: Chronic oral toxicity test on the honey bee (*Apis mellifera* L.) in the laboratory	N/A [redacted], 2017 Report No. IO-2016-0508	HPLC-UV LOQ 0.5 g/L 0.5-30 g/L	No	Method fit-for-purpose	Y
CA 4.1.2/212 (CA 8.3.1.3/001)	[redacted] 2020 Report No. 19 48 BLC 0068	Amended report for MSL0031012: MON 0139 - Repeated exposure of honey bee larvae (*Apis mellifera* L.) under laboratory conditions	N/A [redacted], 2020 Report No. 19 48 BLC 0068	LC-MS/MS LOQ 16.1 mg/L 16.1-1664 mg/L	Yes	-	Y
CA 4.1.2/213 (CA 8.3.1.4/001)	[redacted] 2012 Report No. V7YH1001	Glyphosate: Evaluating potential effects on honeybee brood (*Apis mellifera*) development	N/A [redacted] 2012 Report No. V7YH1001	HPLC-MS/MS LOQ 1.0 mg/kg 1-200 mg/kg or 1-400 mg/kg	No	Method fit-for-purpose	Y
CA 4.1.2/214 (KCP 8.6.2/001)	[redacted] [redacted] 1994 Report No. 93235	Tier 2 vegetative vigor nontarget phytotoxicity study using glyphosate	N/A [redacted] [redacted], 1994 Report No. 93235	HPLC-FD LOQ 200 mg/L 200-13000 mg/L	No	Method fit-for-purpose	Y

Determination of glyphosate in dose formulation

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/147 (CA 8.1.1.1/003)
Report authors	[redacted]
Report year	1991
Report title	Glyphosate technical: Acute oral toxicity (LD_{50}) to the bobwhite quail
Report No	[redacted] 48/91266
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No chromatograms and linearity functions provided • Matrix effects and stability of sample extracts not assessed • Efficacy of derivatisation not assessed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (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	CA 4.1.2/148 (CA 8.1.1.1/007)
Report authors	[redacted]
Report year	1992
Report title	Glyphosate technical: Acute oral toxicity (LD_{50}) to the mallard duck (*Anas platyrhynchos*)
Report No	[redacted] 48/91843
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No chromatograms and linearity functions provided • Matrix effects and stability of sample extracts not assessed • Efficacy of derivatisation not assessed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	[redacted]

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 in dose formulation (1% methylcellulose) by HPLC-UVD with external calibration. An aliquot (1 mL) was dissolved in an appropriate volume of 0.1 M aqueous trimethylamine to provide a solution containing glyphosate in the concentration range of 2.0 – 4.0 µg/mL. For derivatisation, an aliquot (10 mL) of the dilution was mixed with saturated borax solution (10 mL) and 1-fluoro-2,4-dinitrobenzene (DNFB) solution (20 mL), and the mixture was incubated at darkness for 1.5 hours before adding aqueous citrate buffer (pH 3, 25 mL). The derivatised extract was then partitioned twice with ethyl acetate (2 x 50 mL) by shaking vigorously. The ethyl acetate layers were removed and discarded, while the aqueous layer was acidified with phosphoric acid (2 mL) and partitioned again with ethyl acetate (50 mL). The ethyl acetate layer was collected and evaporated to dryness. The derivatised residue was dissolved in 5 mL acetonitrile/aqueous tetraethylammoniom bromide buffer (1/5, v/v) and submitted to analysis by HPLC with UV detection (UVD).

Chromatographic conditions**:**

HPLC system:	HPLC with UV Detector
HPLC column:	Licospher 100 RP-18e (BDH Ltd.), 250 × 4.0 mm i.d., 5 µm particle size
Guard column:	Licospher 100 RP-18e (BDH Ltd.), 4.0 × 4.0 mm i.d., 5 µm particle size
Column temperature:	Not provided
Mobile phase:	Acetonitrile/tetraethylammonium bromide-PO_4-buffer (20/80, v/v)
Flow rate:	1.0 mL/min
Injection volume:	40 µL
Derivatisation agent:	1-fluoro-2,4-dinitrobenzene (DNFB)
Retention time:	Not provided
Detection:	UV at 383 nm

Findings
Recoveries
For method validation, aliquots of control vehicle (1 % **methylcellulose solution**) were fortified with glyphosate technical at two fortification levels, i.e. 2 and 350 g/L. The results are summarised in the table below. The average recovery values were between 70 % and 110 %, with relative standard deviations (RSDs) of < 20 %. Control samples were also analysed without detecting glyphosate acid above the LOQ (< 0.15 g/L).

Table 5.1-141: Results of method validation (spike recovery) for the determination of glyphosate in dose formulation

Report No.	Analyte	Fortification level (g/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Dose formulation	Glyphosate	2	92.0 – 102	98.3	3.0	3.1	9
		350	91.3 – 104	96.3	4.1	4.2	9
		Overall	91.3 – 104	97.3	3.6	3.7	18

[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 freshly prepared dose formulations were analysed for glyphosate content using the analytical method. The results are shown in the table below. The average recovery values were between 70 % and 110 %, with an overall relative standard deviation (RSDs) of < 20 %. Only at one concentration level these criteria were exceeded due to a single high recovery of 127 %. It is noted that these are not true validation recovery data; however, the results show the correct dosing during the test.

Table 5.1-142: Results of dose formulation analyses

Report No.	Matrix	Analyte	Nominal concentration (g/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
[redacted] 48/91266	Dose formulation	Glyphosate	50	96.0 – 107	102	/	/	2
			100	109 – 110	110	/	/	2
			200	96.5 – 104	100	/	/	2
			Overall	96.0 – 110	104	6.2	6.0	6
[redacted] 49/91843	Dose formulation	Glyphosate	100	91.6 – 110	100.8	/	/	2
			200	92.5 – 127	109.8	/	/	2
			400	93.8 – 97.8	95.8	/	/	2
			Overall	91.6 – 127	102.1	13.9	13.7	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.

Specificity

The identification was based on the selected wavelength and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compound. Not assessed, no chromatograms are provided in the report.

Linearity
The linearity of the detector response was tested using five calibration standard concentrations in the range of 2 to 10 µg/mL (duplicate analyses). Calibration standards were prepared in aqueous triethylamine (0.1 M) and submitted to derivatisation. No further details to the calibration function were provided.

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 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 2 g/L fortification level for dosing solution.
The limit of detection (LOD), defined as the concentration of glyphosate in control matrix producing a peak response equivalent to 3x baseline noise, was determined as 0.15 g/L.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed. However, the stability of the test item in dose formulations was demonstrated for 72 hours when stored in the dark at ambient temperature during the day and at +4 °C overnight.

Conclusion
The analytical method was validated for the determination of glyphosate in dosing solutions (1% methylcellulose). Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, the method is considered as fit-for-purpose for the determination of glyphosate in dose formulations.

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 meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no chromatograms and linearity functions provided, matrix effect and stability of sample extracts 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 method is not in agreement with the SANCO 3029/99. The linearity plots, calibration functions, chromatograms are not available, the matrix effect and the derivatisation efficiency were not performed.

However, the precision was demonstrated. The recovery data are in acceptable range and can be compiled as all analysis have been performed in the same laboratory. The recoveries tested are in agreement with the targeted dose. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in methyl cellulose solution at the targeted doses..

Determination of glyphosate in test diet

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/149 (CA 8.1.1.3/001)
Report authors	[redacted]
Report year	1999
Report title	Glyphosate acid: A reproduction study with Northern bobwhite (*Colinus virginianus*)
Report No	123-186

Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Matrix effects and stability of sample extracts not assessed • Efficacy 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:	CA 4.1.2/150 (CA 8.1.1.3/004)
Report authors	[redacted]
Report year	1999
Report title	Glyphosate acid: A reproduction study with the mallard (*Anas platyrhynchos*)
Report No	123-187
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Matrix effects and stability of sample extracts not assessed • Efficacy 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]

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 in avian test diet (major ingredients: 37.5 % fine corn meal, 34.8 % soybean meal, 6 % fish meal, 5 % ground oats, 5 % wheat mids) by HPLC-DAD with external calibration. Aliquots (10 g) were extracted with water by shaking for one hour. The extracts were centrifuged and further diluted if required to achieve a concentration of 1.0 to 5.0 mg/L glyphosate. For derivatisation, an aliquot (10 mL) of this extract was mixed with saturated, aqueous disodium tetraborate solution (10 mL) and 2,4-dinitrofluorobenzene (DNFB) solution (20 mL), and left for one hour before adding aqueous citrate buffer (pH 3, 25 mL). This solution was then partitioned twice with ethyl acetate (50 mL). The aqueous phase was acidified with phosphoric acid (2 mL) and partitioned again with ethyl acetate (50 mL), and this extract was then evaporated to dryness. The derivatised residue was dissolved in acetonitrile/aqueous tetraethylammoniom bromide buffer (1/5, v/v), filtered and submitted to analysis by HPLC with diode array detection (DAD).

Chromatographic conditions**:**

HPLC system:	HPLC (HP 1090) with Diode Array Detector (DAD)
HPLC column:	Phenomenex LUNA C_5 (250 × 4.6 mm i.d., 5 µm particle size)

Column temperature:	40 °C
Mobile phase:	A: Acetonitrile/tetraethylammonium bromide-PO_4-buffer (20/80, v/v) B: Acetonitrile/ tetraethylammonium bromide-PO_4-buffer (50/50, v/v)

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.01	100	0	1.0
1.00	100	0	1.0
10.0	0	100	1.0
12.0	0	100	1.0
12.1	100	0	1.0
16.0	100	0	1.0

Injection volume:	50 µL
Derivatisation agent:	2,4-dinitrofluorobenzene (DNFB)
Retention time:	Glyphosate: ~ 5.2 to 5.5 min
Detection:	UV at 383 nm

Findings

Recoveries

For method validation, aliquots of control diets were fortified with glyphosate acid at three fortification levels, i.e. 50, 1000 and 3500 mg/kg. The results are summarised in the table below. The average recovery values were between 70 % and 110 %, with relative standard deviations (RSDs) of < 20 %. Control samples were also analysed without detecting glyphosate acid above the LOQ (< 50 mg/kg).

Table 5.1-143: Results of method validation (spike recovery) for the determination of glyphosate acid in test diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test diet	Glyphosate acid	50	109 – 119	115	4.1	3.6	5
		1000	93.1 – 119	102	9.9	9.7	5
		3500	81.4 – 106	95.1	10.0	10.5	5
		Overall	81.4 – 119	104	11.5	11.0	15

[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 freshly prepared test dies were collected at weeks 1, 2, 3, 4, 8, 12, 16 and 20, and analysed for glyphosate acid content using the analytical method. Day 1 samples were collected from the top, middle and bottom of the mixing vessel. The results are shown in the table below. The overall mean recovery value was between 70 % and 110 %, with a relative standard deviations (RSDs) of < 20 %. It is noted that these are not true validation recovery data; however, the results show the correct dosing during the tests.

Table 5.1-144: Results of test diet analyses

Matrix	Analyte	Nominal concentration (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test diet	Glyphosate acid	500	91.0 – 117	101.4	7.2	7.1	20
		1000	84.8 – 119	99.4	7.0	7.1	20
		2250	88.4 – 112	97.1	5.6	5.8	20
		Overall	84.8 – 119	99.3	6.8	6.8	60

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

Specificity

The identification was based on the selected wavelength and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compound. No interfering peaks were observed at the retention time of the analyte. Along with the sample analyses, five matrix blanks were analysed to determine possible interferences. No interferences were observed at or above the mg/kg equivalent of the lowest calibration standard during sample analyses.

Linearity

The linearity of the detector response was tested using five calibration standard concentrations in the range of 1.0 to 5.0 µg/mL (duplicate analyses). The equivalency in mg/kg is not available. Calibration standards were prepared by fortification of control diets and extraction/analysis together with the verification sample set. Linear regressions were found with correlation coefficients of > 0.99.

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

Acceptable recoveries were obtained at the lowest fortification level of 50 mg glyphosate acid/kg. The limit of detection (LOD) was set at 50 ng on column based on the injection volume (50 µL) and the lowest calibration standard concentration (1 µg/mL), which was equivalent to a calculated value of 20 mg/kg in the matrix blank extract. LOQ = 50 mg/kg

Matrix effects

Not assessed. Matrix effects were eliminated by preparing calibration standards by fortification of control diets and extraction/analysis together with the verification sample set.

Stability of analytes in sample extracts

Not assessed. However, the stability of the test item in diets was demonstrated for 7 days.

Conclusion

The analytical method was validated for the determination of glyphosate in avian test diet. Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, the method is considered as fit-for-purpose for the determination of glyphosate in test diet.

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 meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (matrix effect and stability of sample extracts 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 is not in agreement with the guidance SANCO 3029/99 rev.4 as the derivatisation efficiency was not assessed

However, the other parameters are considered acceptable. Therefore, the method can be considered as fit-for-purpose for the determination of glyphosate in diet at the targeted doses.

Determination of glyphosate in test medium (well water)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/151 (CA 8.2.1/001)
Report authors	[redacted]
Laboratory	[redacted]
Report year	2003
Report title	MON 78623: A 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)
Report No	139A-310C
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes, minor (SANCO/3029/99 rev. 4): • Limited validation recoveries from spiked samples • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/167 (CA 8.2.4.1/001)
Report authors	[redacted]
Report year	2003
Laboratory	Wildlife International ltd. Easton Maryland USA
Report title	MON 78623: A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)
Report No	139A-309

Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes, minor (SANCO/3029/99 rev. 4): • Limited validation recoveries from spiked samples • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 medium (well water) by HPLC-UVD. The samples were first diluted with freshwater to yield concentrations within the calibration range. For derivatisation, aliquots (2 mL) were mixed with 1 mL aqueous potassium tetraborate (0.37 M) and 2 mL NBD-Cl (7-chloro-4-2-oxa-1,3-diazole) (0.025 M, methanolic). The mixture was heated to about 80 °C and allowed to derivatise for 30 min. After addition of 1 mL HCl (1.2 M, aqueous), the solution was filtered (0.45 µm PTFE Acrodisc) and analysed for glyphosate by HPLC-UVD using external calibration.

This method was used in the following studies:

Annex Point	Report No.	Author	Year	Test
CA 8.2.1/001	139A-310	[redacted]	2003	Rainbow trout, acute toxicity (96 hour)
CA 8.2.4.1/001	139A-309	[redacted]	2003	*Daphnia magna*, acute toxicity (48 hour)

Chromatographic conditions:

HPLC system:	Hewlett-Packard Model 1090 or Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) with an Agilent Model 1100 Variable Wavelength Detector
HPLC column:	YMC-Pack ODS-AM (150 × 4.6 mm, 3 µm particle size)
Column temperature:	40 °C
Mobile phase:	A: 0.01 M KH_2PO_4 (pH 3.6) B: CH_3CN

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.01	95	5	1.0
3.00	95	5	1.0
8.00	30	70	1.0
11.00	30	70	1.0
11.10	95	5	1.0
16.00	95	5	1.0

Injection volume:	25 µL
Derivatisation agent (pre-column):	NBD-Cl (7-chloro-4-2-oxa-1,3-diazole) (0.025 M)
Retention time:	Glyphosate: approx. 3.1 min
Detection:	UV/Vis wavelength: 500 nm

Findings

Recoveries

Test medium (well water) samples were fortified at relevant concentrations of 150, 600 and 2500 mg/L and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 10.5 mg test item/L, < 5 mg a.e./L). The recovery values were between 70 % and 110 %, with a relative standard deviation of < 20 %. The results are summarised in the table below.

Table 5.1-145: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Report	Matrix	Analyte	Fortification level (mg test item/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
139A-310	Well water	Glyphosate	150	99.8 – 105	103	2.6	2.6	3
			600	97.8 – 103	100	2.7	2.7	3
			2500	99.1 – 101	99.9	1.0	1.0	3
139A-309	Well water	Glyphosate	150	101 – 102	102	–	–	2
			600	101 – 103	102	–	–	2
			2500	100 – 102	101	–	–	2

[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.

Duplicate samples of the test water collected at beginning of the test and were analysed for the concentration of the test article using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 10.5 mg test item/L, < 5 mg a.e./L). The recovery values were between 70 % and 110 %, with an overall relative standard deviation of 2.1 %. It is noted that these are not true validation recovery data; however, the results show good performance of the method. The recovery results are shown in the table below.

Table 5.1-146: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concetration (mg test item/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
139A-310	Well water	Glyphosate	156	102 – 104	103	–	–	2
			313	105 – 106	106	–	–	2
			625	101 – 102	102	–	–	2
			1250	99.9 – 107	104	–	–	2
			2500	101 – 102	102	–	–	2

Table 5.1-146: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concetration (mg test item/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
139A-309	Well water	Glyphosate	156	103 – 105	104	–	–	2
			313	99.0 – 99.3	99.2	–	–	2
			625	102 – 102	102	–	–	2
			1250	102 – 102	102	–	–	2
			2500	101 – 102	102	–	–	2

[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.

Linearity
The linearity of the detector response was tested using five calibration standard concentrations in the range of 5.0 to 50 mg glyphosate acid equivalents (a.e.)/L prepared in freshwater. The calibration standards were derivatised as described above and analysed with each sample set. All calibration curves generated had a coefficient of determination (r^2) of > 0.**999.** Details to example calibrations are provided below.

Table 5.1-147: Details to example calibrations

Study	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
139A-310	Glyphosate	Linear	5.0 – 50	5 levels	y = 81.6715 x – 13.21560	0.9998
139A-309	Glyphosate	Linear	5.0 – 50	5 levels	y = 83.01129 x + 2.36742	1.0000

Repeatability (Precision)
The overall relative standard deviations (RSDs) of recovery values 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 both studies was 150 mg test item/Lbased on recovery data. The limit of detection (LOD) was not reported in the study.

Matrix effects
Matrix effects were not assessed.

Stability of the analyte in sample extracts
Stability of glyphosate in sample extracts was not assessed. However, it was shown that the test material was stable test medium for duration of the test.

Conclusion
The analytical method was validated for the determination of glyphosate in test medium (well water). The method validation meets criteria set in SANCO/3029/99 rev. 4 in most relevant points and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) in most aspects (minor deficits: limited true validation data, matrix effects and storage stability of extracts 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 method is not in agreement with the SANCO 3029/99 rev.4 as the precision was not demonstrated and the derivatisation efficacy was not assessed.

However, the linearity plots and calibration function were provided. The recovery data are in acceptable range and in agreement with the targeted dose. The method can be considered as fit for purpose for the determination of glyphosate in well water at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/152 (CA 8.2.1/002)
Report authors	[redacted]
Report year	1995
Report title	Glyphosate acid: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Report No	[redacted]5552/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (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	CA 4.1.2/155 (CA 8.2.1/009)
Report authors	[redacted]
Report year	1995
Report title	Glyphosate acid: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*)

Report No	[redacted]5553/B
Document No	2310926
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (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	CA 4.1.2/168 (CA 8.2.4.1/004)
Report authors	[redacted]
Report year	1996
Report titles	Glyphosate acid: Acute toxicity to *Daphnia magna*
Test facility	Brixham Environmental Laboratory ZENECA Limited Brixham Devon TQS SBA UK
Report No	BL5551/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/189 (CA 8.2.6.1/005)
Report authors	[redacted]
Report year	1995
Report title	Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*
Test facility	Brixham Environmental Laboratory

	ZENECA Limited Brixham Devon TQS SBA UK
Report No	BL5550/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/197 (CA 8.2.6.2/001)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae*
Report No	BL5698/B
Document No	-
Test Facility	Brixham Environmental Laboratory ZENECA Limited Brixham Devon TQ5 8BA UK
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/199 (CA 8.2.6.2/004)
Report authors	[redacted]
Report year	1996

Report title	Glyphosate acid: Toxicity to the freshwater diatom *Navicula pelliculosa*
Report No	BL5673/B
Document No	-
Test Facility	Brixham Environmental Laboratory ZENECA Limited Brixham Devon TQ5 8BA UK
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/201 (CA 8.2.6.2/006)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Test facility	Brixham Environmental Laboratory ZENECA Limited Brixham Devon TQ5 8BA UK
Report No	BL5684/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/206 (CA 8.2.7/005)

Report authors	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to duckweed *(Lemna gibba)*
Test facility	Brixham Environmental Laboratory ZENECA Limited Brixham Devon TQ5 8BA UK
Report No	BL5662/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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-FD. This method is based on Brixham Environmental Laboratory Standard Operating Procedure AL228, version 03. Aqueous samples were quantified against standard solutions of glyphosate acid prepared in deionised water. Prior to analysis, samples and standards were derivatised using fluorenylmethyl chloroformate (FMOC-Cl). No further details to the extraction and derivatisation steps are provided in the analytical sections.

This method was used in the following studies with minor modifications:

Annex point	Report No.	Author	Year	Test
CA 8.2.1/002	[redacted]5552/B	[redacted]	1995	Rainbow trout, acute toxicity (96 hour)
CA 8.2.1/009	[redacted]5553/B	[redacted]	1995	Bluegill sunfish, acute toxicity (96 hour)
CA 8.2.4.1/004	BL5551/B	[redacted]	1996	*Daphnia magna*, acute toxicity (48 hour)
CA 8.2.6.1/005	BL5550/B	[redacted]	1995	*Selenastrum capricornutum*, toxicity (120 hours)
CA 8.2.6.2/001	BL5698/B	[redacted]	1996	*Anabaena flos-aquae*, toxicity (120 hours)
CA 8.2.6.2/006	BL5684/B	[redacted]	1996	*Sceletonema costatum*, toxicity (120 hours)
CA 8.2.6.2/004	BL5673/B	[redacted]	1996	*Navicula pelliculosa*, toxicity (120 hours)
CA 8.2.7/005	BL5662/B	[redacted]	1996	Duckweed (*Lemna gibba*), toxicity (14 days)

Chromatographic conditions:

HPLC system:	HPLC equipped with fluorescence detector

HPLC column:	Spherisorb S5 SAX, 50 × 4.6 mm id
Column oven temperature:	Not provided
Mobile phase:	Acetonitrile/deionised water/buffer (22/18/60, v/v/v) Buffer: Deionised water/glacial acetic acid/orthophosphoric acid (97/2/1, v/v/v)
Flow rate:	2.0 mL/min
Injection volume:	20 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Detection:	Excitation wavelength: 254 nm Emission wavelength: 300 nm
Retention time:	Approx. 3.4 min ([redacted]5552/B) Approx. 2.6 min ([redacted]5553/B) Approx. 1.7 min (BL5551/B) Approx. 1.6 min (BL5550/B) Approx. 2.0 min (BL5698/B) Approx. 2.4 min (BL5684/B) Approx. 2.3 min (BL5673/B) Approx. 2.9 min (BL5662/B)

Findings

Recoveries

True validation recoveries obtained from spiked test water (reconstituted water) were not presented within the report. However, samples of test water were taken from the tanks at start of the experiment using the excess remaining after filling the test vessels and analysed for glyphosate concentration using the analytical method. Control test water samples were also analysed, without detecting glyphosate above the LOD (see below). All average recovery values were between 70 % and 110 %. It is noted that these are not true validation recovery data; however, the results show good performance of the method. The results are provided in the table below.

Table 5.1-148: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
[redacted]5552/B	Test medium (reconstituted water)	Glyphosate acid	32	91	–	–	–	1
			56	95 – 100	97	2.7	2.8	3
			100	94	–	–	–	1
			180	100	–	–	–	1
			320	100	–	–	–	1
			560	98	–	–	–	1
			Overall	91 – 100	97	3.3	3.5	8
[redacted]5553/B	Test medium (reconstituted water)	Glyphosate acid	10	110	–	–	–	1
			18	100 – 106	102	3.2	3.1	3
			32	97	–	–	–	1
			56	98	–	–	–	1
			100	99	–	–	–	1

Table 5.1-148: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			180	100	–	–	–	1
			Overall	97 – 110	101	4.4	4.3	8
BL5551/B	Test medium (reconstituted water)	Glyphosate acid	10	86	–	–	–	1
			18	89 – 94	91	3.2	3.5	3
			32	91	–	–	–	1
			56	88	–	–	–	1
			100	92	–	–	–	1
			180	100	–	–	–	1
			Overall	86 – 100	91	4.5	4.9	8
BL5550/B	Test medium (reconstituted water)	Glyphosate acid	5.6	98	–	–	–	1
			10	99 – 100	100	0.6	0.6	3
			18	106	–	–	–	1
			32	103	–	–	–	1
			56	104	–	–	–	1
			100	100	–	–	–	1
			Overall	98 – 106	99	2.6	2.6	8
BL5698/B	Test medium (reconstituted water)	Glyphosate acid	0.75	97	–	–	–	1
			1.5	93	93	0	0	3
			3.0	97	–	–	–	1
			6.0	97	–	–	–	1
			12	100	–	–	–	1
			24	96	–	–	–	1
			48	90	–	–	–	1
			96	104	–	–	–	1
			Overall	93 – 104	96	4.1	4.2	10
BL5684/B	Test medium (reconstituted water)	Glyphosate acid	1.0	97	–	–	–	1
			1.8	100	100	0	0	3
			3.2	91	–	–	–	1
			5.6	104	–	–	–	1
			10	100	–	–	–	1
			18	100	–	–	–	1
			32	94	–	–	–	1
			56	105	–	–	–	1

Table 5.1-148: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			Overall	91 – 105	99	4.3	4.4	10
BL5673/B	Test medium (reconstituted water)	Glyphosate acid	1.8	106	–	–	–	1
			3.2	106	106	0	0	3
			5.6	109	–	–	–	1
			10	100	–	–	–	1
			18	106	–	–	–	1
			32	106	–	–	–	1
			56	107	–	–	–	1
			100	110	–	–	–	1
			Overall	100 – 110	106	2.6	2.5	10
BL5662/B	Test medium (reconstituted water)	Glyphosate acid	0.75	91	–	–	–	1
			1.5	93	93	0	0	3
			3.0	97	–	–	–	1
			6.0	93	–	–	–	1
			12	100	–	–	–	1
			24	96	–	–	–	1
			48	96	–	–	–	1
			96	96	–	–	–	1
			Overall	93 – 100	95	2.6	2.7	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

No significant interferences were observed at the retention time of the analyte in example chromatograms.
Study BL5550/B: Typical chromatograms of a glyphosate acid standard and of a test sample were provided.
Study BL5698/B and BL5673/B: Chromatogram of blank solution is not provided.
Study BL5684/B and BL5662/B: Chromatograms of standard concentration nd of nominal concentration of glyphosate acid have been provided.

Linearity

No details to calibration functions are provided.

Repeatability (Precision)

The relative standard deviations (RSDs) of the overall recovery values were below 20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection

The limit of quantification (LOQ) was not assessed in these studies.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies were previously evaluated at EU level. They were performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries from spiked samples provided, no calibration details provided, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the ecotox studies concerned.

Assessment and conclusion by RMS:
The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4. The linearity data are not available, the precision was non demonstrated, the derivatisation efficiency is not assessed.

However, considering recovery values on the test medium, the method can be considered as fit for purpose for the determination of glyphosate in Test medium (reconstitued water).

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/153 (CA 8.2.1/004)
Report authors	[redacted]
Report year	1993
Report title	Acute Toxicity Testing in Fish – Test article: “Glyphosate isopropylamine salt”
Report No	80-91-2328-03-93
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No analytical results of control samples presented • Matrix effects and stability of sample extracts not assessed • Derivatisation step insufficiently described and efficacy of derivatisation not assessed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes –
Acceptability/Reliability	Supportive (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	CA 4.1.2/158 (CA 8.2.1/016)
Report authors	[redacted]
Report year	1993
Report title	Acute Toxicity Testing in Fish – Test article: "Glyphosate isopropylamine salt"
Report No	80-91-2328-02-93
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No analytical results of control samples presented • Matrix effects and stability of sample extracts not assessed • Derivatisation step insufficiently described and efficacy of derivatisation not assessed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes –
Acceptability/Reliability	Supportive (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	CA 4.1.2/170 (CA 8.2.4.1/007)
Report authors	[redacted]
Report year	1994
Report title	Acute toxicity study in *Daphnia magna* - Test article: Glyphosate isopropylamine salt
Report No	83-91-0737-00-93
Test facility	IBR Forschungs D-29664 Walsrode Germany
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Recovery experiments for method accuracy not provided • Details to the derivatisation not provided • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/180 (CA 8.2.5.1/003)
Report authors	[redacted]
Report year	1993
Report title	21 d Reproduction test in Daphnia – Test article: "Glyphosate isopropylamine salt"
Test facility	IBR Forschungs GmbH, Feodor-Lynen-Str.5, 30625 Hannover
Report No	80-91-2328-05-93
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No analytical results of control samples presented • Matrix effects and stability of sample extracts not assessed • Derivatisation step insufficiently described and efficacy of derivatisation not assessed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/192 (CA 8.2.6.1/013)
Report authors	[redacted]
Report year	1993
Report title	Algae growth inhibition test – Test article: "Glyphosate isopropylamine salt"
Report No	80-91-2328-01-93
Document No	WL-2010-329
Test facility	IBR Forschungs GmbH&co Feodor-Lynen-Str.5 30625 Hannover
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No analytical results of control samples presented • Matrix effects and stability of sample extracts not assessed • Derivatisation step insufficiently described and efficacy of derivatisation not assessed.
Previous evaluation	Yes, evaluated and accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities

Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 medium (reconstituted water) by HPLC with VIS detection and external calibration. Glyphosate was dansylated before analysis and determined by reverse phase HPLC. No details were provided to sample preparation and derivatisation conditions.

This method was used in the following studies:

Annex Point	Report No.	Author	Year	Test
CA 8.2.1/004	80-91-2328-03-93	[redacted]	1993	*Oncorhynchus mykiss*, 96 hour acute
CA 8.2.1/016	80-91-2328-02-93	[redacted]	1993	*Leuciscus idus*, 96 hour acute
CA 8.2.4.1/007	83-91-0737-00-93	[redacted]	1994	*Daphnia magna*, 48 hour acute
CA 8.2.5.1/003	80-91-2328-05-93	[redacted]	1993	*Daphnia magna*, 21 day reproduction
CA 8.2.6.1/013	80-91-2328-01-93	[redacted]	1993	*Desmodesmus subspicatus*, 72 hour growth inhibition

Chromatographic conditions**:**

HPLC system:	High performance Liquid Chromnatograph (HPLC) with UV-Visible Detector
HPLC column:	Nucleosil 120-5-C18
Column temperature	40 °C
Mobile phase:	A: 25 mM sodiumphosphate buffer pH 6.8/N,N-dimethyl-formamide (960/40, v/v) B: acetonitrile/N,N-dimethylformamide (960/40, v/v)
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Derivatisation agent:	Dabsyl chloride
Retention time:	Glyphosate: approx. 10.2 min
Detection:	VIS at 436 nm

Findings
Recoveries
For precision testing, the intra-assay variation was ascertained by performing six single determinations at concentration levels of 50 and 500 mg/L. The day-to-day variation of this assay was evaluated by running a 100 mg/L standard together with each set of samples on each day of analysis. The results are summarised in the table below. The average recovery values were between 70 % and 110 %, with relative standard deviations of < 20 %.

Table 5.1-149: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Fortification level (mg glyphosate /L)	Recovery[1, 2]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	50	103 – 110	106	2.2	2.1	6
		100	92.3 – 115	102	5.5	5.4	27
		500	99.2 – 107	104	2.7	2.6	6

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

[2] Calculation of recoveries was done based on reported peak areas using the low range calibration for the 50 and 100 mg/L spike level and the high range calibration for the 500 mg/L spike level.

Additionally, samples of the test medium were analysed for the concentration of glyphosate using the analytical method. The results are shown in the table below. The mean recovery values were between 70 % and 110 %, with relative standard deviations of < 20 %. It is noted that these are not true validation recovery data; however the results show good performance of the method and the correct dosing during the tests.

Table 5.1-150: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg glyphosate/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
80-91-2328-03-93	Test medium (reconstituted water)	Glyphosate	45.6	105	105	–	–	1
			456	109	109	–	–	1
			48.8	99.2	99.2	–	–	1
			236	103	103	–	–	1
			1141	95.1	95.1	–	–	1
			Overall	95.1 – 109	102	5.4	5.2	5
80-91-2328-03-93	Test medium (reconstituted water)	Glyphosate	45.6	90.4	90.4	–	–	1
			456	97.6	97.6	–	–	1
			227	81.9	81.9	–	–	1
			720	94.6	94.6	–	–	1
			2282	96.2	96.2	–	–	1
			Overall	81.9 – 97.6	92.1	6.3	6.9	5

Table 5.1-150: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg glyphosate/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
83-91-0737-00-93	Test medium (reconstituted water)	Glyphosate	45.6	104	104	–	–	1
80-91-2328-05-93	Test medium (reconstituted water)	Glyphosate	45.6	87.2	87.2	–	–	1
			456	100	100	–	–	1
			19.6	82.3 – 97.5	91.2	5.7	6.3	5
			94.5	91.9 – 101	96.4	4.4	4.5	3
			208	90.1 – 110	103	8.9	8.6	5
			456	101 – 102	101	0.8	0.8	2
			Overall	82.3 – 110	97.1	7.8	8.0	17
80-91-2328-01-93	Test medium (reconstituted water)	Glyphosate	45.6	94.9	94.9	–	–	1
			456	90.6	90.6	–	–	1
			7.2	108.6	108.6	–	–	1
			22.8	111	111	–	–	1
			72.1	103	103	–	–	1
			198	101	101	–	–	1
			Overall	90.6 – 111	102	7.8	7.7	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.

Specificity

The identification was based on the selected wavelength and the retention time. Study 80-91-2328-05-93: Results of control blank test solution not reported. Only a chromatogram of a spiked sample (aqueous solution containing 500mg/L of glyphosate) was provided. The absence of interference cannot be demonstrated.

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 100 mg/L to 500 mg/L and four calibration standard concentrations in the range of 10 mg/L to 100 mg/L (duplicate injections) with correlation coefficients of > 0.99. Details to the calibration functions are provided below.

Table 5.1-151: Linearity parameters

Analyte	Calibration function	Calibration concentrations (mg/L)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate	Linear	10 – 100	4 levels	y = 0.14331 x + 613.8	0.9983
Glyphosate	Linear	100 – 500	5 levels	y = 0.16557 x – 5863	0.9992

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
Acceptable recoveries were obtained at the lowest fortification level of 50 mg glyphosate/L.

Matrix effects
Not assessed.

Interference

Stability of glyphosate isopropylamine salt in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate in test medium (reconstituted water). Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

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 partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with some deficits (analysis of control specimens not provided, matrix effect and stability of sample extracts not assessed, no details to derivatisation provided, 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 does not fully meet criteria set in SANCO/3029/99 rev. 4 as the absence of interference is not demonstrated, and the derivatisation efficiency was not assessed.
However, the recovery data are in acceptable range. Therefore, the analytical method can be considered as fit-for-purpose for the determination of glyphosate in aqueous test medium at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/154 (CA 8.2.1/005)
Report authors	[redacted]
Report year	1990
Report title	Glyphosate technical: 96-hour acute toxicity study (LC50) in the rainbow trout
Report No	271631

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 recoveries provided • Only 4 calibration determinations • 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	Supportive (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	CA 4.1.2/171 (CA 8.2.4.1/009)
Report authors	[redacted]
Report year	1990
Report title	48-hour acute toxicity of glyphosate technical to *Daphnia magna* (OECD-Immobilization test)
Report No	272968
Test facility	Umweltchemie AG PO Box CH-4452 Itingen Switzerland
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Limited validation recoveries provided • Only 4 calibration determinations • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 medium (reconstituted water) by HPLC-FD. The samples were first diluted with bidistilled water to yield concentrations within the concentration range. For derivatisation, aliquots (0.1 mL) were mixed with 0.9 mL borate buffer (pH 9), 0.9 mL acetone and 0.1 mL FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride) solution (0.1 M in acetone). The mixture was allowed to derivatise for 25 min at room temperature, and then extracted twice with diethyl ether (2 x 1 mL). The diethyl ether extracts were discarded, and an aliquot of the aqueous phase was analysed for glyphosate by HPLC-FD.

This method was used in the following studies with minor modifications:

Annex point	Report No.	Author	Year	Test
CA 8.2.1/005	271631	[redacted]	1990	Rainbow trout, acute toxicity (96 hour)
CA 8.2.4.1/009	272968	[redacted]	1990	*Daphnia magna*, acute toxicity (48 hour)

Chromatographic conditions:

HPLC system:	Spectra 8770 pump with Merck F10-50 flurescence detector SP4290 intergrator Merck 655A 40 sampling unit
HPLC column:	271631: Lichrospher 100 NH_2, 125 mm × 4.6 mm, 5 µm 272968: Lichrospher 100 NH_2, 100 mm × 4 mm, 5 µm
Column oven temperature:	Ambient
Mobile phase:	Acetonitrile/KH_2PO_4 1.5% (pH 4.8) (150/850, v/v)
Flow rate:	1.0 mL/min
Temperature:	Room temperature
Injection volume:	271631: 5 µL 272968: 10 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride)
Detection:	Excitation wavelength: 260 nm Emission wavelength: 310 nm
Retention time (Glyphosate):	271631: ~ 7 min 272968: ~ 3.2 min

Findings

Recoveries

Test medium (reconstituted water) samples were fortified at relevant concentrations of 100, 200, 300, 500 and 1000 mg/L and analysed using the analytical method. The recovery results are shown in the table below**.** The average recovery was 101 %, with a relative standard deviation of 2.8 %.

Table 5.1-152: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Report No.	Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
271631	Test medium (reconstituted water)	Glyphosate	100	99.1	–	–	–	1
			200	105	–	–	–	1
			300	104	–	–	–	1
			500	98.3	–	–	–	1
			1000	99.7	–	–	–	1
			Overall	98.3 – 105	101	2.8	2.8	5

Table 5.1-152: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Report No.	Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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.

Duplicate samples of the test water were analysed for the concentration of the test article using the analytical method. The recovery results are shown in the table below. Control samples were also analysed, without detecting glyphosate above the LOD (see below). All overall average recovery values were between 70 % and 110 %, however mean low recoveries (59.6 – 72.7 %) were detected at the low nominal concentration levels in Study 271631. It is noted that these are not true validation recovery data; however the results show good performance of the method.

Table 5.1-153: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
271631	Test medium (reconstituted water)	Glyphosate	95	79.2 – 80.4	79.8	–	–	2
			171	65.8 – 79.6	72.7	–	–	2
			309	58.6 – 60.5	59.6	–	–	2
			556	94.4 – 95.6	95.0	–	–	2
			1000	101 – 102	102	–	–	2
			Overall	58.6 – 102	82	16.4	20.0	10
272968	Test medium (reconstituted water)	Glyphosate	62.5	81-89	85	–	–	2
			125	78-79	78	–	–	2
			250	92-93	93	–	–	2
			500	95	95	–	–	2
			1000	70-85	78	–	–	2

[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

. Solvent peak is near to the retention time of the analyte. The method consists of a derivatisation step which is considered to be specific to the target compound.Chromatograms were provided.

Linearity
The linearity of the detector response was tested using at least four calibration standard concentrations for glyphosate in the range of 0.075 µg/mL to 4 µg/mL with coefficients of determination > 0.99. The calibration standards were prepared in bi-distilled water. Details to the calibrations are provided below.

Table 5.1-154: Linearity parameters

Report No.	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
271631	Glyphosate	Linear	0.25 – 4.00	4 levels	$y = 1.484 \times 10^{-6} x + 0.107$	0.996
272968	Glyphosate	Linear	0.075 – 0.6	4 levels	$y = 2.144 \times 10^{-6} x + 0.0023$	0.988

Limit of Quantification
The limit of quantification (LOQ) was not assessed in the study. Acceptable recoveries were obtained at the lowest fortification level and test item concentration.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed. The test solutions were proved to be stable for the duration of the tests.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were performed under GLP and partially meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries given, matrix effect and stability of sample extracts 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 is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision and the absence of interference was not demonstrated and the derivatisation efficiency was not assessed.

However, the recovery data are in acceptable range. Therefore the analytical meyhod can be considered as fit-for-purpose for the determination of glyphosate in aqueous test medium at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/156 (CA 8.2.1/010)
Report authors	[redacted]
Report year	1991
Report title	Glyphosate technical: 96-hour acute toxicity study (LC50) in the bluegill sunfish
Report No	271642
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries provided (limited validation recoveries provided in Report No 271631) • Only 4 calibration determinations • 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	Supportive (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	CA 4.1.2/181 (CA 8.2.5.1/004)
Report authors	[redacted].
Report year	1990
Report title	Influence of glyphosate on the reproduction of *Daphnia magna*
Test facility	RCC Umweltchemie AG, P.O. Box, CH -4452 Itingen/BL Switzerland
Report No	250795
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries provided (limited validation recoveries provided in Report No 271631) • Only 4 calibration determinations • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/193 (CA 8.2.6.1/015)
Report authors	[redacted]
Report year	1990
Report title	Acute toxicity of glyphosate to *Scenedesmus subspicatus* (OECD – algae growth inhibition test
Report No	250773
Document No	-
Test facility	RCC Umweltchemie AG P.O. box CH-4452 Itengen /BL Switzerland
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries provided (limited validation recoveries provided in Report No 271631) • Only 4 calibration determinations • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 medium (reconstituted water) by HPLC-FD. The samples were first diluted with bi-distilled water to yield concentrations within the concentration range. For derivatisation, aliquots (0.1 mL) were mixed with 0.9 mL borate buffer (pH 9), 0.9 mL acetone and 0.1 mL FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride) solution (0.1 M in acetone). The mixture was allowed to derivatise for 20 – 25 min at room temperature, and then extracted twice with diethyl ether (2x 1 mL). The diethyl ether extracts were discarded, and an aliquot (20 – 25 µL) of the aqueous phase was analysed for glyphosate by HPLC-FD.

This method was used in the following studies with minor modifications:

Annex point	Report No.	Author	Year	Test
CA 8.2.1/010	271642	[redacted]	1991	Bluegill sunfish, acute toxicity (96 hour)
CA 8.2.5.1/004	250795	[redacted]	1990	*Daphnia magna*, reproduction (21 day)
CA 8.2.6.1/015	250773	[redacted]	1990	*Scenedesmus subspicatus*, acute toxicity (96 hour)

Chromatographic conditions:

HPLC system:	Spectra 8770 pump with Merck F10-50 flurescence detector SP4290 intergrator Merck 655A 40 sampling unit
HPLC column:	271642: Spherisorb NH_2, 250 mm × 4.6 mm, 5 µm 250795: Lichrospher NH_2, 125 mm × 4.6 mm, 5 µm 250773: Lichrospher NH_2, 125 mm × 4.6 mm, 5 µm
Column oven temperature:	Ambient
Mobile phase:	Acetonitrile/KH_2PO_4 1.5% (pH 5.8) (175/825, v/v)
Flow rate:	1.0 mL/min
Temperature:	Room temperature
Injection volume:	271642: 10 µL 250795: 20 µL 250773: 20 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride)
Detection:	Excitation wavelength: 260 nm Emission wavelength: 310 nm
Retention time (Glyphosate):	271642: ~ 9.6 min 250795: ~ 3.8 min 250773: ~ 3.3 min

Findings

Recoveries

Method validation experiments with fortified samples were not performed in these studies. However, such validation data are provided in Report No 271631 (performed in the same laboratory) using the identical method with minor modifications.

Duplicate samples of the test water were analysed for the concentration of glyphosate using the analytical method. The results are shown in the table below. Control test water samples were also analysed, without detecting glyphosate above the LOD (see below). The average recovery values were between 70 % and 110 %, with the exception of study 250773 where recoveries (60.4 – 66.6 %) were detected at all nominal concentration levels. It is noted that these are not true validation recovery data; however, the results show good performance of the method.

Table 5.1-155: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
271642	Test medium (reconstituted water)	Glyphosate	59.3	75.2 – 75.9	75.9	0.0	0.0	2
			88.9	107 – 110	108.4	2.1	2.0	2
			133.3	89.5 – 91.0	90.3	1.1	1.2	2
			200	87.3 – 89.2	88.3	1.3	1.5	2
			300	78.9 – 80.5	79.7	1.1	1.4	2
			Overall	75.2 – 110	88.5	11.9	13.5	10
250795	Test medium	Glyphosate	3.0	74.8 – 113	94.1	27.2	28.9	2

Table 5.1-155: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	(reconstituted water)		30	84.3 – 100	92.4	11.4	12.3	2
			300	126 – 134	130.2	5.4	4.2	2
			Overall	74.8 – 134	105.5	23.3	22.1	6
250773	Test medium (reconstituted water)	Glyphosate	1.6	62.1 – 63.4	62.8	0.9	1.5	2
			40	59.1 – 61.7	60.4	1.8	3.0	2
			1000	64.0 – 69.2	66.6	3.7	5.5	2
			Overall	59.1 – 69.2	63.3	3.4	5.3	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.

Specificity

The identification was based on the selected wavelength and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compound. No significant interferences were observed at the retention time of the analyte in example chromatograms.
In RCC Project 250795, Chromatogams of standards (2), of control sample and spiked samples (3 and 300 mg/L) have been provided.
In study 250773, Chromatograms of standard, blank solution and fortified sample are provided

Linearity

Linearity of detector response was tested using at least 4 calibration standard concentrations for glyphosate in the range of 0.25 to 20 µg/mL with coefficients of determination > 0.99. The calibration standards were prepared in bi-distilled water. Details to the calibrations are provided below.

Table 5.1-156: Linearity parameters

Report No.	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
271642	Glyphosate	Linear	0.25 – 2.0	5 levels	$y = 7.4853\times10^{-7} x - 1.0438$	0.990
250795	Glyphosate	Linear	1.0 – 20	4 levels	$y = 5.44\times10^{-5} x + 0.290$	0.998
250773	Glyphosate	Linear	1.0 – 20	4 levels	$y = 2.61\times10^{-5} x + 0.180$	0.999

Repeatability (Precision)

The relative standard deviations (RSDs) of the overall recovery values were ≥20 % in RCC Project 271642 and RCC Project 250773. In RCC Project 250795 the relative standard deviation RSD of the recovery values at 3 mg/L is above 20 %.

Limit of Quantification and Detection
The limit of quantification (LOQ) was not assessed in the study. The Limit of detection (LOD) was not reported in the study, the concentrations in control test water were reported as <6.25 mg/L (271642), <1.0 mg/L (250795) or <0.5 mg/L (250773).

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed. The test solutions were proved to be stable for the duration of the tests.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no recoveries from fortified samples provided, matrix effect and stability of sample extracts 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 is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision was not demonstrated and the derivatisation efficiency was not assessed.
However, the recovery data are in acceptable range. Therefore, the analytical method can be considered as fit for purpose for the determination of glyphosate in the test medium at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/157 (CA 8.2.1/013)
Report authors	[redacted]
Report year	2006
Report title	Glyphosate technical: Acute toxicity to common carp (*Cyprinus carpio*)
Report No	2060/015
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 recoveries provided • LOQ not validated with sufficient recoveries • Matrix effect and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes –
Acceptability/Reliability	Supportive (with relevance for analytical methods)

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

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 medium (reconstituted water) by HPLC-UVD using external standard. The method was developed by the Department of Analytical Services, Safepharm Laboratories Limited, United Kingdom. The test samples were analysed directly without further treatment.

Chromatographic conditions:

HPLC system:	Agilent Technologies 1100 incorporating autosampler and workstation
HPLC column:	Hamilton PRP-X400 cation exchange (250 × 4.1 mm id)
Column oven temperature:	30 °C
Mobile phase:	5 mM potassium dihydrogen orthophosphate in HPLC grade water adjusted to approximately pH 1.9 with phosphoric acid
Flow Rate:	0.5 mL/min
Injection Volume:	50 µL
Derivatisation agent:	Not applicable (no derivatisation)
Detection:	UV/Vis wavelength: 200 nm
Retention time:	Glyphosate: Approx. 6 min

Findings
Recoveries
Test medium (reconstituted water) samples were fortified at concentrations of 100 mg/L and analysed using the analytical method. The recovery results are shown in the table below. **The average recovery was 95.9 %.**

Table 5.1-157: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	100	94.0 – 97.8	95.9	–	–	2

[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 the test water collected at beginning of the test were analysed for the concentration of glyphosate using the analytical method. The results are shown in the table below. Control test water samples were also analysed, without detecting glyphosate above the LOQ (< 5.3 mg/L). The average recovery value was 96.5 %. It is noted that these are not true validation recovery data; however the results show good performance of the method.

Table 5.1-158: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	100	95.2 – 97.8	96.5	–	–	2

[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 significant interferences were observed at the retention time of the analyte in example chromatograms.

Linearity
The linearity of the detector response was tested using eight calibration standard concentrations of glyphosate in the range of 5.3 to 210 µg/mL with a coefficient of determination of 0.9985. The calibration standards were prepared in bi-distilled water. Details to the calibration is provided below.

Table 5.1-159: Linearity parameters

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate	Linear	5.3 – 210	8 levels	y = 1.3107 x – 4.0766	0.9985

Repeatability (Precision)
The relative standard deviations (RSDs) of the overall recovery values (4 analyses) was below 20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was reported as the lowest calibration concentration in the study, i.e. 5.3 mg/L, this is not considered acceptable. The Limit of detection (LOD) was not reported.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

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 partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries given, LOQ not validated with sufficient spike recoveries, matrix effect and stability of sample extracts 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 is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision was not demonstrated.

However, the linearity and the specificity (interference) were demonstrated. The recovery data are in acceptable range. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in test medium at the targeted dose 100 mg/L.

Determination of AMPA in test medium (reconstituted water)

Studies previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/159 (CA 8.2.1/017)
Report authors	[redacted]
Report year	1998
Report title	96-Hour acute toxicity study in rainbow trout with (aminomethyl)phosphonic acid (static)
Report No	232469
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries with fortified samples provided • Limited calibration data • Limit of detection/quantification not assessed • 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	Supportive (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	CA 4.1.2/172 (CA 8.2.4.1/012)
Report authors	[redacted]
Report year	1998
Report title	Acute toxicity study in *Daphnia magna* with (aminomethyl)phosphonic acid (Static).

Report No	232471
Test facility	Notox 5231 DD Hertogenbosch, Netherlands
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries with fortified samples provided • Limited calibration data • Limit of detection/quantification not assessed • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/194 (CA 8.2.6.1/016)
Report authors	[redacted]
Report year	1998
Report title	Fresh water algal growth inhibition test with (aminomethyl)phosphonic acid
Report No	232458
Document No	-
Test facility	NOTOX B.V. Hambakenwetering 3 5231 DD's-Hertogenbosch The Netherlands
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries with fortified samples provided • Limited calibration data • Limit of detection/quantification not assessed • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of aminomethyl-phosphonic acid (AMPA) in test water by HPLC with fluorescence detection. The samples were first diluted with boric acid solution (0.16 M, pH 9.6) and then derivatised using FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride) solution (0.5 mM in acetonitrile). The mixture was allowed to derivatise for 17 min at 30 °C before halting the reaction using phosphoric acid solution (0.1%). The samples were then cleaned-up by on-line solid phase extraction (SPE). An aliquot of the eluate of each sample was analysed for AMPA by HPLC-FD with external calibration.

This method was used in the following studies:

Annex point	Report No.	Author	Year	Test
CA 8.2.1/017	232469	[redacted]	1998	Rainbow trout, acute toxicity (96 hour)
CA 8.2.4.1/012	232471	[redacted]	1998	*Daphnia magna*, reproduction (21 day)
CA 8.2.6.1/016	232458	[redacted]	1998	*Selenastrum capricornutum*, acute toxicity (72 hour)

Chromatographic conditions:

HPLC system:	HPLC with fluorescence detector
HPLC column:	Lichrosorb NH_2 HIBAR; 250 mm × 4.0 mm (i.d.), 5 µm particle size
Column temperature:	40 °C
Mobile phase:	Acetonitrile/0.2% phosphoric acid (75/25, v/v)
Flow Rate:	1.5 mL/min
Injection volume:	200 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride)
Detector:	Excitation wavelength: 265 nm Emission wavelength: 300 nm
Retention time:	AMPA: ~ 7 min

Findings

Recoveries

Method validation experiments with fortified samples were not performed in this study. However it is stated that such validation data are provided in NOTOX Report No 232482 (not available to the applicant).

Samples of the test water were collected at start of the studies and analysed for the concentration of AMPA using the analytical method. The results are shown in the table below. Control test water samples were also analysed, without detecting AMPA. The average recovery values were between 70 % and 110 %. It is noted that these are not true validation recovery data; however the results show good performance of the method.

Table 5.1-160: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
232469	Test medium (reconstituted water)	AMPA	10	94.5 – 94.7	94.6	–	–	2
			100	105	105	–	–	1
			Overall	94.5– 105	98.1	6.0	6.1	3
232471	Test medium (reconstituted water)	AMPA	100	95.4	95.4	–	–	1
232458	Test medium (reconstituted water)	AMPA	10	98.7	98.7	–	–	1
			46	99.6	99.6	–	–	1
			220	102 – 106	104	–	–	2

Table 5.1-160: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			Overall	98.7 – 106	102	3.2	3.2	4

[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 identification was based on fluorescence detection. The method consists of a derivatisation step which is considered to be specific to the target compound. No chromatograms were provided, specificity was not demonstrated.

Linearity
The linearity of the detector response was tested using two calibration standard concentrations in the range of 0.03 to 0.05 mg/L. No further details are provided.

Limit of Quantification
Not assessed.

Matrix effects
Not assessed.

Stability of AMPA in sample extracts
Not assessed. However it was shown that AMPA is stable in test solution for the duration of the test (72 hours).

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless the method is considered as fit-for-purpose for the determination of AMPA in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no true validation recoveries with fortified samples provided, limited calibration data, limit of detection/quantification not assessed, matrix effect and stability of sample extracts 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 is not in agreement with the guidance SANCO 3029/99 re.4 as the linearity plot and calibration function is not available, the range of linearity does not cover the recovery level tested, the absence of interference is not demonstated, the precision was not demonstrated and the derivatisation efficiency was not assessed.

However, the recovery data are in acceptable range. Therefore, the analytical method can be considered as fit for purpose for the determination of AMPA in test medium at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/160
Report authors	[redacted]
Laboratory	Not precised
Report year	1990
Report title	Results of the analyses of AMPA in a 96-hour acute study with rainbow trout
Report No	[redacted]-90-403
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Insufficient recovery validation data • Insufficient calibration information • Only one chromatogram provided from test sample • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/161 (CA 8.2.1/019)
Report authors	[redacted]
Laboratory	[redacted]
Report year	1991
Report title	Acute toxicity of AMPA to rainbow trout (*Oncorhynchus mykiss*)
Report No	[redacted]-90-402
Document No	-
Guidelines followed in study	OECD Guideline 203 Guideline 72-1; U.S. EPA-FIFRA, 40 CFR, Section 158.145
Deviations from current test guideline	Deviation compared with OECD 203 (2019): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/174 (CA 8.2.4.1/014)
Report authors	[redacted]
Laboratory	ABC bboratories, Inc. Envkonmenti Biology Division 7200 East ABC he P.O. Box 1097 Columbia, Missouri 65205

Report year	1991
Report title	Acute toxicity of AMPA to *Daphnia magna*
Report No	AB-90-401
Document No	-
Guidelines followed in study	Guideline No. 72-2, U.S. EPA-FIFRA 40 CFR. Part 158, 145
Deviations from current test guideline	Deviation from to the guideline OECD 202 (2004): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of amino-methyl-phosphenic acid (AMPA) in test medium (reconstituted water) by HPLC with fluorescence (VIS) detection. Aliquots of water samples were diluted if required and transferred into HPLC vials prior to direct injection into the HPLC system. The analyte was derivatised at post-column with boric acid/sodium hydroxide solution and o-phthalaldehyde (OPA) in the presence of mercaptoethanol.

This method was used in the following studies:

Annex Point	Report No.	Author	Year	Test
CA 8.2.1/017	[redacted]-90-402	[redacted]	1991	*Oncorhynchus mykiss*, 96 hour acute
CA 8.2.4.1/014	AB-90-401	[redacted]	1991	*Daphnia magna*, 48 hour acute

Chromatographic conditions:

HPLC system:	Varian 5500
HPLC column:	Dupont Zorbax 300 SCX, 25 cm × 4.6 mm ID
Column temperature:	Not provided
Mobile phase:	0.01 M KH_2PO_4 in 4% MeOH (pH 2.3 with H_3PO_4)
Flow rate:	0.6 mL/min
Injection volume:	20 µL
Derivatisation agent (post-column):	o-phthalaldehyde (OPA)/mercaptoethanol (MERC)
Detection:	Variable wavelength; 340 nm
Retention time:	AMPA: ~ 5.7 min

Findings
Recoveries
In the summary provided by applicant (Doc M) following results are reported. However, these results are not found in the study report available (report n° [redacted]90-403/[redacted] 90187, [redacted]-90-402 and AB-90-401). Therefore, they cannot be taken into account.

Specificity
The identification was based on the selected wavelength and the retention time. No interfering peaks were observed at the retention time of the analyte. It is reported that analyses of control water samples indicated no significant interfering peaks at the retention time of the analyte.

Linearity
No details to the calibration of the instrument are provided.

Repeatability (Precision)
No data available

Limit of Quantification and detection
Not reported. No LOQ can be set.

Matrix effects
Not assessed.

Stability of glyphosate in sample extracts
Not assessed. However it was shown that the test item was stable in test solution for the duration of the study (4 days).

Conclusion
The analytical method was validated for the determination of AMPA in test medium (reconstituted water). Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, is considered as fit-for-purpose for the determination of AMPA in aqueous test medium.

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 partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with some deficits (insufficient recovery validation data, insufficient calibration information, only one sample chromatogram provided, matrix effects 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:
No validation data are available in the study reports provided by applicant. Therefore, in absence of data, the method cannot be considered as fit for purpose.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/162 (CA 8.2.1/020)
Report authors	[redacted]
Laboratory	[redacted]
Report year	1994
Report title	AMPA: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Report No	[redacted]5070/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium

	• No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/173 (CA 8.2.4.1/013)
Report authors	[redacted]
Laboratory	Brixham environement laboratory, Zeneca, UK
Report year	1994
Report title	AMPA: Acute toxicity to *Daphnia magna*
Report No	BL5061/B
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed in the studies [redacted]5070/B ([redacted]., 1994) and BL5061/B ([redacted] 1994) for the determination of AMPA in test medium (reconstituted water) by HPLC with UV detection. This method is based on Brixham Environmental Laboratory Standard Operating Procedure AL286, version 01. Aqueous samples were derivatised with p-toluenesulphonyl chloride and analysed for AMPA by high performance liquid chromatography (HPLC) with UV detection.

This method was used in the following studies:

Annex Point	Report No.	Author	Year	Test
CA 8.2.1/020	[redacted]5070/B	[redacted]	1994	Rainbow trout, acute toxicity (96 hour)
CA 8.2.4.1/013	BL5061/B	[redacted]	1994	*Daphnia magna*, acute toxicity (48 hour)

Chromatographic conditions:

HPLC system:	HPLC equipped with ultra-violet detector
HPLC column:	Spherisorb 3µm ODS2, 150 mm x 4.6 mm id
Column temperature:	Not reported
Mobile phase:	De-ionised water/acetonitrile/phosphoric acid (85/15/1, v/v/v)

Flow rate:	1.5 mL/min
Injection volume:	10 µL
Derivatisation agent (pre-column):	p-toluenesulphonyl chloride
Detection:	UV at 230 nm
Retention time:	Approx. 4.6 min (BL5070/B) Approx. 4.9 min (BL5061/B)

Findings

Recoveries

True validation recoveries obtained from spiked test water (reconstituted water) were not presented within the report. However, samples of test water were taken at start of the experiments from the centre of the test vessels and analysed for AMPA concentration using the analytical method. Control test water samples were also analysed, without detecting AMPA above the LOD (see below). The average recovery value in study **BL5061/B** was above 110 %, due to high concentrations at the lower levels. It is noted that these are not true validation recovery data; nevertheless the results show good performance of the method. The results are provided in the table below.

Table 5.1-161: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
[redacted]5070/B	Test medium (reconstituted water)	AMPA	18	122	–	–	–	1
			32	103 – 113	108	4.8	4.4	3
			56	104	–	–	–	1
			100	110	–	–	–	1
			180	106	–	–	–	1
BL5061/B	Test medium (reconstituted water)	AMPA	18	189	–	–	–	1
			32	109 – 181	142	36.5	25.8	3
			56	130	–	–	–	1
			100	99	–	–	–	1
			180	94	–	–	–	1

[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 method consists of a derivatisation step which is considered to be specific to the target compound. No significant interferences were observed at the retention time of the analyte in example chromatograms.

Linearity

No details to calibration funcions are provided.

Repeatability (Precision)

The repeatability cannot be assessed.

Limit of Quantification and Detection
The limit of quantification (LOQ) can be set at 32 mg/L based on recovery data.

Matrix effects
Not assessed.

Stability of glyphosate in sample extracts
Not assessed.

Conclusion
The analytical method was developed for the determination of AMPA in test medium (reconstituted water). The validation does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless the method showed acceptable performance and is considered as fit-for-purpose for the determination of AMPA in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies were previously evaluated at EU level. They were performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries from spiked samples provided, no calibration details provided, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed).
Nevertheless, the method is considered as fit-for-purpose to support the ecotox studies concerned.

Assessment and conclusion by RMS:
The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision was not demonstrated, no linearity data are available and matrix effect efficiency of derivatisation are not assessed. However, the recovery data are in acceptable range. Therefore, the method can be considered as fit for purpose for the determination of AMPA in the test water at the targeted doses.

Determination of glyphosate acid in test medium (reconstituted water)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/163 (CA 8.2.2.1/001)
Report authors	[redacted]
Laboratory	[redacted]
Report year	2010
Report title	Glyphosate acid: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
Report No	1005.029.321
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	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)

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 medium (reconstituted water) by HPLC-UVD. Aqueous samples were first diluted with fresh water to yield concentrations within the calibration range. For derivatisation, aliquots (1 mL) were mixed with 2.5 mL boric acid buffer (pH 9) and 1 mL FMOC-Cl (Fluorenylmethoxycarbonyl chloride) (1 g/L in acetonitrile). The mixture was allowed to derivatise at room temperature for 60 min. After addition of 4 mL dichloromethane and mixing, an aliquot of the aqueous phase was analysed for glyphosate by HPLC-UVD using external calibration. During analysis of samples, two slightly different analytical columns were used.

Chromatographic conditions:

HPLC system:	Thermo ACCELA, high speed pump Detector: ACCELA PDA detector Autosampler: ACCELA autosampler Software: ChromQuest 4.2.34 Version 3.1.6
HPLC column:	Hypersil Gold, 2.1 × 50 mm, 1.9 µm (A) Hypersil Gold aQ, 2.1 × 50 mm, 1.9 µm (B)
Column temperature:	Room temperature
Mobile phase:	A: methanol B: 0.1% Tetramethyl ammonium chloride

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.0	10	90	0.35
0.5	10	90	0.35
3.5	70	30	0.35
3.6	90	10	0.35
5.0	90	10	0.35
5.1	10	90	0.35
8.0	10	90	0.35

Flow rate:	0.35 mL/min
Injection volume:	20 µL (A) 50 µL (B)
Derivatisation agent:	FMOC-Cl (Fluorenylmethoxycarbonyl chloride)
Retention time:	Approx. 3.2 minute (A) Approx. 2.6 minute (B)
Detection:	UV wavelength: 265 nm

Findings
<u>Recoveries</u>
Test medium (reconstituted water) samples were fortified with test item (glyphosate acid) at concentrations of 0.064 and 10.7 mg a.s./L. Additionally three unfortified samples were prepared, where glyphosate was not detected above the limit of quantification (< 0.064 mg a.s./L). The mean recovery values were between 70 % and 110 %, with relative standard deviations < 20 %. The results are summarised in the table below.

Table 5.1-162: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	0.064	90.1 – 128	110	16.5	15.0	5
		10.7	104 – 106	105	0.89	0.80	5
		Overall	90.1 – 128	107	11.3	10.5	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 identification was based on the selected wavelength and the retention time. The method includes a derivatisation step which is considered to be specific to the target compound. The average interference signal observed in the unfortified control sample chromatograms was about 13 % of the peak area of the low recovery samples.

Linearity

Calibration standards in the range from 0.040 to 12.4 mg/L were prepared with Milli-Q water. For the analysis of the low recovery samples only calibration standards ranging from 0.040 to 0.62 mg/L were used, whereas for high recovery samples all calibration standards were used. Linear calibration function for analysis of low recovery sample is summarised below.

Table 5.1-163: Details to calibration function for analysis of low recovery samples

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate	Linear	0.04 – 0.62	5 (one sample on 5 levels)	y = 500095 x – 6963.4	0.9998

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 quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20 %. These criteria were fulfilled for the 0.064 mg/kg fortification level test medium. Limit of detection (LOD) was not reported.

Matrix effects

Not assessed.

Stability of glyphosate acid in sample extracts

Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate in test medium (reconstituted water). The method validation meets criteria set in SANCO/3029/99 rev. 4, in most relevant points and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

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) in most aspects (minor deficits: matrix effects and storage stability of extracts 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 method is not in agreement with the SANCO 3029/99 rev.4. The linearity plots and calibration function were provided only for the range 0.04 – 0.62 µg/mL. The quantification is based on derivatisation of the substance and no data have been provided to demonstrated that derivatisation reaction is complete.

However, the recovery data are in acceptable range and in agreement with the targeted dose. The method can be considered as fit for purpose for the determination of glyphosate in test medium.

Determination of AMPA in test medium (freshwater)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/164 (CA 8.2.2.1/004)
Report authors	[redacted]
Laboratory	[redacted]
Report year	2011
Report title	AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*)
Report No	139A-394
Document No	[redacted]-2010-328
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes, minor (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)

Data point	CA 4.1.2/185 (CA 8.2.5.1/007)
Report authors	[redacted]
Laboratory	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601 USA

Report year	2011
Report title	AMPA (Aminomethylphosphonic acid): A semi-static life-cycle toxicity test with the Cladoceran (*Daphnia magna*)
Report No	139A-393
Document No	WL-2010-327
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes, minor (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)

#

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of AMPA (aminomethylphosphonic acid) in freshwater by HPLC-UVD. The samples were first diluted with freshwater to yield concentrations within the calibration range. For derivatisation, aliquots (2 mL) were mixed with 1 mL aqueous potassium tetraborate (0.37 M) and 2 mL NBD-Cl (7-chloro-4-2-oxa-1,3-diazole) (0.025 M, methanolic). The mixture was heated to about 80 °C and allowed to derivatise for 40 min. After addition of 1 mL HCl (1.2 M, aqueous), the solution was filtered (0.45 µm PTFE Acrodisc) and analysed for glyphosate by HPLC-UVD using external calibration.

This method was used in the following studies:

Annex point	Report No.	Author	Year	Test
CA 8.2.2.1/004	139A-394	[redacted]	2011	Fathead minnow, ELS test (AMPA)
CA 8.2.5.1/007	139A-393	[redacted]	2011	*Daphnia magna*, life cycle test (AMPA)

Chromatographic conditions:

HPLC system:	Agilent Series 1100/1200 HPLC equipped with an Agilent Series 1100 Variable Wavelength Detector
HPLC column:	YMC-PACK ODS-AM (150 × 4.6 mm, 3 µm particle size)
Column temperature:	40 °C
Mobile phase:	A: 0.1% H_3PO_4 B: CH_3CN

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.01	95	5	1.0
8.50	80	20	1.0
8.60	1	99	1.0
15.00	1	99	1.0
15.10	95	5	1.0
22.00	95	5	1.0

Injection volume:	25 µL
Derivatisation agent:	NBD-Cl (7-chloro-4-2-oxa-1,3-diazole) (0.025 M)
Retention time:	Approx. 6.5 min (WL-2010-328) Approx. 7.3 min (WL-2010-327)
Detection:	UV/Vis wavelength: 500 nm

Findings

Recoveries

Test medium (freshwater) samples were fortified at relevant concentrations in the range of 0.75 to 120 mg/L AMPA and analysed using the analytical method. Control samples were also analysed, without detecting AMPA above the LOQ (see LOQ values below). The average recovery values were between 70 % and 110 %, with relative standard deviations of < 20 %. The results are summarised in the table below.

Table 5.1-164: Results of method validation (spike recovery) for the determination of AMPA in freshwater

Report No.	Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
139A-394	Freshwater	AMPA	0.75	87.0 – 103	95	5.3	5.5	6
			3.0	92.6 – 106	101	4.9	4.9	6
			12.0	94.8 – 104	100	4.1	4.0	6
139A-393	Freshwater	AMPA	7.5	95.4 – 105	100	4.4	4.4	6
			25	94.3 – 104	100	4.4	4.3	6
			120	87.9 – 103	97	5.1	5.2	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.

Specificity

The method consists of a derivatisation step which is considered to be specific to the target compound. No interfering peaks were observed at the retention time of the analyte.

Linearity

The linearity of the detector response was tested using five calibration standard concentrations in the range of 0.4 to 10 mg/L AMPA prepared in freshwater. The calibration standards were derivatised as described above and analysed with each sample set. All calibration curves generated had a coefficient of determination (r^2) of > 0.99. Details to example calibrations are provided below.

Table 5.1-165: Details to example calibrations

Report No.	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
139A-394	AMPA	Linear	0.4 – 4.0	One sample on 5 levels	y = 113.5335 x – 2.0852	0.99920
139A-393	AMPA	Linear	1.0 – 10	One sample on 5 levels	y = 70.6300 x – 11.4839	> 0.99

Repeatability (Precision)

The relative standard deviations (RSDs) of recovery values at each fortification level 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) of the mehod is set at 0.75 mg/L AMPA, based on recovery data.

Matrix effects
Matrix effects were not assessed. However test water (freshwater) was used to prepare calibration standards.

Stability of AMPA in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of AMPA in test medium (freshwater). The method validation meets criteria set in SANCO/3029/99 rev. 4, in most relevant points and is considered as fit-for-purpose for the determination of AMPA in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) in most aspects (minor deficits: matrix effects and storage stability of extracts 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 method is not in agreement with the SANCO 3029/99 rev.4 as the quantification is based on derivatisation of the substance and no data have been provided to demonstrated that derivatisation reaction is complete.

However, linearity data, specificity (interference), recovery and repeatability data are acceptable and in agreement with the targeted dose. Therefore, the method can be considered as fit for purpose for the determination of AMPA in fresh water.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/165 (CA 8.2.3/001)
Report authors	[redacted]
Laboratory	[redacted]
Report year	2012
Report title	Glyphosate: Fish short-term reproduction assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*)
Report No	707A-102A
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • 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)

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 fluorescence detection. Samples were diluted as appropriate with freshwater. Aliquots (2.0 mL) were mixed with 0.37 M aqueous sodium tetraborate (1.0 mL) and 0.025 M NBD-Cl (7-chloro-4-nitrobenz-2-oxa-1,3-diazole, 2.0 mL). For derivatisation, the mixtures were incubated at 80 °C for 30 minutes. Thereafter, 1.2 M aqueous HCl (1.0 mL) was added and the solution was left for about 10 minutes. An aliquot of the derivatised sample was filtered (0.45 µm PTFE Puradisc) and submitted to analysis by HPLC-FD with external calibration.

Chromatographic conditions:

HPLC system:	Agilent Series 1100/1200 High Performance Liquid Chromatograph (HPLC) with an Agilent Series 1100 Variable Wavelength Detector
HPLC Column:	YMC-PACK ODS-AM, 150 × 4.6 mm, 3 µm particle size
Column temperature:	40 °C
Mobile phase:	A: 0.1% H_3PO_4 B: Acetonitrile

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.01	95	5	1.0
8.50	80	20	1.0
8.60	1	99	1.0
15.00	1	99	1.0
15.10	95	5	1.0
22.00	95	5	1.0

Injection volume:	25 µL
Derivatisation agent (pre-column):	NBD-Cl (7-chloro-4-nitrobenz-2-oxa-1,3-diazole)
Detection:	Fluorescence at 500 nm
Retention time:	Glyphosate: ~ 5.3 min

Findings
Recoveries (accuracy)
Blank samples of test water were fortified with reference item at relevant concentrations of 0.045, 1.0 and 30 mg/L and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 0.03 mg/kg). The average recovery values at each fortification level and overall were between 70 % and 110 %, with an overall relative standard deviation (RSD) of 6.5 %. The detailed results are summarised in the table below.

Table 5.1-166: Results of method validation (spike recovery) for the determination of glyphosate acid in test water

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test water	Glyphosate acid	0.045	90.0 – 112	102	8.6	8.4	5
		1.0	91.7 – 103	98.5	4.9	4.9	5
		30	89.3 – 105	101	6.7	6.6	5

[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 identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds. Four matrix blank samples were analysed to determine possible interferences. No interfering peaks (<30 % LOQ) were observed at the retention time of the analyte.

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 0.03 to 0.3 µg glyphosate acid/mL prepared in well water. The calibration standards were derivatised as described above and fresh calibration standards were prepared analysed with each sample set. Linear calibration curves were found with coefficients of determination (r^2) of >0.99**.** Details to a sample calibration are provided below.

Table 5.1-167: Details to the calibration

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate acid	Linear	0.03 – 0.3	10 (two samples on 5 levels)	y = 75.3698 x – 0.4574	0.99881

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) for the analysis of glyphosate in freshwater was set at 0.045 mg/L based on recovery data.

Matrix effects

Matrix blank sample did not show any peak at the retention time of interest. Calibration standards were prepared in matrix (well water).

Stability of glyphosate acid in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate acid in test water. The method validation meets criteria set in SANCO/3029/99 rev. 4 with very minor deficits and is considered as valid for the determination of glyphosate acid 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 minor deficits (stability of sample extracts not assessed, efficiency of derivatisation not assessed). The method is considered as valid to support the ecotoxicological study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99 as the derivatisation efficiency was not assessed. However, the linearity plots and calibration function were provided. The range of recoveries are in acceptable range and in agreement with the targeted dose. The method can be considered as fit for purpose for the determination of glyphosate in test water.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/166 (CA 8.2.3/002)
Report authors	[redacted]
Laboratory	[redacted]
Report year	2012
Report title	Glyphosate: Amphibian metamorphosis assay for the detection of thyroid active substances
Report No	707A-103
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • 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)

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 fluorescence detection. Samples were diluted as appropriate with freshwater. Aliquots (2.0 mL) were mixed with 0.37 M aqueous sodium tetraborate (1.0 mL) and 0.025 M NBD-Cl (7-chloro-4-nitrobenz-2-oxa-1,3-diazole, 2.0 mL). For derivatisation, the mixtures were incubated at 80 °C for 30 minutes. Thereafter, 1.2 M aqueous HCl (1.0 mL) was added and the solution was left for about 10 minutes. An aliquot of the derivatised sample was filtered (0.45 µm PTFE Puradisc) and submitted to analysis by HPLC-FD with external calibration.

Chromatographic conditions:

HPLC system:	Agilent Series 1100/1200 High Performance Liquid Chromatograph (HPLC) with an Agilent Series 1100 Variable Wavelength Detector			
HPLC Column:	YMC-PACK ODS-AM, 150 × 4.6 mm, 3 µm particle size			
Column temperature:	40 °C			
Mobile phase:	A: 0.1% H_3PO_4 B: Acetonitrile			
Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.01	95	5	1.0
	8.50	80	20	1.0
	8.60	1	99	1.0
	15.00	1	99	1.0
	15.10	95	5	1.0
	22.00	95	5	1.0
Injection volume:	25 µL			
Derivatisation agent (pre-column):	NBD-Cl (7-chloro-4-nitrobenz-2-oxa-1,3-diazole)			
Detection:	Fluorescence at 500 nm			
Retention time:	Glyphosate: ~ 4.8 min			

Findings
Recoveries (accuracy)
Blank samples of test water were fortified with reference item at relevant concentrations of 0.16, 10 and 100 mg/L and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 0.10 mg/kg). The average recovery values at each fortification level and overall were between 70 % and 110 %, with an overall relative standard deviation (RSD) of 2.9 %. The detailed results are summarised in the table below.

Table 5.1-168: Results of method validation (spike recovery) for the determination of glyphosate acid in test water

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test water	Glyphosate acid	0.16	99.4 – 108	103	3.4	3.3	5
		10	96.0 – 101	99.2	1.9	1.9	5
		100	97.3 – 105	102	2.9	2.8	5
		Overall	96.0 – 108	101	2.9	2.9	15

Table 5.1-168: Results of method validation (spike recovery) for the determination of glyphosate acid in test water

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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 identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds. Four matrix blank samples were analysed to determine possible interferences. No interfering peaks (<30 % LOQ) were observed at the retention time of the analyte.

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 0.10 to 1.0 µg glyphosate acid/mL prepared in well water. The calibration standards were derivatised as described above and fresh calibration standards were prepared analysed with each sample set. Linear calibration curves were found with coefficients of determination (r^2) of >0.99. **Details to a sample calibration are provided below.**

Table 5.1-169: Details to the calibration

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate acid	Linear	0.10 – 1.0	10 (2 points on 5 levels)	y = 76.0800 x – 0.3712	0.99988

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) for the analysis of glyphosate in freshwater was set at 0.10 mg/L, calculated as the product of the concentration of the lowest calibration standard (0.10 mg/L) and the dilution factor of the matrix blank samples (1.00).

Matrix effects

Matrix blank sample did not show any peak at the retention time of interest. Calibration standards were prepared in matrix (well water).

Stability of glyphosate acid in sample extracts

Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate acid in test water. The method validation meets criteria set in SANCO/3029/99 rev. 4 with very minor deficits and is considered as valid for the determination of glyphosate acid 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 minor deficits (stability of sample extracts not assessed, efficiency of derivatisation not assessed). The method is considered as valid to support the ecotoxicological study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99 rev.4 as the derivatisation efficiency was not assessed.
However, the linearity plots and calibration function were provided. The recovery data are in acceptable range and in agreement with the targeted dose. The method can be considered as fit for purpose for the determination of glyphosate in test water.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/169 (CA 8.2.4.1/006)
Report authors	[redacted]
Laboratory	Notox BV hertogenbosch NL
Report year	1995
Report title	Acute toxicity study in *Daphnia magna* with glyfosaat
Report No	141863
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries provided • No details to calibration functions provided • No chromatograms provided (interference not assessed) • Matrix effects and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/179 (CA 8.2.5.1/002)
Report authors	[redacted]
Laboatory	Notox BV hertogenbosch NL
Report year	1995
Report title	*Daphnia magna*, reproduction test with glyfosaat

Report No	141874
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries provided • No details to calibration functions provided • No chromatograms provided (interference not assessed) • Matrix effects and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/190 (CA 8.2.6.1/007)
Report authors	[redacted]
Laboratory	Notox BV hertogenbosch NL
Report year	1995
Report title	Fresh water algal growth inhibition test with glyfosaat
Report No	141896
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • No true validation recoveries provided • No details to calibration functions provided • No chromatograms provided (interference not assessed) • Matrix effects and stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 medium (reconstituted water) by HPLC-UVD with external calibration. Samples were transferred quantitatively into suitable volumetric flasks and diluted with mobile phase before direct injection to the HPLC.

This method was used in the following studies:

Annex Point	Report No.	Author	Year	Test
CA 8.2.4.1/006	141863	[redacted]	1995	*Daphnia magna*, acute toxicity (48 hour)
CA 8.2.5.1/002	141874	[redacted]	1995	*Daphnia magna*, reproductive toxicity (21 days)

CA 8.2.6.1/007	141896	[redacted]	1995	Freshwater alga (*Selenastrum capricornutum*), growth inhibition (72 hour)

Chromatographic conditions**:**

HPLC system:	High Performance Liquid Chromatograph (HPLC) equipped with an UV Detector
HPLC column:	Partisil P10SAX, 250 × 4.6 mm i.d. (Hichrom Ltd.)
Mobile phase:	4/96 (v/v) methanol/aqueous buffer, pH 2.2 Aqueous buffer: 1.69 g potassiumdihydrogenphosphate (KH_2PO_4) in 1920 mL Milli-Q water, adjusted to pH 2.2 with phosphoric acid
Flow rate:	1.5 mL/min
Injection volume:	100 µL
Derivatisation agent:	Not applicable, no derivatisation
Detection:	UV at 195 nm
Retention time:	Not provided (no chromatograms available)

Findings

Recoveries

Method validation experiments with fortified samples were not performed in this study.

Samples of test medium (reconstituted water) were taken at the beginning of the experiments and analysed for the concentration of glyphosate using the analytical method. The results are summarised in the table below. Control test water samples were also analysed, without detecting glyphosate. At some low nominal concentration levels the measured concentrations were too high to fulfil EU guideline requirements according to document SANCO/3029/99 rev. 4 (>110 % recoveries). However is noted that these are not true validation recovery data, and the results show good general performance of the method.

Table 5.1-170: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concen-tration level (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
141863	Test medium (reconstituted water)	Glyphosate	100	112	–	–	–	1
141874	Test medium (reconstituted water)	Glyphosate	5	169	–	–	–	1
			10	120	–	–	–	1
			18	118	–	–	–	1
			32	101	–	–	–	1
			56	105	–	–	–	1
			100	100	–	–	–	1
141896	Test medium (reconstituted water)	Glyphosate	10	106	-	-	-	1
			32	109	-	-	-	1
			100	108	-	-	-	1

Table 5.1-170: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concen-tration level (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[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 identification was based on the selected wavelength and the retention time. Not assessed (no chromatograms are provided).

Linearity
No details to the calibration functions are provided.

Repeatability (Precision)
Not assessed.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method was developed for the determination of glyphosate in test media (reconstituted water). Despite the method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points, the results show that the measured concentrations of glyphosate are higher than the nominal concentrations in test media.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies on aquatic ecotoxicology of glyphosate were previously evaluated at EU level. The analytical parts do not meet current requirements (EU guideline SANCO/3029/99 rev. 4) in seversal points (no true validation recoveries with fortified samples provided, no details to calibration functions provided, repeatability, interference and stability of sample extracts not assessed, no chromatograms provided). However, the results show that the measured concentrations of glyphosate are higher than the nominal concentrations in test media.

Assessment and conclusion by RMS:
The method is not in agreement with the SANCO 3029/99 rev.4. The linearity plots and calibration function were not provided, the specificity (interference) and the precision were not demonstrated.

However, the recovery data are in acceptable range for the concentration range 10 – 100 mg/L. The recovery is not acceptable at 5 mg/L.
Therefore, the method can be considered as fit for purpose for the determination of glyphosate at targeted doses in the range 10-100 mg/L.

Determination of HMPA in test medium (well water)

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/175 (CA 8.2.4.1/015)
Report authors	[redacted]
Report year	2011
Report title	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)
Report No	139A-395
Document No	WL-2010-329
Test facility	Wildlife International, Ltd 8598 Commerce Drive Easton, Maryland 21601 USA 1-410-822-8600
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Limited validation recoveries from spiked samples • Stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical Method was developed for the determination of hydroxymethylphosphonic acid (HMPA) in test medium (well water) by LC-MS. The samples were first diluted with freshwater to yield concentrations within the calibration range and then directly submitted to analysis by high performance liquid chromatography with mass selective detection (LC-MS).

Chromatographic conditions:

HPLC system:	Hewlett Packard Series 1100 HPLC equipped with a Perkin-Elmer SCIEX API 100 LC Mass Spectrometer			
HPLC column:	Thermo Prism (50 mm × 2.1 mm, 5 µm particle size)			
Column temperature:	40 °C			
Mobile phase:	A: 0.1% Formic Acid in H_2O B: CH_3CN			
Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (µL/min)
	0.00	98	2	250
	0.10	98	2	250
	0.50	98	2	250
	0.60	98	2	300
	5.00	98	2	300
Injection volume:	5 µL			

Retention time:	HMPA: ~ 2.9 min
Ion source:	Perkin Elmer SCIEX TurboIonSpray
Carrier gas:	Air 60 psi Nitrogen (99.5%) 65 psi; 6 L/min
Monitored mass:	111 amu

Findings

Recoveries

For method validation, samples of test medium (well water) were spiked with the analyte at one fortification level at 100 mg/L, with mean recovery found as 97 %. The recovery value was between 70 % and 110 %. The detailed results are summarised in the table below.

Table 5.1-171: Results of method validation (spike recovery) for the determination of HMPA in test medium

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Well water	HMPA	100	92.9 – 101	97.0	–	–	2

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

Additionally one sample of test medium prepared at 0 hour was analysed using the analytical method. The result of this analysis is provided in the table below. These are not true validation recovery data; however, the results show the good performance of the method.

Table 5.1-172: Results of test medium analyses

Matrix	Analyte	Nominal concentration level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Well water	HMPA	100	85.9	85.9	–	–	1

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

Specificity

Determination by LC-MS is considered to be highly specific. Chromatograms were provided. No interfering peaks were observed at the retention time of the analyte.

Linearity

The linearity of the detector response was tested using five calibration standard concentrations in the range of 1.0 to 10 mg/L prepared in freshwater. A linear function was found (y = 477000 x – 91900, 1/x weighting) with a coefficient of determination (r) of 0.9994.

Repeatability (Precision)
The relative standard deviation (RSD) of all recovery values (n = 3) was 4.2 %, i.e. in compliance with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification
The limit of quantitation (LOQ) in the study was 1.0 mg/L, calculated as the product of the concentration of the lowest calibration standard (1.0 mg/L) and the dilution factor of the matrix blank samples (1.0).

Matrix effects
Matrix effects were eliminated by using freshwater for dilution of test water and preparation of calibration standards.

Stability of HMPA in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of HMPA in test water (freshwater). Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, it is considered as fit-for-purpose for the determination of HMPA 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 partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (limited number of validation measurements, storage stability of extracts not assessed). Nevertheless, the method is considered as fit-for-purpose to support the ecotox study concerned.

Assessment and conclusion by RMS:
The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision was not demonstrated, the linearity does not covered the concentration tested.

However, the recovery data available at 100 mg/L are in acceptable range and the concentration tested corresponds to the targeted dose. Therefore, the method validation can be considered as fit-for-purpose for the determination of HMPA in aqueous test medium at 100 mg/L.

Determination of glyphosate in test medium (seawater)

Study not previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/176 (CA 8.2.4.2/001)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to mysid shrimp (*Mysidopsis bahia*)
Report No	BL5713/B
Test facility	Brixham Environmental Laboratory ZENECA Limited Brixam Devon TQ5 8BA, UK
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/177 (CA 8.2.4.2/003)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to larvae of the Pacific oyster (*Crassostrea gigas*)
Report No	BL5714/B
Test facility	Brixham Environmental Laboratory ZENECA Limited Brixam Devon TQ5 8BA, UK
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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-FD. Aqueous samples were quantified against standard solutions of glyphosate acid prepared in deionised water. Prior to analysis, samples and standards were derivatised using fluorenylmethyl chloroformate. No further details to the extraction and derivatisation steps are provided in the analytical section.

Chromatographic conditions:

HPLC system:	HPLC equipped with fluorescence detector
HPLC Column:	Spherisorb S5 SAX, 50 × 4.6 mm id, 5 µm particvle size
Column oven temperature:	Not provided
Mobile phase:	Acetonitrile/deionised water/buffer (22/18/60, v/v/v) Buffer: Deionised water/glacial acetic acid/orthophosphoric acid (97/2/1, v/v/v)
Flow rate:	2.0 mL/min
Injection volume:	20 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)

Detection:	Excitation wavelength: 254 nm Emission wavelength: 300 nm
Retention time:	Glyphosate: ~ 2.2 min

Findings

Recoveries

Details to true validation recoveries obtained from spiked test water were not presented within the report. The method is based on Brixham Environmental Laboratory Standard Operating Procedure AL228 (version 04), which was used in several studies conducted in this laboratory.

Samples of test water were taken at starting timepoint of test medium preparation (day 0) from the centre of the test vessels and analysed for glyphosate concentration using the analytical method. Control test water samples were also analysed, without detecting glyphosate above the LOQ (< 0.01 mg/L). All average recovery values were between 70 % and 110 %. It is noted that these are not true validation recovery data; however, the results show good performance of the method.

Table 5.1-173: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
BL5713/B	Test medium (seawater)	Glyphosate acid	3.2	150	150.0	–	–	1
			5.6	83.9	83.9	–	–	1
			10	79.0	79.0	–	–	1
			18	88.9	88.9	–	–	1
			32	93.8	93.8	–	–	1
			56	98.2	98.2	–	–	1
			100	98.0	98.0	–	–	1
			180	94.4	94.4	–	–	1
			320	93.8	93.8	–	–	1
			560	94.6	94.6	–	–	1
			1000	94.0	94.0	–	–	1
BL5714/B	Test medium (seawater)	Glyphosate acid	3.2	93.8	93.8	–	–	1
			5.6	102	102	–	–	1
			10	100	100	–	–	1
			18	100	100	–	–	1
			32	100	100	–	–	1
			56	100	100	–	–	1
			100	100	100	–	–	1
			180	100	100	–	–	1

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

Specificity
The method consists of a derivatisation step which is considered to be specific to the target compound. Blank chromatograms were not provided.. . No interfering peaks were observed at the retention time of the analyte

Linearity
No details to calibration functions are provided.

Limit of Quantification
The limit of quantification (LOQ) was not assessed in these studies.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies were previously evaluated at EU level. They were performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries from spiked samples provided, no calibration details provided, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit for purpose to support the ecotox studies concerned.

Assessment and conclusion by RMS:
The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4, as the linearity data are not available, the precisions was not demonstrated, the matrix effect and the derivatisation efficiency was not assessed.

However, the recovery data are in acceptable range except for the concentration of 3.2 mg/L. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in seawater in the range of concentrations 5.6 -1000 mg/L.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/178 (CA 8.2.5.1/001)
Report authors	[redacted]
Report year	1999
Report title	Glyphosate acid: Chronic toxicity to *Daphnia magna*
Report No	BL6535/B
Test facility	Brixham Environmental Laboratory ZENECA Limited Brixam Devon TQ5 8BA, UK
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No validation recoveries from spiked samples provided given (recoveries calculated from analysis of water test medium • No details to calibration provided

	• 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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-FD. Aqueous samples were quantified against standard solutions of glyphosate acid prepared in deionised water. Prior to analysis, samples and standards were derivatised using fluorenylmethyl chloroformate (FMOC-Cl). No further details to the extraction and derivatisation steps are provided in the analytical section.

Chromatographic conditions:

HPLC system:	HPLC equipped with fluorescence detector
HPLC column:	Spherisorb S5 SAX, 50 × 4.6 mm id, 5 µm particvle size
Column temperature:	Not provided
Mobile phase:	Acetonitrile/deionised water/buffer (22/18/60, v/v/v) Buffer: Deionised water/glacial acetic acid/orthophosphoric acid (97/2/1, v/v/v)
Flow rate:	2.0 mL/min
Injection volume:	20 µL
Derivatisation agent (pre-column):	FMOC-Cl (9-Fluorenylmethyl chloroformate)
Detection:	Excitation wavelength: 254 nm Emission wavelength: 300 nm
Retention time:	Glyphosate: ~ 1.7 min

Findings
Recoveries
Details to true validation recoveries obtained from spiked test water (reconstituted water) were not presented within the report. It is stated that spiked samples were prepared in triplicate on each sampling occasion at 5 and 10 mg/L and analysed concurrently with test medium samples. The measured concentrations in these spiked samples ranged from 99 to 100 %, and therefore no corrections were made to measured concentrations.
Samples of test water were taken at each time point of test medium preparation (day 0, 2, 7, 9, 14 and 16) and analysed for glyphosate concentration using the analytical method. Control test water samples were also analysed, without detecting glyphosate above the 30% LOQ area. All average recovery values were between 70 % and 110 %. It is noted that these are not true validation recovery data; however, the results show good performance of the method.

Table 5.1-174: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Test medium	Glyphosate	12.5	96.0 – 104	100	4.4	6

Table 5.1-174: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
(reconstituted water)	acid	25	96.0 – 108	100	4.4	6
		50	98.0 – 102	99.3	1.6	6
		100	99.0 – 100	99.8	0.4	6

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

Specificity
The method consists of a derivatisation step which is considered to be specific to the target compound. No interfering peaks were observed at the retention time of the analyte. Nevertheless, blank chromatograms were not provided.

Linearity
No details to calibration functions are provided.

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

Limit of Quantification
The limit of quantification (LOQ) was not assessed in these studies.

Matrix effects
Not assessed.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no validation recoveries from spiked samples provided, no calibration details provided, matrix effect and stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the ecotox study concerned.

Assessment and conclusion by RMS:
The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4, as the linearity data are not available and the derivatisation efficiency was not assessed.
However the recovery data are in acceptable range. Therefore, the analytical method can be considered as fit for purpose for the determination of glyphosate in test medium at the targeted dose.

1. Information on the study

Data point	CA 4.1.2/182
Report authors	[redacted]
Report year	1989
Report title	Results of the analyses of glyphosate in a 21-day chronic *Daphnia* exposure study
Test facility	Analytical Bio-Chemistry Laboratories
Report No	ML-89-62
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Insufficient recovery validation data • Insufficient calibration information • Only one chromatogram provided from test sample • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/183 (CA 8.2.5.1/005)
Report authors	[redacted]
Report year	1989
Report title	21-day prolonged static renewal toxicity of glyphosate technical to *Daphnia magna*
Test facility	Analytical Bio-Chemistry Laboratories, Inc. Aquatic Toxicology Division 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205
Report No	AB 89-58
Document No	-
Guidelines followed in study	OECD Guideline 202 U.S. Guideline 72-4, (EPA-FIFRA, 40 CFR, Section 158.145)
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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-UV/Vis. Samples were diluted as appropriate with water. Aliquots (2.0 mL) were mixed with 0.37 M aqueous potassium tetraborate (1.0 mL) and 0.025 M NBD-Cl (7-chloro-4-nitrobenz-2-oxa-1,3-diazole, 2.0 mL). For derivatisation, the mixtures were incubated at 80 °C for 15 – 30 minutes. Thereafter, 1.2 M aqueous HCl (1.0 mL) was added and the solution was left for about 10 minutes. An aliquot of the derivatised sample was filtered (0.22 or 0.45 µm) and submitted to analysis by HPLC-FD with external calibration.

Chromatographic conditions:

HPLC system:	Varian 5500 with Spectra Physics 4270 integrator and variable wavelength detector			
HPLC column:	Spherex C-18 (Phenomenex), 250 × 4.6 mm			
Guard column:	Brownlee C-18 (Phenomenex), 30 × 4.6 mm			
Column temperature:	Ambient			
Mobile phase:	A: 0.01 M KH_2PO_4 (pH 3.6) B: Acetonitrile			
Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.01	95	5	0.5
	8.50	50	50	0.5
	9.50	50	50	2.0
	14.00	95	5	2.0
	15.00	95	5	0.5
	16.00	95	5	0.5
Injection volume:	20 µL			
Derivatisation agent (pre-column):	NBD-Cl (7-chloro-4-nitrobenz-2-oxa-1,3-diazole)			
Detection:	Absorbance at 500 nm			
Retention time:	Glyphosate: ~ 7.5 min			

Findings

Recoveries

For method validation, blank samples were fortified to cover the nominal test concentrations and analysed concurrently with the test samples. The results are summarised in the table below. The average recovery values were between 70 % and 110 %.

Table 5.1-175: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Fortification level (mg glyphosate /L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	6.0 / 8.7	86.7 – 101	93.9	–	–	2
		12 / 17	100 – 100	100	–	–	2
		25 / 26	96.2 – 112	104	–	–	2
		50 / 65	95.6 – 102	99.3	–	–	2
		100 / 109	95.4 – 100	97.7	–	–	2

Table 5.1-175: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Fortification level (mg glyphosate /L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	86.7 – 112	99.0	6.4	6.4	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.

In the summary provided by applicant (doc M), additional recovery data are reported. However these values were not found in the study reports available. These data cannot be taken into account.

Specificity
The identification was based on the selected wavelength and the retention time. No interfering peaks were observed at the retention time of the analyte. Only a chromatogram of a typical standard was provided for study ML-89-62. Therefore, the absence of interference is not demonstrated.

Linearity
ML-89-62: No details to the calibration of the instrument are provided. It is stated in the report that two linear regressions were prepared covering the range of 2 – 56 mg/L and 2 – 120 mg/L for the calculations of low and high concentration samples, respectively. No calibration curve was provided.
AB 89-58: linearity data were not reported

Repeatability (Precision)
The precision of the method could not be demonstrated.

Limit of Quantification and detection
Not reported.

Matrix effects
Not assessed.

Stability of glyphosate in sample extracts
Not assessed. However it was shown that the test item was stable in test solution for 2 days under test conditions. Moreover, the stability of the test material in standard solutions was demonstrated for the duration of the study.

Conclusion
The analytical method was validated for the determination of glyphosate in test medium (reconstituted water). Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies were previously evaluated at EU level and considered acceptable. They were performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with some deficits (insufficient recovery validation data, insufficient calibration information, only one sample chromatogram provided, matrix effects 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 method is not in agreement with the guidance SANCO 3029/99 rev.4 as the specificity (interference) was not demonstrated, the linearity plot is not available and the precision were not demonstrated.

However, the recovery data are in acceptable range. Therefore, the analytical method can be considered as fit for purpose for the determination of glyphosate in the test medium (reconstituted water).

1. Information on the study

Data point	CA 4.1.2/184 (CA 8.2.5.1/006)
Report authors	[redacted]
Report year	1982
Report title	Chronic toxicity of glyphosate to *Daphnia magna* under flow-through test conditions
Test facility	Analytical Biochemistry Laboratories, Columbia, Missouri 65205
Report No	AB-82-036
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No detailed reporting in several points • Limited number of fortified samples for validation • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

Analysis of water samples for glyphosate content was accomplished following the Monsanto procedure dated September 4, 1980 entitled, "Analytical Residue Method for N-Phosphonomethyl-glycine, Aminomethylphosphonic Acid and N-nitroso-N-(phosphonomethyl)-glycine in Environmental Water". However, the report detailing this method was not provided. Therefore, these data could not be taken into account.

Aliquots (10 mL) of test water samples were first diluted to 500 mL using deionized water, ion-exchanged and eluted from A-101D resin using 500 mL of 0.2 M NH_4HCO_3. The eluent was evaporated, fractionated on AG 50W-XS ion-exchange resin and derivatised. Residues were then transferred to a known volume of 4 % methanol in tetrahydrofuran before analysis by GLC with flame photometric detection (FPD).

Chromatographic conditions:

GC system:	Gas chromatographic (GC) system (Tractor Model 650) equipped with a flame photometric detector (FPD)
GC column:	Chromosorb W(HP) colum, 6 ft. x 4 mm i.d., 10% DC-200, 80/100 mesh
Inlet temperature:	200 °C
Oven temperature:	178 °C

Carrier gas:	Nitrogen: 40 mL/min Air: 13 mL/min Hydrogen: 10 mL/min
Derivatisation:	No details provided
Detection:	Flame photometric detection (FPD)
Detector temperature:	200 °C

Findings

Recoveries

In the summary provided by applicant, the following results are reported. However, the report from which these data come from has not been provided. Therefore, these data could not be taken into account.

The test concentrations of glyphosate were measured on days 0, 4, 7, 14 and 21 through the use of the analytical method. The results are summarised in the table below. Average recovery values were between 70 % and 110 %. It is noted that these are not true validation recovery data; however, the results show good performance of the method and the correct dosing during the tests.

Table 5.1-176: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg glyphosate/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (well water)	Glyphosate	25	92.0 – 116	105.6	9.6	9.1	5
		50	88.0 – 106	100.0	7.1	7.1	5
		99	84.8 – 107	97.2	8.1	8.3	5
		199	79.4 – 104	93.3	8.7	9.3	5
		397	86.1 – 106	95.3	7.0	7.4	5

[1] Recovery values are 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. The method consists of a derivatisation step which is considered to be specific to the target compound. Some chromatograms were provided. However, they were not readable. Therefore, the absence of interference could not be demonstrated.

Linearity

In the summary provided by applicant, the following results are reported. However the report from which these data come from has not been provided. Therefore, these data could not be taken into account.

Repeatability (Precision)

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

Limit of Quantification and Detection

Not reported.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for standard solution.

Stability of analytes in sample extracts
Not assessed.

Conclusion
The analytical method was developed and validated for the determination of glyphosate in test medium (well water). It does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless, the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

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 meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (no detailed reporting in several points, limited number of fortified samples, matrix effect and stability of sample extracts 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 method is not in agreement with guidance SANCO 3029/99 rev.4 as the specificity (interference) was not demonstrated and the linearity data are not available.
However, considering the acceptable recoveries and precision, the method is considered as fit for purpose for the determination of glyphosate in Test medium (well water).

1. Information on the study

Data point	CA 4.1.2/186 (CA 8.2.5.3/001)
Report authors	[redacted]
Report year	2020
Report title	MON 77973: A study on the toxicity to the sediment dweller *Chironomus riparius* using spiked water
Test facility	ECT Oekotoxikologie GmbH Böttgerstr. 2 - 14 65439 Flörsheim am Main Germany
Report No	20FV2ME (Interim Report – no analytical report presented)
Document No	-
Guidelines followed in study	OECD guideline 219 (2004)
Deviations from current test guideline	To be determined
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

The analytical phase of this report was not yet available to the applicant at the time of submission. A summary of the analytical phase should have been provided as soon as the analytical report was available. However, no analytical phase was available at the time of the assessment.

Assessment and conclusion by RMS: Without any data on the analytical phase, the method cannot be considered as fit for purpose.

Determination of glyphosate and AMPA in test medium (reconstituted water)

1. Information on the study

Data point	CA 4.1.2/187 (CA 8.2.6.1/001)
Report authors	[redacted]
Report year	2002
Report title	A study on the toxicity of glyphosate isopropylamine salt 62.5% to algae (*Pseudokirchneriella subcapitata*)
Test facility	ECT Oekotoxikologie GmbH Böttgerstr. 2 - 14 D-65439 Flörsheim am Main
Report No	A-99-02-04
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 recoveries with fortified samples provided • Repeatability (precision) not assessed • Limit of quantification not reported • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of glyphosate and amino-methyl-phosphenic acid (AMPA) in test medium (reconstituted water) by HPLC with fluorescence detection. Aliquots of water samples were diluted if required and transferred into HPLC vials prior to direct injection into the HPLC system. The analytes were derivatised at post-column (Merck-Hitachi 655A-13 post column reactor) with OCl^- oxidation solution and o-phthalaldehyde (OPA) in the presence of $HS(CH_2)_2OH$.

Chromatographic conditions:

HPLC system:	HPLC with Merck Hitachi F-1050 Fluorescence Detector
HPLC column:	Supelcosil LC-SCX (Agilent), 25 cm × 4.6 mm ID, 5 µm particle size
Column temperature:	60 °C

Mobile phase:	Methanol/Buffer (KH_2PO_4/H_3PO_4) (50/50, v/v) (pH 2)
Flow rate:	0.5 mL/min
Run time:	25 min
Injection volume:	10 and 25 µL, cut volume
Derivatisation agent (post-column):	o-phthalaldehyde (OPA)/$HS(CH_2)_2OH$
Detection:	Excitation wavelength: 335 nm Emission wavelength: 455 nm
Retention time:	Glyphosate: ~ 7 min AMPA: ~ 9 min

Findings

Recoveries

Test medium samples were spiked with the analytes at two fortification levels, *i.e.* 4.02 and 100.5 mg/L for glyphosate and 4.06 and 101.6 mg/L for AMPA and analysed using the analytical method. The results are summarised in the table below. Average recovery values were between 70 % and 110 %, therefore in compliance with EU guideline document SANCO/3029/99 rev. 4.

Table 5.1-177: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in test medium

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstitued water)	Glyphosate	4.020	75.3	–	–	–	1
		100.5	92.1	–	–	–	1
	AMPA	4.06	73.0	–	–	–	1
		101.6	94.3	–	–	–	1

[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 identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds. Representative chromatograms of AMPA and glyphosate standards, of control water sample and fortified control water sample have been provided. No significant interferences were observed at the retention time of the analytes in example chromatograms.

Linearity

Linearity of detector response for glyphosate was tested using six (glyphosate) or seven (AMPA) calibration standard concentrations in the range of 2.5 to 50 mg/L for both analytes, prepared in HPLC grade water. Details to the calibration functions are provided below.

Table 5.1-178: Linearity parameters

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate	Linear	2.5 – 50	6 (5 levels)	y = 0.411700 × x – 0.203638	0.970
AMPA	Linear	2.5 – 50	7 (5 levels)	y = 12.507165 × x – 0.223369	0.978

Repeatability (Precision)
Insufficient validation recoveries available to calculate a reliable RSD.

Limit of Quantification and determination
The Limit of Determination was equivalent to 4 µg/mL or 4 mg/L, equivalent to the smallest concentration in the in-life phase.

Matrix effects
Not assessed.

Stability of glyphosate and AMPA in sample extracts
Not assessed.

Conclusion
The analytical method was partially validated for the determination of glyphosate and AMPA in aqueous test medium. Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, it is considered as fit-for-purpose to support the ecotoxicological study concerned.

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 partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (limited validation recoveries with fortified samples provided, repeatability (precision) not assessed, limit of quantification not reported, matrix effect and stability of sample extracts 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 method is not in agreement with guidance SANCO 3029/99 rev.4 as the precision was not demonstrated and the derivatisation efficiency was not assessed.
However, the recovery data are in acceptable range at the targeted doses. Therefore, the method can be considered as fit for purpose for the determination of AMPA and glyphosate in test medium (reconstitued water) at the targeted doses.

1. Information on the study

Data point	CA 4.1.2/188 (CA 8.2.6.1/002)
Report authors	[redacted]
Report year	2003
Report title	MON 78623: A 72-hour toxicity test with the freshwater alga (*Selenastrum capricornutum*)
Test facility	WildlIfe International, Ltd. 8598 Cormmerce Drive Easton, Maryland 21601

Report No	139A-311
Document No	WL-2002-149
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Limited validation recoveries from spiked samples • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 medium (reconstituted water) by HPLC-UVD. The samples were first diluted with freshwater to yield concentrations within the concentration range. For derivatisation, aliquots (2 mL) were mixed with 1 mL aqueous potassium tetraborate (0.37 M) and 2 mL NBD-Cl (7-chloro-4-2-oxa-1,3-diazole) (0.025 M, methanolic). The mixture was heated to about 80 °C and allowed to derivatise for 30 min. After addition of 1 mL HCl (1.2 M, aqueous), the solution was filtered (0.45 µm PTFE Acrodisc) and analysed for glyphosate by HPLC-UVD using external calibration.

Chromatographic conditions:

HPLC system:	Hewlett-Packard Model 1090 or Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) with an Agilent Model 1100 Variable Wavelength Detector			
HPLC column:	YMC-Pack ODS-AM (150 mm × 4.6 mm, 3 µm particle size)			
Column temperature:	40 °C			
Mobile phase:	A: 0.01 M KH_2PO_4 (pH 3.6) B: CH_3CN			
Gradient:	Time (min)	% A	% B	Flow rate (mL/min)
	0.01	95.0	5.0	1.0
	8.50	80.0	20.0	1.0
	8.60	30.0	70.0	1.0
	11.00	30.0	70.0	1.0
	11.10	95.0	5.0	1.0
	16.00	95.0	5.0	1.0
Injection volume:	25 µL			
Derivatisation agent (pre-column):	NBD-Cl (7-chloro-4-2-oxa-1,3-diazole) (0.025 M)			
Retention time:	Glyphosate: approx. 2.9 min			
Detection:	UV/Vis wavelength: 500 nm			

Findings

Recoveries (accuracy)

Test medium (reconstituted water) samples were fortified at relevant concentrations of 7, 30 and 120 mg test item/L (equivalent to 3.34, 14.31 and 57.24 g glyphosate/L) and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 4.19 mg test item/L, < 2 mg a.e./L). The recovery values were between 70 % and 110 %, with an overall relative standard deviation of 1.3 %. The detailed results are given in the table below.

Table 5.1-179: Results of method validation (spike recovery) for the determination of glyphosate in test medium

Matrix	Analyte	Fortification level (mg a.e./L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	3.339	99.8 – 102	101	–	–	2
		14.31	98.1 – 99.8	99.0	–	–	2
		57.24	99.4 – 100	99.7	–	–	2

[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.

Single samples of the test water collected at beginning of the test and were analysed for the concentration of the test article using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOQ (< 4.19 mg test item/L, < 2 mg a.e./L). The recovery values were between 70 % and 110 %, with an overall relative standard deviation of 1.5 %. It is noted that these are not true validation recovery data; however the results show good performance of the method. The recovery results are shown in the table below.

Table 5.1-180: Results of test medium analyses

Matrix	Analyte	Fortification level (mg a.e./L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	3.578	107	107	–	–	1
		7.155	104	104	–	–	1
		14.31	104	104	–	–	1
		28.62	104	104	–	–	1
		57.24	103	103	–	–	1

[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 method consists of a derivatisation step which is considered to be specific to the target compound. Representative chromatograms of a low-level glyphosate calibration standard, of a high-level glyphosate calibration standard, of a matrix blank sample, of a matrix fortification sample and of a test sample have been provided. No interfering peaks were observed at the retention time of the analyte.

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 2.0 to 20 mg glyphosate/L prepared in test medium. The calibration standards were derivatised as described above and analysed with each sample set. All calibration curves generated had a coefficient of determination (r^2) of > 0.999. Details to an example calibration are provided below.

Table 5.1-181: Details to calibration function

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate	Linear	2.0 – 20	5 levels	y = 89.4659 x – 9.00577	0.9999

Repeatability (Precision)

The precision could not be demonstrated.

Limit of Quantification and Detection

The limit of quantitation (LOQ) was 4.19 mg test item/L, calculated as the product of the concentration of the lowest calibration standard (2.0 mg a.e./L) and the dilution factor of the matrix blank samples (1.0), corrected for the glyphosate content of MON 78623 (47.7 %). The limit of detection (LOD) was not reported in the study.

Matrix effects

Not directly assessed. However matrix blank sample did not show any peak at the retention time of interest.

Stability of glyphosate in sample extracts

Stability of glyphosate in sample extracts was not assessed. However it was shown that the test material was stable test medium for duration of the test (103 % recovery of test item after 72 hours).

Conclusion

The analytical method was validated for the determination of glyphosate in test medium (reconstituted water). The method validation meets criteria set in SANCO/3029/99 rev. 4 in most relevant points and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

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) in most aspects (minor deficits: limited true validation data, matrix effecs and storage stability of extracts 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 method is not in agreement with guidance SANCO 3029/99 re.4 as the precision was not demonstrated and the derivatisation efficiency was not assessed.

However, considering the additional data for recoveries, the method can be considered as fit for purpose for the determination of glyphosate in Test medium (reconstitued water) at the targeted doses.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/191 (CA 8.2.6.1/009)
Report authors	[redacted]
Report year	1987
Report title	Volume I: The toxicity of glyphosate technical to *Selenastrum capricornutum*
Report No	1092-02-1100-1

Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries provided • No details to calibration/linearity provided • No chromatograms provided • Stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	Malcolm Pirnie, Inc. 2 Corporate Park Drive Xhite Plains, NY 10602
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/198 (CA 8.2.6.2/002)
Report authors	[redacted]
Report year	1987
Report title	Volume IV: The toxicity of glyphosate technical to *Anabaena flos-aquae*
Report No	1092-02-1100-4
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries provided • No details to calibration/linearity provided • No chromatograms provided • 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
Test facility	Malcolm Pirnie, Inc. 2 Corporate Park Drive Xhite Plains, NY 10602
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/200 (CA 8.2.6.2/005)
Report authors	[redacted]
Report year	1987
Report title	Volume II: The toxicity of glyphosate technical to *Navicula pelliculosa*
Report No	1092-02-1100-2
Document No	-

Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries provided • No details to calibration/linearity provided • No chromatograms provided • Stability of sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Test facility	Malcolm Pirnie, Inc. 2 Corporate Park Drive Xhite Plains, NY 10602
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/202 (CA 8.2.6.2/008)
Report authors	[redacted]
Report year	1987
Report title	Volume III: The toxicity of glyphosate technical to *Skeletonema costatum*
Test facility	Malcolm Pirnie, Inc. 2 corporate park drive White Plains, NY
Report No	1092-02-1100-3
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries provided • No details to calibration/linearity provided • No chromatograms provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/207 (CA 8.2.7/007)
Report authors	[redacted]
Report year	1987
Report title	Volume V: The toxicity of glyphosate technical to *Lemna gibba*
Test facility	Malcolm Pirnie, Inc. 2 corporate park drive White Plains, NY

Report No	1092-02-1100-5
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries provided • No details to calibration/linearity provided • No chromatograms provided • 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 aqueous growth media by HPLC with fluorescence detection. The analyte was derivatised at post-column where glyphosate was first oxidised with calcium hypochlorite and the product (glycine) was then coupled with o-phthalaldehyde (OPA) in the presence of mercaptoethanol. The test concentrations were of sufficient clarity that no prior clean-up or sample preparation was required other than filtration. The samples were either directly injected or diluted 1:1 with deionized water prior to analysis.

This method was used in the following studies:

Annex point	Report No.	Author	Year	Test
CA 8.2.6.1/009	1092-02-1100-1	[redacted]	1987	*Pseudokirchneriella subcapitata*, 72 h and 168 h
CA 8.2.6.2/005	1092-02-1100-2	[redacted]	1987	*Navicula pelliculosa*, 72 h and 168 h
CA 8.2.6.2/008	1092-02-1100-3	[redacted]	1987	*Skeletonema costatum*, 72 h and 168 h
CA 8.2.6.2/002	1092-02-1100-4	[redacted]	1987	*Anabaena flos-aquae*, 72 h and 168 h
CA 8.2.7/007	1092-02-1100-5	[redacted]	1987	*Lemna gibba*, 14 d static

Chromatographic conditions:

HPLC system:	High Performance Liquid Chromatograph (HPLC) equipped with an OPA post-column reactor and fluorescence detector
HPLC column:	Aminex A-9 cation exchange, 10 cm × 4.6 mm (BioRad)
Column temperature:	Not reported
Mobile phase:	0.005 M KH_2PO_4 in 4% MeOH (acidified to pH 1.9 with H_3PO_4)
Flow rate:	Not reported
Injection volume:	Not reported
Derivatisation agent:	o-phthalaldehyde (OPA)/mercaptoethanol (MERC)
Retention time:	Not reported

Detection:	Fluorescence Excitation wavelength: 340 nm Emission wavelength: 455 nm

Findings

Recoveries

Method validation determinations with specific fortified samples were not performed in these studies**.**

However samples of the test media were collected after preparation and analysed for the concentration of glyphosate using the analytical method. **The results are shown in the table below.** Control test water samples were also analysed, without detecting glyphosate above the LOD (< 0.05 mg/L). All average recovery values were between 70 % and 110 %. It is noted that these are not true validation recovery data; however the results show good performance of the method.

Table 5.1-182: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
1092-02-1100-1	Test medium (reconstituted water)	Glyphosate	10	106	106	–	–	1
			18	104	104	–	–	1
			32	106	106	–	–	1
			56	102 – 105	103	–	–	2
			100	100	100	–	–	1
			Overall	100 – 106	104	2.3	2.2	6
1092-02-1100-2	Test medium (reconstituted water)	Glyphosate	10	105	105	–	–	1
			18	99.4	99.4	–	–	1
			32	97.2	97.2	–	–	1
			56	97.0	97.0	–	–	1
			100	102 – 103	103	–	–	2
			Overall	97.0 – 105	101	3.3	3.2	6
1092-02-1100-3	Test medium (reconstituted water)	Glyphosate	0.1	100	–	–	–	1
			0.2	105	–	–	–	1
			0.4	110	–	–	–	1
			0.8	118	–	–	–	1
			1.6	113	–	–	–	1
			3.2	103 – 107	105	–	–	2
			Overall	100 – 118	108	6.0	5.6	7
1092-02-1100-4	Test medium (reconstituted water)	Glyphosate	10	91.3	91.3	–	–	1
			18	97.2	95.0	–	–	2
			32	95.6	95.6	–	–	1

Table 5.1-182: Results of test medium analyses

Report No.	Matrix	Analyte	Nominal concentration (mg/L)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			56	96.4	96.4	–	–	1
			100	98.4	98.4	–	–	1
			Overall	98.4	95.3	2.7	2.8	6
1092-02-1100-5	Test medium (reconstituted water)	Glyphosate	5	100	–	–	–	1
			9	104	–	–	–	1
			16	105	–	–	–	1
			28	103	–	–	–	1
			50	99.0	–	–	–	1
			Overall	99.0 – 105	102	2.5	2.5	5

[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 identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds. Not assessed. No chromatograms are provided in the reports.

Linearity
No details are provided to the calibration functions. It is stated in the reports that sample quantitation was based on.the peak height of the sample relative to standard peak heights across the range of expected sample concentrations.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recovery values were < 20 %. Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4. However repeatability should be performed with 5 replicate at same concentration.

Limit of Quantification
The limit of quantification (LOQ) was not assessed in these studies. However, the limit of determination was reported as < 0.05 mg/L for all studies.

Matrix effects
It is stated in the reports that fortification experiments showed that there were no sample matrix effects and comparisons of samples to glyphosate standards prepared in deionised water were sufficient.

Stability of glyphosate in sample extracts
Not assessed.

Conclusion
The analytical method was developed for the determination of glyphosate in aqueous test medium (reconstituted water). It does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in several points. Nevertheless the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies were previously evaluated at EU level. They were performed under GLP and partly meet current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (no true validation data, no information to calibration/linearity, no chromatograms provided, stability of sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the ecotoxicological studies concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the guidance SANCO 3029/99 rev.4 as the linearity data are not available, the specificity (interference) and the precision were not demonstrated and the derivatisation efficiency was not assessed.
Howevre, the recovery data are in acceptable range. Therefore the analytical methode can be considered as fit for purpose for the determination of glyphosate in test medium at the targeted doses.

Determination of AMPA in test medium (reconstituted water)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/195 (CA 8.2.6.1/018)
Report authors	[redacted]
Report year	1994
Report title	Testing of toxic effects of Aminomethyl phosphonic acid (AMPA) on the single cell green alga *Scenedesmus subspicatus*
Report No	IFU93006/01-Ss
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Very limited validation recoveries provided • Repeatability (precision) not assessed • Limit of quantification not reported • 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
Test facility	Arbeitsgemeinschaft GAB Biotechnologie GmbH and IFU Umweltanalyttik GmbH Eutinger Str. 24 D-75223 Niefern-Oschelbronn
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
The determination of aminomethyl phosphonic acid (AMPA) in aqueous test medium was performed according to DFG method 405 by HPLC coupled with fluorescence (UV) detection. Aliquots were filtered and directly injected into the HPLC. The analyte was derivatised at post-column with o-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol.

Chromatographic conditions:

HPLC system:	HPLC (Waters 600E) equipped with a post-column derivatisation system (Water 510I and a fluorescence detector (Perkin-Elmer LS-3B)
HPLC column:	Glyphosate analysis column (BioRad No. 125-0104), 300 mm × 4.6 mm i.d., 11.6 µm particle size
Column temperature:	30 °C
Mobile phase:	5 mM KH_2PO_4 + 4% methanol, pH 2.0 (H_3PO_4)
Flow rate:	0.5 mL/min
Injection volume:	50 µL
Derivatisation agent:	o-phthalaldehyde (OPA)/mercaptoethanol (MERC)
Retention time:	AMPA: ~ 32 min
Detection:	Excitation wavelenght: 360 nm Emission wavelenght: 455 nm

Findings
Recoveries (accuracy)
No true validation recoveries with fortified samples were presented within the report. However, an aliquot of aqueous growth medium prepared at 0 hour was analysed using the analytical method. In this sample, nominal AMPA concentration was recovered at 103 %.

Table 5.1-183: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg AMPA/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstitued water)	AMPA	0.96	103	–	–	–	1

[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 identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds.

Linearity
Linearity of detector response was tested using three calibration standard concentrations in duplicate, in the range of 0.76 to 1.52 µg/mL. Details to the calibration functions are provided below.

Table 5.1-184: Linearity parameters

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of data points	Equation[1]	Coefficient of determination[1] (r^2)
AMPA	Linear	0.76 – 1.52	3 (3 levels)	y = 11766 x – 198	0.9997

[1] Calculations of linearity parameters were performed using Excel with peak areas provided in the report.

Repeatability (Precision)
Not assessed.

Limit of Quantification and Detection
Not assessed.

Matrix effects
Not assessed.

Stability of AMPA in sample extracts
Not assessed.

Conclusion
The analytical method was developed for the determination of AMPA in **aqueous test medium**. Despite the method validation did not meet criteria set in SANCO/3029/99 rev. 4 in several points, .

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 partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (very limited validation recoveries provided, repeatability (precision) not assessed, limit of quantification not reported, matrix effect and stability of sample extracts 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 for the determination of AMAP in aqueous test medium is not in agreement with the guidance SANCO/3029/99 rev. 4 as specificity (interference), accuracy, precision were not demonstrated and the derivatisation efficiency was not assessed.
Only one recovery data is available. Even if the result is in acceptable range, considering the very limited data available, the method cannot be considered as fir for purpose

Determination of HMPA in test medium (reconstituted water)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/196 (CA 8.2.6.1/019)
Report authors	[redacted]

Report year	2011
Report title	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)
Report No	139A-396A
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes, minor (SANCO/3029/99 rev. 4): • Limited validation recoveries from spiked samples • Stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
Test facility	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601 USA (410) 822-8600
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/210 (CA 8.2.7/012)
Report authors	[redacted]
Report year	2011
Report title	HMPA (hydroxymethylphosphonic acid): A 7-day static-renewal toxicity test with Duckweed (*Lemna gibba G3*)
Test facility	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601 USA (410) 822-8600
Report No	139A-397
Document No	WL-2010-331
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes, minor (SANCO/3029/99 rev. 4): • Limited validation recoveries from spiked samples • Stability of sample extracts not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical Method was developed for the determination of hydroxymethylphosphonic acid (HMPA) in freshwater test medium (reconstituted water) by LC-MS. The samples were first diluted with test medium to yield concentrations within the calibration range and then directly submitted to analysis by high performance liquid chromatography with mass selective detection (LC-MS).

This method was used in the following studies:

Annex point	Report No.	Author	Year	Test
CA 8.2.6.1/019	139A-396A	[redacted]	2011	*Pseudokirchneriella subcapitata* (72 hour)
CA 8.2.7/012	139A-397	[redacted]	2011	*Lemna gibba* (7 day)

Chromatographic conditions:

HPLC system:	Hewlett Packard Series 1100 HPLC equipped with a Perkin-Elmer SCIEX API 100 LC Mass Spectrometer			
HPLC column:	Thermo Prism (50 mm × 2.1 mm, 5 µm particle size)			
Guard column:	Thermo Prism (20 mm × 2.1 mm)			
Column temperature:	40 °C			
Mobile phase:	A: 0.1% Formic Acid in H_2O B: CH_3CN			
Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (µL/min)
	0.00	95	5	300
	0.10	95	5	300
	6.00	95	5	300
Injection volume:	10 µL			
Retention time:	HMPA: ~ 2.5 min			
Ion source:	Perkin Elmer SCIEX TurboIonSpray			
Carrier gas:	Air 60 psi Nitrogen (99.5%) 65 psi; 6 L/min			
Monitored mass:	111 amu			
Parameters:	NEB:	10	CUR:	8
	DP:	-46	IS:	-4200
	FP:	-270	EP:	-10
	TEM:	500		

Findings

Recoveries

For method validation, samples of test medium (reconstituted freshwater) were spiked with the analyte at three fortification levels from 7.5 to 120 mg/L. The average recovery values were between 70 % and 110 %. The detailed results are summarised in the table below.

Table 5.1-185: Results of method validation (spike recovery) for the determination of HMPA in test medium

Report No.	Matrix	Analyte	Fortification level (mg/L)	Recovery[(a)]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
139A-396A	Test medium (reconstituted water)	HMPA	7.5	98.3 – 101	100	–	–	2
			30	103 – 109	106	–	–	2
			120	99.8 – 103	101	–	–	2
			Overall	98.3 – 109	102	3.7	3.7	6

Table 5.1-185: Results of method validation (spike recovery) for the determination of HMPA in test medium

Report No.	Matrix	Analyte	Fortification level (mg/L)	Recovery[(a)]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
139A-397	Test medium (reconstituted water)	HMPA	7.5	87.5 – 104	98	9.3	9.4	3
			25	85.5 – 107	98	11.0	11.2	3
			120	92.0 – 102	96	5.5	5.7	3
			Overall	85.5 – 107	97	7.8	8.0	9

(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

Determination by LC-MS is considered to be highly specific. No interfering peaks were observed at the retention time of the analyte. Chromatograms of standards, blank and fortified sample are provided for study 139A-396A. and study 139A-397 .

Linearity

The linearity of the detector response was tested using five calibration standard concentrations in the range of 1.0 to 10 mg/L prepared in test medium. Linear calibrations (duplicate injections) were confirmed with correlation coefficients of > 0.99. **Details to the calibration functions are provided below**.

Table 5.1-186: Linearity parameters

Report No.	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
139A-396A	HMPA	Linear (1/x weighting)	1.0 – 10	10 (5 levels)	y = 424000 x – 74200	0.9995
139A-397	HMPA	Linear (1/x weighting)	1.0 – 10	10 (5 levels)	y = 246000 x – 121000	0.9958

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

The limit of quantitation (LOQ) was 1.0 mg/L, calculated as the product of the concentration of the lowest calibration standard (1.0 mg a.s./L) and the dilution factor of the matrix blank samples (1.0). The limit of detection (LOD) is not reported.

Matrix effects

Matrix effects were eliminated by using test water for calibration solutions.

Stability of HMPA in sample extracts
Not assessed. However the test media were proved to be stable for the duration of the studies.

Conclusion
The analytical method was validated for the determination of HMPA in **aqueous test medium**. Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, it is considered as fit-for-purpose for the determination of HMPA in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
These studies were previously evaluated at EU level. They were performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (limited number of validation measurements, storage stability of extracts 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 is not in agreement with the guidance SANCO 3029/99 rev.4 as the derivatisation efficiency was not assessed.

However, the linearity and the specifity are demonstrated. The recovery data are in acceptable range.
Therefore the analytical method can be considered as fit for purpose for the determination of HMPA in the test medium.

Determination of glyphosate in test medium (reconstituted water)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/203 (CA 8.2.6.2/010)
Report authors	[redacted]
Report year	1996
Report title	Glyphosate tec. – Alga, growth inhibition test to *Nitzschia palea*
Test facility	Dr U NOACK6LABORATORIUM FUR ANGEWANDTE BIOLOGIE Kathe-Paulus-Str. 1 D-31157 Sarstedt
Report No	960606FH
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries with fortified samples • Insufficient reporting to derivatisation and calibration procedures • Matrix effects and storage stability of extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, evaluated and accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 aqueous growth medium (Bacillariophyacean medium according to SAG) by HPLC with fluorescence detection. Samples were filtered and diluted with water as required prior to analysis. The analyte was derivatised at post-column where glyphosate was first oxidised with NaOCl and the product was then coupled with o-phthalaldehyde (OPA) for fluorescence detection and quantification based on external calibration.

Chromatographic conditions:

HPLC system:	HPLC (Waters 510, Waters 501) equipped with fluorescence detector (Shimadzu RF-535)
HPLC column:	Zorbax SAX (250 mm × 4 mm i.d.)
Column temperature:	25.0 ± 0.1 °C
Mobile phase:	6.8 g/L KH_2PO_4 in methanol/water (4/96, v/v) adjusted to pH 2.0 with phosphonic acid
Flow rate:	0.7 mL/min
Injection volume:	100 µL
Derivatisation agent:	o-phthalaldehyde (OPA)
Retention time:	Glyphosate: ~ 16 min
Detection:	UVD Excitation wavelength: 320 nm Emission wavelength: 530 nm

Findings
Recoveries
Method validation experiments with fortified samples were not performed in this study. However freshly prepared test media were analysed for glyphosate content using the analytical method. The results are summarised in the table below. Control test water samples were also analysed, without detecting glyphosate above the limit of quantification (<0.18 mg/L). The average recovery values at each concentration level and overall were between 70 % and 110 %, with an overall relative standard deviation (RSD) of 8.6 %. It is noted that these are not true validation recovery data; however the results show good performance of the method.

Table 5.1-187: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg glyphosate/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	0.3094	77.6 – 80.8	79.2	–	–	2
		0.967	99.3 – 100.3	99.8	–	–	2
		3.094	97.6 – 98.9	98.3	–	–	2
		9.67	95.8 – 95.8	95.8	–	–	2
		30.94	99.5 – 101.7	100.6	–	–	2

Table 5.1-187: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg glyphosate/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		96.7	102.9 – 103.7	103.3	–	–	2
		309.4	103.1 – 107.8	105.5	–	–	2
		Overall	77.6 – 107.8	97.5	8.4	8.6	14

[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 identification was based on fluorescence detection. The method consists of a derivatisation step which is considered to be specific to the target compound. No interfering peaks were observed at the retention time of the analyte. Chromatograms of standard, test substance, control have been provided.

Linearity
The linearity of the detector response was tested using six calibration standard concentrations in the range of 0.5 to 3.0 mg/L, prepared in water (duplicate injections). A linear correlation was found without providing details to the calibration functions, however it is stated that the coefficient of determiation (r^2) was satisfactory (0.9692). A calibration plot is provided in the report.

Repeatability (Precision)
It is stated that repeatability was tested by analysing five sub-samples prepared from a single homogeneous sample, at levels of 0.475 and 2.85 mg/L. The results of these determinations are reported in peak areas, which cannot be re-calculated to measured concentrations because calibration equations were not reported. However the RSDs at these concentration levels of 0.475 and 2.85 mg/L based on peak areas were 2.9 % and 2.1 %, respectively, thereby in compliance with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
It is stated in the report that the limit of quantification (LOQ) was 0.28 mg/L. The limit of detection (LOD) was 0.18 mg/L, but these limits are not demonstrated.

Matrix effects
Not assessed.

Stability of glyphosate in sample extracts
Not assessed. However it was shown that the test material was stable in test solutions for 96 hours.

Conclusion
The analytical method was validated for the determination of glyphosate in **aqueous test medium**. Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, it is considered as fit-for-purpose for the determination of glyphosate 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 meets current requirements (EU guideline SANCO/3029/99 rev. 4) with several deficits (no true validation recoveries with fortified samples, insufficient reporting to derivatisation and calibration procedures, matrix effects and storage stability

of extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the ecotox study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the guidance SANCO 3029/99 rev.4 as the derivatisation efficiency was not assessed.
However, the linearity and specificity (interference) are demonstrated. The recovery data are in acceptable range; Therefore, the analytical method can be considered as fit for purpose for the determination of glyphosate in test medium.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/204 (CA 8.2.7/001)
Report authors	[redacted]
Report year	2002
Report title	IPA salt of glyphosate: Effects on *Lemna minor*
Test facility	CEM Analytical Service Limited (CEMAS) Glendale Park Fembank Raod North Ascot Berkshire SL5 8JB United Kingdom
Report No	CEMR-1873
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Method validation data with fortified samples only at one fortification level • Matrix effects 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	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate and aminomethyl phosphonic acid (AMPA) in aqueous growth media (reconstituted water according to OECD 221) by HPLC with fluorescence detection. The method is based on AOAC Method 2000.522. The samples were first diluted with freshwater to yield concentrations within the calibration range. The analytes were derivatised at post-column where they first oxidised with sodium hypochlorite and the products were then coupled with o-phthalaldehyde (OPA) in the presence of methanol. The test concentrations were of sufficient clarity that no prior clean-up or sample preparation was required other than filtration.

Chromatographic conditions:

HPLC system:	HPLC (Waters) equipped with an OPA post-column reactor (Pickering PCX5200) and fluorescence detector (Waters 474)
HPLC column:	Cation exchange, K+ form (Pickeing), 150 × 4.0 mm, 8 µm
Guard column:	Cation exchange, K+ form (Pickeing), 20 × 3.0 mm, 8 µm
Column temperature:	55°C
Mobile phase:	A: Potassium phoshate eluent (Pickering No. K200) B. Column regenerant (Pickering No. RG019)

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.0	100	0	0.4
10.0	100	0	0.4
10.2	0	100	0.4
12.0	0	100	0.4
12.2	100	0	0.4
28	100	0	0.4

Injection volume:	25 µL
Derivatisation agent:	o-phthalaldehyde (OPA)/methanol
Retention time:	Glyphosate: ~ 8.6 min AMPA: ~ 17.6 min
Detection:	Fluorescence Excitation wavelength: 330 nm Emission wavelength: 465 nm

Findings

Recoveries

For method validation, test medium control samples were fortified with glyphosate and AMPA at a level of 1 mg/L and analysed using the analytical method. The recoveries reported below are not reported in the study.

Table 5.1-188: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in test medium

Matrix	Analyte	Fortification level (mg /L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	1	95.9 – 104	99.5	3.7	3.7	4
Test medium (reconstituted water)	AMPA	1	74.7 – 94.7	87.0	8.6	9.9	4

[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, samples of test media freshly prepared on days 0 and 2 of the study using the analytical method. The average recoveries for both analytes at each concentration level and overall were between 70 % and 110 %, except at the highest concentration level. Control test medium samples were also analysed, without detecting glyphosate

above the LOD (< 0.26 mg/L). Levels of AMPA in all test media were also below the LOD (< 0.26 mg/L). The results are summarised in the table below. It is noted that these are not true validation recovery data; however the results show the good performance of the method.

Table 5.1-189: Results of test medium analyses

Matrix	Analyte	Nominal concetration (mg glyphosate/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate	2.16	99.1 – 107	103	5.6	5.4	2
		4.32	97.9 – 100	99.1	1.6	1.7	2
		8.65	97.6 – 102	99.7	3.0	3.0	2
		18	98.3 – 99.4	98.9	0.8	0.8	2
		36	101 – 101	101	0.4	0.4	2
		72	103 – 118	111	10.9	9.9	2
		Overall	97.6 – 118	102	5.7	5.6	12

[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 identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds. No significant interferences were observed at the retention times of the analytes in example chromatograms. Chromatograms of glyphosate and AMPA mixed standard, control and sample have been provided

Linearity

The linearity of the detector response was tested using five calibration standard concentrations in the range of 0.25 to 4.0 mg/L of glyphosate and AMPA prepared in water. Linear correlations were found with coefficients of determination (r^2) of > 0.99, except for one calibration used for quantification of glyphosate in two samples where a r^2 of 0.96 was found due to an increase in the response throughout HPLC determination of this batch. Details to example calibrations are provided below.

Table 5.1-190: Linearity parameters

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination (r^2)
Glyphosate	Linear	0.25 – 4.0	10 (5 levels)	y = 229343.3 x	0.9982
AMPA	Linear	0.25 – 4.0	10 (5 levels)	y = 240404.8 x	0.9922

Repeatability (Precision)

For precision testing, five replicate determinations were made of one low level concentration (nominal 2.16 mg/L glyphosate) were made. The relative standard deviation (RSD) of these analyses was 1.2 %, therefore in compliance with EU guideline document SANCO/3029/99 rev. 4. Moreover, the relative standard deviations (RSDs) from analyses of spiked samples and test water media were all < 20 % as required in the EU guideline.

Limit of Quantification
The limit of quantification (LOQ) was not assessed in this study. The limit of detection was reported as < 0.26 mg/L but not demonstrated. LOQ data is missing.

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Matrix effects
Not assessed.

Stability of the analytes in sample extracts
Not assessed.

Conclusion
The analytical method was developed for the determination of glyphosate and AMPA in aqueous test medium (reconstituted water). It does not fulfil the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) in some points. Nevertheless the method showed good performance and is considered as fit-for-purpose for the determination of glyphosate and AMPA in aqueous test medium.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was previously evaluated at EU level. It was performed under GLP and partly meets current requirements (EU guideline SANCO/3029/99 rev. 4) with some deficits (method validation data with fortified samples only at one fortification level, matrix effects and stability of sample extracts 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 method is not in agreement with the guidance SANCO 3029/99 rev.4 as the derivatisation efficiency was not assessed.

However, the linearity, the specifity (interference) and the precision are demonstrated. The recovery data are in acceptable range. Therefore, the analytical method can be considered as fit for purpose for the determination of glyphosate and AMPA in test medium.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/205 (CA 8.2.7/003)
Report authors	[redacted]
Report year	1999
Report title	Glyphosate 62 % IPA-Salt – Aquatic plant toxicity test using *Lemna gibba*
Test facility	Dr. U. NOACK-LABORATORIUM FUR ANGEWANDTE BIOLOGIE Kathe-Paulus-STr.1 D-31157 Sarstedt
Report No	TLA60871
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • No true validation recoveries with fortified samples • Insufficient reporting to derivatisatio and calibration procedures

	• Matrix effects and storage stability of extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 aqueous growth medium (20x AAP medium) by HPLC with fluorescence (UV) detection. Samples were diluted with water as required prior to analysis. The analyte was derivatised at post-column where glyphosate was first oxidised and the product was then coupled with o-phthalaldehyde (OPA) for fluorescence detection. Details to the derivatisation are not provided.

Chromatographic conditions:

HPLC system:	HPLC (WATERS 510) equipped with fluorescence detector (WATERS 470 Fluorescence Detector)
HPLC column:	Zorbax SAX (250 mm × 4 mm i.d.)
Column temperature:	25.0 ± 0.1 °C
Mobile phase:	6.8 g/L KH_2PO_4 in methanol/water (4/96, v/v) adjusted to pH 2.0 with phosphonic acid
Flow rate:	0.3 mL/min
Injection volume:	100 µL
Derivatisation agent:	o-phthalaldehyde (OPA)
Retention time:	Glyphosate: ~ 12.0 min
Detection:	UVD Excitation wavelength: 320 nm Emission wavelength: 530 nm

Findings
Recoveries
Method validation experiments with fortified samples were not performed in this study. However freshly prepared test media at days 4 and 11 were analysed for glyphosate content using the analytical method. The results are summarised in the table below. Control test water samples were also analysed, without detecting glyphosate above the limit of quantification (< 0.9 mg/L for glyphosate IPA salt). The average recovery values at each concentration level and overall were between 70 % and 110 %. The relatively high RSDs are based on generally lower measured concentrations at the day 4 prepared test media. It is noted that these are not true validation recovery data; however the results show good performance of the method.

Table 5.1-191: Results of test medium analyses

Matrix	Analyte	Nominal concentration (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	Glyphosate IPA salt (for glyphosate levels correct with factor 0.741)	3.9	82.3 – 113	96.6	14.6	15.1	4
		7.8	84.2 – 106	95.4	11.8	12.3	4
		15.6	80.4 – 102	90.6	11.1	12.2	4
		31.2	84.4 – 102	93.2	9.2	9.9	4
		62.4	78.0 – 100	89.3	9.7	10.8	4
		Overall	78.0 – 113	93.0	10.5	11.3	20

[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 identification was based on fluorescence detection. The method consists of a derivatisation step which is considered to be specific to the target compound. No interfering peaks were observed at the retention time of the analyte. Chromatograms of standard, test solution and control have been provided.

Linearity
The linearity of the detector response was tested using seven calibration standard concentrations in the range of 0.5 to 3.5 mg/L, prepared in water (duplicate injections). A linear correlation was found without providing details to the calibration functions, however it is stated that correlation coefficients (r) were satisfactory (> 0.98). A calibration plot is provided in the report.

Repeatability (Precision)
It is stated that repeatability was tested by analysing five sub-samples prepared from a single homogeneous sample, at levels of 0.5 and 3.5 mg/L. The results of these determinations are reported in peak areas, which cannot be re-calculated to measured concentrations because calibration equations were not reported. However the RSDs at these concentration levels of 0.5 and 3.5 mg/L based on peak areas were 2.2 % and 2.1 %, respectively, thereby in compliance with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 3.9 mg/L could be used, 4 replicates with acceptable recoveries.

Matrix effects
Not assessed.

Stability of glyphosate in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate in **aqueous test medium**. Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, it is considered as fit-for-purpose for the determination of glyphosate 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 meets current requirements (EU guideline SANCO/3029/99 rev. 4) with several deficits (no true validation recoveries with fortified samples, insufficient reporting to derivatisation and calibration procedures, matrix effects and storage stability of extracts 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 method is not in agreement with the guidance SANCO 3029/99 rev.4 as the derivatisation efficiency was not assessed.
However, the linearity, the specificity and the precision are demonstrated. The recovery data are in acceptable range. Therefore, the method can be considered as fir for purpose for the determination of glyphosate in test medium.

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/208 (CA 8.2.7/010)
Report author	[redacted]
Report year	2012
Report title	Effect of MON77973 (glyphosate acid) on the growth of *Myriophyllum aquaticum* in the presence of sediment. Test with a subsequent recovery period.
Test facility	Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME) 57377 Schmallenberg, Germany
Report No	CHE-015/4-80/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, minor (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	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 analyzed 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: ~ 2.25 min Gluphosinate ammonium (IS): ~ 1.9 min
Detection mode:	MS/MS
Scan type:	MRM
Ionisation mode:	ES negative
Mass transition for evaluation:	Glyphosate acid: *m/z* 168.0→150.0 Gluphosinate 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 5.1-192: 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 and self-confirmatory detection technique. The specificity of the method is shown by LC-MS/MS. No significant interferences were observed at the retention time of the analyte in example chromatograms. Chromatograms of untreated fortification samples (blanks) and control samples of the investigated matrix.

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 (1/x weighting) was found with a correlation coefficient (r) of 0.9992 (y = 468.914 x – 9.1028).

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.

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:
Specificity, precision and linearity are acceptable, the method is validated according to the guidance SANCO 3029/99 rev.4.

Determination of AMPA in test medium (reconstituted water)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/209 (CA 8.2.7/011)
Report author	[redacted]
Report year	2012
Report title	Effect of AMPA (aminomethyl phosphonic acid) on the growth of *Myriophyllum aquaticum* in the presence of sediment, with a subsequent recovery period.
Test facility	Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME) 57377 Schmallenberg, Germany
Report No	CHE-022/4-80/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, minor (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	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

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 AMPA in aqueous growth medium (reconstituted water) by LC-MS/MS with a limit of quantification (LOQ) of 0.5 mg/L. Sample aliquots of 1000 µL of the aqueous test medium, 100 µL methanol and 50 µL of the IS-solution (Glufosinate-ammonium in a water/methanol mixture (9/1, v/v), concentration: 200 mg/L) 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 analyzed 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:	Thermo HyPurity C8, 150 mm × 3.0 mm, 5 µm particle size
Guard column:	Thermo HyPurity C8, 10 mm × 3.0 mm, 5 µm particle size
Column temperature:	30 °C
Mobile phase:	A: Acetonitrile, containing 10 mmol/L ammonium acetate and 1 % formic acid B: Water, containing 10 mmol/L ammonium acetate and 1 % formic acid
Gradient:	Time (min) / % A / % B / Flow rate (mL/min) 0.0 / 0 / 100 / 0.5 1.0 / 0 / 100 / 0.5 1.5 / 100 / 0 / 0.5 2.5 / 100 / 0 / 0.5 2.6 / 0 / 100 / 0.5 6.0 / 0 / 100 / 0.5
Injection volume:	20 µL
Retention time:	AMPA: ~ 1.9 min Glufosinate ammonium (IS): ~ 2.0 min
Detection mode:	MS/MS
Scan type:	MRM
Ionisation mode:	ES negative
Mass transition for evaluation:	AMPA: *m/z* 109.8 → 62.5 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 0.50 and 5.0 mg/L. The mean recovery values at each fortification level and overall were between 70 % and 110 %. Control samples were also analysed without detecting the analyte above the LOQ. The detailed results are summarised in the table below.

Table 5.1-193: Results of method validation (spike recovery) for the determination of AMPA in test medium

Matrix	Analyte	Fortification level (mg/L)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test medium (reconstituted water)	AMPA	0.5	101.0 – 106.8	104.2	2.38	2.28	5
		5.0	98.3 – 100.7	99.6	1.08	1.08	5
		Overall	98.3 – 106.8	101.9	3.0	2.9	10

Specificity
The method allows the determination of AMPA using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. The specificity of the method is shown by LC-MS/MS. No significant interferences were observed at the retention time of the analyte in example chromatograms. Chromatograms of untreated fortification samples (blanks) and control samples of the investigated matrix.

Linearity
The linearity of the detector response was tested using seven calibration standard concentrations in the range of 0.25 to 20 mg/L, which were prepared by diluting an intermediate analyte solution with blank test medium. A linear correlation (1/x weighting) was found with a correlation coefficient (r) of 0.9995 (y = 154.885 x + 3.87454).

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 0.5 mg/L fortification level for aqueous growth medium.

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

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

Conclusion
The analytical method was validated for the determination of AMPA in **aqueous** test medium at a limit of quantification (LOQ) of 0.50 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 meet current requirements (EU guideline SANCO/3029/99 rev. 4). The method is considered as fully validated to support the ecotoxicological study.

Assessment and conclusion by RMS:

Specificity, precision and linearity are acceptable, the method is validated according to the guidance SANCO 3029/99 rev.4

Determination of glyphosate in sugar solution

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/211 (CA 8.3.1.2/001)
Report authors	[redacted].
Report year	2017
Report title	MON 0139: Chronic oral toxicity test on the honey bee (*Apis mellifera* L.) in the laboratory
Report No	(118401136; MSL0029007)
Document No	IO-2016-0508
Guidelines followed in study	SANCO/3029/99 rev. 4
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4): • Matrix effects not assessed • Stability in solvents not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test facility	Ibacon GmbH Arheilger Weg 17 64380 Rossdorf 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 bee feeding (sugar) solution by HPLC-UV. The samples were diluted with solvent mixture (deionized water); final analysis was performed by HPLC-UV without further sample treatment.

Chromatographic conditions:

HPLC system:	VWR Hitachi
HPLC column:	PerfectChrom SAX 100A (250 mm × 4.6 mm)
Column oven temperature:	30 °C
Mobile phase:	Approx. 1.688 g potassium dihydrogen- phosphate dissolved in 1920 mL pure water. 40 mL methanol added and pH of the solution adjusted to 1.9 using phosphoric acid
Flow rate:	1 mL/min
Injection volume:	50 µL
Derivatisation agent:	Not applicable (no derivatisation)
Detection:	UV wavelength: 200 nm
Retention time:	Glyphosate: approx. 4 min

Findings

Recoveries

For method validation, samples of sugar solution were spiked with the analyte at two fortification levels, i.e. at the LOQ of 0.5 g a.s./L and one higher level, with mean recoveries found as 83 **to** 95 %. The recovery values were between 70 % and 110 %. Control samples were also analysed without detecting the analyte at the limit of detection (<3 mg/L). The detailed results are summarised in the table below.

Table 5.1-194: Results of method validation (spike recovery) for the determination of glyphosate in feeding solution

Matrix	Analyte	Fortification level (g a.s./L)	Recovery[(a)]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Feeding solution (sugar solution)	Glyphosate	0.5	81 – 85	83	1.6	1.9	5
		30	91 – 100	95	3.4	3.6	5
		Overall	81 – 100	89	6.7	7.5	10

(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 interfering peaks (< 30 % LOQ) were observed at the retention time of the analyte.

Linearity

The linearity of the detector response was tested using six calibration standard concentrations in the range of 10 to 100 mg/L prepared in deionised water. A linear function was found (y = 3075 x - 6851) with a coefficient of determination (r) of > 0.999.

Repeatability (Precision)

The relative standard deviations (RSD) of all recovery values (n = 5) 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 0.5 g glyphosate/L (diluted by factor 10). The limit of detection (LOD) was 3 mg glyphosate/L.

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Matrix effects

Not assessed.

Stability of glyphosate in sample extracts

Not assessed.

Conclusion

The analytical method was validated for the determination of glyphosate in sugar solution. The method validation largely 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 largely meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (matrix effect and stability in solvents not assessed). The method is considered suitable to support the ecotoxicological study concerned.

Assessment and conclusion by RMS:
The method is not in agreement with the guidance SANCO 3029/99 rev.4 as the matrix effects were not assessed.
However the other parameters are acceptable. Therefore, the method can be considered as fit for purpose for the determination of glyphosate in the feeding solution for bees.

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/212 (CA 8.3.1.3/001)
Report authors	[redacted]
Report year	2020
Report title	Amended report for MSL0031012: MON 0139 - Repeated exposure of honey bee larvae (*Apis mellifera* L.) under laboratory conditions
Report No	19 48 BLC 0068
Document No	BI-2018-0721; TRR0000053
Guidelines followed in study	SANCO/3029/99 rev. 4 (analytical phase)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Stability in extracts 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	BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstr. 6, 04827 Machern OT Gerichshain, 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 bee larvae final diet (aqueous sugar solution + royal jelly) by LC-MS/MS. The samples were extracted with acetonitrile/water containing 0.1 % HFo (1/1, v/v) and diluted with blank extract and water containing 5 mM EDTA. Final analysis was performed by LC-MS/MS.

Chromatographic conditions:

HPLC system:	Agilent 1200 HPLC system equipped with a 6470 triple quadrupole mass spectrometric detector
HPLC column:	Torus DEA (150 mm × 2.1 mm; 5 µm)
Column temperature:	40 °C
Mobile phase:	A: Water containing 50 mM ammonium formate, pH 3 B: Acetonitrile containing 0.9% formic acid

Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (µL/min)
	0.00	10	90	500
	4.50	60	40	500
	8.00	60	40	500
	11.00	Stop		500
Injection volume:	25 µL			
Retention time:	6.74 min			
Ionization mode (polarity):	ESI (-); MRM			
Ion transition:	168 → 63 (quantifier) 168 → 150 (qualifier) 168 → 81 (qualifier)			

Findings

Recoveries

For method validation, samples of final diet were spiked with the analyte at two fortification levels, i.e. at the LOQ of 16.1 mg/kg and one higher level, with mean recoveries found as 91.2-106 %. The recovery values were between 70 % and 110 %. The detailed results are summarised in the table below.

Table 5.1-195: Results of method validation (spike recovery) for the determination of glyphosate in final diet

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Final diet (aqueous sugar solution + royal jelly)	Glyphosate	16.1	103 – 108	106	1.8	1.7	5
		1664	85.0 – 97.3	91.2	5.3	5.8	5
		Overall	85.0 – 108	98.4	8.5	8.6	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

.. Determination by LC-MS/MS is considered to be highly specific. Three ion transitions were measured. No interfering peaks (<30 % LOQ) were observed at the retention time of the analyte.

Linearity

The linearity of the detector response was tested using six calibration standard concentrations in the range of 278 to 2141 µg/L (the eq in mg/kg is not available) prepared in blank extract/water containing 5 mM EDTA (77/23, v/v), covering 47 % of the lowest concentration to 134 % of the highest concentration. A linear function was found ($y = 0.115168 x + 0.748603$) with a coefficient of determination (r) of >0.99.

Repeatability (Precision)

The relative standard deviations (RSD) of all recovery values (n = 5) 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 16.1 mg/kg, corresponding to 595 µg/L in diluted extracts. The limit of detection (LOD) was not reported.

Matrix effects
Matrix-matched standards were used to account for potential matrix effects.

Stability of glyphosate in sample extracts
Not assessed.

Conclusion
The analytical method was validated for the determination of glyphosate in final bee larvae diet (aqueous sugar solution + royal jelly). The method validation largely 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 largely meets current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (stability in extracts not assessed). The method is considered suitable to support the ecotoxicological study concerned.

Assessment and conclusion by RMS:
The method is considered validated according to guidance SANCO 3029/99 rev.4 for the determination of glyphosate in final diet for bees.

Determination of glyphosate acid in larvae and sucrose solution

Study previously submitted to the EU

1. Information on the study

Data point:	CA 4.1.2/213 (CA 8.3.1.4/001)
Report authors	[redacted]
Report year	2012
Report title	Glyphosate: Evaluating potential effects on honeybee brood (*Apis mellifera*) development
Report No	V7YH1001 (Analytical Phase Code S11-01136)
Document No	-
Guidelines followed in study	SANCO/3029/99 rev. 4
Deviations from current test guideline	None
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	Environmental Risk Team Food and Environmental Safety Programme The Food and Environment Research Agency Sand Hutton York YO41 1LZ, UK

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 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.
The analytical method used for the determination of glyphosate in sucrose solution was fully validated during the current study. The samples were extracted with acetonitrile/water (1/4, v/v), derivatised with FMOC-Cl and cleaned up by solid-phase extraction (SPE) over Oasis HLB phase; 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:	30 µL
Derivatisation agent:	FMOC-Cl
Retention time:	Glyphosate: approx. 3.3 min
Ionization mode (polarity):	ESI (-)
Ion transitions:	390.0 → 149.8 (quantifier) 390.0 → 167.8 (quantifier)

Findings

Recoveries

For method validation, samples of larvae and sucrose solution were spiked with the analyte at two fortification levels, i.e. at the LOQ of 1 mg/kg and one higher level, with mean recoveries found as 92 to 104 %. The recovery values were between 70 % and 110 %. The detailed results are summarised in the table below.
A reduced set of recoveries was analysed for larvae due to study comparability of study No. V7YH1002 (Analytical phase No. S11-01135).

Table 5.1-196: Results of method validation (spike recovery) for the determination of glyphosate in larvae and sucrose solution

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Larvae	Glyphosate	1	93 – 111	100	9.9	9.9	3
		200	103 – 105	104	1.2	1.1	3
		Overall	93 – 111	102	6.8	6.6	6

Table 5.1-196: Results of method validation (spike recovery) for the determination of glyphosate in larvae and sucrose solution

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Sucrose solution	Glyphosate	1	89 – 96	92	3.3	3.5	5
		400	88 – 96	92	3.6	3.9	5
		Overall	88 – 96	92	3.2	3.5	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
Determination by LC-MS/MS is considered to be highly specific. A second ion transition was measured. No interfering peaks (< LOD) were observed at the retention time of the analyte.

Linearity
The linearity of the detector response was tested using at least five calibration standard concentrations in the range of 2.0 to 3000 µg/L (larvae) or to 4000 µg/L (sucrose solution) (eq in mg/kg is not available) prepared in acetonitrile/water (1/4, v/v). A linear function was found (1/x weighting, larvae: $y = 0.00813 x + 0.00779$, sucrose solution: $y = 0.00791 x + 0.0101$) with a coefficient of determination (r) of >0.999.

Repeatability (Precision)
The relative standard deviations (RSD) of all recovery values (n = 3-5) 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.

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
Since all samples were analysed within 24 hours after extraction, the storage stability in the final extract was not tested.

Conclusion
The analytical method was validated for the determination of glyphosate in larvae and sucrose solution. 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 method is validated according to the guidance SANCO 3029/99 rev.4 for sucrose solution. Concerning larvae, the precisions was not demonstrated. However the method can be considered as fit for purpose for the determination of glyphosate in larvae.

Determination of glyphosate in test water

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/214 (CA 8.6.2/001)
Report authors	[redacted]
Report year	1994
Report title	Tier 2 vegetative vigor nontarget phytotoxicity study using glyphosate
Report No	93235
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 • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)
Test Facility	Pan-Agricultural Labs, Inc., 32380 Avenue 10, Madera, California 93638

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 fluorescence detection. The analyte was derivatised at post-column where glyphosate was first oxidised with sodium hypochlorite and the product (glycine) was then coupled with o-phthalaldehyde (OPA) in the presence of mercaptoethanol. The test concentrations were of sufficient clarity that no prior clean-up or sample preparation was required other than filtration. The samples were either directly injected or diluted appropriately with deionized water prior to analysis.

Chromatographic conditions:

HPLC system:	Waters 510 pumps, Waters automated gradient controller, Waters TCM temperature controller and column heaters, SSI model 241 PCR System post-column pump and reaction coil, Waters 470 Scanning Fluorescence detector
HPLC Column:	Bio-Rad HRLC glyphosate analysis column (100 × 4.6 mm)
Column temperature:	50 °C
Mobile phase:	0.005 M KH_2PO_4 in 4 % methanol (pH = 2.1)
Flow rate:	0.5 mL/min
Injection volume:	60 µL

Reactor coil temperature	40 °C
Derivatisation agent (post-column):	OPA (o-phthalaldehyde)
NaOCl flow rate:	0.25 – 0.5 mL/min
OPA flow rate:	0.5 mL/min
Retention time:	Glyphosate: ~ 8.7 min
Detection:	Fluorescence Excitation: 340 nm Emission: 455 nm

Findings

Recoveries (accuracy)

Blank samples of test water were fortified with reference item at relevant concentrations of 200, 1000 and 13000 mg/L and analysed using the analytical method. Control samples were also analysed, without detecting glyphosate above the LOD (LOD not reported). The average recovery values at each fortification level and overall were between 70 % and 110 %, with an overall relative standard deviation of 1.5 %. The detailed results are summarised in the table below.

Table 5.1-197: Results of method validation (spike recovery) for the determination of glyphosate in test water

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Test water	Glyphosate	200	101 – 102	101.5	–	–	2
		1000	99.3 – 103	101.2	–	–	2
		13000	99.2 – 101	100.0	–	–	2
		Overall	99.2 – 103	100.9	1.5	1.5	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.

Specificity

The identification was based on fluorescence detection and the retention time. The method consists of a derivatisation step which is considered to be specific to the target compounds. No interfering peaks (<30 % LOQ) were observed at the retention time of the analyte.

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 1.0 to 20 µg glyphosate/mL prepared in water. The calibration standards were derivatised as described above and analysed with each sample set. All calibration curves generated had a coefficient of correlation (r) of > 0.99. **Details to a sample calibration are provided below.**

Table 5.1-198: Details to the calibration

Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of correlation (r)
Glyphosate	Linear (no weghting)	1.0 – 20	10 (5 levels)	y = 0.00011468 x – 0.0036859	0.99888

Repeatability (Precision)
The overall relative standard deviation (RSDs) of recovery values was < 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 as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at with a relative standard deviation (RSD) of < 20 % with residues in the control samples not exceeding 30 % of the proposed LOQ. These criteria were fulfilled for the 200 mg/L fortification level. The limit of detection (LOD) was nor reported.

Matrix effects
Not directly assessed. However matrix blank sample did not show any peak at the retention time of interest.

Stability of glyphosate in sample extracts
The stability of the analyte glyphosate in test water was assessed by analysing fortified samples at 1.0 mg/L and stability was confirmed for three hours.

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 some 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 some deficits (limited validation data, 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 method is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision was not demonstrated and the derivatisation efficiency was not assessed.
However, the linearity and specificity are acceptable. The recovery data are in acceptable range. Therefore, the analytical method can be considered as fir for purpose for the determination of glyphosate in test water.

B.5.1.2.4 Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Overview Table for Analytical Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

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
CA 4.1.2/001 (CA 7.1.2.1.2/003)	[redacted] 2017 Report No. S16-04460	Aminomethylphosphonic acid (AMPA) rate of degradation of AMPA in one acidic soil incubated under aerobic conditions	N/A [redacted] 2017 Report No. S16-04460	HPLC-MS/MS LOQ 0.05 mg/kg 0.05-3.1 mg/kg	Yes	-	Y
CA 4.1.2/002 (CA 7.1.2.2.1/005)	[redacted] 1993 Report No. MSL-12605	The terrestrial field dissipation of glyphosate in Canadian soil	N/A [redacted] 1993 Report No. MSL-12605	HPLC-FD LOQ 0.05 mg/kg 0.05-5 mg/kg	No	Method fit-for-purpose	Y
CA 4.1.2/003 (CA 7.1.2.2.1/006)	[redacted] 1993 Report No. MSL-12651	The terrestrial field dissipation of glyphosate	N/A [redacted] 1993 Report No. MSL-12651	HPLC-FD LOQ 0.05 mg/kg 0.05-5 mg/kg			
CA 4.1.2/004 (CA 7.1.2.2.1/007)	[redacted] 1993 Report No. MSL-12682	Storage stability of Glyphosate and AMPA in soil and stream sediment	N/A [redacted] 1993 Report No. MSL-12682	HPLC-FD LOQ 0.05 mg/kg 0.05-5 mg/kg			
CA 4.1.2/005 (CA 7.1.2.2.1/008)	[redacted] 1992 Report No. RCC 273565	Field soil dissipation rate determination of glyphosate 360 (Diegten, Switzerland)	N/A [redacted] 1992 Report No. RCC 273565	HPLC-FD LOQ 0.02 mg/kg 0.02-3 mg/kg	No	Fit-for-purpose but clarification to be required	Y

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
CA 4.1.2/006 (CA 7.1.2.2.1/009)	[redacted] 1992 Report No. RCC 280416	Field soil dissipation rate determination of glyphosate 360 (Egerkingen, Switzerland)	N/A [redacted] 1992 Report No. RCC 280416	HPLC-FD LOQ 0.02 mg/kg 0.02-2.0mg/kg			
CA 4.1.2/007 (CA 7.1.2.2.1/010)	[redacted] 1992 Report No. RCC 280427	Field soil dissipation rate determination of glyphosate 360 (Bad Krozingen, Germany)	N/A [redacted] 1992 Report No. RCC 280427	HPLC-FD LOQ 0.02 mg/kg 0.02-2.5mg/kg			
CA 4.1.2/008 (CA 7.1.2.2.1/011)	[redacted] 1992 Report No. RCC 280438	Field soil dissipation rate determination of glyphosate 360 (Menslage, Germany)	N/A [redacted] 1992 Report No. RCC 280438	HPLC-FD LOQ 0.02 mg/kg 0.02-2.5mg/kg			
CA 4.1.2/009 (CA 7.1.2.2.1/012)	[redacted] 1995 Report No. RCC 303625	Storage stability of glyphosate and AMPA in soil	N/A [redacted] 1995 Report No. RCC 303625	HPLC-FD LOQ 0.02 mg/kg 0.02-2.5mg/kg			
CA 4.1.2/010 (CA 7.1.2.2.1/013)	[redacted] 1992 Report No. RJ1294B	Glyphosate-Trimesium: Soil dissipation study (Germany, 1990-1992)	WRC 85-34 [redacted] 1992 Report No. RJ1294B	HPLC-FD LOQ 0.05 mg/kg 0.05-2.5 mg/kg	No	-	N
CA 4.1.2/011 (CA 7.1.2.2.1/014)	[redacted] 1992 Report No. RJ1225B	Glyphosate-Trimesium: Soil dissipation study (Canada, 1988-1990)	N/A [redacted] 1992 Report No. RJ1225B	HPLC-FD LOQ 0.05 mg/kg 0.3-0.7 mg/kg			

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
CA 4.1.2/012 (CA 7.1.2.2.1/016)	[redacted] 1989 Report No. WRC 89-37	ICIA 0224-Field dissipation study for terrestrial uses California, 1987-1988 Residue data to support registration of Touchdown	N/A [redacted] 1989 Report No. WRC 89-37	HPLC-FD LOQ 0.05 mg/kg 0.05-0.5 mg/kg			
CA 4.1.2/013 (CA 7.1.2.2.1/017)	[redacted] 1989 Report No. WRC 89-40	ICIA 0224-Field dissipation study for terrestrial uses Mississippi, 1987-1988 Residue data to support registration of Touchdown	N/A [redacted] 1989 Report No. WRC 89-40	HPLC-FD LOQ 0.05 mg/kg 0.05-2.0mg/kg	No	-	No
CA 4.1.2/014 (CA 7.1.2.2.1/018)	[redacted] 1989 Report No. WRC 89-23	ICIA 0224-Field dissipation study for terrestrial uses Georgia, 1987-1988 Residue data to support registration of Touchdown	N/A [redacted] 1989 Report No. WRC 89-23	HPLC-FD LOQ 0.05 mg/kg 0.05-2.0mg/kg			
CA 4.1.2/015 (CA 7.1.2.2.1/019)	[redacted] 1986 Report No. RRC 86-61	Frozen storage stability of touchdown in soil	N/A [redacted] 1986 Report No. RRC 86-61	HPLC-FD LOQ 0.05 mg/kg 0.2-0.5mg/kg			
CA 4.1.2/016 CA 4.1.2/017 (CA 7.1.3.1.2/003)	[redacted] 2002 Report No. PR02/007	Adsorption/desorption behaviour of AMPA on soil according OECD 106 (adopted January 2000)	N/A [redacted] 2001 Report No. PR01/006 [redacted] 2002 Report No. PR02/007	GC-MS LOQ 0.05 mg/kg 0.05-0.5 mg/kg GC-MS LOQ 0.03 mg/L 0.03-10 mg/L	No	Method PR01/006 Fit-for-purpose Method PR02/007, not validated	Y only for method PR01/006

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
CA 4.1.2/018 CA 4.1.2/019 (CA 7.1.4.1.1/005)	[redacted] 1991 Report No. PR90/002 Part 4	Behaviour of glyphosate in water and soil, Part 4, leaching behaviour	iCD033E [redacted] 1991 Report No. PR90/002 Appendix	GC-ECD LOQ 0.1 µg/L 0.1-1000 µg/L	No	Method fit-for-purpose	Y
CA 4.1.2/018 CA 4.1.2/020 (CA 7.2.1.1/006)	[redacted] 1991 Report No. PR90/002 Part 1	Behaviour of glyphosate in water and soil, Part 1, hydrolysis as a function of pH	iCD033E [redacted] 1991 Report No. PR90/002 Appendix	GC-ECD LOQ 0.1 µg/L 0.1-1000 µg/L			
CA 4.1.2/021 CA 4.1.2/022 (CA 7.2.2.3/022)	[redacted] 1989 Report No. MSL-8626	Storage stability of glyphosate in environmental water	N/A [redacted] 1987 Report No. MSL-7200 [redacted] 1989 Report No. MSL-8626	HPLC-FD LOQ 1 µg/L 1-5000 µg/L	N	Fit for purpose for lab 1, 2, 4 and 6 No validated for lab 3 and 5	Y
CA 4.1.2/023 (CA 7.3.1/004)	[redacted] 1996 Report No. PR94/032	Glyphosate volatilisation in the field	N/A [redacted] 1996 Report No. PR94/032	GC-MS LOQ 0.4 µg/L 0.4-20 µg/L	N	Fit for purpose	Y
CA 7.1.2.1.2/002	[redacted] [redacted] 2020 3202599	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil	[redacted] [redacted] 2020 3202599	LC-MS/MS LOQ 0.14mg/kg	Y	Fit for purpose	Y

Determination of AMPA in soil

Study not previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/001 (CA 7.1.2.1.2/003)
Report author	[redacted]
Report year	2017
Report title	Aminomethylphosphonic acid (AMPA) rate of degradation of AMPA in one acidic soil incubated under aerobic conditions
Report No	S16-04460
Document No	EPS-2016-0309-
Guidelines followed in study	OECD 307 SANCO/3029/99 rev.4, 11/07/2000
Deviations from current test guideline	None (SANCO/3029/99 rev. 4)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Test facility	Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn. Germany
Acceptability/Reliability	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of aminomethyl phosphonic acid (AMPA) in soil by HPLC-MS/MS with a limit of quantification (LOQ) of 0.05 mg/kg.

AMPA is extracted from soil samples using 1 N NaOH solution. After centrifugation, an aliquot of the supernatant is filtered through a single syringe filter (0.45 µm pore size). After the addition of internal standard solution to the filtrated extract, approximately 1 mL of each sample extract and calibration standard are transferred to a second clean-up through a SPE cartridge. Final extracts were determined by HPLC-MS/MS using internal standard procedures.

This analytical method is similar to the analytical method developed for the determination of glyphosate and AMPA from soil ([redacted] 2015).

Chromatographic conditions**:**

HPLC-MS/MS:	Agilent 1290 Infinity II, API 6500+ LC-MS/MS system (Sciex)
HPLC column:	Bio-Rad Aminex Fast Acid, No. 1250100, 100 × 7.8 mm i.d., 25 µm
Guard column:	HPLC guard column (KJ0-4282, Phenomenex) with C18 cartridge (AJ0-4287, Phenomenex)
Column oven temperature:	25 °C
Injection volume:	40 µL
Mobile phase:	Eluent A: 0.1% formic acid in water Eluent B: Isopropanol
Flow rate:	2.2 mL/min
Evaporation solvent (Eluent B):	Isopropanol at 0.700 mL/min, combined to the aqueous eluent from analytical column and used for better vaporisation

Retention time:	AMPA: ~ 10.9 min (d2) ^{13}C,^{15}N-AMPA: ~ 10.9 min				
Scan type:	Negative Ion MRM				
Ion source:	ESI				
IonSpray voltage (IS):	-4500 V		Ionspray turbo heater (TEM):	500 °C	
Curtain gas (CUR):	40 (arbitrary units)		Gas flow 1 (GS1):	40 (arbitrary units)	
Collision gas (CAD):	12 (arbitrary units)		Gas flow 2 (GS2):	60 (arbitrary units)	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
AMPA	109.9	62.9	-35	-26	-9
AMPA (IS)	113.9	63.0	-35	-26	-9
Confirmatory ions					
AMPA	109.9	78.9	-35	-40	-9
AMPA (IS)	113.9	78.9	-35	-38	-9

Characteristics of soil tested :
Soil : Warsop
Soil ID : WS
Origin : UK
Texture : Loamy sand
pH (CaCl2) = 3.9
TOC (%) : 1.76

Findings
Recoveries
The method proved to be suitable to determine residues of AMPA in soil.
Soil samples were spiked with AMPA at two fortification levels ranging from LOQ to approximately 62 × LOQ. All average recovery values (mean of 5 replicates per fortification level) were between 70 % and 110 %. The method complies with EU guideline document SANCO/3029/99 rev. 4. The detailed results are given in the table below.

Table 5.1-199: Results of method validation (spike recovery) for the determination of AMPA in soil (Quantification: 109.9 > 62.9 *m/z*)

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Soil WS	AMPA	0.05	96.8 – 104	99.2	2.8	2.8	5
		3.08	102 – 106	105	1.9	1.8	5

[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.

Table 5.1-200: Results of method validation (spike recovery) for the determination of AMPA in soil (Confirmation: 109.9 > 78.9 *m/z*)

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Soil WS	AMPA	0.05	97 – 102	99.8	2.1	2.2	5
		3.08	104 – 106	104	1.1	1.1	5

[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 method allows the determination of AMPA using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required.. Under the described conditions the method is highly specific for the determination of AMPA in soil. Control samples did not reveal any peaks in the chromatogram, which would interfere with the determination of AMPA.

Linearity

Linearity of the detector response was tested using 7 calibration standard concentrations in the range of 1.0 to 500 ng/mL (equivalent to 0.01 – 5.88 mg/kg in the samples) with correlation coefficients of > 0.98. The lower margin of the linearity test was 20 % of the LOQ and the upper margin was higher by at least 20 % of the highest analyte concentration detected. The calibration solutions also contained isotopically enriched internal standards of AMPA**.** The calibration standards were prepared freshly in water containing 0.1 % formic acid by volumetric dilution of the stock solution of AMPA and 100 µL standard solution of (d2) ^{13}C, ^{15}N AMPA, making seven calibration solutions in all. Details to the calibration are provided below.

Table 5.1-201: Calibration parameters

Mass transition	Calibration function	Calibration concentrations (ng/mL)	Number of determinations	Equation	Coefficient of correlation (r)
109.9 > 62.9 *m/z*	Linear	1.0 – 500	7 levels	y = 1.01 x – 0.00345	0.9999
109.9 > 78.9 *m/z*	Linear	1.0 – 500	7 levels	y = 0.957 x – 0.00412	0.9999

Repeatability (Precision)

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

Accuracy

Acceptable mean recovery values at LOQ and higher levels between 70% and 110% for AMPA were found for soil. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection

The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20 %. These criteria were fulfilled for the 0.05 mg/kg fortification level for soil. The limit of detection (LOD) was 0.01 mg/kg.

Matrix effects
Chromatogrames of standard solution, Soil Warsop solution and blank soil matrix were presented for two transitions (Quantification and confirmation)As no significant interferences at the elution time of the analytes of interest were observed, no adverse effects of any matrix occurred. The residues of AMPA in blank samples were less than 20 % of the assigned LOQ of the test item.

Stability of analytes in sample extracts
The soil samples were extracted on the same day of collection and the soil extracts were stored in a freezer ≤ -18 °C and analysed by HPLC-MS/MS within ten days of collection. At every sampling interval concurrent recoveries were worked up and analysed simultaneously. All concurrent recoveries were between 92.7 % and 109 %, so no storage stability check was necessary.

Conclusion
The analytical method was successfully validated for the determination of AMPA in soil at a limit of quantification (LOQ) of 0.05 mg/kg. The analytical method fulfils the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000).

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 valid to support the environmental fate study concerned.

Assessment and conclusion by RMS:
The analytical method is validated according to the guidance SANCO 3029/99 rev.4 with an LOQ of 0.05 mg/kg.

Determination of glyphosate and AMPA in soil

Study previously submitted to the EU

1. Information on the studies

Data point	CA 4.1.2/002 (CA 7.1.2.2.1/005)
Report author	[redacted]
Report year	1993
Report title	The terrestrial field dissipation of glyphosate in Canadian soil
Report No	MSL-12605
Document No	-
Author of analytical part	[redacted] August 1991
Guidelines followed in study	None specified (with respect to analytical methods)
Test facility	The Agricultural Group of the Monsanto Company Environmental ScienceDepartement, Missouri
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve not reported • Matrix effects and stability of extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/003 (CA 7.1.2.2.1/006)
Report author	[redacted]
Report year	1993
Report title	The terrestrial field dissipation of glyphosate
Report No	MSL-12651
Document No	-
Guidelines followed in study	None specified
Test facility	The Agricultural Group of the Monsanto Company Environmental ScienceDepartement, Missouri
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve not reported • Matrix effects and stability of extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/004 (CA 7.1.2.2.1/007)
Report author	[redacted]
Report year	1993
Report title	Storage stability of Glyphosate and AMPA in soil and stream sediment
Report No	MSL-12682
Document No	-
Guidelines followed in study	None specified
Test facility	The Agricultural Group of the Monsanto Company Environmental ScienceDepartement, Missouri And Pan-Agricultural Laboratoiries, Inc. California
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Calibration curve not reported • Detailed recovery data not reported • Matrix effects ~~and stability of extracts~~ not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

Analytical Method was developed in the studies MSL-12605 ([redacted] 1993), MSL-12651 ([redacted] 1993), and MSL-12682 ([redacted] 1993) for the determination of glyphosate and aminomethylphosphonic acid (AMPA) in soil by HPLC-FD with a limit of quantification (LOQ) of 0.05 mg/kg.
The two analytes, glyphosate and AMPA are extracted by the addition of 0.5 N KOH solution. After shaking and centrifugation the resulting extract is adjusted to pH 2.0 ± 0.4 by dilution with deionized water and the addition of HCl. Glyphosate and AMPA are isolated from the pH adjusted matrix extracts by chelation to Chelex® 100 resin in the Fe(III) form. Glyphosate and AMPA iron salts are eluted from the resin with hydrochloric acid (6 N HCl) to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness to remove the hydrochloric acid samples are analysed using a two-column-switching high pressure liquid chromatograph equipped with an o-phtalaldehyde (OPA) post column reactor and a fluorescence detector. Final extracts were determined by HPLC-FD using external calibration procedures.

Chromatographic conditions:

HPLC system:	Water's model 510 buffer pumps (2), Water's WISP model 712 or 717 autosampler, Water's temperature controller/ column heater for HPLC columns and oxidation reaction coil, Water's model 470-AC fluorescence detector, Water's automated column switching valve
Post column rector pumps:	SSI
Reaction coil:	Applied Biosystems 1400-1324
Pre-column:	RP-18 Spheri-10 (Brownlee Labs), 15 × 3.2 mm ID
HPLC olumn:	Aminex Glyphosate Analysis column (Bio-Rad), 100 × 4.6 mm ID and 300 × 4.6 mm ID
Column temperature:	50 °C ± 1 °C
Derivatisation agent:	o-phtalaldehyde (OPA)
Reactor coil temperature:	40°C
Buffer flow rate:	0.50 mL/min
NaOCl flow rate:	Approximately 0.2 mL/min
OPA flow rate:	Approximately 0.50 mL/min
Injection volume:	60 µL
Detection:	Fluorescence Excitation: 340 nm Emission: 455 nm
Retention times:	Glyphosate: ~ 22 min AMPA: ~ 42 min
Notes:	Typical equipment and chromatographic parameters which were adapted to the laboratory equipment.

Findings

Recoveries

The method proved to be suitable to determine residues of glyphosate and AMPA in the tested soils from each location, namely Yuma, Hawkinsville, Madera, Danville, Lamberton, Phelps, New Holland, Snook, Alberta, Oakville Manitoba, Ayr Ontario, Georgia Iowa and Oregon.
Samples were spiked with glyphosate and AMPA at fortification levels ranging from 0.05 to 5 mg/kg. The overall average recovery values for glyphosate and AMPA were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-202: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
MSL-12605	Alberta soil (Loam/ Sandy clay loam)	Glyphosate	0.05	71.2 – 126	104	23	22	4
			0.1	75.7 – 98.8	87.5	12	13	3
			0.5	–	100	–	–	1
			2	–	88.6	–	–	1
	Oakville Manitoba soil (Loam/Sandy Loam)	Glyphosate	0.05	–	71.4	–	–	1
			0.1	63.5 – 73.9	68.7	–	–	2
			0.5	63.7 – 84.7	73.7	6.5	8.9	8
	Ayr Ontario (Loamy sand/ sand)	Glyphosate	0.05	80.0 – 91.9	85.3	6.0	7.1	3
			0.07	–	85.4	–	–	1
			0.1	83.4 – 94.1	88.7	–	–	2
			0.25	–	86.0	–	–	1
			0.5	79.3 – 82.9	81.1	–	–	2
			1	–	77.9	–	–	1
	Alberta soil (Loam/ Sandy Clay loam)	AMPA	0.05	85.1 – 92.7	89.7	3.5	3.9	4
			0.1	75.5 – 87.6	83.2	6.7	8.0	3
			0.5	–	94.1	–	–	1
			2	–	87.0	–	–	1
	Oakville Manitoba soil (Loam/Sandy Loam)	AMPA	0.05	–	89.8	–	–	1
			0.1	68.8 – 88.9	78.8	–	–	2
			0.5	65.0 – 91.9	78.8	11	13	8
	Ayr Ontario soil (Loamy Sand/ Sand)	AMPA	0.05	80.8 – 87.2	83.6	3.3	4.0	3
			0.07	–	85.4	–	–	1
			0.1	49.8 – 82.6	66.2	–	–	2
			0.25	–	83.5	–	–	1
			0.5	–	77.2	–	–	1
			1	–	77.3	–	–	1
MSL-12651	Yuma soil/ Arizona (Clay Loam for the top)	Glyphosate	0.05	61.3 – 104.3	86.8	16	19	6
			0.25	44.0 – 93.2	61.4	22	35	4
			1	62.0 – 75.7	68.9	4.8	6.9	6
			5	59.0 – 88.1	70.3	9.9	14	7

Table 5.1-202: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	Hawkinsville soil/ Georgia (sand in the top)	Glyphosate	0.05	62.5 – 116	95.1	16	17	7
			0.25	66.7 – 90.3	82.6	8	9	7
			1	83.1 – 102	90.8	8	9	5
			5	73.0 – 91.3	83.7	10	11	3
	Madera soil/ Califrnia (a loamy sand for the top)	Glyphosate	0.05	52.2 – 121	85.9	23	27	9
			0.25	71.6 – 84.6	80.0	7	9	3
			1	83.8 – 102	89.1	8	8	5
			5	73.9 – 84.6	79.9	4.7	5.9	4
	Danville soil/ Iowa (Clay loam in the top)	Glyphosate	0.05	50.8 – 83.0	65.0	14	22	4
			0.25	61.4 – 71.1	64.3	4.5	7.0	4
			1	46.9 – 84.2	66.1	11	17	9
			5	56.5 – 76.6	63.3	7.8	12	5
	Lamberton soil/ California (a loamy sand for the top)	Glyphosate	0.05	66.6 – 117	84.8	17	20	6
			0.25	56.6 – 84.3	76.9	12	15	5
			1	63.2 – 83.0	75.8	11	14	3
			5	66.7 – 81.2	73.9	6.5	8.8	4
	Phelps soil/ New York (clay loam in the top)	Glyphosate	0.05	55.7 – 99.7	79.8	15	19	8
			0.1	–	80.7	–	–	1
			0.25	75.8 – 89.6	84.1	5.4	6.4	5
			1	62.4 – 93.1	79.2	13	16	8
			5	55.2 – 88.1	76.0	13	18	6
	New Holland soil/ Ohio (loam for the top)	Glyphosate	0.05	75.5 – 91.9	84.0	5.1	6.0	7
			0.25	69.5 – 89.6	77.1	7.9	10	5
			1	63.1 – 80.1	70.3	8.8	13	3
			5	76.2 – 76.7	76.4	–	–	2
	Snook soil/ Texas (silt loam for the top)	Glyphosate	0.05	65.9 – 102	82.4	13	16	6
			0.25	52.3 – 81.7	70.4	12	17	5
			1	63.1 – 98.1	74.5	13	17	6
			5	65.0 – 66.6	65.8	1.2	1.8	2

Table 5.1-202: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	Yuma soil/ Arizona (clay loam for the top)	AMPA	0.05	66.5 – 138	104	28	27	6
			0.25	64.8 – 90.0	74.7	13	18	3
			1	74.9 – 88.9	81.9	5.7	6.9	6
			5	69.8 – 95.2	77.4	9.2	12	6
	Hawkinsville soil/ Georgia (sand in the top)	AMPA	0.05	66.5 – 124	94.6	18	19	7
			0.25	58.8 – 83.6	73.6	11	15	6
			1	78.1 – 101	89.8	10	11	5
			5	71.1 – 86.7	80.7	8.4	10	3
	Madera soil/ California (Loamy Sand)	AMPA	0.05	59.4 – 128	93.0	26	27	9
			0.25	70.7 – 82.4	76.6	5.9	7.7	3
			1	69.9 – 88.3	78.4	7.1	9.0	5
			5	71.1 – 111	85.4	18	21	4
	Danville soil/ Iowa (Clay loam in the top)	AMPA	0.05	31.6 – 88.4	68.7	32	47	3
			0.25	61.7 – 86.9	76.2	13	17	3
			1	77.1 – 95.8	87.6	6.1	7.0	8
			5	71.9 – 92.4	80.4	8.7	10.9	4
	Lamberton soil/ Minnoesota (Loam)	AMPA	0.05	73.6 – 174	104	37	35	6
			0.25	65.8 – 83.4	75.3	8.9	12	3
			1	68.2 – 82.9	77.9	8.4	11	3
			5	76.3 – 80.3	78.5	1.8	2.3	4
	Phelps soil/ New York (clay loam in the top)	AMPA	0.05	48.9 – 141	98.7	34	35	6
			0.1	–	69.9	–	–	1
			0.25	85.3 – 94.6	88.0	4.4	5.1	4
			1	64.2 – 99.5	78.5	14	18	8
			5	74.0 – 91.5	80.7	6.2	7.7	6
	New Holland soil/ Ohio (loam for the top)	AMPA	0.05	83.9 – 110	96.8	9.3	9.6	5
			0.25	74.0 – 83.8	78.0	4.0	5.1	5
			1	78.4 – 95.2	87.8	8.6	9.7	3
			5	73.6 – 78.2	75.9	–	–	2

Table 5.1-202: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	Snook soil/ Texas (silt loam for the top)	AMPA	0.05	74.4 – 101	88.0	11	12	4
			0.25	81.7 – 102	86.2	8.8	10	5
			1	78.9 – 106	89.2	10	12	6
			5	75.7 – 84.9	80.3	–	–	2

[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.

For study MSL-12682, soil from Georgia (sandy loam soil) and Iowa (silt loam soil) and one stream sediment from Oregon (sandy clay loam soil) were used.
In study MSL-12682 samples were spiked with glyphosate and AMPA at 1 mg/kg (one fortification and two determinations for each compound). The average recoveries of the Georgia control soil fortified on the date of extraction (Day 0 samples) were 0.82 mg/kg (82 %, mean of tow determinations) and 0.80 mg/kg (80 %, mean of two determinations) for glyphosate and AMPA, respectively. The average recoveries of the Iowa control soil fortified on Day 0 were 0.76 mg/kg (80 %, mean of two determinations) and 0.73 mg/kg (73 %, mean of two determinations) for glyphosate and AMPA respectively. The average recoveries of the Oregon check sediment fortified on the date of extraction were 0.81 mg/kg (81 %, mean of two determinations) and 0.80 mg/kg (80 % mean of two determinations) for glyphosate and AMPA, respectively. All recovery values were between 70 % and 110 %. The detailed procedural recovery results are not reported.
Results after frozen storage these samples for 65 days to 975 days (6 determinations in duplicate for each soil sample)
The average recoveries of the Georgia soil after frozen for 65 to 975 days were 75% and 78% for glyphosate and AMPA respectively
The average recoveries of the Iowa soil after frozen for 65 to 975 days were 72% and 78% for glyphosate and AMPA respectively
The average recoveries of the Oregon soil after frozen for 65 to 975 days were 73% and 78% for glyphosate and AMPA respectively.

Specificity
The UV-wavelengths chosen are specific for the analytes glyphosate and AMPA. The identification was based on the selected wavelengths and the retention times. No interference > LOQ was observed at the retention times of interest in the provided chromatograms. Under the described conditions the method is specific for the determination of glyphosate and AMPA in soil matrices.. The representative chromatograph of blank, standard (glyphosate and AMPA) and typical chromatograph of soil fortified sample were presented. Presented control samples did not reveal any peaks > LOQ in the chromatogram, which would interfere with the determination of glyphosate and AMPA.

Linearity
Linearity of detector response was tested using at least 5 calibration standard concentrations prepared in the range of 0.25 to 10 µg/mL (eq in mg/kg is not availabl). External standards prepared in deionised water were used for quantification with an allowed linearity requirement of $r \geq 0.99$. Further details to calibration functions are not reported.

Repeatability (Precision)
The overall relative standard deviations (RSDs) of recovery values were ≤ 20 % for the determination of glyphosate and AMPA in soil.

Accuracy
Acceptable overall mean recovery values for all soils between 70 % and 110 % were found for glyphosate and AMPA.

Limit of Quantification and Detection
The limit of quantification (LOQ) was validated at 0.05 mg/kg. The limit of detection (LOD) was 0.02 mg/kg for glyphosate and 0.04 mg/kg for AMPA.

Matrix effects
Not assessed.

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

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level. They were performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (calibration curve not reported, matrix-effects and stability of extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the environmental fate study concerned.

Assessment and conclusion by RMS:
The method validation does not fully meet criteria set in SANCO/3029/99 rev. 4 as calibration function are not available.
The matrix effect was not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

Concerning accuracy, as studies have been performed in the same laboratory the recovery results can be compiled in order to have 5 sample by fortification level. The recoveries and repeatability are in acceptable range.
The linearity are only available in µg/mL, the equivalence in mg/kg is missing. However, as accuracy are acceptable we can consider that the linearity range covers the fortification levels.

The specificity (interference) was demonstrated. .
Therefore, the method can be considered as fit-for-purpose for the determination of glyphosate and AMPA in soil.

Study previously submitted to the EU

1. Information on the studies

Data point	CA 4.1.2/005 (CA 7.1.2.2.1/008)
Report author	[redacted]
Report year	1992

Report title	Field soil dissipation rate determination of glyphosate 360 (Diegten, Switzerland)
Report No	273565
Performing laboratory	R C C UMWELTCHEMIE AG Itingen BL Switzerland
Document No	-
Guidelines followed in study	None stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Recoveries at LOQ not sufficient • Non-linear regression without justification • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/006 (CA 7.1.2.2.1/009)
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of glyphosate 360 (Egerkingen, Switzerland)
Report No	280416
Document No	-
Performing laboratory	R C C UMWELTCHEMIE AG Itingen BL Switzerland
Guidelines followed in study	None stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Recoveries at LOQ not sufficient • Non-linear regression without justification • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/007 (CA 7.1.2.2.1/010)
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of glyphosate 360 (Bad Krozingen, Germany)
Report No	280427
Document No	-
Performing laboratory	R C C UMWELTCHEMIE AG Itingen BL Switzerland
Guidelines followed in study	None stated (with relevance to analytical methods)

Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Recoveries at LOQ not sufficient • Non-linear regression without justification • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/008 (CA 7.1.2.2.1/011)
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of glyphosate 360 (Menslage, Germany)
Report No	280438
Document No	-
Performing laboratory	R C C UMWELTCHEMIE AG Itingen BL Switzerland
Guidelines followed in study	None stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Recoveries at LOQ not sufficient • Non-linear regression without justification • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/009 (CA 7.1.2.2.1/012)
Report author	[redacted]
Report year	1995
Report title	Storage stability of glyphosate and AMPA in soil
Report No	303625
Document No	-
Performing laboratory	R C C UMWELTCHEMIE AG Itingen BL Switzerland
Guidelines followed in study	None stated (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Recoveries at LOQ not sufficient (n = 4) • Non-linear regression without justification • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developped in the studies RCC Project 273565 ([redacted] 1992), RCC Project 280416 ([redacted] 1992), RCC Project 280427 ([redacted] 1992), RCC Project 280438 ([redacted] 1992) and RCC Project 303625 ([redacted] 1995) for the determination of glyphosate and AMPA in soil by HPLC-FD with a limit of quantification (LOQ) of 0.02 mg/kg.
The analytes are extracted from soil with ammonium hydroxide solution. The extract is adjusted to pH 2.0 and cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA are eluted with hydrochloric acid and the co-eluted Fe (III) ions were removed from the eluates using and ion-exchange resin. Afterwards, the resulting eluate is concentrated to dryness by means of a rotary-evaporator. Glyphosate and AMPA are quantified separately by HPLC, equipped with a post column derivatization unit and a fluorescence detector. Glyphosate is oxidized with sodium hypochlorite to obtain glycine. Glycine and AMPA are coupled with o-phthaldialdehyde (OPA) in presence of mercaptoethanol to give fluorescent compounds.

Chromatographic conditions:

HPLC system:	High pressure pump: Merck/Hitachi L-6200; Reaction pump: Merck/Hitachi 655A-13; Sampling unit: Merck/Hitachi AS-4000
Detector:	Fluorescence detector: Hitachi F1000
HPLC column:	Bio Rad Aminex A-9 (K^+), 300 × 6 mm ID
Dimension of reaction coil:	7 m × 0.25 mm
Mobile phase:	50 mM KH_2PO_4 adjusted to pH 2 with H_3PO_4 / Methanol (960/40, v/v)
Flow rates:	Mobile phase: 0.5 mL/min Oxidative solution: about 0.25 mL/min Derivatization solution: about 0.25 mL/min
Temperatures:	Column: ambient Reaction coil: 30 °C
Injection volume:	50 µL
Derivatisation agent:	o-phthaldialdehyde (OPA)/mercaptoethanol (MERC)
Detection:	Fluorescence Excitation: 330 nm Emission: 445 nm
Retention time:	Glyphosate: ~ 9.2 – 10.7 min AMPA: ~ 10.9 – 12.3 min
Notes:	Typical equipment and chromatographic parameters which were adapted to the laboratory equipment.

Findings
Recoveries
Soil samples were spiked with glyphosate and AMPA at LOQ and higher levels ranging from 0.02 mg/kg to 3.0 mg/kg. Recovery values for glyphosate and AMPA were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-203: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
RCC 273565	Soil (Sandy clay soil)	Glyphosate	0.02	–	119	–	–	1
			0.10	68.0 – 72.7	70.4	–	–	2
			0.50	–	98.7	–	–	1
			1.0	–	75.8	–	–	1
			1.5	–	82.5	–	–	1
			2.5	–	64.2	–	–	1
			3.0	–	77.3	–	–	1
			Overall	64.2 – 119	82.2	18	21.9	8
		AMPA	0.02	–	105	–	–	1
			0.10	69.3 – 70.2	69.8	–	–	2
			0.50	–	97.1	–	–	1
			1.0	–	76.7	–	–	1
			1.5	–	95.6	–	–	1
			2.5	–	75.9	–	–	1
			3.0	–	82.4	–	–	1
			Overall	69.3 – 105	84.1	13.6	16.1	8
RCC 280416	Soil (clay loam soil)	Glyphosate	0.02	–	118	–	–	1
			0.10	–	84.2	–	–	1
			0.50	–	68.9	–	–	1
			1.00	–	64.0	–	–	1
			1.50	–	71.3	–	–	1
			2.00	–	69.8	–	–	1
			Overall	64.0 – 118	79.3	20.0	25.2	6
		AMPA	0.02	–	82.9	–	–	1
			0.10	–	56.8	–	–	1
			0.50	–	82.9	–	–	1
			1.00	–	79.7	–	–	1
			1.50	–	78.1	–	–	1
			2.00	–	92.9	–	–	1
			Overall	56.8 – 92.9	78.9	12.0	15.2	6
RCC	Soil	Glyphosate	0.050	–	82.6	–	–	1

Table 5.1-203: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
280427			0.1	–	80.3	–	–	1
			0.5	–	86.7	–	–	1
			1	68.1 – 81.0	74.6	–	–	2
			1.5	–	85.9	–	–	1
			2.5	–	84.8	–	–	1
			Overall	68.1 – 86.7	81.3	6.3	7.8	7
		AMPA	0.05	–	94.7	–	–	1
			0.1	–	87.0	–	–	1
			0.5	–	86.3	–	–	1
			1	80.2 – 81.6	80.9	–	–	2
			1.5	–	87.1	–	–	1
			2.5	–	86.7	–	–	1
			Overall	80.2 – 94.7	86.2	4.7	5.4	7
RCC 280438	Soil sandy soil	Glyphosate	0.020	–	72.7	–	–	1
			0.050	–	71.9	–	–	1
			0.10	–	69.1	–	–	1
			0.20	–	60.3	–	–	1
			0.50	70.2 – 104	87.0	–	–	2
			1.00	–	70.8	–	–	1
			1.50	–	76.5	–	–	1
			2.50	–	77.1	–	–	1
			Overall	60.3 – 104	74.7	11.9	16.0	9
		AMPA	0.020	–	68.1	–	–	1
			0.050	–	68.2	–	–	1
			0.10	–	66.6	–	–	1
			0.20	–	64.2	–	–	1
			0.50	75.5 – 112	94.0	–	–	2
			1.00	–	87.1	–	–	1
			1.50	–	85.7	–	–	1
			2.50	–	80.7	–	–	1
			Overall	64.2 – 112	78.7	15.2	19.3	9

Table 5.1-203: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
RCC 303625	Soil	Glyphosate	1.0	67.6 – 90.1	78.6	9.7	12	4
			Overall	67.6 – 90.1	78.6	9.7	12	4
		AMPA	0.5	55.7 – 80.0	71.6	14	19	3
			Overall	55.7 – 80.0	71.6	14	19	3
Overall Glyphosate				60.3 – 119	79.1	14	17	34
Overall AMPA				55.7 – 112	81.0	12	15	33

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

A confirmatory method is not considered necessary and is not specifically required for data generation methods. The UV-wavelengths chosen are specific for the analytes glyphosate and AMPA. The identification was based on the selected wavelengths and the retention times. No interferences ≥ LOQ were observed at the retention times of interest in the control samples. The method consists of a derivatisation step which is considered to be specific to the target compounds. Typical chromatograms of standard glyphosate and standard AMPA solution, Chromatograms of fortified and untreated soil samples were presented. No interference was showen at the retention time of glyphosate and AMPA compound. Control soils did not reveal any peaks ≥ LOQ in the chromatograms, which would interfere with the determination of glyphosate and AMPA.

Linearity

Linearity of detector response was tested using at least 5 calibration standard concentrations for glyphosate and AMPA in the range of 0.025 µg/mL to 1 µg/mL (eq in mg/kg is not available) with coefficients of determination > 0.99. Calibration solutions were prepared in 0.001 M ethylenediaminetetraacetic acid disodium salt (EDTA) solution. Details to the calibrations are provided below.
Calibration curve was only reported for study RCC 280438, for another studies curves were missing.

Table 5.1-204: Calibration parameters

Study	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination(r^2)
RCC 273565	Glyphosate	Non-linear	0.025 – 1.00	7 levels	$y = e^{(-5\ 2+1\ 01*lnx)}$	> 0.99
	AMPA	Non-linear	0.025 – 1.00	7 levels	$y = e^{(-4\ 74+1\ 00*lnx)}$	> 0.99
RCC 280416	Glyphosate	Non-linear	0.025 – 1.00	7 levels	$y = e^{(-5\ 11+1\ 01*lnx)}$	> 0.99
	AMPA	Non-linear	0.025 – 1.00	7 levels	$y = e^{(-4\ 96+1\ 02*lnx)}$	> 0.99
RCC 280427	Glyphosate	Non-linear	0.05 – 1.00	6 levels	$y = e^{(-4\ 38+0\ 94*lnx)}$	> 0.99
	AMPA	Non-linear	0.05 – 1.00	6 levels	$y = e^{(-4\ 57+0\ 98*lnx)}$	> 0.99

Table 5.1-204: Calibration parameters

Study	Analyte	Calibration function	Calibration concentrations (µg/mL)	Number of determinations	Equation	Coefficient of determination(r^2)
RCC 280438	Glyphosate	Non-linear	0.025 – 1.00	7 levels	$y = e^{(-4\ 76+0\ 98*lnx)}$	> 0.99
	AMPA	Non-linear	0.025 – 1.00	7 levels	$y = e^{(-5\ 27+0\ 99*lnx)}$	> 0.99
RCC 303625	Glyphosate	Non-linear	0.025 – 1.00	6 levels	lny = -4.961 + 1.011 × lnx	> 0.99
	AMPA	Non-linear	0.025 – 1.00	5 levels	lny = -4.981 + 1.027 × lnx	> 0.99

Repeatability (Precision)
The overall relative standard deviations (RSDs) of recovery values was < 20 % for glyphosate and AMPA.
Therefore, the method complies with EU guideline document SANCO/3029/99 rev. 4 for the determination of glyphosate and AMPA in soil.

Accuracy
Acceptable overall mean recovery values between 70 % and 110 % for glyphosate and AMPA were found for soil.

Limit of Quantification and Detection
The limit of quantification (LOQ) was validated at 0.02 mg/kg for both analytes. The limit of detection (LOD) for glyphosate and AMPA with 0.01 mg/kg was calculated from the lowest calibration point.

Matrix effects
Not assessed.

Stability
The stability of glyphosate and AMPA in calibration solutions was tested within the field dissipation studies. The calibration solutions of glyphosate and AMPA are considered to be stable during the time period in which the experimental work was performed.

Efficiency of derivatisation:
Not assessed

Conclusion
The analytical method was validated for the determination of glyphosate and AMPA in soil at a limit of quantification (LOQ) of 0.02 mg/kg. Despite the method validation did not fully meet criteria set in SANCO/3029/99 rev. 4, it is fit-for-purpose to support the environmental fate study concerned.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level. They were performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev. 4) with minor deficits (recoveries at LOQ not sufficient, non-linear regression without justification, ~~matrix effects not assessed~~, efficiency of derivatisation not assessed). The method is fit-for-purpose to support the environmental fate 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 :The matrix effect was not demonstrated. However as the accuracy are in acceptable range we consider that no matrix effect was observed.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

The equivalence of the linearity range in mg/kg us missing and no justification was provided concerning the non linearity of the calibration curve .

The specificity (interference) was demonstrated. Concerning the accuracy, as studies have been performed in the same laboratory, the data can be compiled in order to have 5 samples by fortification level. Mean recoveries and repeatability were in the acceptable range.

The method can be considered as fit-for-purpose for the determination of glyphosate and AMPA in soil. However, the equivalence of the linearity range in mg/kg, a justification concerning the non linearity of the calibration curve should be provided

Study previously submitted to the EU

1. Information on the studies

Data point	CA 4.1.2/010 (CA 7.1.2.2.1/013)
Report author	[redacted]
Report year	1992
Report title	Glyphosate-Trimesium: Soil dissipation study (Germany, 1990-1992)
Report No	RJ1294B
Test facility	Jealotts Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Linearity data not reported • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/011 (CA 7.1.2.2.1/014)
Report author	[redacted]
Report year	1992
Report title	Glyphosate-Trimesium: Soil dissipation study (Canada, 1988-1990)
Report No	RJ1225B
Test facility	Jealotts Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Linearity data not reported • Matrix effects not assessed • Efficiency of derivatisation not assessed

Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/012 (CA 7.1.2.2.1/016)
Report author	[redacted]
Report year	1989
Report title	ICIA 0224-Field dissipation study for terrestrial uses California, 1987-1988 Residue data to support registration of Touchdown
Report No	WRC 89-37
Test facility	ICI Americas Inc. Western Research Center, Environmental sciences department, 1200 South 47th Street Box number 4023 Richmond, CA 94804-0023
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Linearity data not reported • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/013 (CA 7.1.2.2.1/017)
Report author	[redacted]
Report year	1989
Report title	ICIA 0224-Field dissipation study for terrestrial uses Mississippi, 1987-1988 Residue data to support registration of Touchdown
Report No	WRC 89-40
Test facility	ICI Americas Inc. Western Research Center, Environmental sciences department, 1200 South 47th Street Box number 4023 Richmond, CA 94804-0023
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Linearity data not reported • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)

GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/014 (CA 7.1.2.2.1/018)
Report author	[redacted]
Report year	1989
Report title	ICIA 0224-Field dissipation study for terrestrial uses Georgia, 1987-1988 Residue data to support registration of Touchdown
Report No	WRC 89-23
Test facility	ICI Americas Inc. Western Research Center, Environmental sciences department, 1200 South 47th Street Box number 4023 Richmond, CA 94804-0023
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Linearity data not reported • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/015 (CA 7.1.2.2.1/019)
Report author	[redacted]
Report year	1986
Report title	Frozen storage stability of touchdown in soil
Report No	RRC 86-61
Test facility	Stauffer Chemical company (SCC) 1200 South 47th Street Richmond, CA 94804
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Linearity data not reported • Matrix effects not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Supportive (with relevance for analytical methods)

Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method

The analytical method WRC 85-34 was validated within the studies RJ1294B ([redacted] RJ1225B [redacted]), WRC 89-37 ([redacted].), WRC 89-40 ([redacted].), WRC 89-23 ([redacted] and RRC 86-61 [redacted].) for the determination of glyphosate and AMPA in soil by HPLC-FD with a limit of quantification (LOQ) of 0.05 mg/kg. The analytical method WRC 85-34 supersedes method WRC 83-44.

Glyphosate and AMPA are extracted from soil samples using 0.5 M ammonium hydroxide. After centrifugation, an aliquot of the supernatant is filtered and taken to dryness using a rotary evaporator. If required, a cation resin cleanup was performed. Therefore, the evaporated extract was dissolved in 0.08 N acid eluate (0.08 N hydrochloric acid in 10 % methanol) at this point. However, according to the method WRC 85-34 the cation exchange cleanup is usually not required for soil).

After re-dissolving the residue in 0.05 M borate buffer the glyphosate and AMPA are then derivatised with 9-fluorenylmethyl chloroformate (FMOC-Cl). The derivates were determined by HPLC using an S5-AX column and fluorescence detection.

Chromatographic conditions:

HPLC system:	Injector: Water intelligent sample processor (WISP 710B) Pump: Waters 590
Detector:	Perkin-Elmer LS-4 Fluorescence Spectrometer
HPLC column:	HICHROM™ S5-AX Spherisorb analytical column (250 × 4.6 mm ID)
Mobile phase:	Acetonitrile/water/1% orthophosphoric acid (50/30/20, v/v/v)
Flow rate:	1 mL/min
Derivatisation agent:	FMOC-Cl (9-fluorenylmethyl chloroformate)
Detection:	Fluorescence Excitation: 254 nm Emission: 300-315 nm
Retention time:	AMPA: ~ 13 min Glyphosate: ~ 15 min
Notes:	Typical equipment and chromatographic parameters which were adapted to the laboratory equipment.

Findings

Characteristics of different soil types used in report RJ 1294B:
RS-9027/B1:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 30	6.4	80	14	6	2.8	12.72	6.44	6.5	Loamy Sand
30 - 60	6.5	80	12	8	2.1	9.33	5.35	5.5	Loamy Sand
60 - 100	6.7	81	15	4	0.8	6.95	3.26	3.5	Loamy Sand

RS-9027/B2:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 30	7.0	66	21	13	1.9	13.71	7.15	7.7	Sandy Loam
30 - 60	7.0	68	15	17	1.2	14.60	7.55	8.7	Sandy Loam
60 - 100	7.3	62	19	19	0.2	15.19	8.35	10.4	Sandy Loam

RS-9027/E1:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 30	6.7	48	39	13	1.8	15.57	7.26	6.6	Loam
30 - 60	5.4	53	31	16	0.6	16.50	8.16	6.1	Sandy Clay Loam
60 - 90	5.3	44	37	19	0.3	16.88	8.92	6.2	Loam

RS-9027/E2:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 25	8.5	12	77	11	1.8	23.10	11.78	12.7	Silt Loam
25 - 35	8.5	13	60	27	0.5	21.28	11.30	12.1	Silt Loam
35 - 105	8.7	15	70	15	0.1	18.95	6.41	5.4	Silt Loam

RS-9027/G1:

Soil # Horizon	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
Upper Horizon	8.0	23	47	30	2.8	24.31	12.68	14.4	Clay Loam
Lower Horizon	8.4	21	58	21	0.8	21.18	8.90	9.3	Silt Loam

RS-9027/G2:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 30	7.2	25	51	24	2.1	24.53	8.98	14.0	Silt Loam

Characteristics of different soil types used in report RJ 1125B:

Soil – St David's, Ontario:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 30	7.9	11	49	41	4.3	30.63	22.53	15.8	Silty Clay
30 - 50	7.9	1	19	80	3.8	43.44	32.47	25.3	Clay
50 +	7.7	14	46	40	0.8	26.77	19.26	12.0	Silty Clay Loam

Soil-Carman, Manitoba:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 15	7.8	80	10	10	2.9	10.26	6.14	10.8	Loamy Sand
15 - 30	8.1	81	9	10	2.6	10.34	5.51	10.4	Loamy Sand

Soil-Grandora, Saskatchewan:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 12.5	7.1	42	30	28	3.3	21.44	10.26	15.3	Clay Loam
12.5-22.5	7.9	36	34	30	2.0	22.51	10.61	15.5	Clay Loam
22.5 +	8.6	45	29	27	1.0	20.01	11.10	15.8	Clay Loam

Soil-Speers, Saskatchewan:

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 12	7.1	12	55	34	9.1	34.71	22.33	22.0	Silty Clay Loam
12 - 24	7.8	7	60	33	2.0	24.68	12.13	16.7	Silty Clay Loam
24 +	8.2	8	59	33	0.9	24.49	12.09	17.5	Silty Clay Loam

Soil-Brooks, Alberta:

Table 25 : Soil Physico-chemical Properties - Brooks, Alberta.

Soil Horizon (cm)	pH	Particle Size Analysis (%)			Organic Matter (%)	Moisture Holding Capacity		Cation Exchange Capacity (meq/100g)	Soil Classification
		Sand	Silt	Clay		0.33 Bar	15 Bar		
0 - 15	7.6	36	42	22	1.7	18.84	9.57	13.2	Loam
15 - 30	7.3	39	38	23	1.7	18.56	9.68	13.6	Loam

Physico-chemical properties of the different soil types, used in reports WRC 89-37, WRC 89-40, WRC 89-23 and RRC 86-61, were not provided.

Recoveries

Soil samples were fortified with glyphosate and AMPA at LOQ and higher levels. The average recovery values per fortification level and analyte were between 70 % and 110 %, except for the 1.25 mg/kg fortification level in study RJ 1294B ([redacted] 1992) with 69 % recovery for AMPA**.**

The detailed results are given in the table below.

Table 5.1-205: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Forti-fication level (mg/kg)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
RRC 86-61 (Method RRC 85-34)	Soil	Glyphosate	0.2	61 – 111	83.8	22	26	4
			0.5	82 – 100	91.7	9.1	9.9	3
		AMPA	0.2	79 – 98	89	7.8	8.8	4
			0.5	83 – 92	87.5	–	–	2
RJ 1294B	Soil	Glyphosate	0.05	65 – 90	75.7	9.4	12.4	7
			0.1	63 – 98	80.5	10.7	13.2	18
			0.5	66	66	–	–	1
			0.75	83 – 94	88.8	4.6	5.2	5
			1.25	77	77	–	–	1
			1.5	80	80	–	–	1

Table 5.1-205: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Forti-fication level (mg/kg)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
			2.5	68 – 94	83.5	9.5	11.4	6
		AMPA	0.05	78 – 106	93.8	10.6	11.3	6
			0.1	53 – 111	89.5	16.1	17.9	19
			0.5	70	70	–	–	1
			0.75	89-99	95.0	3.8	4.0	5
			1.25	69	69	–	–	1
			1.5	77	77	–	–	1
			2.5	71 – 105	88.2	11.2	12.7	6
RJ 1225B	Ontario soil	Glyphosate	0.7	71	71	–	14	N/A
		AMPA	0.3	82	82	–	15	N/A
	Manitoba soil	Glyphosate	0.7	93	93	–	16	N/A
		AMPA	0.3	86	86	–	12	N/A
	Saskatchewan soil	Glyphosate	0.7	80	80	–	20	N/A
		AMPA	0.3	87	87	–	16	N/A
	Alberta soil	Glyphosate	0.7	81	81	–	14	N/A
		AMPA	0.3	90	90	–	14	N/A
WRC 89-37	Soil	Glyphosate	0.05	70 – 118	94.2	13	14	18
			0.1	71 – 90	81.0	9.5	12	3
			0.2	73 – 90	82.6	6.9	8.3	5
			0.5	71 – 74	72.5	–	–	2
		AMPA	0.05	64 – 120	93.9	16	17	18
			0.1	70 – 92	82.7	11	14	3
			0.2	79 – 95	87.2	5.8	6.6	5
			0.5	73 – 74	73.5	–	–	2
WRC 89-40	Soil	Glyphosate	0.05	60 – 94	76.9	10	13	12
			0.25	88	88	–	–	1
			0.5	85	85	–	–	1
			1	80 – 84	82.0	–	–	2
			2	76	76	–	–	1

Table 5.1-205: Results of method validation (spike recovery) for the determination of glyphosate and AMPA in soil

Report No.	Matrix	Analyte	Forti-fication level (mg/kg)	Recovery				
				Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		AMPA	0.05	86 – 108	95.1	6.4	6.7	12
			0.1	74	74.0	–	–	1
			0.5	93	93.0	–	–	1
			1	88 – 97	92.5	–	–	2
			2	81	81.0	N/A	N/A	1
WRC 89-23	Soil	Glyphosate	0.05	68 – 88	76.4	6.6	8.7	11
			0.1	71	71.0	–	–	1
			0.2	79	79.0	–	–	1
			0.3	115	115	–	–	1
			0.5	86	86.0	–	–	1
			1	92	92.0	–	–	1
			2	81 – 85	83.0	–	–	2
		AMPA	0.05	70 – 118	81.9	12.4	15.1	12
			0.1	76	76.0	–	–	1
			0.2	70	70.0	–	–	1
			0.3	102	102	–	–	1
			1	84	84.0	–	–	1
			2	76 – 77	76.5	–	–	2

Specificity

The UV-wavelengths chosen are specific for the analytes glyphosate and AMPA. The identification was based on the selected wavelengths and the retention times. The method consists of a derivatisation step which is considered to be specific to the target compounds. Chromatograms of control soils presented in the studies RJ1294B and RJ1225B did not reveal any peaks in the chromatograms, which would interfere with the determination of glyphosate and AMPA. Chromatograms were not provided for unfortified (blank) and fortified soil samples in reports WRC 89-37, WRC 98-40 and WRC 89-23.

Linearity

Calibration solutions were prepared in acidified ultra-pure water and derivatised with 9-fluorenylmethyl chloroformate prior HPLC-FD analysis.
The detailed linearity data were not provided.

Repeatability (Precision)

The overall relative standard deviations (RSDs) of recovery values was < 20 % for glyphosate and AMPA.
Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4 for the determination of glyphosate and AMPA in soil.

Accuracy
Acceptable mean recovery values at LOQ and higher levels between 70 % and 110 % for glyphosate and AMPA were found for soil, except for the 1.25 mg/kg fortification level in study RJ 1294B (BOD95-00424) with 69 % recovery for AMPA. Therefore the method complies mainly with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤20 %. These criteria were fulfilled for the 0.05 mg/kg fortification level for soil. The limit of detection (LOD) was not determined.

Matrix effects
Not assessed.

Stability
Calibration solutions prepared in ultra-pure water are stable at least one year at ambient temperatures in an amber bottle when acidified (one (1) drop HCl in 100 mL water).-Not evaluated

Conclusion

The analytical method was successfully validated for the determination of glyphosate and AMPA in soil at a limit of quantification (LOQ) of 0.05 mg/kg. The analytical method fulfils the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000) with deficits ; the linearity data were not provided, the efficiency of derivatisation and matrix effect were not assessed.
Nevertheless the method is considered as fit-for-purpose for the determination of glyphosate and AMPA in soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were mostly performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (insufficient linearity data, matrix effects not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the environmental fate studies concerned.

Assessment and conclusion by RMS:

The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4 as the linearity plots and calibration function are not available, the specificity (interference) is not completely demonstrated, the matrix effect and the derivatiation efficiency were not assessed.

By compilation of results obtained for different soils, the number of sample by fortification level can be considered as sufficient. The recovery and precision data are in acceptable range.

The method cannot be considered as fit for pupose for the determination of AMPA in soil.

Determination of glyphosate and AMPA in soil and aqueous solutions

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/016
Report author	[redacted]
Report year	2001
Report title	Validation of an analytical method for the determination of glyphosate in soil
Report No	PR01/006
Test facility	UCL GmbH, NL Koln, Eupener Str. 150 50933 Koln
Document No	-
Guidelines followed in study	SANCO/825/00 rev.6
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Derivatisation efficiency not addressed
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)

Data point	CA 4.1.2/017 (CA 7.1.3.1.2/003)
Report author	[redacted]
Report year	2002
Report title	Adsorption/desorption behaviour of AMPA on soil according OECD 106 (adopted January 2000)
Report No	PR02/007
Test facility	UCL GmbH, NL Koln, Eupener Str. 150 50933 Koln
Document No	-
Guidelines followed in study	OECD 106 SANCO/825/00 rev. 6
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Number of procedural recoveries at LOQ not sufficient, n=3 • Derivatisation efficiency not addressed
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)

2. Full summary of the study according to OECD format

(A) Determination of glyphosate and AMPA in soil (report PR01/006)

Principle of the method

An analytical method was developed for the determination of glyphosate and aminomethyl phosphonic acid (AMPA) in soil by GC-MS with a limit of quantification (LOQ) of 0.05 mg/kg. Analysis of the samples was performed according to the previously (GLP) validated analytical method PR01/006, with slight modifications.
Soil samples are spiked with internal standard (^{13}C-glyphosate and ^{15}N-AMPA). For pre-treatment the soil samples are blended with water and alkalised with sodium hydroxide. The centrifuged extract were subjected to a clean-up by an anion exchange resin. A charcoal clean-up followed then the samples were derivatised with trifluoroacetic acid, trifluoroacetic acid anhydride and trifluoroethanol at 70 °C. Afterwards another clean-up followed up by means of a liquid-liquid extraction. Then samples were submitted to analysis by gas liquid chromatography with mass selective detection (GC-MSD) in the select ion monitoring (SIM) mode, monitoring the fragment ions of > 100 mass units (glyphosate target ion: 411 *m/z*; AMPA target ion: 302 *m/z*). For the current study sample work up was simplified with respect to the clean-up steps. The clean-up steps could be left out, because this study works at a higher concentration level than study PR01/006.
For method validation specificity, linearity and accuracy of the method were performed and the limit of detection and the limit of quantification were determined. The method is highly specific under investigation as three typical fragmentation ions of glyphosate and AMPA were used for detection.

Chromatographic conditions**:**

GC:	Dani 86.10 with autosampler LS 32
Column:	50 m CP-SIL 19 c.b. (corr OV1701), I.D. 0.25 mm, df=04 µm)
Column oven temperature:	120 C° (3 min) with 10 C°/min to 140 C° (10 min) with 5 C°/min to 260 C° (15 min)
Injector:	PTV, split/splitless
Injection volume:	3 µL
Carrier gas:	Helium (4.6 bar)
Mass spectrometer:	HP 5970 MSD with ChemStation Vers. 3..2
Scan mode:	SIM
Ions monitored:	Glyphosate: 411/412 (quantification), 238/239, 384/385 AMPA: 302/303 (quantification), 126/127, 109/110
Retention time:	Glyphosate: ~ 19.2 min AMPA: ~ 17.1 min

Findings

Recoveries

Samples were spiked with Glyphosate and AMPA at two fortification levels ranging from LOQ to approximately 10 × LOQ. All average recovery values (mean of 5 replicates per fortification level) were between 70 % and 110 %. The detailed results are given in the table below.

Table 5.1-206: Results of the method validation for the determination of glyphosate and AMPA in soil

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Soil	Glyphosate	0.05	102 – 110	105	3.3	3.2	5

Table 5.1-206: Results of the method validation for the determination of glyphosate and AMPA in soil

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.5	108 – 110	109	0.4	0.4	5
	AMPA	0.05	100 – 107	104	3.3	3.2	5
		0.5	103 – 117	108	5.5	5.1	5

[1] Recovery values of AMPA are 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

Mass selective detection in the select ion monitoring (SIM) mode with a target ion and two qualifier ions per analyte is considered a highly specific technique and no confirmatory analytical method is required. The specificity was tested with control (untreated) samples of soil. Blank values were detected for AMPA in the matrix soil, for glyphosate no blank values > 20 % LOQ were detected. For AMPA the blank values exceed 30 % of the LOQ in one case. This value was not taken for calculation because it is possible that AMPA was carried over by the use of clean but old glassware.

Linearity

Linearity of detector response was tested using 5 calibration standard concentrations in the range of 1.99 µg/mL to 29.9 µg/mL (25 µg/mL ISTD; eq in mg/kg is not available) with correlation coefficients of > 0.99. The calibration standards were prepared in water containing.

Repeatability (Precision)

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

Accuracy

Acceptable mean recovery values at LOQ and higher levels between 70 % and 110 % for glyphosate and AMPA were found for soil. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection

The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20 %. These criteria were fulfilled for the 0.05 mg/kg fortification level for soil. The limit of detection (LOD) was set at 0.01 mg/kg (20 % of LOQ).

Matrix effects

Not required as stable-isotope labelled internal standards were used for quantification.

Conclusion

The analytical method was successfully validated for the determination of glyphosate and AMPA in soil at a limit of quantification (LOQ) of 0.05 mg/kg. The analytical method fulfils the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits; derivatisation efficiency was not assessed.

(B) Determination of AMPA in water (PR2/007)

Principle of the method

The analytical procedure is based on the validated method PR01/004. In this study the limit of quantification was determined to be 0.1 µg/L for AMPA in water.

Samples are spiked with internal standard (^{15}N-AMPA). The liquid is than reduced to dryness in a rotary evaporator. The dried samples are derivatised with trifluoroacetic acid, trifluoroacetic acid anhydride and trifluoroethanol at 70°C. Afterwards a clean-up was carried out by means of a liquid-liquid extraction. The determination was performed by means of GC-MS in the SIM mode using selective mass fragment-ions > 100.

For the current study sample work up was simplified with respect to the clean up steps. The clean up steps could be let out, because this study works at a higher concentration level than study PR01/004.

Chromatographic conditions**:**

GC:	Dani 86.10 with autosampler LS 32
Column:	50 m CP-SIL 19 c.b. (corr OV1701), I.D. 0.25 mm, df = 04 µm)
Column oven temperature:	120 °C (3 min) with 10 °C/min to 140 °C (10 min) with 5 °C/min to 260 °C (15 min)
Injector:	PTV, split/splitless
Injection volume:	3 µL
Carrier gas:	Helium (1.5 bar)
Derivatisation agent (pre-column):	Trifluoroacetic acid, trifluoroacetic acid anhydride, trifluoroethanol
Mass spectrometer:	HP 5970 MSD with ChemStation Vers. 3..2
Scan mode:	SIM
Ions monitored:	AMPA: 302/303 (quantification), 126/127, 109/110
Retention time:	AMPA: ~ 18.5 min

Findings

Recoveries

The method proved to be suitable to determine residues of AMPA in HPLC water, 0.01 M $CaCl_2$ solutions and 0.01 M $CaCl_2$ soil extracts**.**

Samples were spiked with AMPA at several fortification levels ranging from 0.03 mg/L to 10 mg/L. All average recovery values were between 70 % and 110 %. The detailed results are given in the table below**.**

Table 5.1-207: Results of the method validation for the determination of AMPA in HPLC water, 0.01 M $CaCl_2$ solutions and 0.01 M $CaCl_2$ soil extracts

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
0.01 M $CaCl_2$ soil extracts	AMPA	0.03	93.9 – 102	98.5	4.3	4.4	3
HPLC water		1	N/A	101	–	–	1
0.01 M $CaCl_2$		1	N/A	102	–	–	1
0.01 M $CaCl_2$		10	106 – 108	107	0.7	0.7	14

[1] Recovery values of AMPA 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
Mass selective detection in the select ion monitoring (SIM) mode with a target ion and two qualifier ions is considered a highly specific technique and no confirmatory analytical method is required. The specificity was tested with control (untreated) samples containing only 0.01 M $CaCl_2$ solution in the absence of AMPA. No interferences > 30 % LOQ were observed in samples containing only 0.01 M $CaCl_2$ solution. Chromatograms of blank samples were not provided

Linearity
Linearity of detector response was tested using 5 calibration standard concentrations in the range of 0.998 µg/mL to 99.8 µg/mL (50 µg/mL ISTD) with $r^2 > 0.98$. The calibration standards were prepared in water.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were < 20% (for $n \geq 3$). Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Accuracy
Acceptable mean recovery values at LOQ and higher levels between 70% and 110% for AMPA were found for HPLC water, 0.01 M $CaCl_2$ solutions and 0.01 M $CaCl_2$ soil extracts. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20%. These criteria were fulfilled for the 0.03 mg/L fortification level for 0.01 M $CaCl_2$ soil extracts. LOD was not determined.

Matrix effects
Not required as stable-isotope labelled internal standards were used for quantification.

Conclusion
The analytical method was successfully validated for the determination of AMPA in HPLC water, 0.01 M $CaCl_2$ solutions and 0.01 M $CaCl_2$ soil extracts at a limit of quantification (LOQ) of 0.03 mg/L. The analytical method fulfils the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits ; derivatisation efficiency was not assessed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of Glyphosate and AMPA in soil was previously evaluated at EU level.
The validation of the method for analysis of AMPA in aqueous solutions was not previously evaluated at EU level.
Both methods were performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev. 4). The methods are fit-for-purpose to support the environmental fate study concerned.

Assessment and conclusion by RMS:
The analytical method used in report PR01/006 was successfully validated for the determination of glyphosate and AMPA in soil at a limit of quantification (LOQ) of 0.05 mg/kg. The analytical method fulfils requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4. However the derivatisation efficiency was not assessed and the equivalence of the linearity range in mg/kg is missing.

The analytical method used in report PR02/007 is not in agreement with the guidance SANCO 3029/99 rev.4 as the precision was non demonstrated, the derivatisation efficiency was not assessed and the equivalence of the linearity range in mg/kg is missing.

However, the recovery data are in acceptable range .

Therefore, the method PR01/006 can be considered as fir for purpose for the determination of AMPA in solution at the concentration tested. However, the derivatisation efficiency and the equivalence of the linearity range in mg/kg should be provided.
The method PR02/007 cannot be considered as fir for purpose

Determination of glyphosate and AMPA in water

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/018
Report author	[redacted]
Report year	1991
Report title	Estimation of glyphosate residues and aminmethylphosphonic acid (AMPA) residues in water, tapwater
Report No	PR90/002 Appendix
Test facility	Dr. Gerhard Krebs Analytik Eupener Strabe 150 5000 Koln 41
Document No	-
Guidelines followed in study	None (with relevance to analytical methods)
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Insufficient number of procedural recoveries at LOQ (n = 3– 4) • No chromatograms provided • Detailed linearity data is missing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/019 (CA 7.1.4.1.1/005)
Report author	[redacted]
Report year	1991
Report title	Behaviour of glyphosate in water and soil, Part 4, leaching behaviour
Report No	PR90/002 Part 4
Test facility	Dr. Gerhard Krebs Analytik Eupener Strabe 150 5000 Koln 41
Document No	-
Guidelines followed in study	BA-guideline for testing of pesticides Part IV 4-2
Deviations from current test guideline	see [redacted] 1991 (PR90/002 Appendix)
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)

Data point	CA 4.1.2/020 (CA 7.2.1.1/006)
Report author	[redacted]
Report year	1991
Report title	Behaviour of glyphosate in water and soil, Part 1, hydrolysis as a function of pH
Report No	PR90/002 Part 1
Test facility	Dr. Gerhard Krebs Analytik Eupener Strabe 150 5000 Koln 41
Document No	-
Guidelines followed in study	BBA-Guideline "Prüfung des Verhaltens von Pflanzenschutzmitteln in Wasser" (Merkblatt 55, part I and II)
Deviations from current test guideline	see [redacted] 1991 (PR90/002 Appendix)
Previous evaluation	Not 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)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate and AMPA in water by GC-ECD with a limit of quantification (LOQ) of 0.1 µg/L.
Acidified samples are spiked with internal standard (N-phosphonomethyl)-ß-alanine and evaporated to dryness. The remaining residue is derivatised with trifluoracetanhydride and trifluorethanol. An aliquot of the solution is concentrated by means of an evaporator. KH_2PO_4 buffer solution is added. Thereafter extracts are cleaned up using RP8-HPLC. To the collected fraction HPLC water is added and another clean up step using RP 18 cartridge is performed. The derivatised molecules are eluted with ethylacetate. Final extracts were determined by GC-ECD using internal standard procedures.

Chromatographic conditions**:**

GC:	Carlo Erba 2900
Column:	50 m OV 1701, 0.32 mm I.D., 0.6 µm
Carrier gas:	H_2, 0.6 bar
Temperature programm:	70 °C with 10 °C/min up to 140 °C with 5 °C/min to 200 °C (5 min)
Derivatisation agent (pre-column):	Trifluoracetanhydride, trifluorethanol
Injector:	On column
Detector:	ECD, 275 °C
Retention time:	Glyphosate: ~ 15.5 min AMPA: ~ 13.8 min

Findings

Recoveries

Water samples were spiked with AMPA at two fortification levels ranging from LOQ to approximately 10000 × LOQ. All average recovery values were between 70 % and 110 % with exception of the 1.0 µg/L fortification level for glyphosate with 113 % and the 0.1 µg/L fortification level for AMPA with 147 %. The detailed results are given in the table below.

Table 5.1-208: Results of method validation for the determination of glyphosate and AMPA in water

Matrix	Analyte	Fortification level (µg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Water	Glyphosate	0.1	90 – 120	108	15	14	4
		1.0	100 – 120	113	9.6	8.5	4
		10.0	86 – 125	106	17	16	4
		1000	92.6 – 97.2	94.5	2.0	2.1	4
	AMPA	0.1	90 – 200	147	55	38	3
		1.0	80 – 90	85.0	5.8	6.8	4
		10.0	83 – 86	85.0	1.4	1.7	4
		1000	84.8 – 103	94.8	8.9	9.4	4

[1] Single recovery values were calculated on the basis of the observed analytical values in [µg/L] with regard to the reported nominal values in [µg/L]

Specificity

The method consists of a derivatisation step which is considered to be specific to the target compounds. No residues ≥ LOQ were observed in the control samples, which would interfere with the determination of glyphosate and AMPA in water. Chromatograms of control samples were not provided.

Linearity

For calibration a standard solutions containing glyphosate, AMPA and the internal standard are prepared in tap water and treated in the same way as the sample. Calibration of the GC-system is made according to the internal standard method by means of the single point calibration procedure. Detailed linearity data were not provided.

Repeatability (Precision)

The relative standard deviations (RSDs) of recovery values at each fortification level were < 20 % with exception of the 0.1 µg/L fortification level of AMPA with 38 %. Therefore the method complies largely with EU guideline document SANCO/3029/99 rev. 4.

Accuracy

Acceptable mean recovery values at LOQ and higher levels between 70% and 110% for glyphosate and AMPA were found for water with exception of the 1.0 µg/L fortification level for glyphosate with 113 % and the 0.1 µg/L fortification level for AMPA with 147 %. Therefore the method complies largely with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection

The limit of quantification (LOQ) was set at 0.1 µg/L for glyphosate.

The LOQ at 0.1 µg/L for AMPA is not accepted as the recovery and precision data are higher than the maximum limits. The LOQ for AMPA can be set at 1 µg/L.
The LOD for glyphosate and AMPA was 0.02 µg/L and 0.06 µg/L, respectively.

Matrix effects
Standard solutions were prepared in the test system media (water) and treated in the same way as the samples. Furthermore, the internal standard procedure for quantification was performed. Both techniques in combination are suitable to cover any potential matrix effects.

Conclusion
The analytical method was successfully validated for the determination of glyphosate and AMPA in water at a limit of quantification (LOQ) of 0.1 µg/L and 1 µg/L, respectively. The analytical method fulfils the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits ; the detailed linearity data and chromatograms were not provided and derivatisation efficiency was not assessed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The studies were previously evaluated at EU level and considered acceptable. They were mostly performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (insufficient number of procedural recoveries at LOQ (n = 3 – 4), no chromatograms presented, detailed linearity data is missing). Nevertheless, the method is considered as fit-for-purpose to support the environmental fate studies concerned.

Assessment and conclusion by RMS:
The analytical method is not in agreement with the guidance SANCO 3029/99 rev.4 as the linearity plots and calibration function are not available, the specificity (interference) is not demonstrated.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

The recovery and precision are in acceptable range.

The method cannot be considered as fit for purpose for the determination of glyphosate and AMPA in water.

Study previously submitted to the EU

1. Information on the studies

Data point	CA 4.1.2/021
Report author	[redacted]
Report year	1987
Report title	Interlaboratory study for method validation of glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA) in environmental water
Report No	MSL-7200
Test facility	1/ Analytical biochemistry Laboratories P.O. Box 1097 Columbia, MO 65201 2/ Lab Services Division Oregon Department of Agriculture 635 Capital St. NE Salem, OR 97310-0110

	3/Monsanto Agricultural Company 700 Chesterfield village Parkway Chesterfield, Mo. 63198 4/ A&S Environmental Testing Maidencreek Industrial Park R.D. #2 Park Road Reading, PA 19605 5/ Califronia Analytical Laboratories 2544 Industrial Blvd. West Sacramento, CA 95691 6/ Craven Laboratories, Inc. 2800 Longhorn Blvd, Suite 103 Austin, TX 78759
Document No	-
Guidelines followed in study	None specified
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Mean recovery values in some cases not according to guideline requirements • Detailed linearity data is missing • Matrix effects and stability of analytes in sample extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Data point	CA 4.1.2/022 (CA 7.2.2.3/022)
Report author	[redacted]
Report year	1989
Report title	Storage stability of glyphosate in environmental water
Report No	MSL-8626
Test facility	Monsanto Agricultural Company 800 N. Lindbergh Blvd. St.Louis, Missouri 63167
Document No	-
Guidelines followed in study	Guideline 171-4
Deviations from current test guideline	No guideline available
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

An inter-laboratory validation study was conducted for validation of glyphosate and AMPA in water. The repeatability and reproducibility of the method was determined for six laboratories collaborating in this study. Glyphosate and AMPA are subsequently detected by HPLC with post-column reaction detection. The method was validated over the range 0.5 µg/L to 5000 µg/L and was used in the storage stability study MSL-8626.

Principle of the method
Analytical method was validated in study MSL-7200 for the determination of glyphosate and aminomethylphosphonic acid (AMPA) in water by HPLC-FD with a limit of quantification (LOQ) of 1 µg/L.
Water samples are evaporated to dryness by rotary evaporation and reconstituted in buffered solution (0.005 M KH_2PO_4 in 4% methanol/deionized water adjusted to pH 2.1 with concentrated phosphoric acid). EDTA is added to complex any species which may interfere with the chromatography. An aliquot of the concentrate is filtered and injected onto an Aminex A-9 cation exchange HPLC column to separate the glyphosate and AMPA for post-colum derivatization. A calcium hypochlorite solution was introduced into the stream to oxidize glyphosate to a primary amine prior to fluorogenic derivatization with o-phtalaldehyde (OPA). OPA also reacted with the metabolite, AMPA. This post-column reactor system is used to oxidize glyphosate to glycine and couple AMPA and glycine to o-phtalaldehyde. The formed fluorophors are determined by HPLC-FD with excitation at 340 nm and emission measured at 455 nm using external standard procedures.
Chromatographic conditions:

HPLC-FD:	Solvent buffer reservoir, HPLC pumps (2), Temperature controller for HPLC columns and oxidation reaction coil (Kratos URA 200 or equivalent), $Ca(OCl)_2$ solution reservoir, OPA solution reservoir, Post column derivatization system (Kratos URS 051-dual pump reagent delivery module or equivalent), 1 mL reaction coil (2), Fluorescence spectrometer (Perkin-Elmer LS-4 or equivalent)
Pre-column:	RP-18 Spheri-10, 3.6 cm x 4.6 mm I.D., Brownlee Labs Inc.
HPLC column:	Aminex A–9, 30 cm x 4.6 mm I.D., Bio-Rad Laboratories
Column temperature:	50 °C
Injection volume:	200 µL
Buffer flow rate:	0.5 mL/min
Derivatisation agent (post-column):	OPA/MERC
$Ca(OCl)_2$ flow rate:	0.5 mL/min
OPA flow rate:	0.5 mL/min
Detection wavelengths:	Excitation approx. 340 nm Emission: approx. 455 nm
Retention time:	Glyphosate: ~ 16 min AMPA: ~ 30 min

Findings
Recoveries
The method was initially validated by six different laboratories by analysis of freshly fortified samples at levels ranging from 0.5 µg/L to 5000 µg/L. The results of the analyses of glyphosate and AMPA are summarised in the table below. The average recovery values for glyphosate and AMPA were between 70 % and 110 %, with the exception of the determination of glyphosate at the lowest fortification level. Relatively high relative standard deviations were found at some fortification levels, this is because of outlying recovery values determined in laboratory 5, and also non-detect values for the lowest fortification levels. These outlying values were not considered for statistical evaluations.

Table 5.1-209: Results of the initial inter-laboratory method validation for the determination of glyphosate and AMPA in water by different laboratories (freshly fortified samples)

Matrix	Analyte	Fortification level (µg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses[2] (n)
Water	Glyphosate	0.5	90.0 – 154	119.2	22.9	19.2	10 (5)
		1	86.0 – 140	105.4	16.5	15.6	10 (5)
		5	95.6 – 136	108.6	12.8	11.8	11 (6)
		10	88.0 – 128	104.9	13.2	12.6	12 (6)
		50	86.0 – 134	101.8	12.5	12.3	12 (6)
		100	70.0 – 120	99.3	14.6	14.7	12 (6)
		500	90.4 – 134	104	13.5	13.0	12 (6)
		1000	79.2 – 130	94.6	13.3	14.1	12 (6)
		5000	67.2 – 132	97.4	16.2	16.6	11 (6)
Water	AMPA	0.5	72.0 – 108	86.3	11.8	13.7	8 (4)
		1	74.0 – 102	86.9	8.5	9.8	10 (5)
		5	86.0 – 164	100.1	21.5	21.4	11 (6)
		10	78.0 – 180	99.7	28.0	28.0	12 (6)
		50	78.0 – 96.4	89.9	6.5	7.3	12 (6)
		100	80.6 – 130	93.3	13.4	14.4	12 (6)
		500	88.2 – 110	95.5	6.2	6.5	12 (6)
		1000	82.8 – 120	93.4	10.6	11.4	12 (6)
		5000	67.2 – 106	93.0	10.9	11.7	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.

[2] Number of laboratories involved in the analyses is provided in brackets.

Results presented in the table above shows a wide range of recovery percentages. In order to verify the validation of the method in the different laboratory, results should be presented for each laboratory as follow:

Analyte Glyphosate	Recovery % (mean) (n≥2)					
Fortification level (µg/L)	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
0.5	115.5	93.5	190.5	103.5	n.d.	103
1	101.5	93	130	112.5	n.d.	90
5	96.8	98	156	101.5	92.7	94.85

10	96.5	93.5	125	107.5	115	91.9
50	93	99	121	108.6	92	97
100	88	99.5	103.75	98.675	106.3	89.275
500	94.8	101.9	108.6	94.4	129	95
1000	85.2	102.8	90.35	86.2	115	88.25
5000	92.4	99.15	95.98	92.31	108.7	96.55

Analyte AMPA	Recovery % (mean) (n≥2)					
Fortification level (µg/L)	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
0.5	90	90	117	95	n.d.	100
1	93.5	87	84	82.5	n.d.	87.5
5	89.5	82.5	125	83.5	154,0	95
10	99.5	84.5	82	90.5	150	91.9
50	92	91	87	93.1	83	93.2
100	103.75	95.75	88.25	89.075	90.5	84
500	91.2	96	96	93.5	104	92.5
1000	87	99.45	92.45	84.15	110	87.1
5000	91.8	97.65	88.14	95.265	107.3	95.4

The recovery percentages presented in the table above for each laboratory show that results are globally in acceptable limits for lab 1, 2, 4 and 6. However recovery percentages for lab 3 and 5 show results outside the acceptable range 70%-110 %. Thus the method cannot be validated for laboratory 3 and 5.

Additionally, procedural recoveries were determined concurrently with sample analysis of storage stability samples. The results are presented in the table below. The average recovery values for glyphosate and AMPA were between 70 % and 110 %, with the exception of the determination of glyphosate at the lowest fortification level. Standard deviations were not provided as the number of samples per level was lower than 3 samples.

Table 5.1-210: Results of the method validation for the determination of glyphosate and AMPA in water (procedural recoveries from fortified samples)

Matrix	**Analyte**	**Fortification level (µg/L)**	**Recovery[(a)]**				
			Range (%)	**Mean (%)**	**Standard deviation (%)**	**Relative standard deviation (%)**	**Number of analyses (n)**
Water	Glyphosate	0.5	118 – 146	132	–	–	2
		1	96.0 – 107	102	–	–	2
		5	96.0 – 103	99.6	–	–	2
		10	95.0 – 98.0	96.5	–	–	2
		50	92.0 – 94.0	93.0	–	–	2
		100	70.0 – 94.0	82.0	–	–	2
		500	93.6 – 96.0	94.8	–	–	2

Table 5.1-210: Results of the method validation for the determination of glyphosate and AMPA in water (procedural recoveries from fortified samples)

Matrix	Analyte	Fortification level (µg/L)	Recovery[(a)]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number of analyses (n)
		1000	79.2 – 91.2	85.2	–	–	2
		5000	67.2 – 103	85.2	–	–	2
Water	AMPA	0.5	76.0 – 108	92.0	–	–	2
		1	85.0 – 102	93.5	–	–	2
		5	94.0 – 96.0	95.0	–	–	2
		10	89.0 – 110	99.5	–	–	2
		50	92.0 – 92.0	92.0	–	–	2
		100	92.0 – 102	97.0	–	–	2
		500	88.8 – 93.6	91.2	–	–	2
		1000	82.8 – 91.2	87.0	–	–	2
		5000	67.2 – 106	86.4	–	–	2

(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

A confirmatory method is not considered necessary and is not specifically required for data generation methods. The UV-wavelengths chosen are specific for the analytes glyphosate and AMPA. The identification was based on the selected wavelengths and the retention times.. Under the described conditions the method is specific for the determination of glyphosate and AMPA in water. Presented control samples did not reveal any peaks > LOQ in the chromatogram, which would interfere with the determination of glyphosate and AMPA. Chromatograms have been provided for control, standard and recovery samples (report MSL-7200 and MSL-8626).

Linearity

Linearity of detector response was tested using at least 4 calibration standard concentrations prepared in the range of 0.25 µg/mL to 50 µg/mL. External standards prepared in 0.001 M EDTA solution were used for quantification with a coefficient of determination (r^2) of ≥ 0.99.
Detailed data for linearity were not provided (report MSL-7200 and MSL-8626).

Repeatability (Precision)

The relative standard deviations (RSDs) of recovery values at each fortification level were < 20% (report MSL-7200). Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.
No precision data were provided in the report MSL-8626.

Accuracy

Acceptable mean recovery values at LOQ and higher levels between 70 % and 110 % for glyphosate and AMPA were found for water in most cases. With exception of the 0.5 µg/L fortification level of glyphosate which accounts for 119 % (Report MSL-7200) and 132% (Report MSL-8626).

Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤20 %. These criteria were fulfilled for the 1 µg/L fortification level for water.

Matrix effects
Not assessed.

Conclusion
The analytical method does fulfil the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits; detailed linearity data not provided, derivatisation and matrix effect not assessed. The method is considered as fit-for-purpose for the determination of glyphosate and AMPA in water. However this method is not validated for the laboratory 3 and 5 in the inter-laboratory study.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The storage stability study was previously evaluated at EU level and not considered acceptable. Both studies were performed under GLP and meet current analytical requirements (EU guideline SANCO/3029/99 rev. 4) with deficits (mean recovery values in some cases not according to guideline requirements, detailed linearity data is missing, matrix effects and stability of analytes in sample extracts not assessed, efficiency of derivatisation not assessed). Nevertheless, the method is considered as fit-for-purpose to support the environmental fate studies concerned as the presented analytical data show the good performance of the method.

Assessment and conclusion by RMS:
The analytical method fulfills the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) with minor deficits; detailed linearity data not provided.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

For laboratory 3 and 5 the recoveries are not un acceotable range for several fortification levels.

For laboratory 1, 2, 4 and 6, the method is considered as fit-for-purpose for the determination of glyphosate and AMPA in water. However, the linearity range should be provided.
Concerning the laboratory 3 and 5, this method is not validated study

Determination of glyphosate in air (washing water)

Study previously submitted to the EU

1. Information on the studies

Data point	CA 4.1.2/023 (CA 7.3.1/004)
Report author	[redacted]
Report year	1996
Report title	Glyphosate volatilisation in the field
Report No	PR94/032
Test facility	Dr. Gerhard Krebs Analytik Eupener Str. 150 D-50933 Koln

Document No	-
Guidelines followed in study	None specified
Deviations from current test guideline	Yes (SANCO/3029/99 rev. 4) • Modifications in method • Limited calibration data • Stability of extracts not assessed • Efficiency of derivatisation not assessed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the analysis of glyphosate in water from gas washing bottles by GC-MS. Analysis of the samples was performed according to the previously (GLP) validated analytical method DrK097, with modifications.
For collection of air samples, air was sucked with water pumps through adsorbent water. This procedure was also done with fortified water samples, which means that recovery experiments were performed under enrichment conditions (35 °C, 80 % RH). After enrichment, internal standard (N-phosphomethyl-β-alanine) was added and the solution was acidified with phosphoric acid and evaporated to dryness. For derivatisation, trifluoracetic anhydride and trifluorethanol was added to the residue and the solution was incubated at 70 °C for 60 min. The derivatised sample was then cleaned up on silica gel column (in the original method, clean up was performed by HPLC). The eluate was further enriched using a RP18 cartridge. Finally the sample is eluted from the cartridge using acetic ester. Samples were then submitted to analysis by GC-MS (in the original method, analysis was done by GC-ECD).

Chromatographic conditions:

GC system:	Dani 86.10 with HP 5970 mass spectrometer
GC column:	OV 1701, 50 m, 0.32 mm ID, 0.6 µm film thickness
Injector:	PTV (Programmed Temperature Vaporising)
PTV program:	Total 80 °C with 300 °C/min to 270 °C (10 min) Split open after 4 min
Temperature program:	70 °C (4 min) with 10 °C/min to 250 °C (10 min)
Injection volume:	1 µL
Derivatisation agent:	Trifluoracetic anhydride (TFAA)
Retention times:	Glyphosate: ~ 18.5 min N-phosphomethyl-β-alanine (IS): ~ 20.4 min
Run time:	24 min
MS scan mode:	SIM
Monitored ions:	*m/z* 113
Dwell time:	400 msec
Multiplier voltage:	1400 V

Findings

Recoveries

All recovery experiments from spiked samples were conducted under worst-case enrichment conditions (35 °C, 80 % RH).

The original method DrK079 (GC-ECD) was validated for the study PR94/032 und GLP. Adsorbent water was fortified with glyphosate IPA salt at levels of 0.4 to 20 µg/L and enriched by air flow-through for 1 hour (2 samples at highest level for 6 hours, test of retention capacity). The average recovery values for glyphosate at each fortification levels and overall were between 70 % and 110 %. The detailed results are summarised in the table below. Control samples were analysed in triplicate without detecting glyphosate IPA salt above the LOD (< 0.2 µg/L).

Table 5.1-211: Results of method validation (spike recovery) for the determination of glyphosate IPA salt in adsorbent water (recoveries obtained from analysis using GC-ECD)

Matrix	Analyte	Fortification level (µg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Adsorbent water	Glyphosate IPA salt	0.4	75 – 105	90	12.2	13.6	4
		2	80 – 82	82	1.0	1.2	4
		20	83 – 108	95	11.0	11.6	4

[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.

Using the GC-MS method, one recovery sample was analysed at 20 µg/L with a 24 h enrichment time under worst-case enrichment conditions (35 °C, 80 % RH) concurrently with analyses of field samples. The fortified analyte was recovered at a rate of 74 %.

Specificity

The method consists of a derivatisation step which is considered to be specific to the target compound. Presented control samples did not reveal any peaks >LOQ in the chromatograms, which would interfere with the determination of glyphosate IPA salt.

Linearity

The linearity of the detector response was tested using 3 calibration standard concentrations prepared in in the range of 0.4 to 20 ng/mL prepared in water. Peak ratios of the analyte and internal standard were plotted. A linear response was found ($y = 0.0011 x – 0.0044$) with a coefficient of determination (r^2) of 1.000 (linearity parameters were calculated using Excel with peak areas provided in the report).

Repeatability (Precision)

The relative standard deviations (RSDs) of recovery values at each fortification level and overall were ≤20 % for the determination of glyphosate IPA salt in adsorbent water.

Accuracy

Acceptable overall mean recovery values of between 70 % and 110 % were found for glyphosate IPA salt.

Limit of Quantification and Detection

The limit of quantification (LOQ) was validated at 0.4 µg/L. The limit of detection (LOD) was stated as 0.2 µg/L.

Matrix effects

Not relevant for the analysis of adsorbent water.

Efficiency of derivatisation was not assessed.

Conclusion

The analytical method does fulfil the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000) with deficits; efficiency of derivatisation not assessed. Nevertheless the method is considered as fit-for-purpose for the determination of glyphosate IPA salt in adsorbent water.

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) with deficits (modifications of method, limited calibration data, stability of extracts not assessed, efficiency of derivatisation not assessed). Nevertheless the method is considered as fit-for-purpose to support the environmental fate study concerned.

Assessment and conclusion by RMS:

The method is validated according to SANCO/3029/99 rev.4. However, the equivalence of the linearity range in mg/kg are missing and should be provided.

Concerning derivatisation step, as the derivatisation step is an online part of the detection system, we can consider that the calibration has been done on derivatised species. Therefore, no further data required.

Data point:	CA 7.1.2.1.2/002
Report author	[redacted]
Report year	2020
Report title	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil
Report No	3202599
Document No	
Guidelines followed in study	OECD 307 EPA 835.4100 Commission Regulation (EU) No. 283/2013 Regulation (EC) No. 1107/2009 (2009)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Smithers ERS Limited, North Yorkshire, HG1 4LS, UK

Principle of the method

20 g (or 100 g for incubation vessels) dry weight equivalent of soil sample was transferred to plastic pots (recovery vessels fortified with known amounts of AMPA) and extracted with 200 mL (1000 mL for incubation vessels) 1M NaOH(aq) (minus the volume of water already present in the soil) for 20 minutes via mechanical agitation. A portion of extract was transferred into a centrifuge tube centrifuged at 1455 g for 5 minutes.

A portion of the resulting supernatant (3 mL) was cleaned-up via filtration (passed through a Macherery-Nagel™ Chromafil™ MV Cellulose Mixed Esters syringe filter; 2.5 mm diameter, 0.45 µm pore). The filtrate (1.7 mL) was acidified with ≥ 98 % formic acid (0.1 mL) and spiked with 0.5 µg/mL internal reference standard (0.2 mL). An aliquot (1 mL) was cleaned-up further by solid phase extraction, SPE (Strata-X 33u Polymeric RP 3 mL; 60 mg) prior to LC-MS/MS analysis.

Findings

Recoveries

The method proved to be suitable to determine residues of AMPA in soil.

Soil samples were spiked with AMPA at two fortification levels ranging from LOQ to approximately 62 × LOQ. All average recovery values (mean of 5 replicates per fortification level) were between 70 % and 110 %. The method complies with EU guideline document SANCO/3029/99 rev. 4. The detailed results are given in the table below.

Table 5.1-212: Results of method validation (spike recovery) for the determination of AMPA in soil (Quantification: 109.9 > 62.9 *m/z*)

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Soil WS	AMPA	0.05	96.8 – 104	99.2	2.8	2.8	5
		3.08	102 – 106	105	1.9	1.8	5

[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.

Table 5.1-213: Results of method validation (spike recovery) for the determination of AMPA in soil (Confirmation: 109.9 > 78.9 *m/z*)

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Soil WS	AMPA	0.05	97 – 102	99.8	2.1	2.2	5
		3.08	104 – 106	104	1.1	1.1	5

[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

Chromatograms of blank, of standards solution, of samples are provided. Control (blank) soil extracts were free from components that interfered with the analysis of AMPA. There were no interferences at the retention time of AMPA > 30 % of the LOQ therefore, the analytical procedure was considered specific for AMPA.

Linearity

The LC-MS/MS detector response for AMPA was found to be linear in the range of 0.001 to 0.4 µg/mL (equivalent to sample concentrations over the range of 0.0118 to 4.7 mg/kg, using a dilution factor of 11.8). The correlation coefficient (r) for the calibration lines was ≥ 0.995 (equivalent to a coefficient of determination (r2) of ≥ 0.99)

Repeatability (Precision)

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

Accuracy

The mean recovery ranged from 99.2 to 105 % and the % RSD ranged from 0.98 to 4.7 % for each soil and for each concentration.

18 Acres Soil: Transition 109.9->62.9m/z

Sequence/ Batch ID	Sample ID	Fortified Concentration (mg/kg)	Measured Concentration (mg/kg)	Recovery (%)
Val 1	Con A	0	0.00636	---
Val 1	Con B	0	0.00660	---
Val 1	F0.14 A	0.140	0.144	103
Val 1	F0.14 B	0.140	0.144	103
Val 1	F0.14 C	0.140	0.144	103
Val 1	F0.14 D	0.140	0.147	105
Val 1	F0.14 E	0.140	0.146	104
			Mean	**104**
			SD	1.01
			% RSD	0.975
Val 1	F1.4 A	1.40	1.43	102
Val 1	F1.4 B	1.40	1.45	104
Val 1	F1.4 C	1.40	1.47	105
Val 1	F1.4 D	1.40	1.47	105
Val 1	F1.4 E	1.40	1.47	105
			Mean	**104**
			SD	1.28
			% RSD	1.23
Val 1	F3.1 A	3.10	3.21	104
Val 1	F3.1 B	3.10	3.22	104
Val 1	F3.1 C	3.10	3.20	103
Val 1	F3.1 D	3.10	3.28	106
Val 1	F3.1 E	3.10	3.19	103
			Mean	**104**
			SD	1.14
			% RSD	1.10
		Overall	**Mean**	**104**
			SD	1.09
			% RSD	**1.05**

18 Acres Soil: Transition 109.9->78.8

Sequence/ Batch ID	Sample ID	Fortified Concentration (mg/kg)	Measured Concentration (mg/kg)	Recovery (%)
Val 1	Con A	0	0.00608	---
Val 1	Con B	0	0.00753	---
Val 1	F0.14 A	0.140	0.146	104
Val 1	F0.14 B	0.140	0.141	101
Val 1	F0.14 C	0.140	0.145	104
Val 1	F0.14 D	0.140	0.146	104
Val 1	F0.14 E	0.140	0.149	106
			Mean	**104**
			SD	2.06
			% RSD	1.98
Val 1	F1.4 A	1.40	1.41	101
Val 1	F1.4 B	1.40	1.46	104
Val 1	F1.4 C	1.40	1.49	106
Val 1	F1.4 D	1.40	1.48	106
Val 1	F1.4 E	1.40	1.48	106
			Mean	**105**
			SD	2.29
			% RSD	2.19
Val 1	F3.1 A	3.10	3.21	104
Val 1	F3.1 B	3.10	3.11	100
Val 1	F3.1 C	3.10	3.12	101
Val 1	F3.1 D	3.10	3.24	105
Val 1	F3.1 E	3.10	3.23	104
			Mean	**103**
			SD	**2.01**
			RSD %	**1.96**
		Overall	**Mean**	**104**
			SD	2.13
			% RSD	**2.05**

Brierlow soil: transition 109.9->62.9m/z

Sequence/ Batch ID	Sample ID	Fortified Concentration (mg/kg)	Measured Concentration (mg/kg)	Recovery (%)
Val 2	Con C	0	0.0161	---
Val 2	Con D	0	0.0147	---
Val 2	F0.14 F	0.140	0.142	101
Val 2	F0.14 G	0.140	0.144	103
Val 2	F0.14 H	0.140	0.148	106
Val 2	F0.14 I	0.140	0.143	102
Val 2	F0.14 J	0.140	0.150	107
			Mean	**104**
			SD	2.45
			% RSD	2.36
Val 2	F1.4 F	1.40	1.40	100
Val 2	F1.4 G	1.40	1.34	95.7
Val 2	F1.4 H	1.40	1.42	101
Val 2	F1.4 I	1.40	1.37	97.9
Val 2	F1.4 J	1.40	1.50	107
			Mean	**100**
			SD	4.33
			% RSD	4.31
Val 2	F3.1 F	3.10	3.10	100
Val 2	F3.1 G	3.10	3.14	101
Val 2	F3.1 H	3.10	3.12	101
Val 2	F3.1 I	3.10	3.04	98.1
Val 2	F3.1 J	3.10	3.04	98.1
			Mean	**99.6**
			SD	1.49
			% RSD	1.49
		Overall	**Mean**	**101**
			SD	3.37
			% RSD	**3.34**

Brierlow soil: transition 109.9->62.9m/z

Sequence/ Batch ID	Sample ID	Fortified Concentration (mg/kg)	Measured Concentration (mg/kg)	Recovery (%)
Val 2	Con C	0	0.0139	---
Val 2	Con D	0	0.0146	---
Val 2	F0.14 F	0.140	0.140	100
Val 2	F0.14 G	0.140	0.152	109
Val 2	F0.14 H	0.140	0.145	104
Val 2	F0.14 I	0.140	0.139	99.3
Val 2	F0.14 J	0.140	0.154	110
			Mean	**104**
			SD	4.87
			% RSD	4.67
Val 2	F1.4 F	1.40	1.42	101
Val 2	F1.4 G	1.40	1.41	101
Val 2	F1.4 H	1.40	1.44	103
Val 2	F1.4 I	1.40	1.37	97.9
Val 2	F1.4 J	1.40	1.38	98.6
			Mean	**100**
			SD	2.06
			% RSD	2.05
Val 2	F3.1 F	3.10	3.08	99.4
Val 2	F3.1 G	3.10	3.05	98.4
Val 2	F3.1 H	3.10	3.14	101
Val 2	F3.1 I	3.10	3.02	97.4
Val 2	F3.1 J	3.10	3.08	99.4
			Mean	**99.2**
			SD	1.44
			% RSD	1.45
		Overall	**Mean**	**101**
			SD	3.71
			% RSD	**3.67**

Limit of Quantification and Detection

The LOQ corresponded to the lowest fortification level where an acceptable mean recovery (70 to 110 %) and % RSD of ≤ 20 % was achieved. The LOQ is 0.14 mg/kg in dry soil (equivalent to 5 % of the nominal applied amount of test item)

Matrix effects

he matrix effect was significant (> 20 %) for Brierlow soiwhen comparing peak areas. Since AMPA and the internal reference standard were equally suppressed correcting for matrix effects, peak area ratios were compared, resulting in a protocol deviation; which states to compare peak area to assess soil matrix effects. The matrix effect was not significant (< 20 %) for either soil when comparing peak area ratios, with and without soil matrix. Since peak area ratios and not peak areas are used for quantification, the matrix effect had no significant effect on the analysed sample concentration, therefore, non-matrix matched standards were used for the quantification of AMPA.

Conclusion

The analytical method was successfully validated for the determination of AMPA in soil at a limit of quantification (LOQ) of 0.14 mg/kg. The analytical method fulfils the European requirements for risk assessment methods as outlined in SANCO/3029/99 rev. 4 (11/July/2000).

Assessment and conclusion by RMS:
The analytical method is validated according to the guidance SANCO 3029/99 rev.4 with an LOQ of 0.14mg/kg.

B.5.1.2.5 Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

During the conduct of physical and chemical properties tests analyses of the administered doses are necessary in some cases of study types. Analytical results provided were reviewed according SANCO/3029/99 rev.4, probable deviations from current guideline were reported.

All submitted physical and chemical properties tests were searched for analytical results. Where analytical results were available within the reports a summary of the analytical method used was written. In some cases analytical determinations were expected but no analytical information was presented within the relevant study reports. Furthermore in some cases analysis was performed but the given information was insufficient to write a summary.

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**
CA 4.1.2/215 (CA 2.5/001)	[redacted] 2020 Report No. 139K-101	Determination of the water solubility of glyphosate by the shake flask method	N/A [redacted] 2020 Report No. 139K-101	LC-MS/MS LOQ 60 mg/L 60-120 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/216 (CA 2.5/004)	[redacted] 2020 Report No. 139K-107	Determination of the water solubility of glyphosate ammonium salt by the shake flask method	N/A [redacted] 2020 Report No. 139K-107	LC-MS/MS LOQ 60 mg/L 60-120 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/217 (CA 2.5/008)	[redacted] 2020a Report No. 89593	Determination of the water solubility of HMPA (hydroxymethylphosphonic acid) by the shake flask method	N/A [redacted] 2020a Report No. 89593	LC-MS/MS LOQ 500 µg/L 500 - 990 µg/L LOQ 510 µg/L 510 - 1000 µg/L	No	Method fit-for-purpose	Y

CA 4.1.2/218 (CA 2.7/001)	[redacted] 2020 Report No. 139K-102	Determination of the n-octanol/water partition coefficient of glyphosate using the shake flask method	N/A [redacted] 2020 Report No. 139K-102	LC-MS/MS LOQ 100 mg/L 100-600 mg/L LOQ 0.3-1.2 µg/L 0.3-6.0 µg/L	No	Method fit-for-purpose	Y
CA 4.1.2/219 (CA 2.7/006)	[redacted] 2012 Report No. 497741	Determination of physico-chemical properties of glyphosate potassium salt	[redacted] 2012 Report No. 497741	HPLC-UV LOQ 5 mg/L 5-100 mg/L	Yes		Y
CA 4.1.2/220 (CA 2.7/008)	[redacted] 2020 Report No. 139K-104	Determination of the n-octanol / water partition coefficient of N-acetyl glyphosate using the shake flask method	[redacted] 2020 Report No. 139K-104	LC-MS/MS LOQ 100 mg/L 100-500 mg/L LOQ 0.024-0.08 µg/L 0.024-0.4 µg/L	No	Method fit-for-purpose	Y
CA 4.1.2/221 (CA 2.7/009)	[redacted] 2020 Report No. 139K-103	Determination of the n-octanol / water partition coefficient of AMPA using the shake flask method	[redacted] 2020 Report No. 139K-103	LC-MS/MS LOQ 40 mg/L 40-600 mg/L LOQ 1.5-5 µg/L 1.5-22.5 µg/L	No	Method fit-for-purpose	Y
CA 4.1.2/222 (CA 2.7/010)	[redacted] 2020 Report No. 89592	Determination of the n-octanol/water partition coefficient of HMPA using the shake flask method	[redacted] 2020 Report No. 89592	LC-MS/MS LOQ 50 mg/L 500-1000 mg/L LOQ 0.57 mg/L 0.57-5.7 mg/L	No	Method fit-for-purpose	Y
CA 4.1.2/143 (CA 6.5.1/001)	[redacted] 2020 Report No. S19-22457	AMPA and N-Acetyl AMPA hydrolysis under typical conditions (pH, temperature and time) of processing	N/A [redacted] 2020 Report No. S19-22457	LC-MS/MS *LOQ 0.05 mg/kg* *0.05-1.1 mg/kg*	Yes		Y

Determination of glyphosate in buffered water (pH 9)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/215 (CA 2.5/001)
Report authors	[redacted]
Report year	2020
Report title	Determination of the water solubility of glyphosate by the shake flask method
Report No	139K-101
Document No	-
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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	Eurofins EAG Agroscience, LLC 8598 Commerce Drive Easton, Maryland 21601 USA

2. Full summary of the study according to OECD format

Principle of the method
Analytical method was developed for the determination of glyphosate in buffered water (pH 9) by LC-MS/MS. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Agilent 1200 Infinity High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 5000 LC-MS/MS (and QJet Ion Guide) operated in the multiple reaction monitoring (MRM) mode
HPLC column:	Thermo Acclaim Trinity Q1 (100 × 3.0 mm ID, 3 µm particle size)
Column temperature:	40 °C
Mobile phase:	A: 50 mM Ammonium Formate in HPLC grade water (pH 2.9) B: 0.1% Formic Acid in Acetonitrile

Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.00	100	0	0.35
	3.00	100	0	0.35
	3.01	0	100	0.35
	4.00	0	100	0.35
	4.01	100	0	0.35
	8.00	100	0	0.35
Injection volume:	5.0 µL			
Detection:	Multiple reaction monitoring (MRM) mode			
Ion source:	Turbo V Ion Source			
Monitored transitions:	168.0 → 63.0 (dwell time 200.0 msec for all transitions)			
Retention time:	Glyphosate: approx. 3.0 min			

Findings

Recoveries

The method proved to be suitable to determine residues of **glyphosate** in buffered water (pH 9). Samples were spiked with the analyte at two fortification levels from 60 mg/L to 120 mg/L. The recovery values were between 70 % and 110 % at fortification level of 60 mg/L. For the high level fortification samples at a nominal concentration of 120 mg/mL, the slightly low recoveries (<70 %, with a mean of 65.4 %) were attributed to prepared concentrations that approached the solubility limit, and the recoveries did not reflect a limitation of the analytical methodology. The detailed results are given in the table below.

Table 5.1-214: Recovery results of the method validation for the determination of glyphosate in buffered water (pH 9)

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Buffered water (pH 9)	Glypohsate	Blank control	< LOQ	< LOQ	–	–	3
		60	92.4 – 99.1	95.9	2.6	2.7	7
		120	64.9 – 66.1	65.4	0.6	1.0	3
		Overall	64.9 – 99.1	86.7	14.9	17.2	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 method allows the determination of Glyphosate acid using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required. Under the described conditions, the method is highly specific for the determination of glyphosate acid in buffed water (pH 9). No significant interference was observed in chromatograms (chromatograms of glyphosate standard, representative chromatograms of low level and high level calibration standards, representative chromatogram for matrix blanck sample : pH 9 buffer, representative chromatogram of glyphosate at pH 9 shake flask solubility sample)

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 1.0 mg/L to 10.0 mg/L with correlation coefficient of > 0.99. The calibration standards were prepared in reagent water. No

information regarding dilution for samples outside the calibration range. Liner curve: y = 220598 x + 7914.66 (x: concentration (mg/L), y: peak area, r = 0.9989890).

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level 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 as the concentration of the lowest level fortification as 60.0 mg/L. Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Conclusion
The analytical method was successfully validated for the determination of glyphosate in buffered water (pH 9) as test medium. The method validation meets criteria set in SANCO/3029/99 rev. 4, it is adequate to support the water solubility study concerned.

3. Assessment and conclusion

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

Assessment and conclusion by RMS:
The analytical method for the determination of glyphosate in water solubility samples was not fully in agreement with the SANCO 3029/99 rev.4. Indeed the number of replicates by fortification level is < 5 for the high level. However, the number of replicates for both fortification levels is equal to 10 and it is considered sufficient to validate the recovery and the repeatability. The method is considered as fit for purpose with LOQ = 60 mg/L The method is considered as adequate to support the water solubility concerned.

Determination of glyphosate ammonium salt in buffered water (pH 5, 7 and 9)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/216 (CA 2.5/004)
Report authors	[redacted]
Report year	2020
Report title	Determination of the water solubility of glyphosate ammonium salt by the shake flask method
Report No	139K-107
Document No	MSL0030983
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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	Eurofins EAG Agroscience, LLC 8598 Commerce Drive Easton, Maryland 21601 USA

2. Full summary of the study according to OECD format

Principle of the method
Analytical method was developed for the determination of Glyphosate ammonium salt in buffered water (pH 5, 7 and 9) by LC/MS/MS. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Agilent 1200 Infinity High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 5000 LC-MS/MS (and QJet Ion Guide) operated in the multiple reaction monitoring (MRM) mode
HPLC column:	Thermo Acclaim Trinity Q1 (100 × 3.0 mm ID, 3 µm particle size)
Column temperature:	40 °C
Mobile phase:	A: 50 mM Ammonium Formate in HPLC grade water (pH 2.9) B: 0.1% Formic Acid in Acetonitrile

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.00	100	0	0.35
3.00	100	0	0.35
3.01	0	100	0.35
4.00	0	100	0.35
4.01	100	0	0.35
8.00	100	0	0.35

Injection volume:	5.0 µL
Ion source:	Turbo V Ion Source
Detection:	Multiple reaction monitoring (MRM) mode
Monitored transitions:	168.0 → 63.0 (dwell time 200.0 msec for all transitions)
Retention time:	Glyphosate: approx. 3.0 min

Findings
Recoveries
The method proved to be suitable to determine residues of glyphosate ammonium salt in buffered water (pH 5, 7 and 9). Samples were spiked with the analyte at two fortification levels from 60 mg/L to 120 mg/L. The recovery values were between 70 % and 110 % at both fortification levels and overall. The detailed results are given in the table below.

Table 5.1-215: Recovery results of the method validation for the determination of glyphosate ammonium salt in buffered water (pH 5, 7 and 9)

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Buffered water (pH 5)	Glypohsate ammonium salt (measured as glyphosate)	Blank control	< LOQ	< LOQ	–	–	2
		60	87.1 – 105	95.3	5.5	5.8	7
		120	87.8 – 91.0	90.0	1.8	2.1	3
		Overall	87.1 – 105	93.7	5.3	5.7	10
Buffered water (pH 7)	Glypohsate ammonium salt (measured as glyphosate)	Blank control	< LOQ	< LOQ	–	–	2
		60	91.6 – 106	96.7	4.5	4.7	7
		120	94.4 – 98.4	96.5	2.0	2.1	3
		Overall	91.6 – 106	96.6	3.8	3.9	10
Buffered water (pH 9)	Glypohsate ammonium salt (measured as glyphosate)	Blank control	< LOQ	< LOQ	–	–	3
		60	90.7 – 97.3	94.4	2.8	3.0	7
		120	89.6 – 92.7	91.3	1.6	1.7	3
		Overall	89.6 – 97.3	93.5	2.6	3.0	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 method allows the determination of glyphosate ammonium using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required. Under the described conditions, the method is highly specific for the determination of glyphosate ammonium in buffed water (pH 5, 7 and 9). No significant interference was observed in chromatograms. (chromatograms of glyphosate standard, representative chromatograms of low level and high level calibration standards, representative chromatogram for matrix blanck sample : pH 5, pH 7 and pH 9 buffer, representative chromatogram of glyphosate at pH 5, 7 and 9 after 48-hr solubility)

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 1.0 mg/L to 10.0 mg/L with correlation coefficient of > 0.99. The calibration standards were prepared in reagent water. No information regarding dilution for samples outside the calibration range.
Linear curve: y = 203000 x + 1990 (x: concentration (mg/L), y: peak area, r = 0.9986)

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 as the concentration of the lowest level fortification as 60.0 mg/L. Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Conclusion
The analytical method was successfully validated for the determination of glyphosate ammonium salt in buffered water (pH 5, 7 and 9) as test mediums. The method validation meets criteria set in SANCO/3029/99 rev. 4, it is adequate to support the water solubility study concerned.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was not previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev.4). The method is considered as adequate to support the water solubility.

Assessment and conclusion by RMS:
The analytical method for the determination of glyphosate in water solubility samples was not fully in agreement with the SANCO 3029/99 rev.4. Indeed the number of replicates by fortification level is < 5 for the high level. However, the number of replicates for both fortification levels is equal to 10 and it is considered sufficient to validate the recovery and the repeatability. The method is considered as fit for purpose with LOQ = 60 mg/L The method is considered as adequate to support the water solubility concerned.

Determination of HMPA in buffered water (pH 4, 7 and 9)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/217 (CA 2.5/008)
Report authors	[redacted]
Report year	2020
Report title	Determination of the water solubility of HMPA (hydroxymethylphosphonic acid) by the shake flask method
Report No	89593
Document No	MSL0030981
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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	Eurofins EAG Agroscience, LLC 7200 E. ABC Lane Columbia, Missouri 65202

2. Full summary of the study according to OECD format

Principle of the method
Analytical method was developed for the determination of HMPA (hydroxymethylphosphonic acid) in buffered water (pH 4, 7 and 9) by LC-MS/MS. The analytical method consisted of diluting the samples in water. The test

samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Applied Biosystems/Sciex API 5000
HPLC column:	Waters Torus DEA (100 × 3.0 mm ID, 1.7 µm particle size)
Column temperature:	40 °C
Mobile phase:	A: 0.9% Formic Acid + 50 mM Ammonium Formate in water B: 0.9% Formic Acid in Acetonitrile

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.00	100	0	0.5
3.00	100	0	0.5
3.01	5	95	0.5
5.00	5	95	0.5
5.01	100	0	0.5
7.00	100	0	0.5

Injection volume:	10 µL
Ion source:	Turbo Spray (negative polarity)
Detection:	Multiple reaction monitoring (MRM) mode
Monitored transitions:	MS: 111.0 → 63.0 (primary) MS: 111.0 → 79.0 (confirmatory) (dwell time 500.0 msec for all transitions)
Retention time:	HMPA: approx. 2.0 min

Findings

Recoveries (accuracy)

The method proved to be suitable to determine residues of **HMPA** in buffered water (pH 4, 7 and 9). Samples were spiked with the analyte at two fortification levels from 60 mg/L to 120 mg/L. The recovery values were between 70 % and 110 % at both fortification levels and overall. The detailed results are given in the table below.

Table 5.1-216: Recovery results of the method validation for the determination of HMPA in buffered water (pH 4, 7 and 9)

Matrix	Analyte	Fortification level (µg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Buffered water (pH 4)	HMPA	Blank control	< LOQ	< LOQ	–	–	1
		990	90.8 – 96.9	94.1	2.7	2.6	5
		500	100.6 – 104.6	103.1	2.2	2.2	3
		Overall	90.8 – 104.6	97.5	5.2	5.4	8
Buffered water (pH 7)	HMPA	Blank control	< LOQ	< LOQ	–	–	1
		1000	100.7 – 110.4	103.9	3.8	4.0	5
		510	106.6 – 113.2	109.4	3.1	3.4	3
		Overall	91.6 – 106	106.0	4.5	4.2	8

Table 5.1-216: Recovery results of the method validation for the determination of HMPA in buffered water (pH 4, 7 and 9)

Matrix	Analyte	Fortification level (µg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Buffered water (pH 9)	HMPA	Blank control	< LOQ	< LOQ	–	–	1
		1000	92.1 – 95.6	94.1	1.5	1.4	5
		510	105.5 – 111.8	108.6	2.9	3.2	3
		Overall	92.1 – 111.8	99.5	7.8	7.8	8

[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 method allows the determination of HMPA using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required. Under the described conditions, the method is highly specific for the determination of HMPA in buffed water (pH 4, 7 and 9). No significant interference was observed in chromatograms. (chromatograms of definitive test samples (pH4, 7 and 9), representative chromatograms of low level and high level calibration standards, representative chromatogram for matrix blanck sample : pH 4, pH 7 and pH 9 buffer)

Linearity
Linearity of detector response was tested using five calibration standard concentrations in the range of 26 ng/L to 1000 ng/L with correlation coefficient of >0.99. The calibration standards were prepared in reagent water. No information regarding dilution for samples outside the calibration range.
Linear curve: y = 977.439 x + 45014.35 (x: concentration (mg/L), y: peak area, r = 0.99782262)

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level 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 as the concentration of the lowest level fortification as 500 mg/L under pH 4 buffered water, 510 mg/L under pH 7 buffered water and pH 9 buffered water. Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solutions.

Conclusion
The analytical method was successfully validated for the determination of HMPA in buffered water (pH 4,7 and 9) as test mediums. The method validation meets criteria set in SANCO/3029/99 rev. 4, it is adequate to support the water solubility study concerned.

3. Assessment and conclusion

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

Assessment and conclusion by RMS:
The analytical method for the determination of glyphosate in water solubility samples was not fully in agreement with the SANCO 3029/99 rev.4. Indeed the number of replicates by fortification level is < 5 for the high level. However, the number of replicates for both fortification levels is equal to 8 and it is considered sufficient to validate the recovery and the repeatability. The method is considered as fit for purpose with LOQ = 500 mg/L for pH 4 and 510mg/L for pH 7 and 9 The method is considered as adequate to support the water solubility concerned.

Determination of glyphosate in buffered *n*-octanol/water (pH 5, 7 and 9)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/218 (CA 2.7/001)
Report authors	[redacted]
Report year	2020
Report title	Determination of the *n*-octanol/water partition coefficient of glyphosate using the shake flask method
Report No	139K-102
Document No	
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
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	Eurofins EAG Agroscience, LLC 8598 Commerce Drive Easton, Maryland 21601 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 *n*-octanol/water (buffered at pH 5, 7 and 9) by LC-MS/MS. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Agilent 1200 Infinity High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 5000 LC/MS/MS (and QJet Ion Guide) operated in the multiple reaction monitoring (MRM) mode
HPLC column:	Thermo Acclaim Trinity Q1 (100 × 3.0 mm, 3 µm particle size)
Column temperature:	40 °C

Mobile phase:	A: 50 mM Ammonium Formate in HPLC grade water (pH 2.9) B: 0.1% Formic Acid in Acetonitrile
Gradient:	Time (min) / Eluent A (%) / Eluent B (%) / Flow rate (mL/min) 0.00 / 100 / 0 / 0.35 3.00 / 100 / 0 / 0.35 3.01 / 0 / 100 / 0.35 4.00 / 0 / 100 / 0.35 4.01 / 100 / 0 / 0.35 8.00 / 100 / 0 / 0.35
Injection volume:	50.0 µL or 100 µL
Ion source:	Turbo V Ion Source
Detection:	Multiple reaction monitoring (MRM) mode
Monitored transitions:	168.0 → 63.0 (dwell time 200.0 msec)
Retention time:	Glyphosate: approx. 3.1 min

Findings

Recoveries (accuracy)

The method proved to be suitable to determine residues of **glyphosate** in buffered *n*-octanol/water (pH 5, 7 and 9). Samples were spiked with the analyte at two fortification levels in aqueous buffer saturated with *n*-octanol (pH 5, 7 and 9) and *n*-octanol saturated with aquous buffer (pH 5, 7 and 9), respectively. Matrix fortification samples aqueous phase buffer saturated with *n*-octanol gave good recoveries of 105 % of the nominal fortified concentration for each pH**.** Matrix fortification samples in *n*-octanol saturated with buffer yielded lower than expected recoveries of 56.2 %, 61.7 % and 42.4 % for *n*-octanol saturated with pH 5, 7 and 9 buffer, respectively. Due these low recoveries, the measured concentrations in the *n*-octanol phase of the definitive test vessels were adjusted for the overall mean *n*-octanol recovery of 53.4 % for determination of the partition coefficients and other calculations. The detailed results are given in the table below.

Table 5.1-217: Recovery results of the method validation for the determination of glyphosate in *n*-octanol/water (pH 5, 7 and 9)

Matrix	Analyte	Fortification level	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous buffer saturated with *n*-octanol (pH 5)	Glyphosate	Blank control	< LOQ	< LOQ	–	–	3
		100 mg/L	93.1 – 106	99.2	5.2	5.2	7
		600 mg/L	103 – 103	103	-	-	3
		Overall	93.1 – 106	100.4	4.6	4.6	10
Aqueous buffer saturated with *n*-octanol (pH 7)	Glyphosate	Blank control	< LOQ	< LOQ	–	–	3
		100 mg/L	91.0 – 113	102	7.1	7.0	7
		600 mg/L	98.9 – 113	107	6.1	5.7	6
		Overall	91.0 – 113	104	6.9	6.6	13
Aqueous buffer	Glyphosate	Blank control	< LOQ	< LOQ	–	–	3
		100 mg/L	90.9 – 106	101	5.2	5.1	7

Table 5.1-217: Recovery results of the method validation for the determination of glyphosate in *n*-octanol/water (pH 5, 7 and 9)

Matrix	Analyte	Fortification level	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
saturated with *n*-octanol (pH 9)		600 mg/L	99.3 – 105	101	0.6	1.0	3
		Overall	90.9 – 106	101	4.5	4.4	10
n-octanol saturated with pH 5 aqueous buffer	Glyphosate	Blank control	< LOQ	< LOQ	–	–	3
		1.20 µg/L	42.8 – 57.9	47.1	5.5	12	7
		6.0 µg/L	49.4 – 58.2	53.0	4.6	8.7	3
		Overall	42.8 – 58.2	48.8	5.7	11.7	10
n-octanol saturated with pH 7 aqueous buffer	Glyphosate	Blank control	< LOQ	< LOQ	–	–	3
		0.14 µg/L	52.3 – 68.9	59.6	7.1	12	7
		0.6 µg/L	62.8 – 65.6	64.2	1.4	2.2	3
		Overall	52.3 – 68.9	61.0	6.2	10.2	10
n-octanol saturated with pH 9 aqueous buffer	Glyphosate	Blank control	< LOQ	< LOQ	–	–	3
		0.3 µg/L	62.1 – 85.7	73.3	8.5	12	7
		3.0 µg/L	70.3 – 82.2	75.7	6.0	8.0	3
		Overall	62.1 – 85.7	74.0	7.6	10.3	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 method allows the determination of glyphosate using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required. Under the described conditions, the method is highly specific for the determination of glyphosate acid in buffered *n*-octanol/water (pH 5, 7 and 9)**.** No significant interference was observed in chromatograms. (chromatograms of low and high level glyphosate calibration standard, representative chromatograms of pH 5, 7 and 9 n-octanol quality control samples, representative chromatograms of pH 5, 7 and 9 aqueous quality control samples, representative chromatograms of pH 5, 7 and 9 partition test samples)

Linearity

Initially, six calibration standards of glyphosate in reagent water ranging in concentration from 1.00 to 40.0 µg a.i./L, were analysed concurrently with the feasibility trial samples, and an additional low standard at 0.500 µg a.i./L was also used for analysis of pH 5 definitive test *n*-octanol and aqueous samples and for the first run of pH 7 definitive test samples. However, pH 7 and pH 9 *n*-octanol sample concentrations were too low for this range, so all *n*-octanol samples from the pH 7 and pH 9 definitive tests were analysed separately from aqueous phase samples using six calibration standards ranging from 0.100 to 4.00 µg a.i./L. The LC-MS/MS injection volume was also increased to 100 µL for the pH 7 and pH 9 *n*-octanol sample analysis.
Linear curve (in pH 7 analysis): y = 16400 x+ 403 (x: concentration (mg/L), y: peak area, r = 0.9997)

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level 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 as the concentration of the lowest level fortification level for each matrix as following:

Aqueous buffer saturated with *n*-octanol (pH 5): 100 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 7): 100 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 9): 100 mg/L;
n-octanol saturated with pH 5 aqueous buffer: 1.20 µg/L;
n-octanol saturated with pH 7 aqueous buffer: 0.14 µg/L;
n-octanol saturated with pH 9 aqueous buffer: 0.3 µg/L.
Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Conclusion
The analytical method was successfully validated for the determination of glyphosate in buffered *n*-octanol/water (pH 5, 7 and 9)**.** Matrix fortification samples in n-octanol saturated with buffer yielded lower than expected recoveries. Due these low recoveries, the measured concentrations in the *n*-octanol phase of the definitive test vessels were adjusted for the overall mean *n*-octanol recovery of 53.4 % for determination of the partition coefficients and other calculations. The method validation meets criteria set in SANCO/3029/99 rev. 4, it is fit for purpose to support the *n*-octanol/water partition coefficients study concerned.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was not previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev.4). The method is considered as fit-for-purpose to support the *n*-octanol/water partition coefficients study concerned.

Assessment and conclusion by RMS:
The analytical method for the determination of glyphosate in **buffered *n*-octanol/water (pH 5, 7 and 9)** by LC-MS/MS is not fully in agreement with SANCO/3029/99 rev.4. Indeed, the number of replicates by fortification level is < 5 for the high level. However the number of replicates for both levels us equal to 10 and it is considered as sufficient. For n-octanol saturated with aqueous buffer at pH 5 and 7, the mean recoveries are below the acceptable range.
The method is considered as fit for purpose to support the aqueous buffer saturated with n-octanol for all pH and n-octanol saturated with aqueous buffer at pH 9. For n-octanol saturated with aqueous buffer at pH 7 and 5, the method can be considered fit for purpose with an adjustement of the measured concentration.

Determination of glyphosate potassium salt in buffered water (pH 7)

Study previously submitted to the EU

1. Information on the study

Data point	CA 4.1.2/219 (CA 2.7/006)
Report authors	[redacted]
Report year	2012
Report title	Determination of physico-chemical properties of glyphosate potassium salt
Report No	497741

Document No	-
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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	NOTOX B.V. Hambakenwetering 7 5231 DD s-Hertogenbosch The Netherlands

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of glyphosate potassium in 0.01 M phosphate buffer pH 7 by HPLC-UV. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analyzed in HPLC-UV.

Chromatographic conditions**:**

HPLC system:	Alliance Separation Module 2695 (Waters, Milford, MA, USA)
HPLC column:	Symmetry Shield RP-18, (150 × 3.0 mm, 5 µm particle size)
Column temperature:	40 °C ± 1 °C
Mobile phase:	Acetonitrile/water with 0.5% H_3PO_4 (40/60, v/v)
Flow rate:	0.45 mL/min
Injection volume:	25 µL
Detection:	UV at wavelength: 200 nm
Retention time:	Approx. 1.7 min

Findings
Recoveries
The method proved to be suitable to determine residues of glyphosate in phosphate buffer pH 7 in the target concentration range of 5.01 to 100 mg/L. The recovery values were between 70 % and 110 % at both fortification levels and overall.

Table 5.1-218: Recovery results of the method validation for the determination of glyphosate potassium salt in buffered water (pH 7)

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Buffered water (pH 7)	Glyphosate potassium	5.01	84 – 88	86	1.5	1.9	5
		100	92 – 96	95	1.7	1.8	5

Table 5.1-218: Recovery results of the method validation for the determination of glyphosate potassium salt in buffered water (pH 7)

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	salt (analyzed as acid form)	Overall	84 – 96	90	5.2	5.7	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 chromatogram of the blank sample showed no peak at the retention time of the test substance. Since no interferences were detected, the specificity requirements were met and the analytical method was found to be specific for the test substance. No significant interference was observed in chromatograms (chromatogram of the blank accuracy and 1000 mg/L test substance solution)

Linearity
Five calibration solutions in the concentration range of 1.6 to 100 mg/L were prepared from two stock solutions. The end solution of the calibration solutions was 40/60 (v/v) acetonitrile/phosphate buffer pH 7 containing 0.5 % H_3PO_4.
Linear curve: y = 3610 x+ 7660 (x: test substance concentration, y: response, r = 0.9995)

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

Limit of Quantification and Detection
The limit of quantification (LOQ) was assessed at 5 mg/L in phosphate buffer pH 7. Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Stability of the analytical system and end solutions
Since the coefficient of variation at both concentration levels was ≤ 20 % the analytical system and end solutions were stable over at least a 3.49 hour time interval.

Stability of stock solutions
The coefficient of variation on the response factors of the calibration solutions prepared with fresh and stored stock solutions was 2.7 %. Since the value was ≤ 10 % the stock solutions were stable when stored at room temperature for at least 9 days.

Conclusion
The analytical method was successfully validated for the determination of glyphosate potassium salt in **in buffered pH 7 water**. The method validation meets criteria set in SANCO/3029/99 rev. 4,

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was not previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev.4). The method is considered as adequate to support the *n*-octanol/water partition coefficient study concerned.

Assessment and conclusion by RMS:

The analytical method for the determination of glyphosate potassium salt (analyzed as acid form) in buffered water (pH 7) by HPLC-UV was validated for linearity, accuracy, precision and specificity based on SANCO/3029/99 rev.4.

Determination of *N*-acetyl glyphosate in buffered *n*-octanol/water (pH 5, 7 and 9)

Study submitted to the EU for the first time

1. Information on the study

Data point	CA 4.1.2/220 (CA 2.7/008)
Report authors	[redacted]
Report year	2020
Report title	Determination of the n-octanol / water partition coefficient of N-acetyl glyphosate using the shake flask method
Report No	139K-104
Document No	-
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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	Eurofins EAG Agroscience, LLC 8598 Commerce Drive Easton, Maryland 21601 USA

2. Full summary of the study according to OECD format

Principle of the method
An analytical method was developed for the determination of *N*-acetyl glyphosate in *n*-octanol/water (buffered at pH 5, 7 and 9) by LC-MS/MS. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Agilent 1200 Infinity High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 5000 LC/MS/MS (and QJet Ion Guide) operated in the multiple reaction monitoring (MRM) mode

HPLC column:	Thermo Hypercarb (100 × 2.1 mm, 3 µm particle size)
Column temperature	40 °C
Mobile phase:	A: 50 mM Ammonium formate in HPLC grade water (pH 2.9) B: 0.1% Formic acid in acetonitrile

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.00	100	0	0.2
2.00	100	0	0.2
3.00	30	70	0.2
4.00	30	70	0.2
4.01	100	0	0.2
9.00	100	0	0.2

Injection volume:	100 µL for octanol sample analyses 50 µL for aqueous sample analyses
Ion source:	Turbo V Ion Source
Detection:	Multiple reaction monitoring (MRM) mode
Monitored transitions:	*m/z* 210.0 → 63.0 (dwell time 500.0 msec)
Retention time:	*N*-acetyl glyphosate: 2.4 minutes (octanol phase sample analyses) *N*-acetyl glyphosate: 2.1 minutes (aqueous phase sample analyses)

Findings

Recoveries (accuracy)

The method proved to be suitable to determine residues of *N*-acetyl **glyphosate** in buffered *n*-octanol/water (pH 5, 7 and 9). Samples were spiked with the analyte at two fortification levels in aqueous buffer saturated with *n*-octanol (pH 5, 7 and 9) and *n*-octanol saturated with aquous buffer (pH 5, 7 and 9), respectively**.** Mass balance was obtained for *N*-acetyl glyphosate in all test vessels, with mean recoveries of nominal fortified *N*-acetyl glyphosate mass of 103 %, 108 % and 110 % for pH 5, 7 and 9, respectively. Matrix fortification samples in aqueous phase buffer saturated with *n*-octanol gave good recoveries of 104 %, 106 %, and 111 % of the nominal concentrations for pH 5, 7 and 9, respectively. Matrix fortification samples in *n*-octanol saturated with buffer yielded lower than expected recoveries of 61.3 %, 54.0 % and 55.5 % for *n*-octanol saturated with pH 5, 7 and 9 buffer, respectively. Due to these low recoveries, the measured concentrations in the *n*-octanol phase of the definitive test vessels were adjusted for the overall mean recovery of 56.9 % for determination/calculation of the partition coefficients.

Table 5.1-219: Recovery results of the method validation for the determination of *N*-acetyl glyphosate in *n*-octanol/water (pH 5, 7 and 9)

Matrix	Analyte	Fortification level	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous buffer saturated with *n*-octanol (pH 5)	*N*-acetyl glypohsate	Blank control	< LOQ	< LOQ	–	–	3
		100 mg/L	91.3 – 95.6	93.7	1.6	1.7	7
		500 mg/L	88.7 – 95.5	91.3	3.7	4.0	3
		Overall	88.7 – 95.6	93.0	2.5	2.7	10
Aqueous buffer saturated with *n*-octanol	*N*-acetyl glypohsate	Blank control	< LOQ	< LOQ	N/A	N/A	3
		100 mg/L	61.1 – 97.0	79.3	14	18	7
		500 mg/L	65.7 – 94.5	77.1	15	20	3

Table 5.1-219: Recovery results of the method validation for the determination of *N*-acetyl glyphosate in *n*-octanol/water (pH 5, 7 and 9)

Matrix	Analyte	Fortification level	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
(pH 7)		Overall	61.1 – 97.0	78.6	13.7	17.4	10
Aqueous buffer saturated with *n*-octanol (pH 9)	*N*-acetyl glypohsate	Blank control	< LOQ	< LOQ	–	–	3
		100 mg/L	90.8 – 102	95.3	4.0	4.2	7
		500 mg/L	72.2 – 98.4	87.5	14	16	3
		Overall	72.2 – 102	93.0	8.1	8.8	10
n-octanol saturated with pH 5 aqueous buffer	*N*-acetyl glypohsate	Blank control	< LOQ	< LOQ	–	–	3
		0.05 µg/L	74.0 – 95.9	82.3	6.7	8.2	7
		0.4 µg/L	66.7 – 71.2	68.9	2.3	3.3	3
		Overall	66.7 – 95.9	68.9	2.3	3.3	10
n-octanol saturated with pH 7 aqueous buffer	*N*-acetyl glypohsate	Blank control	< LOQ	< LOQ	–	–	3
		0.08 µg/L	66.3 – 79.8	75.6	6.4	4.8	7
		0.3 µg/L	58.3 – 65.0	61.8	3.4	5.4	3
		Overall	58.3 – 79.8	71.5	7.9	11.0	10
n-octanol saturated with pH 9 aqueous buffer	*N*-acetyl glypohsate	Blank control	< LOQ	< LOQ	–	–	3
		0.024 µg/L	41.4 – 64.5	53.8	8.6	16	7
		0.2 µg/L	46.7 – 59.0	51.5	6.6	13	3
		Overall	41.4 – 64.5	53.1	7.8	14.7	10

[1] Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity

The method allows the determination of *N*-acetyl glyphosate using LC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required. Under the described conditions, the method is highly specific for the determination of *N*-acetyl glyphosate acid in buffed ***n***-octanol/water (pH 5, 7 and 9)**.** No significant interference was observed in chromatograms. (chromatograms of low and high level N-acetyl-glyphosate calibration standard, representative chromatograms of pH 5, 7 and 9 n-octanol quality control samples, representative chromatograms of pH 5, 7 and 9 aqueous quality control samples, representative chromatograms of pH 5, 7 and 9 partition test samples)

Linearity

For quantitation of *N*-acetyl glyphosate in the aqueous phase samples, five calibration standards of N-acetyl glyphosate in reagent water ranging in concentration from 2.00 to 40.0 µg/L, were analysed concurrently with each set of partition samples. For quantitation of N-acetyl glyphosate in the pH 5 and pH 7 *n*-octanol phase samples, six calibration standards of *N*-acetyl glyphosate in reagent water ranging in concentration from 0.025 to 1.00 µg/L, were analyzed concurrently with each set of partition samples. For the pH 9 *n*-octanol phase sample analysis, the additional low-level calibration standard at a concentration of 0.010 µg/L was used, and a total of six standard were analysed ranging in concentration from 0.010 to 0.500 µg/L (the 1.00 µg/L standard was not used).

Linear curve (*n*-octanol phase analysis): y = 31260.9 x – 11.164 (x: concentration (mg/L), y: peak area, r = 0.9976531);
Linear curve (aqueous phase analysis): y = 17005.4 x – 1836.3 (x: concentration (mg/L), y: peak area, r = 0.9998647)

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level 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 as the concentration of the lowest level fortification level for each matrix as following:

Aqueous buffer saturated with *n*-octanol (pH 5): 100 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 7): 100 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 9): 100 mg/L;
n-octanol saturated with pH 5 aqueous buffer: 0.05 µg/L;
n-octanol saturated with pH 7 aqueous buffer: 0.08 µg/L;
Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Conclusion
The analytical method was successfully validated for the determination of *N*-acetyl glyphosate in buffered *n*-octanol/water (pH 5, 7 and 9)**.** Matrix fortification samples in *n*-octanol saturated with buffer yielded lower than expected recoveries of 61.3 %, 54.0 % and 55.5 % for *n*-octanol saturated with pH 5, 7 and 9 buffer, respectively. Due to these low recoveries, the measured concentrations in the *n*-octanol phase of the definitive test vessels were adjusted for the overall mean recovery of 56.9 % for determination/calculation of the partition coefficients. The method validation meets criteria set in SANCO/3029/99 rev. 4, it is fit for purpose to support the ***n*-octanol/water partition coefficients** study concerned.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev.4). The method is considered as fit for purpose to support the n-octanol/water partition coefficients study concerned as the presented analytical data show the good performance of the method.

Assessment and conclusion by RMS:
The analytical method for the determination of N-acetyl-glyphosate in **buffered *n*-octanol/water (pH 5, 7 and 9)** by LC-MS/MS is not fully in agreement with SANCO/3029/99 rev.4. Indeed, the number of replicates by fortification level is < 5 for the high level. However the number of replicates for both levels us equal to 10 and it is considered as sufficient. For n-octanol saturated with aqueous buffer at pH 5, 7 and 9, the mean recoveries are below the acceptable range.

The method is considered as fit for purpose to support the aqueous buffer saturated with n-octanol for all pH. For n-octanol saturated with aqueous buffer at pH 7, 5 and 9, the method can be considered fit for purpose with an adjustement of the measured concentration.

Determination of AMPA in buffered *n*-octanol/water (pH 5, 7 and 9)

Data point	CA 4.1.2/221 (CA 2.7/009)
Report authors	[redacted]
Report year	2020
Report title	Determination of the n-octanol / water partition coefficient of AMPA using the shake flask method
Report No	139K-103
Document No	
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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)
Trst facility	Eurofins EAG USA

Principle of the method
An analytical method was developed for the determination of AMPA in *n*-octanol/water (buffered at pH 5, 7 and 9) by LC-MS/MS. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Agilent 1200 Infinity High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 5000 LC-MS/MS (and QJet Ion Guide) operated in the multiple reaction monitoring (MRM) mode
HPLC Column:	Thermo Acclaim Trinity Q1 (100 × 3.0 mm, 3 µm particle size)
Column temperature	40 °C
Mobile phase:	A: 50 mM Ammonium formate in HPLC grade water (pH 2.9) B: 0.1 % Formic acid in acetonitrile
Gradient:	Time (min) / Eluent A (%) / Eluent B (%) / Flow rate (mL/min) 0.00 / 100 / 0 / 0.35 3.00 / 100 / 0 / 0.35 3.01 / 0 / 100 / 0.35 4.00 / 0 / 100 / 0.35 4.01 / 100 / 0 / 0.35 8.00 / 100 / 0 / 0.35
Injection volume:	50.0 µL
Ion source:	Turbo V Ion Source
Detection:	Multiple reaction monitoring (MRM) mode
Monitored transitions:	*m/z*: 110.0 → 63.0 (dwell time 200.0 msec)
Retention time:	AMPA: ~ 1.8 min

Findings

Recoveries

The method proved to be suitable to determine residues of AMPA in buffered *n*-octanol/water (pH 5, 7 and 9). Samples were spiked with the analyte at two fortification levels in aqueous buffer saturated with *n*-octanol (pH 5, 7 and 9) and *n*-octanol saturated with aqueous buffer (pH 5, 7 and 9), respectively. Mass balance was obtained for AMPA in all test vials, with mean recoveries of nominal fortified AMPA mass of 109 %, 109 % and 97.6 % for pH 5, 7 and 9, respectively. Matrix fortification samples in aqueous phase buffer saturated with *n*-octanol gave good recoveries of 106 %, 109 %, and 94.1 % of the nominal concentrations for pH 5, 7 and 9, respectively. Matrix fortification samples in *n*-octanol saturated with buffer yielded lower than expected recoveries of 44.8 %, 62.3 % and 54.8 % for *n*-octanol saturated with pH 5, 7 and 9 buffer, respectively. Due these low recoveries, the measured concentrations in the *n*-octanol phase of the definitive test vials were adjusted for the overall mean recovery of 54.0 % for determination/calculation of the partition coefficients.

Table 5.1-220: Recovery results of the method validation for the determination of AMPA in *n*-octanol/water (pH 5, 7 and 9)

Matrix	Analyte	Fortification level	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous buffer saturated with *n*-octanol (pH 5)	AMPA	Blank control	< LOQ	< LOQ	–	–	3
		40 mg/L	92.1 – 99.9	97.4	2.7	2.8	7
		600 mg/L	92.3 – 99.5	95.0	3.9	4.2	3
Aqueous buffer saturated with *n*-octanol (pH 7)	AMPA	Blank control	< LOQ	< LOQ	–	–	3
		40 mg/L	89.4 – 106	99.6	7.1	7.2	7
		600 mg/L	94.8 – 104	97.9	5.3	5.4	3
Aqueous buffer saturated with *n*-octanol (pH 9)	AMPA	Blank control	< LOQ	< LOQ	–	–	3
		40 mg/L	64.6 – 107	90.4	14	15	7
		600 mg/L	67.4 – 86.3	76.6	9.5	12	3
n-octanol saturated with pH 5 aqueous buffer	AMPA	Blank control	< LOQ	< LOQ	–	–	3
		5.0 µg/L	38.7 – 72.4	52.2	10	19	7
		22.5 µg/L	60.1 – 74.5	65.8	7.6	12	3
n-octanol saturated with pH 7 aqueous buffer	AMPA	Blank control	< LOQ	< LOQ	–	–	3
		5.0 µg/L	52.4 – 68.3	62.3	6.4	10.0	7
		22.5 µg/L	76.1 – 86.4	80.8	5.2	6.5	3
n-octanol saturated with pH 9 aqueous buffer	AMPA	Blank control	< LOQ	< LOQ	–	–	3
		1.5 µg/L	48.3 – 66.8	59.5	6.4	11.0	7
		7.5 µg/L	50.3 – 62.0	56.7	5.9	10.0	3

[1] Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity
Chromatograms of standards solution, of control sample od different pH, of sample at different pH are provided. No chromatographic interferences were observed in any of the n-octanol and aqueous phase matrix blank samples. No significant interference was observed in chromatograms. (chromatograms of low and high level AMPA calibration standard, representative chromatograms of pH 5, 7 and 9 n-octanol quality control samples, representative chromatograms of pH 5, 7 and 9 aqueous quality control samples, representative chromatograms of pH 5, 7 and 9 partition test samples)

Linearity
For quantitation of AMPA in both partition phase samples, six calibration standards of AMPA in reagent water ranging in concentration from 1.00 to 40.0 µg/L, were analysed concurrently with each set of partition samples. The calibration set was injected at the beginning and end of each analytical sequence with one calibration standard injected after a maximum of every five study samples. Linear regression equations (weighted 1/x) were generated for each analytical sequence using the peak area response versus the respective AMPA concentrations of the calibration standards.
Linear curve: y = 9010 x – 540 (x: concentration (mg/L), y: peak area, r = 0.9999)

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level 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 as the concentration of the lowest level fortification level for each matrix as following:

Aqueous buffer saturated with *n*-octanol (pH 5): 40 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 7): 40 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 9): 40 mg/L;
n-octanol saturated with pH 5 aqueous buffer: 5.0 µg/L;
n-octanol saturated with pH 7 aqueous buffer: 5.0 µg/L;
n-octanol saturated with pH 9 aqueous buffer: 1.50 µg/L;
Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Conclusion
The analytical method was successfully validated for the determination of AMPA in buffered *n*-octanol/water (pH 5, 7 and 9)**.** Matrix fortification samples in *n*-octanol saturated with buffer yielded lower than expected recoveries of 44.8%, 62.3% and 54.8% for *n*-octanol saturated with pH 5, 7 and 9 buffer, respectively. Due to these low recoveries, the measured concentrations in the *n*-octanol phase of the definitive test vials were adjusted for the overall mean recovery of 54.0 % for determination/calculation of the partition coefficients**.** The method validation meets criteria set in SANCO/3029/99 rev. 4, it is valid to support the *n*-octanol/water partition coefficient study concerned.

Assessment and conclusion by applicant:
This study was not previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev.4). The method is considered as valid to support the *n*-octanol/water partition coefficient study concerned.

Assessment and conclusion by RMS:
The analytical method for the determination of N-acetyl-glyphosate in **buffered *n*-octanol/water (pH 5, 7 and 9)** by LC-MS/MS is not fully in agreement with SANCO/3029/99 rev.4. Indeed, the number of replicates by fortification level is < 5 for the high level. However the number of replicates for both levels us equal to 10 and it is considered as sufficient. For n-octanol saturated with aqueous buffer at pH 5, 7 and 9, the mean recoveries are below the acceptable range.

The method is considered as fit for purpose to support the aqueous buffer saturated with n-octanol for all pH. For n-octanol saturated with aqueous buffer at pH 7, 5 and 9, the method can be considered fit for purpose with an adjustement of the measured concentration.

Determination of HMPA in buffered *n*-octanol/water (pH 4, 7 and 9)

1. Information on the study

Data point	CA 4.1.2/222 (CA 2.7/010)
Report authors	[redacted]
Report year	2020
Report title	Determination of the *n*-octanol/water partition coefficient of HMPA using the shake flask method
Test facility	Eurofins EAG Agroscience, LLC 7200 E. ABC Lane Columbia, Missouri 65202
Report No	89592
Document No	MSL0030982
Guidelines followed in study	EU guideline SANCO/3029/99 rev.4
Deviations from current test guideline	None
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)

2. Full summary of the study according to OECD format

Principle of the method

An analytical method was developed for the determination of HMPA in *n*-octanol/water (buffered at pH 4, 7 and 9) by LC-MS/MS. The analytical method consisted of diluting the samples in water. The test samples were diluted in water and directly analysed in LC-MS/MS. The sample is diluted to bring sample concentration into range of the calibration standards

Chromatographic conditions**:**

HPLC system:	Agilent 1200 Infinity High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 5000 LC-MS/MS (and QJet Ion Guide) operated in the multiple reaction monitoring (MRM) mode
HPLC Column:	Waters Torus DEA (100 × 3.0 mm, 1.7 µm particle size)
Column temperature	40 °C
Mobile phase:	A: 0.9% Formic Acid + 50 mM Ammonium Formate in water B: 0.9% Formic Acid in Acetonitrile

Gradient:	Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
	0.00	100	0	0.5
	3.00	100	0	0.5
	3.01	5	95	0.5
	5.00	5	95	0.5
	5.01	100	0	0.5
	7.00	100	0	0.5
Injection volume:	5 µL			
Ion source:	Turbo Spray (negative polarity)			
Detection:	Multiple reaction monitoring (MRM) mode			
Monitored transitions:	*m/z* 110.0 → 63.0 (primary) *m/z* 110.0 → 79.0 (confirmatory) (dwell time 500.0 msec)			
Retention time:	HMPA: approx. 2.0 min			

Findings

Recoveries (accuracy)

The method proved to be suitable to determine residues of HMPA in buffered *n*-octanol/water (pH 4, 7 and 9). Samples were spiked with the analyte at two fortification levels in aqueous buffer saturated with *n*-octanol (pH 4, 7 and 9) and *n*-octanol saturated with aquous buffer (pH 4, 7 and 9), respectively. The recovery of HMPA for the octanol QC samples at pH 7 and pH 9 ranged from 60.5 to 69.1 % and 63.7 to 66.4 %, respectively. While these recoveries are lower than desired, possibly due to poor solubility in octanol, they are considered acceptable for the purposes of this study since determined LOQ values were used for calculation of the P_{OW}. All other recovery values were within an acceptable range and verified the analysis for the quantitation of HMPA.

Table 5.1-221: Recovery results of the method validation for the determination of HMPA in *n*-octanol/water (pH 4, 7 and 9)

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Aqueous buffer saturated with *n*-octanol (pH 4)	HMPA	Blank control	< LOQ	< LOQ	–	–	1
		1000	98.4 – 108	102	5.6	5.7	3
		500	91.5 – 110	99.4	6.9	6.8	5
		Overall	91.5 – 110	100	6.1	6.1	8
Aqueous buffer saturated with *n*-octanol (pH 7)	HMPA	Blank control	< LOQ	< LOQ	–	–	1
		1000	106 – 110	107	2.5	2.7	3
		500	98.1 – 109	104	3.9	4.1	5
		Overall	98.1 – 110	105	3.8	3.6	8
Aqueous buffer saturated with *n*-octanol (pH 9)	HMPA	Blank control	< LOQ	< LOQ	–	–	1
		1000	97.8 – 102	99.8	2.2	2.2	3
		500	92.4 – 106	101	5.8	5.9	5
		Overall	92.4– 106	100	4.6	4.6	8
n-octanol	HMPA	Blank control	< LOQ	< LOQ	–	–	2

Table 5.1-221: Recovery results of the method validation for the determination of HMPA in *n*-octanol/water (pH 4, 7 and 9)

Matrix	Analyte	Fortification level (mg/L)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
saturated with pH 4 aqueous buffer		5.7	71.2 – 81.6	76.9	6.9	5.3	3
		0.57	**58.2 – 62.5**	**60.8**	2.9	1.7	5
		Overall	58.2 – 81.6	66.8	8.9	13.4	8
n-octanol saturated with pH 7 aqueous buffer	HMPA	Blank control	< LOQ	< LOQ	–	–	2
		5.7	88.4 – 92.9	91.2	2.7	2.5	3
		0.57	78.4 – 86.9	82.7	3.8	3.2	5
		Overall	78.4 – 92.9	85.9	5.2	6.0	8
n-octanol saturated with pH 9 aqueous buffer	HMPA	< LOQ	< LOQ	–	–	-	4
		5.7	95.0 – 99.1	96.4	2.4	2.3	3
		0.57	**63.9** – 97.3	78.7	16.5	13.0	5
		Overall	63.9 – 99.1	85.3	13.5	15.8	8

[1] Calculations of mean, SDs, RSDs and overall values were performed using Excel with individual concentration values as given in the report.

Specificity

The method allows the determination of HMPA using LC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required. Under the described conditions, the method is highly specific for the determination of HMPA in buffed *n*-octanol/**water (pH 4, 7 and 9)**. Representative chromatograms of a high and low standard and of test samples in all matrices have been provided. No significant interference was observed in chromatograms.

Linearity

For quantitation of HMPA in both partition phase samples, six calibration standards of HMPA in reagent water ranging in concentration from 26 to 1000 µg/L, were analysed concurrently with each set of partition samples.

Linear curve: y = 416.6482 x – 4690.926 (x: concentration (ng/mL), y: peak area, r = 0.99545085)

Repeatability (Precision)

The relative standard deviations (RSDs) of recovery values at each fortification level 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 as the concentration of the lowest level fortification level for each matrix as following:

Aqueous buffer saturated with *n*-octanol (pH 4): 500 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 7): 500 mg/L;
Aqueous buffer saturated with *n*-octanol (pH 9): 500 mg/L;
n-octanol saturated with pH 4 aqueous buffer: 5.7 mg/L (as the mean recovery is <70%)
n-octanol saturated with pH 7 aqueous buffer: 0.57 mg/L;
n-octanol saturated with pH 9 aqueous buffer: 0.57 mg/L.
Limit of detection (LOD) is not reported in the study.

Matrix effects
Matrix effects were eliminated by using the test water as solvent for test solution.

Conclusion
The analytical method was successfully validated for the determination of HMPA in buffered *n*-octanol/water (pH 5, 7 and 9). The recovery of HMPA for the *n*-octanol QC samples at pH 7 and pH 9 ranged from 60.5 to 69.1 % and 63.7 to 66.4 %, respectively. While these recoveries are lower than desired, possibly due to poor solubility in *n*-octanol, they are considered acceptable for the purposes of this study since determined LOQ values were used for calculation of the P_{OW}. The method validation meets criteria set in SANCO/3029/99 rev. 4, it is valid to support the *n*-octanol/water partition coefficient study concerned.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study was not previously evaluated at EU level. It was performed under GLP and meet current requirements (EU guideline SANCO/3029/99 rev.4). The method is considered as valid to support the *n*-octanol/water partition coefficient study concerned.

Assessment and conclusion by RMS:
The analytical method for the determination of N-acetyl-glyphosate in **buffered *n*-octanol/water (pH 5, 7 and 9)** by LC-MS/MS is not fully in agreement with SANCO/3029/99 rev.4. Indeed, the number of replicates by fortification level is < 5 for the high level. However the number of replicates for both levels us equal to 10 and it is considered as sufficient. For n-octanol saturated with aqueous buffer at pH 5, 7 and 9, the mean recoveries are below the acceptable range.

The method is considered as fit for purpose to support the aqueous buffer saturated with n-octanol for all pH. For n-octanol saturated with aqueous buffer at pH 7, 5 and 9, the method can be considered fit for purpose only with an adjustement of the measured concentration.

Determination of AMPA and N-Acetyl AMPA in buffered solutions

Information on the study

Data point	CA 4.1.2/143 (CA 6.5.1/001)
Report authors	[redacted]
Report year	2020
Report title	AMPA and N-Acetyl AMPA hydrolysis under typical conditions (pH, temperature and time) of processing
Test facility	Eurofins Agroscience Services EcoChem GmbH Eutinger Straße 24 75223 Niefern-Öschelbronn, Germany
Report No	S19-22457
Document No	Not applicable
Guidelines followed in study	OECD 507 SANCO/3029/99, rev. 4
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	Valid (with relevance for analytical methods)
Category study in AIR 5 dossier (L docs)	Category 1 (with relevance for analytical methods)

Full summary of the study according to OECD format
An analytical method for the determination of AMPA and N-Acetyl AMPA in buffered solutions was successfully validated according SANCO/3029/99, rev. 4 in order to support the hydrolysis study. The hydrolysis study was conducted to investigate the stability of AMPA and N-Acetyl-AMPA (two metabolites of glyphosate) under hydrolytic conditions representative of processing as defined in the guideline OECD 507.

Principle of the method
The method was validated for the determination of AMPA and N-Acetyl AMPA in buffered solutions by HPLC-MS/MS with a limit of quantification (LOQ) of 0.05 mg/L.
Aliquots of 0.05 mL are taken from test vessels before and after the respective processing and are diluted 20-fold with water + 0.1 % formic acid prior to HPLC-MS/MS analysis.
Analyte determination was performed using matrix matched calibration standards.

Chromatographic conditions for AMPA:

HPLC system:	Shimadzu HPLC system, Software: Analyst 1.6.3
HPLC Column:	Bio-Rad Cation-H Guard Column, 30 mm x 4.6 mm, (Part No. 1250129)
Column oven temperature:	40 °C
Injection volume:	5 µL
Mobile phase:	Eluent A: Water + 0.1 % formic acid Eluent B: Acetonitrile
Flow rate:	0.5 mL/min
Retention time:	4.3 min
MS system:	API 5500™ LC-MS/MS System (Sciex)
Ionisation type:	Electrospray (ESI, TurboIon Spray)
Polarity:	Negative Ion Mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Mass transition for evaluation	m/z 109.9 → 78.9 for quantification m/z 109.9 → 62.9 for confirmation

Chromatographic conditions for N-Acetyl AMPA:

HPLC system:	Agilent 1290 Infinity II HPLC system, Software: Analyst 1.6.3
Column oven temperature:	25 °C
Injection volume:	20 µL
Mobile phase:	Water + 0.1 % formic acid, isocratic
Retention time:	1.4 min
MS system:	API 6500+™ LC-MS/MS System (Sciex)
Ionisation type:	Electrospray (ESI, TurboIon Spray)
Polarity:	Negative Ion Mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Mass transition for evaluation	m/z 151.9 → 109.9 for quantification m/z 151.9 → 62.9 for confirmation

Findings
Recoveries
The method proved to be suitable to determine residues of AMPA and N-Acetyl AMPA in buffered solutions. Samples were spiked with the analyte at 2 fortification levels at LOQ and 22 x LOQ. All average recovery values

(mean of 5 replicates per fortification level and analyte) were between 70 % and 110 %. The detailed results are given in the table below.

Results of the method validation for the determination of AMPA and N-Acetyl AMPA in buffered solutions

Matrix	Analyte	Fortification level (mg/L)	Recovery			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Buffered solution (pH 4)	AMPA	0.05	87.4 – 107	96.9	8.8	5
		1.1	89.0 – 101	95.5	5.6	5
Buffered solution (pH 5)	AMPA	0.05	93.3 – 106	96.7	6.3	5
		1.1	93.4 – 111	100	7.0	5
Buffered solution (pH 6)	AMPA	0.05	102 – 105	103	1.6	5
		1.1	92.1 – 105	94.5	7.4	5
Buffered solution (pH 4)	N-Acetyl AMPA	0.05	102 – 108	105	2.3	5
		1.1	94.4 – 101	99.0	2.7	5
Buffered solution (pH5)	N-Acetyl AMPA	0.05	96.4 – 100	98.5	1.4	5
		1.1	94.0 – 102	98.6	2.9	5
Buffered solution (pH6)	N-Acetyl AMPA	0.05	100 – 105	103	1.9	5
		1.1	100 – 104	102	2.1	5

Specificity / Interference

The method allows the determination of AMPA and N-Acetyl AMPA using HPLC-MS/MS, which is a highly selective and self-confirmatory detection technique. The specificity of the detection is provided by monitoring two mass transitions per analyte.
Therefore, no confirmatory technique is required. Under the described conditions the method is highly specific for the determination of AMPA and N-Acetyl AMPA in buffered solutions.
Chromatograms of AMPA and N-Acetyl AMPA standards, of blank buffer samples and treated sample in buffered solutions for AMPA and N-Acetyl AMPA have been provided. Control samples did not reveal any peaks ≥ 30 % LOQ in the chromatogram, which would interfere with the determination of AMPA and N-Acetyl AMPA.

Linearity
Linearity of detector response was tested using 8 matrix matched calibration standards in the range of 0.5 ng/mL to 100 ng/mL (equivalent to 0.01 mg/L to 2 mg/L in the samples) and covers the range from no more than 20 % of the limit of quantification (LOQ) and at least + 20 % of the highest analyte concentration detected in a diluted sample. The correlation coefficients (r) were > 0.99. The matrix matched calibration standards were prepared in 20-fold diluted buffer solutions (diluted with water + 0.1 % formic acid).

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

Accuracy
Acceptable mean recovery values at LOQ and 22 x LOQ between 70 % and 110 % for AMPA and N-Acetyl AMPA were found for buffered solutions. Therefore the method complies with EU guideline document SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20%. These criteria were fulfilled for the 0.05 mg/L fortification level for buffered solutions. The limit of detection (LOD) was set to 1/5 of the LOQ (LOD = 0.01 mg/L), while the signal to noise ratio was ≥ 3.

Matrix effects
Matrix effects were tested by comparing the HPLC-MS/MS responses of standards prepared in solvent to those prepared in matrix. The mean matrix effects were between -3 % and 4 % for AMPA and between -26 % and -13 % for N-Acetyl AMPA. Therefore, calibration was performed with standards in matrix (20-fold diluted buffer solutions).

Stability
Regarding stability of the samples before analysis, all buffer solutions were analysed on the same day after preparation. Therefore, no storage stability was shown.

Conclusion
The analytical method was successfully validated for the determination of AMPA and N-Acetyl AMPA in buffered solutions at a limit of quantification (LOQ) of 0.05 mg/L. The analytical method fulfils the European requirements for risk assessment methods as outlined SANCO/3029/99 rev. 4 (11/July/2000).

Assessment and conclusion

Assessment and conclusion by applicant:
The validation of the method for analysis of AMPA and N-Acetyl AMPA 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 suitable to support the residue study concerned.

Assessment and conclusion by RMS:
The analytical method is considered as validated for the determination of AMPA and N-Acetyl AMPA in buffered solutions with a LOQ of 0.05 mg/L.

B.5.1.2.6 Methods in soil, water and any additional matrices used in support of efficacy studies

Analytical methods used in support of efficacy studies are not submitted.

B.5.1.2.7 Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

Analytical methods used in support of operator, worker, resident and bystander exposure studies are not submitted.

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

Note: Some of the described analytical methods use the internal radiolabelled standards glyphosate/N-acetylglyphosate/AMPA. Applicant confirms that labelled isotope standards used in monitoring methods are commercially available.

New analytical methods for monitoring purposes are owned by one or more of the member companies of the European Glyphosate Renewal Group (GRG) with the members Bayer Agriculture BV, Barclay Chemicals Manufacturing Ltd., CIECH Sarzyna S.A., Albaugh Europe SARL, Nufarm GmbH & Co KG, SINON Corporation, Industrias Afrasa S.A., Syngenta Crop Protection AG and/or affiliated entities.

B.5.2.2 Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

B.5.2.1.1 Plant matrices

Residue definition
Enforcement of conventional crops: glyphosate.
Enforcement of GMO crops: sum of glyphosate, AMPA and N-acetyl-glyphosate, expressed as glyphosate.

New analytical methods for monitoring purposes have been developed and validated according to current EU guidelines for the determination of residues in crop matrices. No multiresidue method was provided, this is a data gap A summary of these methods is provided below for all residue definition and summarized below:

Matrix	Analyte(s)	Method	LOQ	Reference	Validation	Data point
	Plant matrices					
Cereals and other dry crops	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.05 mg/kg	[redacted] (2016) Report no.: MSL0027298	Validated	CA 4.2/001
		ILV: LC-MS/MS	0.05 mg/kg	[redacted] (2015) Report no.: S14-05172	Validated as ILV	CA 4.2/002
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027300	Validated	CA 4.2/004
		ILV: LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027695, S15-04467	Validated as ILV	CA 4.2/005
Commodities with high water content	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.05 mg/kg	[redacted] (2016) Report no.: MSL0027298	Validated	CA 4.2/001
		ILV: LC-MS/MS	0.05 mg/kg	[redacted] (2012) Report no.: S11-03331	Validated as ILV	CA 4.2/003
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027300	Validated	CA 4.2/004

Matrix	Analyte(s)	Method	LOQ	Reference	Validation	Data point
		ILV: LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027695, S15-04467	Validated as ILV	CA 4.2/005
Commodities with high fat content	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.05 mg/kg	[redacted] (2016) Report no.: MSL0027298	Validated	CA 4.2/001
		ILV: LC-MS/MS	0.05 mg/kg	[redacted] (2015) Report no.: S14-05172	Validated as ILV	CA 4.2/002
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027300	Validated	CA 4.2/004
		ILV: LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027695, S15-04467	Validated as ILV	CA 4.2/005
Fruits with high acid content	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.05 mg/kg	[redacted] (2016) Report no.: MSL0027298	Validated	CA 4.2/001
		ILV: LC-MS/MS	0.05 mg/kg	[redacted] (2015) Report no.: S14-05172	Validated as ILV	CA 4.2/002
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027300	Validated	CA 4.2/004
		ILV: LC-MS/MS	0.025 mg/kg	[redacted] (2016) Report no.: MSL0027695, S15-04467	Validated as ILV	CA 4.2/005
Commodities which are difficult to analyse		Not required (no intended use in difficult matrices)				

Glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Data point	CA 4.2/001
Report author	[redacted]
Report year	2016
Report title	Analytical method for the determination of Glyphosate and AMPA in matrices of plant origin

Report No	MSL0027298
Document No	Not available
Guidelines followed in study	US EPA OCSPP 860.1340 OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance document on residue analytical methods, 2007
Deviations from current test guideline	None (in line with SANCO/825/00 rev. 8.1, though not specifically referenced)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (According to Guidance Document 7109/VI/94-Rev. 6.c1 the development and validation of an analytical method for monitoring purposes and post-registration control is not subject to GLP)
Acceptability/Reliability	not validated
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company On behalf of the Glyphosate Task Force Environmental Sciences 800 N. Lindbergh Blvd. St. Louis, MO 63167 USA

The analytical method AG-ME-1294-01 was validated for the determination of residues of glyphosate and AMPA in various crop matrices, including plant matrices representing high water content (sugar beet tops), high oil content (undelinted cotton seeds, soybean seeds), dry (corn grain and corn stover) and fruits with high acid content (oranges). Glyphosate and AMPA were determined by HPLC-MS/MS using two mass transitions and were quantitated by the use of internal standards. The LOQ for both analytes was established at 0.05 mg/kg, defined as the lowest validated fortification level. The ILV available present a validation range reduce in comparison to the primary method. In consequence the method is considered as fully validated in the range 0.05 – 0.5 mg/kg.

Principle of the method
Glyphosate and AMPA were isolated from crop matrices by high speed blender extraction using 100mL of 0.1% formic acid in water and 100 ml methylene chloride. Following centrifugation, an aliquot of the aqueous phase extract is filtered and mixed with stable isotope labelled glyphosate and AMPA internal standards then passed through solid phase extraction media for final clean-up.
Two different stable isotope labeled analogs of glyphosate were used as internal standards during the course of the validation ($^{13}C^{15}N$-Glyphosate, $^{13}C_3{}^{15}N$-Glyphosate). Both compounds performed equally well and thus could be used in the method. One stable isotope labelled analogs of AMPA was used as internal standards.
Glyphosate and AMPA residues were determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) was 0.05 mg/kg for both analytes for all crops.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Agilent Series 1200 HPLC (Agilent Technologies) AB – Sciex API 5000 tandem mass spectrometer
Column:	Bio – Rad Fast Acid 100 x 7.8 mm, 9 µm
Column oven temperature:	22 °C
Injection volume:	40 µL
Mobile phase:	0.1 % formic acid in water (isocratic)
Flow rate:	1.5 mL/min
Evaporation solvent (post column):	Methanol at 0.70 mL/min

Split ratio:		The flow of 0.1 % formic acid in water + methanol (1.50 mL/min + 0.70 mL/min) is split 1:1 prior to entering the mass spectrometer resulting a flow of 1.10 mL/min			
Retention time:		Glyphosate: ~ 2.7 min Glyphosate IS: ~ 2.7 min AMPA: ~ 14.2 min AMPA IS: ~ 14.2 min			
Scan type:		Negative Ion MRM			
Ion source:		ESI			
Ion Spray Voltage (IS):	-4500 V		Ion Spray turbo heater (TEM):	600 °C	
Curtain gas (CUR):	15		Gas flow 1 (GS1):	40	
Collision Gas (CAD):	6		Gas flow 2 (GS2):	30	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-70	-31	-25
Glyphosate IS	172	63	-70	-31	-25
AMPA	110	63	-70	-30	-20
AMPA IS	114	63	-70	-30	-20
Confirmatory ions					
Glyphosate	168	79	-70	-50	-31
Glyphosate IS	172	79	-70	-50	-31
AMPA	110	79	-70	-40	-20
AMPA IS	114	79	-70	-40	-20

For certain matrices like canola seed and alfalfa hay more consistent results could be obtained by using a different HPLC column, which allows also the use of a modified mobile phase and do not require split and evaporation solvent. The mass spectrometric conditions remained the same.

Column:	Bio-Rad Fast Acid 100 x 7.8 mm, 9 µm
Column oven temperature:	50 °C
Injection volume:	10 µL
Mobile phase A:	A: 0.1 % formic acid in water B: acetonitrile
Flow rate:	0.5 mL/min, isocratic with 80 % A and 20 % B

Findings

Recoveries (accuracy)

The samples were fortified with glyphosate and AMPA at fortification levels in range of 0.05 mg/kg to 100 mg/kg. . The sample is diluted to bring sample concentration into range of the calibration standards

All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-1: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortificat-ion level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Sugar beet	Tops	Glyphosate	168→63	0.05	82 – 102	88	8	7
				5	83 – 88	85	2	7
				10	84 – 87	86	1	5
			168→79	0.05	80 – 104	88	9	7
				5	83 – 87	84	2	7
				10	85 – 86	85	1	5
		AMPA	110→63	0.05	82 – 109	88	11	7
				5	83 – 86	84	1	7
				10	83 – 86	84	1	5
			110→79	0.05	80 – 111	87	12	7
				5	83 – 86	85	1	7
				10	84 – 86	85	1	5
Corn	Grain	Glyphosate	168→63	0.05	92 – 96	94	1	7
				5	87 – 92	90	2	8
				25	92 – 97	95	2	5
			168→79	0.05	92 – 95	94	1	7
				5	87 – 91	89	1	8
				25	93 – 98	96	2	5
		AMPA	110→63	0.05	85 – 124	96	14	7
				5	90 – 93	92	1	8
				25	95 – 97	96	1	5
			110→79	0.05	85 – 125	97	13	7
				5	90 – 94	92	1	8
				25	94 – 100	97	2	5
Soybean	Seed	Glyphosate	168→63	0.05	91 – 95	94	2	7
				5	92 – 94	93	1	7
				25	95 – 97	96	1	5
			168→79	0.05	92 – 99	94	3	7
				5	91 – 94	93	1	7
				25	94 – 98	96	2	5
		AMPA	110→63	0.05	87 – 95	91	3	7
				5	90 – 92	91	1	7

Table 5.2-1: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortificat-ion level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
				25	92 – 96	94	2	5
			110→79	0.05	87 – 95	92	3	7
				5	90 – 92	91	1	7
				25	92 – 96	94	2	5
Cotton	Seed (undelinted)	Glyphosate	168→63	0.05	89 – 92	90	1	7
				5	81 – 91	88	4	7
				40	85 – 88	87	2	5
			168→79	0.05	87 – 97	90	4	7
				5	81 – 90	87	4	7
				40	86 – 89	87	1	5
		AMPA	110→63	0.05	86 – 89	87	1	7
				5	78 – 87	85	4	7
				40	84 – 86	85	1	5
			110→79	0.05	84 – 93	88	4	7
				5	79 – 87	85	3	7
				40	85 – 86	85	1	5
Orange	Fruit (whole)	Glyphosate	168→63	0.05	61 – 84	78	10	7
				0.50	80 – 85	83	2	7
				5	81 – 85	82	2	5
			168→79	0.05	62 – 86	79	10	7
				0.50	80 – 83	83	1	7
				5	80 – 84	83	2	5
		AMPA	110→63	0.05	62 – 82	79	10	7
				0.50	81 – 82	82	1	7
				5	82 – 83	82	1	5
			110→79	0.05	62 – 83	79	10	7
				0.50	79 – 83	81	1	7
				5	82 – 84	83	1	5
Corn	Stover	Glyphosate	168→63	0.05	87 – 95	91	3	7
				5	90 – 93	92	1	7
				100	90 – 92	91	1	5
			168→79	0.05	87 – 91	88	2	7

Table 5.2-1: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortificat-ion level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
				5	89 – 93	91	1	7
				100	88 – 91	89	1	5
		AMPA	110→63	0.05	84 – 89	87	2	7
				5	84 – 91	88	3	7
				100	89 – 91	90	1	5
			110→79	0.05	84 – 90	88	2	7
				5	82 – 91	88	4	7
				100	89 – 90	89	1	5

Specificity
For both transition and for glyphosate and AMPA, chromatograms of standards solutions, of samples, of untreated control (for all matrices) and fortified samples at LOQ are provided. No interference is observed at the retention of glyphosate and AMPA. Extracts of control samples showed that no signals above 30 % of the LOQ indicating that no significant interferences were present. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity
The linearity of the detector response was confirmed by making triplicate measurements of seven concentrations covering the ranges 2.5 to 600 ng/mL (equivalent to 0.025 to 6.0 mg/kg). The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A quadratic fit with 1/x weighting was used.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20 %.
Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all matrices investigated. The limit of detection (LOD) was set at 30 % of the LOQ, which is 0.015 mg/kg.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate and AMPA.

Matrix effects
Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.

Extraction efficiency
See pages below for the assessement of the extraction efficiency

Stability of glyphosate and AMPA in sample extracts
Extract stability was investigated for corn grain and stover matrices by re-injection of a processed set of fortification samples, that had been stored at +1 to +10 °C. The test indicated no decline of the recoveries during

a storage period of at least 7 days for the two matrices under cool conditions. Glyphosate and AMPA are stable in corn grain and stover extracts at +1 to +10 °C in the dark.

Conclusion
The method AG-ME-1294-01 was successfully validated for the analysis of residues of glyphosate and AMPA in plant matrices representing high water content (sugar beet tops), high oil content (undelinted cotton seeds, soybean seeds), dry (corn grain and corn stover) and fruits with high acid content (oranges) at 0.05 mg/kg (LOQ) and higher fortification levels and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010)

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level and it was not performed under GLP (in line with Guidance Document 7109/VI/94-Rev. 6.c1 for analytical method for monitoring purposes). It meets current requirements (EU guideline SANCO/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in all tested matrix groups (high oil, high acid, high water containing and dry commodities).

Assessment and conclusion by RMS: The method AG-ME-1294-01 is validated for specificity, linearity and accuracy, to be used for monitoring glyphosate and AMPA residues in all tested matrix groups (high oil, high acid, high water containing and dry commodities) with an LOQ of 0.05 mg/kg for both glyphosate and AMPA.

The extraction solvent used is 100 mL 0.1% formic acid in water + 100 mL methylene chloride, consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate , AMPA and N-acetylglyphosate in dichloromethane (see extraction efficiency part p 660). It si not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Data point	CA 4.2/002
Report authors	[redacted]
Report year	2015
Report title	Independent laboratory validation of an analytical method for determination of glyphosate and AMPA in different matrices of plant origin
Report No	S14-05172
Document No	Not available
Guidelines followed in study	Council Directive 1107/2009 SANCO/825/00 rev. 8.1, OECD GLP
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Eurofins, D-21079 Hamburg Germany

The analytical method AG-ME-1294-01 was independently validated for the determination of residues of glyphosate and AMPA in cereal (grain), sunflower (seed) and grape (bunches). No addition or modification to the original method other than optimization of instrumental parameters was made. Glyphosate and AMPA were determined by HPLC-MS/MS using two mass transitions and were quantitated by the use of internal standards. The LOQ for both analytes was established at 0.05 mg/kg, defined as the lowest validated fortification level.

Principle of the method
Glyphosate and AMPA were isolated from crop matrices by high speed blender extraction using 100mL of 0.1% formic acid in water and 100 mL methylene chloride. Following centrifugation, an aliquot of the aqueous phase extract is filtered and mixed with stable isotope labelled glyphosate and AMPA internal standards then passed through solid phase extraction media for final clean-up.
Glyphosate and AMPA residues were determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) was 0.05 mg/kg for both analytes for all crops.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Agilent Series 1260 HPLC (Agilent Technologies) AB-Sciex API 5500 tandem mass spectrometer				
Column:	Bio-Rad Fast Acid 100 x 7.8 mm, 9 µm				
Column oven temperature:	25 °C				
Injection volume:	40 µL				
Mobile phase:	0.1% formic acid in water (isocratic)				
Evaporation solvent (post column):	Methanol at 0.70 mL/min				
Split ratio:	The flow of 0.1 % formic acid in water + methanol is split 1:1 prior to entering the mass spectrometer.				
Retention time:	Glyphosate: ~ 2.9 min Glyphosate IS: ~ 2.9 min AMPA: ~ 13.7 min AMPA IS: ~ 13.7 min				
Scan type:	Negative Ion MRM				
Ion source:	ESI				
Ion Spray Voltage (IS):	-4500 V		Ion Spray turbo heater (TEM):	400°C	
Curtain gas (CUR):	30 (arbitrary units)		Gas flow 1 (GS1):	40 (arbitrary units)	
Collision Gas (CAD):	7 (arbitrary units)		Gas flow 2 (GS2):	60 (arbitrary units)	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-60	-32	-19
Glyphosate IS	171	63	-85	-30	-5
AMPA	110	63	-25	-26	-21
AMPA IS	114	63	-75	-28	-23
Confirmatory ions					
Glyphosate	168	79	-60	-54	-9
Glyphosate IS	171	79	-85	-50	-15
AMPA	110	79	-25	-34	-15
AMPA IS	114	79	-75	-36	-7

Findings

Recoveries (accuracy)

The samples were fortified with glyphosate and AMPA at fortification levels of 0.05 mg/kg and 0.5 mg/kg. The sample is diluted to bring sample concentration into range of the calibration standards

All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-2: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortifica-tion level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Cereal	Grain	Glyphosate	168→63	0.05	98 – 102	101	1.7	5
				0.5	99 – 103	100	1.5	5
			168→79	0.05	96 – 104	100	2.9	5
				0.5	97 – 100	99	1.3	5
		AMPA	110→63	0.05	98 – 102	100	1.8	5
				0.5	100 – 103	101	1.3	5
			110→79	0.05	99 – 103	101	1.9	5
				0.5	99 – 103	101	1.7	5
Sun-flower	Seed	Glyphosate [1]	168→63	0.05	74 – 86	82	6.3	5
				0.5	80 – 90	85	5.1	5
			168→79	0.05	74 – 90	85	7.3	5
				0.5	82 – 97	90	8.0	5
		AMPA	110→63	0.05	86 – 94	89	3.3	5
				0.5	86 – 92	89	3.1	5
			110→79	0.05	87 – 96	91	3.7	5
				0.5	88 – 92	90	2.3	5
Grape	Bunch	Glyphosate	168→63	0.05	78 – 89	85	4.9	5
				0.5	74 – 82	79	3.8	5
			168→79	0.05	76 – 86	82	5.0	5
				0.5	76 – 93	84	7.7	5
		AMPA	110→63	0.05	80 – 91	85	4.8	5
				0.5	86 – 97	90	4.8	5
				0.05	84 – 94	89	4.2	5
				0.5	85 – 97	90	5.0	5

Specificity
For both transition and for glyphosate and AMPA, chromatograms of standards solutions, of samples, of blank, of untreated samples (for all samples) and fortified samples at LOQ are provided. No signals above 30 % of the LOQ is observed. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity
The linearity of the detector response was confirmed by making single measurements of seven concentrations covering the ranges 1.0 to 100 ng/mL (equivalent to 0.01 to 1.0 mg/kg). The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20 %.
Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all matrices investigated. The limit of detection (LOD) was set at 30 % of the LOQ, which is 0.015 mg/kg.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate and AMPA.

Matrix effects
Since matrix effects on detection are generally corrected by the use of response ratio of analyte to internal standards no matrix effects were determined in this study.

Stability of glyphosate and AMPA in sample extracts
Extract stability was investigated for cereal grain, sunflower seed and grape bunches matrices by re-injection of a processed set of fortification samples, that had been stored at +1 to +10 °C. The test indicated no decline of the recoveries during a storage period of at least 9 days for investigated matrices under cool conditions. Glyphosate and AMPA are stable in extracts of cereal grain, sunflower seed and grape bunches at +1 to +10 °C in the dark for at least 9 days.

Conclusion
The method AG-ME-1294-01 was successfully and independently validated for the determination of glyphosate and AMPA residues in crops for cereal grain (dry matrix), sunflower seeds (high oil content matrix) and grape bunches (high acid content matrix) at the LOQ (0.05 mg/kg) and 10 × LOQ (0.5 mg/kg). The method fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in all tested matrix groups (high oil, high acid and dry commodities).

Assessment and conclusion by RMS: The HPLC-MS/MS method is considered acceptable as ILV of AG-ME-1294-01 [redacted] 2016 (CA 4.2/001)) method for the determination of Glyphosate and AMPA in high acid, high oil and dry commodities.

ILVs available are not performed at the same fortification levels than the primary method. Based on that, the validity range of the method is reduced to the range 0.05 -0.5 mg/kg..

Data point	CA 4.2/003
Report authors	[redacted]
Report year	2012
Report titles	Validation of an analytical method for the determination of glyphosate and AMPA in Raw Agricultural Commodities using LC/MS/MS
Report No	S11-03331
Document No	Not available
Guidelines followed in study	Regulation (EC) No. 1107/2009 SANCO/825/00 Rev. 8.1 OECD GLP
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Test facility	Eurofins - D-21079 Hamburg Germany

The validation of the analytical method AG-ME-1294-01 within the presented study can be used as independent laboratory validation for the determination of glyphosate and AMPA in high water containing crops. Within this study glyphosate and AMPA were determined in potato (tubers), carrot (roots), onion (bulbs), cucumber (fruit), cabbage (heads), cauliflower (heads), lettuce (leaves), leek (plants) and tomato (fruit). Glyphosate and AMPA were determined by HPLC-MS/MS using two mass transitions and were quantitated by the use of internal standards. The LOQ for both analytes was established at 0.05 mg/kg, defined as the lowest validated fortification level.

Principle of the method
Glyphosate and AMPA were isolated from crop matrices by high speed blender extraction using 100mL of 0.1% formic acid in water and 100 mL methylene chloride. Following centrifugation, an aliquot of the aqueous phase extract is filtered and mixed with stable isotope labelled glyphosate and AMPA internal standards then passed through solid phase extraction media for final clean-up.
Glyphosate and AMPA residues were determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) was 0.05 mg/kg for both analytes for all crops.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Agilent Series 1200 HPLC (Agilent Technologies) AB-Sciex API 5000 tandem mass spectrometer
Column:	Bio-Rad Fast Acid 100 x 7.8 mm, 9 µm
Column oven temperature:	30 °C
Injection volume:	40 µL
Mobile phase:	0.1% formic acid in water (isocratic)
Flow rate:	1.5 mL/min
Evaporation solvent (post column):	Methanol at 0.70 mL/min

Split ratio:	The flow of 0.1 % formic acid in water + methanol (1.50 mL/min + 0.70 mL/min) is split 1:1 prior to entering the mass spectrometer				
Retention time:	Glyphosate: ~ 2.7 min $^{13}C_3$,^{15}N – glyphosate: ~ 2.7 min AMPA: ~ 14.2 min (D2) ^{13}C,^{15}N – AMPA: ~ 14.2 min				
Scan type:	Negative Ion MRM				
Ion source:	ESI				
Ion Spray Voltage (IS): -4500 V			Ion Spray turbo heater (TEM): 600 °C		
Curtain gas (CUR): 35 (arbitrary units)			Gas flow 1 (GS1): 40 (arbitrary units)		
Collision Gas (CAD): Not specified			Gas flow 2 (GS2): 30 (arbitrary units)		
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-60	-32	-19
Glyphosate IS	172	63	-65	-34	-7
AMPA	110	63	-25	-26	-21
AMPA IS	114	63	-75	-28	-23
Confirmatory ions					
Glyphosate	168	79	-60	-54	-9
Glyphosate IS	172	79	-65	-52	-9
AMPA	110	79	-25	-34	-15
AMPA IS	114	79	-75	-36	-7

Findings

Recoveries (accuracy)

The samples were fortified with glyphosate and AMPA at fortification levels of 0.05 mg/kg and 0.5 mg/kg. The sample is diluted to bring sample concentration into range of the calibration standards

All average recoveries were between 70 % and 110 % with RSD ≤ 20%. The detailed results are given in the table below.

Table 5.2-3: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	**Commo-dity**	**Analyte**	**Target ion**	**Fortification level (mg/kg)**	**Recovery**			
					Range (%)	**Mean (%)**	**Relative standard deviation (%)**	**Number of analyses (n)**
Potato	Tubers	Glyphosate	168→63	0.05	89 – 92	91	1.4	5
				0.5	85 – 88	87	1.8	5
			168→79	0.05	90 – 98	93	3.5	5
				0.5	87 – 91	88	2.0	5

Table 5.2-3: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortification level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
		AMPA	110→63	0.05	87 – 90	88	1.7	5
				0.5	88 – 90	89	1.1	5
			110→79	0.05	80 – 90	87	5.0	5
				0.5	90 – 94	93	1.6	5
Carrot	Roots	Glyphosate	168→63	0.05	87 – 95	90	3.4	5
				0.5	89 – 92	91	1.4	5
			168→79	0.05	87 – 94	90	3.3	5
				0.5	89 – 90	90	0.6	5
		AMPA	110→63	0.05	85 – 90	86	2.4	5
				0.5	86 – 90	88	1.7	5
			110→79	0.05	80 – 87	84	3.4	5
				0.5	89 – 92	91	1.2	5
Onion	Bulbs	Glyphosate	168→63	0.05	86 – 90	88	2.1	5
				0.5	84 – 86	85	1.3	5
			168→79	0.05	89 – 92	91	1.6	5
				0.5	82 – 88	85	3.0	5
		AMPA	110→63	0.05	82 – 87	84	2.2	5
				0.5	83 – 86	85	1.5	5
			110→79	0.05	78 – 86	82	3.6	5
				0.5	84 – 88	86	2.1	5
Cucumber	Fruit	Glyphosate	168→63	0.05	86 – 88	86	1.0	5
				0.5	88 – 90	89	1.0	5
			168→79	0.05	88 – 92	90	1.8	5
				0.5	90 – 91	91	0.6	5
		AMPA	110→63	0.05	81 – 87	84	2.6	5
				0.5	93 – 94	93	0.6	5
			110→79	0.05	77 – 84	82	3.4	5
				0.5	88 – 94	91	2.8	5
Cabbage	Heads	Glyphosate	168→63	0.05	86 – 90	88	1.7	5
				0.5	89 – 92	91	1.3	5
			168→79	0.05	87 – 92	90	2.4	5
				0.5	90 – 93	91	1.4	5

Table 5.2-3: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortification level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
		AMPA	110→63	0.05	82 – 87	85	2.1	5
				0.5	90 – 94	92	1.9	5
			110→79	0.05	82 – 86	84	2.2	5
				0.5	92 – 97	94	1.9	5
Cauli-flower	Heads	Glyphosate	168→63	0.05	88 – 89	88	0.6	5
				0.5	91 – 95	93	1.8	5
			168→79	0.05	82 – 92	87	4.3	5
				0.5	88 – 92	90	2.0	5
		AMPA	110→63	0.05	83 – 87	85	1.7	5
				0.5	90 – 96	92	2.5	5
			110→79	0.05	82 – 86	84	2.2	5
				0.5	90 – 95	92	2.1	5
Lettuce	Leaves	Glyphosate	168→63	0.05	90 – 99	94	4.6	5
				0.5	88 – 91	90	1.6	5
			168→79	0.05	94 – 102	96	5.0	5
				0.5	89 – 92	90	1.7	5
		AMPA	110→63	0.05	84 – 93	88	5.0	5
				0.5	91 – 95	93	2.2	5
			110→79	0.05	90 – 95	92	3.0	5
				0.5	90 – 96	94	2.5	5
Leek	Plants	Glyphosate	168→63	0.05	86 – 89	87	1.5	5
				0.5	89 – 92	90	1.3	5
			168→79	0.05	89 – 94	91	2.1	5
				0.5	89 – 94	91	2.1	5
		AMPA	110→63	0.05	84 – 86	85	1.0	5
				0.5	91 – 93	92	.9	5
			110→79	0.05	80 – 85	83	2.5	5
				0.5	90 – 94	92	1.6	5
Tomato	Fruit	Glyphosate	168→63	0.05	84 – 90	87	3.0	5
				0.5	82 – 92	89	4.7	5
			168→79	0.05	83 – 89	86	2.8	5
				0.5	80 – 90	87	4.8	5

Table 5.2-3: Results of method validation for the determination of glyphosate and AMPA in plant matrices using the method AG-ME-1294-01

Crop	Commo-dity	Analyte	Target ion	Fortification level (mg/kg)	Recovery			
					Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
		AMPA	110→63	0.05	82 – 90	86	3.5	5
				0.5	84 – 92	89	3.5	5
			110→79	0.05	80 – 89	84	4.4	5
				0.5	85 – 92	90	3.1	5

Specificity
For both transition and for glyphosate and AMPA, chromatograms of standards solution, of control sample, of fortified sample at LOQ are provided. No signals above 30 % of the LOQ is observed. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity
The linearity of the detector response was confirmed by making single measurements of eight concentrations covering the ranges 1.25 ng/mL to 250 ng/mL (0.014 mg/kg to 2.78 mg/kg). The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20 %.
Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg for both glyphosate and AMPA was established for all matrices investigated. The limit of detection (LOD) was set at 30 % of the LOQ, which is 0.015 mg/kg.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate and AMPA.

Matrix effects
Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.

Stability of glyphosate and AMPA in sample extracts
Extract stability was investigated for the matrices by re-injection of a processed set of fortification samples, that had been stored at +3 to +8 °C. The test indicated no decline of the recoveries during a storage period of at least 7 days for investigated matrices under cool conditions. Glyphosate and AMPA are stable in extracts of potato (tubers), carrot (roots), onion (bulbs), cucumber (fruit), cabbage (heads), cauliflower (heads), lettuce (leaves), leek (plants) and tomato (fruit) at +3 to +8 °C in the dark for at least 7 days.

Conclusion
The method AG-ME-1294-01 was successfully and independently validated for the determination of glyphosate and AMPA residues in crops for potato (tubers), carrot (roots), onion (bulbs), cucumber (fruit), cabbage (heads), cauliflower (heads), lettuce (leaves), leek (plants) and tomato (fruit) at the LOQ (0.05 mg/kg) and 10 × LOQ (0.5

mg/kg). The method fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in commodities with high water content.

Assessment and conclusion by RMS: The validation of the analytical method AG-ME-1294-01 within the presented study can be used as independent laboratory validation for the determination of glyphosate and AMPA in high water containing crops.

N-acetyl glyphosate in plant matrices using the method ME-2000-01

Data point	CA 4.2/004
Report author	[redacted]
Report year	2016
Report title	Analytical method for the determination of N-Acetyl glyphosate in matrices of plant origin
Report No	MSL0027300
Document No	Not available
Guidelines followed in study	US EPA OCSPP 860.1340 OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance document on residue analytical methods, 2007 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP/Officially recognised testing facilities (According to Guidance Document 7109/VI/94-Rev. 6.c1 the development and validation of an analytical method for monitoring purposes and post-registration control is not subject to GLP)
Acceptability/Reliability	not validated
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company On behalf of the Glyphosate Task Force Environmental Sciences 800 N. Lindbergh Blvd. St. Louis, MO 63167, USA

The analytical method ME-2000-01 was validated for the determination of residues of N-acetyl glyphosate in various crop matrices, including plant matrices representing high water content (corn forage), high oil content (soybean and canola seed), dry (corn grain) and fruits with high acid content (oranges). N-acetyl glyphosate was determined by HPLC-MS/MS using two mass transitions and was quantitated by the use of an internal standard. The LOQ was established at 0.025 mg/kg, defined as the lowest validated fortification level.

Principle of the method
N-acetyl glyphosate was isolated from crop matrices by extraction using high speed shaking with 100mL of 0.1 % formic acid in water and 100 mL of methylene chloride containing stable isotope labelled internal standards (($^{13}C_2{}^{15}N$)N-Acetyl glyphosate). Following centrifugation, an aliquot of the aqueous phase extract is filtered prior to analysis.
N-acetyl glyphosate residue was determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (quantifier: *m/z* 210→63, qualifier: *m/z* 210→124 or *m/z* 210→150) and quantitated using internal standards. Depending on the analysed matrix different confirmatory ions should be used.

The limit of quantification (LOQ) was 0.025 mg/kg for both analytes for all crops.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Shimadzu Prominence 20A AB Sciex API 5000/5500				
Column:	Thermo Scientific Hypercarb, 50 mm × 2.1 mm				
Gradient:	Time (min)	%A	%B	A+B Flow (mL/min)	Divert
	0.0	100	0	0.5	To waste
	0.5	100	0	0.5	To MS
	2.5	100	0	0.5	To MS
	5.0	0	100	0.5	To waste
	7.0	0	100	0.5	To waste
	7.01	100	0	0.5	To waste
	12.0	100	0	0.5	To waste
	12.0	Controller stop			
Column oven temperature:	40 °C				
Injection volume:	5 µL				
Mobile phase:	A: 0.5 % formic acid in H_2O B: Acetonitrile				
Retention time:	N-acetyl glyphosate: ~ 1.9 min N-acetyl glyphosate IS: ~ 1.9 min				
Scan type:	Negative Ion MRM				
Ion source:	ESI				

Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	600 °C
Curtain gas (CUR):	15 (arbitrary units)	Entrance Potential:	-10 V
Collision Gas (CAD):	8 (arbitrary units)	Interface heater:	On
Gas flow 1 (GS1):	50 (arbitrary units)	Scan Time:	150 ms
Gas flow 2 (GS2):	50 (arbitrary units)		

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
N-acetyl glyphosate	210	63	-55	-50	-10
N-acetyl glyphosate IS	213	63	-55	-50	-10
Confirmatory ion (corn grain, corn forage, oranges)					
N-acetyl glyphosate	210	124	-55	-25	-10
N-acetyl glyphosate IS	213	126	-55	-25	-10
Confirmatory ion (soybean seed and canola seed)					
N-acetyl glyphosate	210	150	-55	-20	-10
N-acetyl glyphosate IS	213	153	-55	-20	-10

Findings

Recoveries (accuracy)

The samples were fortified with N-acetyl glyphosate at fortification levels in range of 0.025 mg/kg to 5.0 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-4: Results of method validation for the determination of N-acetyl glyphosate in plant matrices using the method ME-2000-01

Crop	Commodity	Target ion	Fortification level (mg/kg)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Corn	grain	210→63	0.025	90 – 94	92	1.9	6
			0.25	94 – 100	97	2.3	6
			5.0	89 – 92	91	1.3	6
		210→124	0.025	92 – 100	96	3.5	6
			0.25	92 – 97	93	2.0	6
			5.0	91 – 94	92	0.9	6
Corn	forage	210→63	0.025	92 – 99	96	2.6	6
			0.25	98 – 101	99	1.1	6
			5.0	91 – 94	93	1.4	6
		210→124	0.025	94 – 100	97	2.3	6
			0.25	92 – 94	93	1.0	6
			5.0	89 – 93	91	1.9	6
Soybean	seed	210→63	0.025	84 – 99	92	6.6	6
			0.25	89 – 94	92	2.2	6
			5.0	88 – 99	92	4.4	6
		210→150	0.025	89 – 115	97	9.2	6
			0.25	86 – 96	90	3.5	6
			5.0	84 – 94	90	4.6	6
Canola	seed	210→63	0.025	91 – 100	94	3.7	6
			0.25	88 – 96	92	3.0	6
			5.0	84 – 90	88	3.2	6
		210→150	0.025	87 – 93	90	2.9	6
			0.25	89 – 94	91	1.9	6
			5.0	83 – 92	89	3.8	6
Orange	fruit (whole)	210→63	0.025	96 – 100	98	1.6	6
			0.25	88 – 92	90	1.8	6
			5.0	83 – 93	87	3.5	6
		210→124	0.025	91 – 97	95	2.6	6

Table 5.2-4: Results of method validation for the determination of N-acetyl glyphosate in plant matrices using the method ME-2000-01

Crop	Commodity	Target ion	Fortification level (mg/kg)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
			0.25	89 – 94	91	2.2	6
			5.0	86 – 91	88	2.1	6

Specificity

For each transition, chromatograms of standards solution, of fortified sample at the LOQ and 10xLOQ, of untreated samples (for all samples) are provided. No interference is observed at the retention time of N-Acetyl glyphosate. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity

The linearity of the detector response was confirmed by making single measurements of ten concentrations covering the ranges 1.5 to 1200 ng/mL (equivalent to 0.0075 to 6.0 mg/kg). The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %.

Accuracy

Acceptable mean recovery values over all fortification levels between 70 % and 110 % for N-acetyl glyphosate were found for the analysed matrices.

Limit of Quantification and Detection

The limit of quantification (LOQ) of 0.025 mg/kg was established for all matrices investigated. The limit of detection (LOD) was determined separately for each matrix, but all values were ≤ 30 % of the LOQ.

Interference

No significant interferences from the specimen matrix were detected at the retention times corresponding to N-acetyl glyphosate.

Matrix effects

Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.

Stability of N-acetyl glyphosate in sample extracts

Extract stability was investigated for all analysed matrices by re-injection of a processed set of fortification samples, that had been stored at approximately 4 °C. The test indicated no decline of the recoveries during a storage period of at least 4 days under cool conditions. N-acetyl glyphosate is stable in all matrices tested at approximately 4 °C for at least 4 days.

Conclusion

The method ME-2000-01 was successfully validated for the analysis of residues of N-acetyl glyphosate in plant matrices representing high water content (corn forage), high oil content (canola seeds, soybean seeds), dry (corn grain) and fruits with high acid content (oranges) at 0.025 mg/kg (LOQ) and higher fortification levels and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level and it was not performed under GLP (in line with Guidance Document 7109/VI/94-Rev. 6.c1 for analytical method for monitoring purposes). It meets current requirements (EU guideline SANCO/825/00 rev. 8.1). The method is considered valid to be used for monitoring N-acetyl glyphosate residues in all tested matrix groups (high oil, high acid, high water containing and dry commodities).

Assessment and conclusion by RMS: The method ME-2000-01 for analysis of residues of N-acetyl glyphosate in different crop matrices, i.e. corn forage (high water), soybean and canola seed (high oil), oranges (high acid content matrix and corn grain (dry matrix) is validated for specificity, linearity and accuracy according to the current guidance.

The extraction solvent used is 100 mL 0.1% formic acid in water + 100 mL methylene chloride, consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate, AMPA and N-acetylglyphosate in dichloromethane (see extraction efficiency part p 660). It si not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Data point	CA 4.2/005
Report author	[redacted]
Report year	2016
Report title	Independent Laboratory Validation of an Analytical Method for the Determination of N-Acetyl glyphosate in Matrices of Plant Origin
Report No	S15-04467 MSL0027695
Document No	Not available
Guidelines followed in study	Regulation (EC) No. 1107/2009 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Eurofins, D-21079 Hamburg Germany

The analytical method ME-2000-01 was independently validated for the determination of residues of N-acetyl glyphosate in tomatoes (fruit), orange (fruit), wheat (grain) and oilseed rape (seed). Only very minor modifications to the original methods were made, which include the optimization of instrumental parameters and the use of cellulose filters instead of GHB membranes. These modifications have no impact on the validity of the method validation by an independent laboratory. N-acetyl glyphosate was determined by HPLC-MS/MS using two mass transitions and was quantitated by the use of an internal standard. The LOQ was established at 0.025 mg/kg, defined as the lowest validated fortification level.

Principle of the method
For analysis of N-acetyl glyphosate, stable isotope labelled internal standard ($^{13}C_2{}^{15}N$ N-Acetyl glyphosate) solution was added to samples of tomatoes (fruit), orange (fruit), wheat (grain) and oilseed rape (seed). Samples were extracted with a 100 mL mixture of 0.1% formic acid and 100 mLdichloromethane and filtered through a cellulose filter. The analytes were analysed by LC-MS/MS detection. Two selected ion mass transitions were evaluated.

N-acetyl glyphosate residue was determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (quantifier: *m/z* 210→63, qualifier: *m/z* 210→124) and quantitated using internal standards. Depending on the analysed matrix different confirmatory ions should be used.
The limit of quantification (LOQ) was 0.025 mg/kg for both analytes for all crops.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Agilent 1260 Infinity Binary HPLC System AB Sciex API 5000				
Column:	Thermo Scientific Hypercarb, 50 mm × 2.1 mm, 3 µm				
Gradient:	Time (min)	%A	%B	Flow (mL/min)	
	0.0	100	0	500	
	2.5	100	0	500	
	5.0	0	100	500	
	7.0	0	100	500	
	7.01	100	0	500	
	12.0	100	0	500	
Column oven temperature:	40 °C				
Injection volume:	5 µL				
Mobile phase:	A: 0.5 % formic acid in H_2O B: Acetonitrile				
Retention time:	N-acetyl glyphosate: ~ 1.6 min N-acetyl glyphosate IS: ~ 1.6 min				
Scan type:	Negative Ion MRM				
Ion source:	ESI				
Ion Spray Voltage (IS):	-4500 V		Ion Spray turbo heater (TEM):	600 °C	
Curtain gas (CUR):	25 (arbitrary units)		Gas flow 1 (GS1):	50 (arbitrary units)	
Collision Gas (CAD):	8 (arbitrary units)		Gas flow 2 (GS2):	50 (arbitrary units)	

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
N-acetyl glyphosate	210	63	-60	-44	-11
N-acetyl glyphosate IS	213	63	-55	-50	-10
Confirmatory ion (corn grain, corn forage, oranges)					
N-acetyl glyphosate	210	124	-60	-26	-15
N-acetyl glyphosate IS	213	126	-55	-25	-10

Findings

Recoveries (accuracy)

The samples were fortified with N-acetyl glyphosate at fortification levels in range of 0.025 mg/kg to 0.5 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-5: Results of method validation for the determination of N-acetyl glyphosate in plant matrices using the method ME-2000-01

Crop	Commodity	Target ion	Fortification level (mg/kg)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Tomato	Fruit	210→63	0.025	101 – 105	104	1.5	5
			0.25	104 – 107	105	1.3	5
		210→124	0.025	98 – 105	102	2.6	5
			0.25	101 – 107	104	2.1	5
Orange	Fruit (whole)	210→63	0.025	98 – 102	101	1.8	5
			0.25	100 – 105	102	2.2	5
		210→124	0.025	98 – 101	99	1.1	5
			0.25	95 – 103	100	3.2	5
Wheat	Grain	210→63	0.025	90 – 98	93	3.2	5
			0.25	94 – 99	98	2.2	5
		210→124	0.025	92 – 98	94	2.5	5
			0.25	95 – 100	98	1.9	5
Oilseed rape	Seed	210→63	0.025	102 – 107	105	1.7	5
			0.25	102 – 104	103	1.1	5
		210→124	0.025	103 – 111	106	2.9	5
			0.25	105 – 109	106	1.5	5

Specificity

For each transition chromatograms of blank, of standards solution, of untreated sample (for all samples) and fortified sample at LOQ and 10x LOQ are provided. No interference is observed at the retention time of analyte. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity

The linearity of the detector response was confirmed by making single measurements of eight concentrations covering the ranges 1.0 to 200 ng/mL (equivalent to 0.005 to 1.0 mg/kg). The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %.

Accuracy

Acceptable mean recovery values over all fortification levels between 70 % and 110 % for N-acetyl glyphosate were found for the analysed matrices..

Extraction efficiency

See pages below for the assessement of the extraction efficiency

Limit of Quantification and Detection

The limit of quantification (LOQ) of 0.025 mg/kg was established for all matrices investigated. The limit of detection (LOD) was set as ≤ 30 % of the LOQ, which is 0.0075 mg/kg.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to N-acetyl glyphosate.

Matrix effects
Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.

Stability of N-acetyl glyphosate in sample extracts
Extract stability was investigated for all analysed matrices by re-injection of a processed set of fortification samples, that had been stored at +1 to +10 °C. The test indicated no decline of the recoveries during a storage period of at least 10 days under cool conditions. N-acetyl glyphosate is stable in all matrices tested for at least 10 days.

Conclusion
The analytical method ME-2000 validated for the determination of N-acetyl glyphosate residues in crops was successfully and independently validated for tomato (fruit), orange (whole fruit), wheat (grain) and oilseed rape (seed) at concentrations levels of LOQ at 0.025 mg/kg and 10 x LOQ at 0.25 mg/kg. The method fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring N-acetyl glyphosate residues in all tested matrix groups (high oil, high acid, high water containing and dry commodities).

Assessment and conclusion by RMS : The analytical method ME-2000 for the determination of N-acetyl glyphosate residues in crops was successfully and independently validated considering specificity, linearity and accuracy for tomato (fruit), orange (whole fruit), wheat (grain) and oilseed rape (seed) at concentrations levels of LOQ at 0.025 mg/kg and 10x LOQ at 0.25 mg/kg.

Extraction efficiency of methods for plant materials

The analytical methods for monitoring in plant material are using **0.1 % aqueous formic acid in water and dichloromethane in proportion 50/50** as extraction solvent. The applicant indicated that the dichloromethane during the sample preparation for glyphosate, AMPA, or *N*-acetyl glyphosate from plant matrices is for clean-up purposes only and is not necessary for extraction. However, the addition of or dichloromethane is done at the same time of 0.1% aqueous formic acid water, therefore the dichloromethane should be considered in thextraction solvent.

, the extraction of glyphosate and its major metabolites with **water or 0.1 % aqueous formic acid in methanol (96/4, v/v)** is sufficiently covered by existing data from metabolism studies (an overview is presented below).

Details on the efficiency of different solvents to extract incurred residues from plant materials measured as extracted part of the total radioactive residue (TRR) are outlined in section CA 6.2 and summarised in the table below.

Table 5.2-6: Extractability of the total radioactive residue with different solvents of different plant materials

Commodity category	Matrix	Extractable residue % TRR (mg/kg)]	Extraction solvent	Reference
High acid	Grape vine fruit (soil treatm.)	69.1 (0.0049)	Water	CA 6.2.1/005: [redacted] 1991, Glyphosate – Trimesium. Uptake and metabolism in USA grape vines, Report No. RJ1002B
	Grape vine fruit (overspray)	96.4 (1.20)		
High water content	Sugar beet, tops (1 pre-emergence treatm.)	59.22 (0.003)	Water	CA 6.2.1/018: [redacted] 2000a, Metabolism of Glyphosate in Roundup Ready Sugarbeet, Report No. MSL-16247
	Sugar beet, tops (2 post-emergence treatm.)	86.65 (2.978)		
	Sugar beet, roots (1 pre-emergence treatm.)	85.56 (0.007)		
	Sugar beet, roots (2 post-emergence treatm.)	103.30 (1.442)		
High water content	Corn, forage (1 soil protected treatm.)	96.2 (12.8)	Water	CA 6.2.1/20: [redacted] 1995, Nature of glyphosate residues in corn plants which are tolerant to Roundup® herbicide, Report No. MSL-14018
	Corn, forage (1 soil non-protected treatm.)	93.0 (10.0)		
(No group)	Corn, silage (1 soil protected treatm.)	93.5 (8.52)		
	Corn, silage (1 soil non-protected treatm.)	86.6 (8.33)		
High water content	Corn, fodder (1 soil protected treatm.)	95.2 (14.2)		
	Corn, fodder (1 soil non-protected treatm.)	94.4 (18.0)		
Dry com.	Corn, grain (1 soil protected treatm.)	77.7 (0.532)		
	Corn, grain (1 soil non-protected treatm.)	81.1 (0.843)		
High water content	Soybean, forage[1]	17.7 (0.024) to 104.2 (24.637)	Water	CA 6.2.1/022: [redacted] 1994, Nature of Glyphosate residues in soybeans tolerant to Roundup herbicide, Report No. MSL-13520
(No group)	Soybean, hay[1]	24.3 (0.029) to 79.9 (0.436) or 77.0 (8.015)		
Dry com.	Soybean, seeds[1]	20.6 (0.092) to 83.3 (14.545)		

Table 5.2-6: Extractability of the total radioactive residue with different solvents of different plant materials

Commodity category	Matrix	Extractable residue % TRR (mg/kg)]	Extraction solvent	Reference
High water content	Cotton, forage (unprotected)	98.5 (30.0)	Water	CA 6.2.1/023: [redacted] 1997, Nature of Glyphosate residues in cotton plants tolerant to Roundup herbicide, Report No. MSL-14113
	Cotton, forage (protected)	96.9 (14.7)		
High oil content	Cotton, seeds (unprotected)	18.6 (0.034)		
	Cotton, seeds (protected)	31.9 (0.034)		
High water content	Corn, foliage	87.0 (3.204)	0.1 % aqueous formic acid in methanol (96/4, v/v)	CA 6.2.1/024: [redacted] 2007, The metabolism of [^{14}C]Glyphosate in Optimum GAT (Event DP- Ø9814Ø-6) field corn, DuPont-19529
	Corn, forage	31.0 (0.007)		
(No group)	Corn, stover	85.0 (10.406)		
	Corn, cobs	69.3 (0.475)		
Dry com.	Corn, grain	71.0 (0.195)		
High water content	Canola, foliage (first harvest)	97.3 (5.818)	0.1 % aqueous formic acid in methanol (96/4, v/v)	CA 6.2.1/025: [redacted] 2010, The metabolism of [^{14}C]Glyphosate in 0827 canola, DuPont-26109
	Canola, foliage (second harvest)	93.0 (1.442)		
(No group)	Canola, immature pods	79.6 (1.013)		
High oil content	Canola, mature seeds	78.4 (1.690)		
High water content	Soybean, forage	28.7 (0.123)	0.1 % aqueous formic acid in methanol (96/4, v/v)	CA 6.2.1/026: [redacted] 2007, The metabolism of [^{14}C]Glyphosate in GAT/GM-HRA (DP- 356Ø43-5, PHP20163a) soybeans, DuPont-19530
(No group)	Soybean, hay	95.9 (12.893)		
Dry com.	Soybean, grain (first harvest)	88.9 (1.694)		
	Soybean, grain (second harvest)	88.0 (2.765)		
(No group)	Soybean, foliage/pods	86.2 (9.676)		
(No group)	Soybean, pods	88.1 (15.639)		
High water content	Soybean, foliage	88.2 (19.481)		

Dry com. Stands for dry commodities (high protein/starch content)
TRR: total radioactive residue
Treatm.: treatment (application)
1 Given ranges represent different application scenarios (pre-emergence, early post-emergence, sequential post-emergence)

In general glyphosate and AMPA are the major metabolites in tolerant and non-tolerant crops. In the tolerant crops with GAT modification *N*-acetyl glyphosate is also found.

In the grape study ([redacted], **1991**) glyphosate and AMPA were identified as the largest part of found radioactivity (in sum 79.6 % of the TRR). Glyphosate and *N*-acetyl-glyphosate are identified as the major residue components in stover, cobs, silage, and forage from glyphosate tolerant corn ([redacted] **1995** and [redacted] **2007**). Also in matrices of soybean (grain, foliage, hay, pods) and cotton forage glyphosate and *N*-acetyl-glyphosate (plus AMPA in soybean grain) represent the largest part of the TRR ([redacted], and [redacted], **2007**). In sugar beet tops only glyphosate formed the major residue ([redacted], **2000**) and in foliage, immature pods and mature seeds of tolerant canola 51 – 93 % of TRR were identified as *N*-acetyl-glyphosate ([redacted], **2010**). Therefore, in these matrices the non-extractable part of the total radioactive residue (≤ 20 %) has not to be considered.

By consideration of all available metabolism studies which reflect the proposed residue definition for enforcement (for plants with glyphosate tolerant genetically modified varieties currently available on the market (sweet corn, cotton seeds, sugar beets, rapeseeds, maize and soybeans): sum of glyphosate, AMPA and *N*-acetyl glyphosate, expressed as glyphosate; for all other plant commodities: glyphosate), all relevant matrix groups (according to SANCO/825/00/rev. 8.1: dry commodities, commodities with high water/oil/acid content) are covered.
In most cases the results show an extraction of more than 70 % of TRR with either water as the only extraction solvent or with 0.1 % aqueous formic acid in methanol (96/4, v/v) as extraction solvent. A lower extraction efficiency for some matrices (containing only very little TRRs) is connected to very low residue level (0.007 to 0.123 mg/kg). It is considered that acidic water extraction is sufficient to extract the majority of the TRR and data on the individual extraction efficiency of glyphosate, AMPA or *N*-acetyl glyphosate are not necessary.

Assessment and conclusion by RMS :
The extraction solvent used in monitoring methods available is 0.1% formic acid in water **and dichloromethane in proportion 50/50**.

The metabolism studies available used water or 0.1 % aqueous formic acid in methanol (96/4, v/v) for extraction solvent. According to the SANTE/2017/10632.rev3, solvent mixtures are considered as being identical if their composition varies by not more than 20 %. However, as the extraction solvent used in monitoring method is the 0.1% formic acid in water and dichloromethane in proportion 50/50, it cannot be considered identical to the solvent used in the metabolism studies.
However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate, AMPA and N-acetyl glyphosate in dichloromethane. It is not expected that dichloromethane modifies the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction solvent used for monitoring and risk assessment methods, can be considered identical to the extraction solvent validated for metabolism studies.
Nevertheless, in order to confirm this hypothesis, a cross validation with incurred residue in plants to compare extraction with dichloromethane and without dichloromethane taking into account the ratio netween solvent and sample should be provided during the peer review.

B.5.2.1.2 Animal matrices

Residue definition

Residue definition for enforcement:
Sum of glyphosate, AMPA and N-acetyl glyphosate, expressed as glyphosate
An overview on the proposed monitoring methods for analysis of glyphosate in animal matrices is given in the following table. No multiresidue method was provided. This is a data gap.

Matrix	Analyte(s)	Method	LOQ	Validation	Reference	Data point
Milk	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027299 Not previously submitted	CA 4.2/006
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027301 Not previously submitted	CA 4.2/007
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
Egg	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027299 Not previously submitted	CA 4.2/006
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027301 Not previously submitted	CA 4.2/007

Matrix	Analyte(s)	Method	LOQ	Validation	Reference	Data point
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
Meat	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027299 Not previously submitted	CA 4.2/006
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027301 Not previously submitted	CA 4.2/007
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
Fat	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027299 Not previously submitted	CA 4.2/006
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008

Matrix	Analyte(s)	Method	LOQ	Validation	Reference	Data point
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027301 Not previously submitted	CA 4.2/007
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
Kidney/liver	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027299 Not previously submitted	CA 4.2/006
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
	N-acetyl-glyphosate	**Primary method:** LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027301 Not previously submitted	CA 4.2/007
		ILV: LC-MS/MS	0.025 mg/kg	Validated	[redacted] (2016) Report no.: MSL0027696, S15-04468 Not previously submitted	CA 4.2/008
Honey	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.025 mg/kg	Not validated	[redacted] (2019) Report no.: MSL 0030583, SGS-19-01-01 Not previously submitted	CA 4.2/009

Matrix	Analyte(s)	Method	LOQ	Validation	Reference	Data point
		ILV: LC-MS/MS	0.025 mg/kg	/	[redacted] (2020) Report no.: S19-04663 Not previously submitted	CA 4.2/010

Primary method for the determination of Glyphosate and AMPA in matrices of animal origin

Data point	CA 4.2/006
Report author	[redacted]
Report year	2016
Report title	Analytical Method for the Determination of Glyphosate and AMPA in Matrices of Animal Origin
Report No	MSL0027299
Document No	Not available
Guidelines followed in study	US EPA OCSPP 860.1340 OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance document on residue analytical methods, 2007 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (According to Guidance Document 7109/VI/94-Rev. 6.c1 the development and validation of an analytical method for monitoring purposes and post-registration control is not subject to GLP)
Acceptability/Reliability	not validated
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company On behalf of the Glyphosate Task Force Environmental Sciences 800 N. Lindbergh Blvd. St. Louis, MO 63167 USA

Principle of the method

Glyphosate and AMPA were isolated from animal matrices samples (2 g) by extraction using high speed shaking with 100 mL of 0.1 % formic acid in water and 100 mL of methylene chloride containing stable glyphosate and AMPA isotope labelled internal standards. Following centrifugation, samples derived from liver and egg were purified using solid phase extraction additionally. Subsequently all samples were analysed by HPLC-MS/MS.
Glyphosate and AMPA residues were determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). For liver as matrix an alternate confirmatory ions (168→150 instead of 168→79) for glyphosate was proposed due to potential interferences that were recognized while using the first confirmatory ion.
The limit of quantification (LOQ) was 0.025 mg/kg for both analytes for all animal matrices.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Shimadzu Prominence 20A
Column:	Bio-Rad Cation-H Guard Column, 30 mm × 4.6 mm
Mobile phase:	A: 0.1 % formic acid in H_2O

B: Acetonitrile
C: 0.2 % phosphoric acid in H_2O

Gradient:	Time (min)	%A	%B	A+B Flow (mL/min)	C Flow (mL/min)	Divert
	0.0	80	20	0.5	0.0	To waste
	0.5	80	20	0.5625	0.0	To MS
	1.0	80	20	0.625	0.0	To MS
	2.5	100	0	0.8125	0.0	To MS
	4.0	100	0	1.0	0.0	To MS
	6.9	100	0	1.0	0.0	To waste
	7.0	100	0	0.5	0.5	To waste
	10	100	0	0.5	0.5	To waste
	10.1	80	20	0.5	0.0	To waste
	12.6	Controller stop				

Injection volume:	5 µL
Autosampler temp:	4 °C
Column oven temp:	40 °C

Mass spectrometer:	AB Sciex API 5000/5500
Scan type:	Negative Ion MRM
Ion source:	ESI, Turbo-V

Period 1

Duration:	3.0 min	IonSpray Voltage (IS):	-4500 V
Curtain gas (CUR):	15 (arbitrary units)	Entrance Potential:	-10 V
Collision Gas (CAD):	8 (arbitrary units)	Interface heater:	On
Gas 1:	80 (arbitrary units)	Temp:	600°C
Gas 2:	60 (arbitrary units)	Scan Time:	150 ms

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-70	-33	-23
Glyphosate IS	172	63	-70	-33	-23
Confirmatory ions (muscle, fat, liver, milk, egg)					
Glyphosate	168	79	-70	-53	-31
Glyphosate IS	172	79	-70	-53	-31
Confirmatory ions (liver, alternate confirmation)					
Glyphosate	68	150	-70	-15	-10
Glyphosate IS	172	154	-70	-15	-10

Period 2

Duration:	3.5 min	IonSpray Voltage (IS):	-4500 V

Curtain gas (CUR):	15 (arbitrary units)	Entrance Potential:	-10 V
Collision Gas (CAD):	8 (arbitrary units)	Interface heater:	On
Gas 1:	80 (arbitrary units)	Temp:	600°C
Gas 2:	60 (arbitrary units)	Scan Time:	150 ms

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
AMPA	110	63	-100	-28	-24
AMPA IS	114	63	-100	-28	-24
Confirmatory ions					
AMPA	110	79	-100	-40	-15
AMPA IS	114	79	-100	-40	-15

Validation

Recoveries (accuracy)
The samples were fortified with glyphosate and AMPA at fortification levels in range of 0.025 mg/kg to 5.0 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-7: Results of method validation for the determination of glyphosate and AMPA in animal matrices using the method ME-1951-01

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Meat	Glyphosate	168→63	0.025	93 – 98	96	2.0	6
			0.25	95 – 97	96	1.1	6
			5.0	93 – 98	96	2.1	6
		168→79	0.025	94 – 105	99	4.0	6
			0.25	90 – 95	92	2.0	6
			5.0	90 – 94	92	2.0	6
	AMPA	110→63	0.025	100 – 104	102	1.4	6
			0.25	99 – 102	100	1.0	6
			5.0	97 – 101	99	1.1	6
		110→79	0.025	99 – 104	101	1.6	6
			0.25	98 – 102	100	1.8	6
			5.0	97 – 102	99	2.0	6
Liver	Glyphosate	168→63	0.025	100 – 106	103	2.4	6
			0.25	96 – 98	97	1.1	6

Table 5.2-7: Results of method validation for the determination of glyphosate and AMPA in animal matrices using the method ME-1951-01

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
			5.0	94 – 98	96	1.6	6
		168→79	0.025	92 – 101	96	3.9	6
			0.25	87 – 94	91	2.7	6
			5.0	87 – 102	91	7.2	6
		168→150	0.025	100 – 111	103	4.6	6
			0.25	94 – 97	96	1.1	6
			5.0	92 – 96	94	1.5	6
	AMPA	110→63	0.025	96 – 104	100	2.8	6
			0.25	99 – 102	101	1.5	6
			5.0	98 – 102	100	1.5	6
		110→79	0.025	94 – 100	97	2.5	6
			0.25	92 – 100	96	3.2	6
			5.0	98 – 99	98	0.7	6
Fat	Glyphosate	168→63	0.025	94 – 97	96	1.1	6
			0.25	94 – 98	95	1.9	5
			5.0	95 – 99	96	1.3	6
		168→79	0.025	99 – 106	103	2.7	6
			0.25	92 – 98	95	2.7	5
			5.0	95 – 100	97	2.3	6
	AMPA	110→63	0.025	96 – 104	100	2.7	6
			0.25	97 – 103	100	2.3	5
			5.0	98 – 102	99	1.5	6
		110→79	0.025	100 – 107	103	2.4	6
			0.25	98 – 102	100	1.6	5
			5.0	96 – 99	98	1.5	6
Egg	Glyphosate	168→63	0.025	94 – 101	99	2.7	6
			0.25	97 – 99	98	0.8	6
			5.0	95 – 101	98	2.0	6
		168→79	0.025	93 – 103	98	4.4	6
			0.25	95 – 101	98	2.2	6
			5.0	96 – 103	98	2.9	6
	AMPA	110→63	0.025	99 – 105	101	3.0	6

Table 5.2-7: Results of method validation for the determination of glyphosate and AMPA in animal matrices using the method ME-1951-01

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
			0.25	98 – 104	101	2.3	6
			5.0	98 – 102	100	1.4	6
		110→79	0.025	96 – 105	102	3.5	6
			0.25	99 – 103	101	1.5	6
			5.0	102 – 103	102	0.4	6
Milk	Glyphosate	168→63	0.025	97 – 102	99	1.6	6
			0.25	95 – 99	96	1.4	6
			5.0	92 – 96	94	1.5	6
		168→79	0.025	91 – 98	94	2.8	6
			0.25	91 – 99	94	3.3	6
			5.0	90 – 94	92	1.9	6
	AMPA	110→63	0.025	92 – 104	97	4.1	6
			0.25	99 – 103	101	1.1	6
			5.0	98 – 101	100	1.4	6
		110→79	0.025	100 – 103	102	1.0	6
			0.25	99 – 104	101	2.2	6
			5.0	98 – 102	101	1.6	6

1 Recovery values are corrected for the mean peak area of the control sample extracts.

Specificity

For both transition, chromatograms of standards solutions, of samples, of untreated control (in milk, egg, muscle, fat and liver) and fortified samples at LOQ and 10xLOQ are provided. No interference is observed at the retention of glyphosate and of AMPA

Extracts of control samples showed no signals above 30 % of the LOQ. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity

For both transitions, the linearity of the detector response was confirmed by making single measurements of ten concentrations corresponding to 0.0075 to 6.0 mg/kg of glyphosate and AMPA in all animal matrices investigated. The coefficient of determination (r^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.025 mg/kg for both glyphosate and AMPA was established for all matrices investigated. The limit of detection (LOD) was determined separately for each matrix, but all values were ≤ 30 % of the LOQ.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate and AMPA. During the method development it was recognized that the confirmatory ion for glyphosate and glyphosate is demonstrated elevated background levels, therefor an alternate confirmatory ion with lower background level for the determination of glyphosate in liver was proposed (168→150).

Matrix effects
Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.

Stability of glyphosate and AMPA in sample extracts
Extract stability was investigated for all matrices investigated by re-injection of a processed set of fortification samples, that had been stored at approximately 4 °C. The test indicated no decline of the recoveries during a storage period of at least 3 days under cool conditions. Glyphosate and AMPA are stable in all extracts at approximately 4 °C for at least 3 days.

Conclusion
The method ME-1951-01 was successfully validated for the analysis of residues of glyphosate and AMPA in beef muscle, liver, fat, chicken egg and cow milk at 0.025 mg/kg (LOQ) and higher fortification levels and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010), SANCO/3029/99 rev. 4 (11/July/2000) and OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17 (13/Aug/2007).

Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level and it was not performed under GLP (in line with Guidance Document 7109/VI/94-Rev. 6.c1 for analytical method for monitoring purposes). It meets current requirements (EU guideline SANCO/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in all tested animal matrices.

Assessment and conclusion by RMS: The analytical method ME-1951-01 using LC-MS/MS is considered validated with a LOQ of 0.025 mg/kg for the determination of residues of glyphosate and AMPA in matrices of animal origin (beef muscle, liver, fat, chicken egg and cow milk). As the method is validated on 2 mass transitions, a confirmatory method is not necessary.

The extraction solvent used is 100 mL 0.1% formic acid in water + 100 mL methylene chloride, consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 691). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Primary method for the determination of N-acetyl glyphosate in matrices of animal origin

Data point	CA 4.2/007
Report author	[redacted]
Report year	2016
Report title	Analytical Method for the Determination of N-Acetyl Glyphosate in Matrices of Animal Origin
Report No	MSL0027301
Document No	Not available
Guidelines followed in study	US EPA OCSPP 860.1340 OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance document on residue analytical methods, 2007 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (According to Guidance Document 7109/VI/94-Rev. 6.c1 the development and validation of an analytical method for monitoring purposes and post-registration control is not subject to GLP)
Acceptability/Reliability	not validated
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company On behalf of the Glyphosate Task Force Environmental Sciences 800 N. Lindbergh Blvd. St. Louis, MO 63167 USA

Principle of the method

N-acetyl glyphosate was isolated from animal matrices by extraction using high speed shaking with 100 mL of 0.1 % formic acid in water and 100 mL of dichloromethane containing stable isotope labelled internal standards (($^{13}C_2{}^{15}N$)N-Acetyl glyphosate). Following centrifugation, an aliquot of the aqueous phase extract is filtered prior to analysis.

N-acetyl glyphosate residue was determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (quantifier: *m/z* 210→63, qualifier: *m/z* 210→124 or *m/z* 210→150) and quantitated using internal standards. Depending on the analysed matrix different confirmatory ions should be used.

The limit of quantification (LOQ) was 0.025 mg/kg for both analytes for all animal matrices.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Shimadzu Prominence 20A AB Sciex API 5000/5500				
Column:	Thermo Scientific Hypercarb, 50 mm × 2.1 mm				
Gradient:	Time (min)	%A	%B	A+B Flow (mL/min)	Divert
	0.0	100	0	0.5	To waste
	0.5	100	0	0.5	To MS
	2.5	100	0	0.5	To MS
	5.0	0	100	0.5	To waste
	7.0	0	100	0.5	To waste

	7.01	100	0	0.5	To waste
	12.0	100	0	0.5	To waste
	12.0	Controller stop			
Column oven temperature:	40 °C				
Injection volume:	5 µL				
Mobile phase:	A: 0.5 % formic acid in H_2O B: Acetonitrile				
Retention time:	N-acetyl glyphosate: ~ 2.0 min N-acetyl glyphosate IS: ~ 2.0 min				
Scan type:	Negative Ion MRM				
Ion source:	ESI				

Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	600 °C
Curtain gas (CUR):	15 (arbitrary units)	Entrance Potential:	-10 V
Collision Gas (CAD):	8 (arbitrary units)	Interface heater:	On
Gas flow 1 (GS1):	50 (arbitrary units)	Scan Time:	150 ms
Gas flow 2 (GS2):	50 (arbitrary units)		

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
N-acetyl glyphosate	210	63	-55	-50	-10
N-acetyl glyphosate IS	213	63	-55	-50	-10
Confirmatory ion (muscle, fat, milk, egg)					
N-acetyl glyphosate	210	124	-55	-25	-10
N-acetyl glyphosate IS	213	126	-55	-25	-10
Confirmatory ion (liver)					
N-acetyl glyphosate	210	150	-55	-20	-10
N-acetyl glyphosate IS	213	153	-55	-20	-10

Validation

Recoveries (accuracy)

The samples were fortified with N-acetyl glyphosate at fortification levels in range of 0.025 mg/kg to 5 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-8: Results of method validation for the determination of N-acetyl glyphosate in animal matrices using the method ME-1999-01

Matrix	Target ion	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Meat	210→63	0.025	93 – 102	98	4.2	6

Table 5.2-8: Results of method validation for the determination of N-acetyl glyphosate in animal matrices using the method ME-1999-01

Matrix	Target ion	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
		0.25	90 – 100	95	4.6	6
		5.0	86 – 92	89	2.7	6
	210→124	0.025	91 – 103	96	5.5	6
		0.25	93 – 102	98	3.8	6
		5.0	84 – 94	90	4.0	6
Liver	210→63	0.025	93 – 97	94	2.1	6
		0.25	94 – 96	95	0.7	6
		5.0	88 – 93	91	2.0	6
	210→150	0.025	81 – 97	88	6.9	6
		0.25	90 – 95	93	2.1	6
		5.0	90 – 96	93	2.4	6
Fat	210→63	0.025	88 – 99	95	4.6	6
		0.25	90 – 96	93	2.7	6
		5.0	87 – 93	89	2.5	6
	210→124	0.025	94 – 103	99	4.2	6
		0.25	84 – 91	87	3.2	6
		5.0	84 – 91	87	3.2	6
Egg	210→63	0.025	92 – 96	94	1.7	6
		0.25	93 – 97	95	1.5	6
		5.0	90 – 94	92	1.6	6
	210→124	0.025	92 – 98	95	2.8	6
		0.25	92 – 94	93	0.9	6
		5.0	89 – 91	90	1.1	6
Milk	210→63	0.025	93 – 96	94	1.2	6
		0.25	92 – 93	93	0.4	6
		5.0	88 – 90	89	1.1	6
	210→124	0.025	92 – 97	95	2.1	6
		0.25	91 – 93	92	0.7	6
		5.0	87 – 90	89	1.2	6

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

Specificity

For both transition, chromatograms of standards solutions, of samples, of untreated control (in milk, egg, musle, fat and liver) and fortified samples at LOQ and 10xLOQ are provided. No interference is observed at the retention of N-acetyl-glyphosate.

Extracts of control samples showed no signals above 30 % of the LOQ. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity

For both transition, the linearity of the detector response was confirmed by making single measurements of ten concentrations covering the ranges 0.0075 to 6.0 mg/kg for N-acetyl glyphosate in all animal matrices investigated. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Accuracy

Acceptable mean recovery values over all fortification levels between 70 % and 110 % for N-acetyl glyphosate were found for the analysed matrices. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Interference

No significant interferences from the specimen matrix were detected at the retention times corresponding to N-acetyl glyphosate.

Limit of Quantification and Detection

The limit of quantification (LOQ) of 0.025 mg/kg was established for all matrices investigated. The limit of detection (LOD) was determined separately for each matrix, but all values were ≤30 % of the LOQ.

Matrix effects

The use of stable isotope labelled internal standards (($^{13}C_2{}^{15}N$)N-Acetyl glyphosate) compensates for any difference in response between samples and standards.

Stability of N-acetyl glyphosate in sample extracts

Extract stability was investigated for all analysed matrices by re-injection of a processed set of fortification samples, that had been stored at approximately 4 °C. The test indicated no decline of the recoveries during a storage period of at least 5 days under cool conditions. N-acetyl glyphosate is stable in all matrices tested at approximately 4 °C for at least 5 days.

Conclusion

The method ME-1999-01 was successfully validated for the analysis of residues of N-acetyl glyphosate in beef muscle, liver, fat, chicken egg and cow milk at 0.025 mg/kg (LOQ) and higher fortification levels and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010), SANCO/3029/99 rev. 4 (11/July/2000) and OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17 (13/Aug/2007).

Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level and it was not performed under GLP (in line with Guidance Document 7109/VI/94-Rev. 6.c1 for analytical method for monitoring purposes). It meets current requirements (EU guideline SANCO/825/00 rev. 8.1). The method is considered valid to be used for monitoring **N-acetyl glyphosate** residues in all tested animal matrices.

Assessment and conclusion by RMS: The analytical method ME-1999-01 using LC-MS/MS is considered validated with a LOQ of 0.025 mg/kg for the determination of residues of N-acetyl glyphosate in matrices of animal origin (beef muscle, liver, fat, chicken egg and cow milk). As the method is validated on 2 mass transitions, a confirmatory method is not necessary.

The extraction solvent used is 100 mL 0.1% formic acid in water + 100 mL methylene chloride, consequently according to the guidance document SANTE 2017/10632 that cannot be considered identical. However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate and AMPA in dichloromethane (see extraction efficiency part p 691). It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Therefore the extraction efficiency can be considered as demonstrated.

Independent laboratory validation for the determination of Glyphosate and its metabolites N-acetyl glyphosate and AMPA in matrices of animal origin

Data point	CA 4.2/008
Report author	[redacted]
Report year	2016
Report title	Independent Laboratory Validation of Analytical Methods for the determination of Glyphosate and its Metabolites N-Acetyl Glyphosate and AMPA in matrices of animal origin
Report No	S15-04468 MSL0027696
Document No	Not available
Guidelines followed in study	Regulation (EC) No. 1107/2009 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Eurofins Agroscience Service, D-21079 Hamburg Germany

Principle of the method

Stable glyphosate, N-acetyl glyphosate and AMPA isotope labelled internal standard solution was added to samples of meat, fat, liver, milk and egg. Samples were extracted with 100 mLof mixture of 0.1% formic acid in water and 100 mL of dichloromethane and filtered through a cellulose filter. In the case of glyphosate and AMPA sample extracts of liver and egg were additionally cleaned up by solid phase extraction (SPE).
Analytes were determined by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79 or 168→150; AMPA: quantifier: 110→63, qualifier: 110→79; N-acetyl glyphosate: quantifier: 210→63, qualifier: 210→124). For liver and egg as matrix an alternate confirmatory ions (168→150 instead of 168→79) for glyphosate was proposed due to potential interferences that were recognized while using the first confirmatory ion.
The limit of quantification (LOQ) was 0.025 mg/kg for all analytes for all animal matrices.

Instrumentation and Chromatographic Conditions for the analysis of glyphosate and AMPA:

HPLC – MS/MS:	Agilent Series 1260 HPLC (Agilent Technologies) AB-Sciex API 5000 tandem mass spectrometer

Column:	Bio-Rad Micro Guard Cation H+, 30 x 4.6 mm
Column oven temperature:	40 °C
Injection volume:	5 µL
Mobile phase:	A: 0.1 % formic acid in H_2O B: Acetonitrile

Gradient:

Time (min)	%A	%B	Flow (mL/min)
0.0	80	20	500
0.5	80	20	563
1.0	80	20	625
2.5	100	0	813
4.0	100	0	1000
6.9	100	0	1000
7.0	80	20	500
9.05	80	20	500

Retention time:	Glyphosate: ~ 1.5 min Glyphosate IS: ~ 1.5 min AMPA: ~ 4.7 min AMPA IS: ~ 4.7 min
Scan type:	Negative Ion MRM
Ion source:	ESI

Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	600 °C
Curtain gas (CUR):	25 (arbitrary units)	Gas flow 1 (GS1):	50 (arbitrary units)
Collision Gas (CAD):	8 (arbitrary units)	Gas flow 2 (GS2):	50 (arbitrary units)

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-60	-32	-19
Glyphosate IS	171	63	-20	-30	-5
AMPA	110	63	-25	-26	-21
AMPA IS	114	63	-75	-28	-23
Confirmatory ions (muscle, fat, milk)					
Glyphosate	168	79	-60	-54	-9
Glyphosate IS	171	79	-20	-50	-15
AMPA	110	79	-25	-34	-15
AMPA IS	114	79	-75	-36	-7
Confirmatory ions (liver, egg)					
Glyphosate	168	150	-60	-14	-21
Glyphosate IS	171	153	-20	-15	-10
AMPA	110	79	-25	-34	-15

AMPA IS	114	79	-75	-36	-7

Instrumentation and Chromatographic Conditions for the analysis of N-acetyl glyphosate:

HPLC – MS/MS:	Agilent 1260 Infinity Binary HPLC System AB Sciex API 5000			
Column:	Thermo Scientific Hypercarb, 50 mm × 2.1 mm, 3 µm			
Column oven temperature:	40 °C			
Injection volume:	5 µL			
Mobile phase:	A: 0.5 % formic acid in H_2O B: Acetonitrile			
Gradient:	Time (min)	%A	%B	Flow (mL/min)
	0.0	100	0	500
	2.5	100	0	500
	5.0	0	100	500
	7.0	0	100	500
	7.01	100	0	500
	12.0	100	0	500
Retention time:	N-acetyl glyphosate: ~ 2.5 min N-acetyl glyphosate IS: ~ 2.5 min			
Scan type:	Negative Ion MRM			
Ion source:	ESI			

Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	600 °C
Curtain gas (CUR):	25 (arbitrary units)	Gas flow 1 (GS1):	50 (arbitrary units)
Collision Gas (CAD):	8 (arbitrary units)	Gas flow 2 (GS2):	50 (arbitrary units)

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
N-acetyl glyphosate	210	63	-60	-44	-11
N-acetyl glyphosate IS	213	63	-55	-50	-10
Confirmatory ion (corn grain, corn forage, oranges)					
N-acetyl glyphosate	210	124	-60	-26	-15
N-acetyl glyphosate IS	213	126	-55	-25	-10

Validation

Recoveries (accuracy)

The samples were fortified with glyphosate and AMPA at fortification levels in range of 0.025 mg/kg and 0.25 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-9: Results of method validation for the determination of glyphosate and AMPA in animal matrices using the method ME-1951-01

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Meat	Glyphosate	168→63	0.025	95 – 102	99	2.6	5
			0.25	102 – 104	103	0.8	5
		168→79	0.025	88 – 98	93	4.6	5
			0.25	104 – 109	107	1.9	5
	AMPA	110→63	0.025	93 – 102	98	4.2	5
			0.25	102 – 106	104	1.6	5
		110→79	0.025	93 – 104	98	4.7	5
			0.25	102 – 105	104	1.1	5
Liver	Glyphosate	168→63	0.025	94 – 99	96	1.9	5
			0.25	93 – 103	98	4.1	5
		168→150	0.025	77 – 85	82	4.1	5
			0.25	88 – 98	92	4.4	5
	AMPA	110→63	0.025	104 – 109	108	1.9	5
			0.25	108 – 110	109	0.6	5
		110→79	0.025	103 – 114	107	3.9	5
			0.25	108 – 110	109	0.8	5
Fat	Glyphosate	168→63	0.025	98 – 104	101	2.4	5
			0.25	104 – 107	105	1.0	5
		168→79	0.025	92 – 105	98	5.4	5
			0.25	104 – 109	106	2.0	5
	AMPA	110→63	0.025	98 – 99	98	0.5	5
			0.25	104 – 105	105	0.4	5
		110→79	0.025	98 – 102	100	1.7	5
			0.25	106 – 108	106	0.8	5

Table 5.2-9: Results of method validation for the determination of glyphosate and AMPA in animal matrices using the method ME-1951-01

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Egg	Glyphosate	168→63	0.025	86 – 91	88	2.2	5
			0.25	101 – 106	103	1.9	5
		168→150	0.025	95 – 102	98	3.2	5
			0.25	91 – 110	102	8.1	5
	AMPA	110→63	0.025	100 – 104	102	1.8	5
			0.25	97 – 109	102	5.3	5
		110→79	0.025	95 – 101	97	2.4	5
			0.25	100 – 109	104	3.4	5
Milk	Glyphosate	168→63	0.025	101 – 102	101	0.5	5
			0.25	102 – 104	103	1.0	5
		168→79	0.025	99 – 109	104	4.4	5
			0.25	107 – 110	109	1.0	5
	AMPA	110→63	0.025	101 – 104	102	1.3	5
			0.25	102 – 104	103	0.8	5
		110→79	0.025	98 – 106	101	3.1	5
			0.25	101 – 104	103	1.1	5

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

Table 5.2-10: Results of method validation for the determination of N-acetyl glyphosate in animal matrices using the method ME-1999-01

Matrix	Target ion	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Meat	210→63	0.025	88 – 92	89	1.9	5
		0.25	91 – 101	95	4.4	5
	210→124	0.025	80 – 92	86	5.1	5
		0.25	85 – 91	89	2.6	5
Liver	210→63	0.025	93 – 96	94	1.4	5
		0.25	91 – 99	94	3.3	5
	210→150	0.025	89 – 104	98	5.7	5
		0.25	92 – 98	95	2.5	5

Table 5.2-10: Results of method validation for the determination of N-acetyl glyphosate in animal matrices using the method ME-1999-01

Matrix	Target ion	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Fat	210→63	0.025	95 – 100	97	2.2	5
		0.25	96 – 100	98	1.7	5
	210→124	0.025	94 – 99	97	2.2	5
		0.25	94 – 97	95	1.4	5
Egg	210→63	0.025	90 – 96	94	2.7	5
		0.25	93 – 96	94	1.6	5
	210→124	0.025	92 – 99	94	2.9	5
		0.25	92 – 97	94	2.3	5
Milk	210→63	0.025	85 – 89	86	1.9	5
		0.25	91 – 94	93	1.2	5
	210→124	0.025	92 – 99	96	2.9	5
		0.25	90 – 98	94	3.1	5

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

Specificity

For both transition, chromatograms of standards solutions, of samples, of untreated control and fortified samples at LOQ and 10xLOQ are provided. No interference is observed at the retention for glyphosate, AMPA and N-acetyl-glyphosate. Extracts of control samples showed no signals above 30 % of the LOQ.
LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity

For both transitions, the linearity of the detector response was confirmed by making single measurements of eight concentrations in the range of 1.0 ng/mL to 200 ng/mL (equivalent to 0.005 to 1.0 mg/kg) for glyphosate and its metabolite AMPA as well as for N-acetyl glyphosate in all plant matrices investigated.
The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Accuracy

Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Limit of Quantification and Detection

The limit of quantification (LOQ) of 0.025 mg/kg for both glyphosate, N-acetyl glyphosate and AMPA was established for all matrices investigated. The limit of detection (LOD) was set as ≤ 30 % of the LOQ, which is 0.0075 mg/kg.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding within the proposed analytical conditions.

Matrix effects
Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.
Stability of glyphosate, N-acetyl glyphosate and AMPA in sample extracts
Extract stability was investigated for all analysed matrices by re-injection of a processed set of fortification samples, that had been stored at +1 to +10 °C. The test indicated no decline of the recoveries during a storage period of at least 13 days under cool conditions. Glyphosate, N-acetyl glyphosate and AMPA are stable in all matrices tested for at least 13 days when stored at +1 to +10 °C.

Conclusion
The analytical methods ME-1951-01 and ME-1999-01 were successfully and independently validated for the determination of residues of glyphosate and its metabolite AMPA, and of N-acetyl glyphosate residues, respectively in beef muscle, liver, fat, chicken egg and cow milk at the LOQ of 0.025 mg/kg and 10x LOQ of 0.25 mg/kg.
The method fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010), SANCO/3029/99 rev. 4 (11/July/2000) and OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17 (13/Aug/2007).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate, N-acetyl glyphosate and AMPA residues in all tested animal matrices.

Assessment and conclusion by RMS: The analytical method ME-1951-01 for the determination of residues of glyphosate and AMPA and the analytical method ME-1999-01 for the determination of residues N-acetyl glyphosate are considered independently validated in matrices of animal origin (beef muscle, liver, fat, chicken egg and cow milk) with a LOQ of 0.025 mg/kg.

Data point	CA 4.2/009
Report author	[redacted]
Report year	2019
Report title	Validation of Monsanto ME-2220 analytical method for the determination of glyphosate and AMPA residues in honey
Report No	SGS-19-01-01 MSL 0030583
Document No	ME-2220
Guidelines followed in study	US EPA OCSPP 860.1340 SANCO/825/00 rev. 8.1 OECD GLP
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	not validated
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	SGS North America, Inc. GLP Laboratory 1405 32nd Avenue Brookings, SD 57006

The purpose of this study was to validate Monsanto analytical method ME-2220 for glyphosate and AMPA in honey. The matrices consisted of three varieties of honey: extremely raw blueberry honey (variety 1), sweet clover raw honey (variety 2), and certified organic honey (variety 3). The method was validated at a limit of quantitation (LOQ) of 0.025 mg/kg for both analytes in all varieties.

Principle of the method
For analysis of glyphosate and AMPA in all honey varieties, stable isotope labeled internal standard solution was added to the sample (2.0 g ± 0.02 g) followed by extraction with 0.1 % formic acid in water in a high-speed shaker. After centrifugation and filtration samples were submitted to HPLC-MS/MS detection using at least 2 mass transitions per analyte in negative multiple reaction monitoring (MRM) mode.
The reference substances (analytical reference standards) used in this validation were glyphosate, AMPA, ($^{13}C3^{15}N$)Glyphosate and (D2$^{13}C^{15}N$)AMPA

Details to the HPLC using MS/MS detection and chromatographic parameters are summarised below.

HPLC – MS/MS: Shimadzu Nexera XR HPLC
Sciex Triple Quadrupole 6500+

Column: Bio – Rad Cation – H Guard Column, 20 mm x 4.6 mm

Gradient:

Time (min)	%A	%B	%C	Flow rate (mL/min)
0.01	80	20	0	0.5
1.00	80	20	0	0.5
2.50	100	0	0	↓
4.00	100	0	0	gradient increase
5.50	0	0	100	1.0
8.50	100	0	0	1.0
15.00	100	0	0	1.0
15.10	80	20	0	1.0
15.10	80	20	0	0.5
17.60	0	0	0	0

Column oven temperature: 40 °C

Injection volume: 5 µL

Mobile phase: A: 0.1 % formic acid in H_2O
B: Acetonitrile
C: 0.2 % phosphoric acid in water/0.1 % formic acid in water (1/1)

Retention time: Glyphosate: ~ 1.6 min
Glyphosate IS: ~ 1.6 min
AMPA: ~ 4.5 min
AMPA IS: ~ 4.5 min

Scan type: Negative Ion MRM

Ion source:	ESI				
Ion Spray Voltage (IS):	-4500 V		Ion Spray turbo heater (TEM):	600 °C	
Curtain gas (CUR):	20 (arbitrary units)		Gas flow 1 (GS1):	80 (arbitrary units)	
Collision Gas (CAD):	8 (arbitrary units)		Gas flow 2 (GS2):	60 (arbitrary units)	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-70	-33	-23
Glyphosate IS	172	63	-70	-33	-23
AMPA	110	63	-40	-24	-5
AMPA IS	114	63	-100	-28	-24
Confirmatory ions					
Glyphosate	168	79	-70	-53	-31
Glyphosate IS	172	79	-70	-53	-31
AMPA	110	79	-40	-30	-9
AMPA IS	114	79	-100	-40	-15
Confirmatory ions					
Glyphosate	168	150	-70	-15	-10
Glyphosate IS	172	154	-70	-15	-10

Findings

Recoveries

The samples were fortified with glyphosate and AMPA at fortification levels in the range of 0.025 mg/kg to 20 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 % with the exception of the 0.025 mg/kg fortification level of glyphosate in honey variety 1 with 116 % and the 20 mg/kg fortification level of AMPA in honey variety 1 with 111 %. The detailed results are given in the table below.

Table 5.2-11: Results of the method validation for the determination of glyphosate and AMPA in honey

Matrix	**Analyte**	**Target ion**	**Fortification level (mg/kg)**	**Recovery**			
				Range (%)	**Mean (%)**	**Relative standard deviation (%)**	**Number analyses (n)**
Variety 1	Glyphosate	168→63	0.025	93 – 104	100	4.0	7
			0.25	91 – 101	96	3.4	6
			20	97 – 114	106	6.1	6
Variety 2			0.025	92 – 102	97	4.2	7
			0.25	96 – 102	99	2.3	6
			20	99 – 111	106	4.7	6
Variety 3			0.025	92 – 107	100	5.3	7

Table 5.2-11: Results of the method validation for the determination of glyphosate and AMPA in honey

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
			0.25	96 – 103	99	2.3	6
			20	97 – 107	103	3.4	6
Variety 1	Glyphosate	168→79	0.025	107 – 120	116	4.1	7
			0.25	98 – 104	101	2.5	6
			20	91 – 109	103	6.4	6
Variety 2			0.025	87 – 101	96	6.0	7
			0.25	95 – 102	98	2.9	6
			20	92 – 120	105	10	6
Variety 3			0.025	81 – 101	91	6.7	7
			0.25	91 – 100	94	4.0	6
			20	94 – 118	104	9.8	6
Variety 1	Glyphosate	168→150	0.025	95 – 108	101	5.1	7
			0.25	101 – 104	102	1.7	6
			20	88 – 102	98	5.3	6
Variety 2			0.025	93 – 98	96	2.0	7
			0.25	91 – 101	96	4.7	6
			20	98 – 112	103	4.8	6
Variety 3			0.025	97 – 114	103	5.5	7
			0.25	95 – 102	99	2.6	6
			20	88 – 109	99	7.7	6
Variety 1	AMPA	110→63	0.025	72 – 110	89	14	7
			0.25	86 – 102	96	6.5	6
			20	98 – 119	111	9.4	6
Variety 2			0.025	83 – 103	91	7.9	7
			0.25	89 – 118	103	10	6
			20	70 – 98	88	12	6
Variety 3			0.025	72 – 102	88	12	7
			0.25	95 – 103	100	2.7	6
			20	90 – 106	97	6.7	6
Variety 1	AMPA	110→79	0.025	81 – 104	95	8.9	7
			0.25	97 – 107	100	3.8	6
			20	84 – 120	101	15	6

Table 5.2-11: Results of the method validation for the determination of glyphosate and AMPA in honey

Matrix	Analyte	Target ion	Fortification level (mg/kg)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Variety 2			0.025	76 – 101	85	9.9	7
			0.25	94 – 109	101	5.5	6
			20	81 – 110	95	10	6
Variety 3			0.025	74 – 115	91	14	7
			0.25	89 – 105	96	5.7	6
			20	82 – 119	100	15	6

Specificity
The concentration of the analytes in prepared samples was determined by liquid chromatography with MS/MS detection. The specificity of the detection is provided by monitoring three mass transitions for glyphosate and two mass transitions for AMPA. Product ion mass spectra of glyphosate, ($^{13}C_3{}^{15}N$) Glyphosate, AMPA and ($D_2{}^{13}C_3{}^{15}N$)AMPA are provided in the report. For each variety of honey, for each transition and for each substance, chromatograms of standards solution, of blank, of fortified samples at LOQ, 10xLOQ and 800xLOQ are provided. No interference (below 30% of the LOQ) is observed at the retention time of each analyte.

Linearity
Linearity of detector response was tested for glyphosate and AMPA using at least 10 matrix equivalent calibration standards covering the range of 0.0075 to 6.0 mg/kg (standards were prepared so expected residues would fall within the linear range of the calibration curve). Linear regression was performed with 1/x weighting and correlation coefficients > 0.99 for glyphosate and AMPA in all matrices were observed.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were <20 %. Therefore the method complies with EU guideline document SANCO/825/00 rev.8.1 and SANCO/3029/99 rev 4.

Accuracy
Average recoveries were between 70 % and 110 % except for the fortification level of 0.025 mg/kg of glyphosate in honey variety 1 with 116 % and the fortification level of 20 mg/kg of AMPA in honey variety 1 with 111 %. This two values only slightly exceed the range of 70 % to 110 % and the other two honey varieties analysed showed acceptable recoveries at these fortification levels. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4.
Note: fortification samples with the highest concentration were diluted and re-measured to be in the linear range

Limit of Quantification and Detection
The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 120 % at a relative standard deviation (RSD) of ≤20 %. These criteria were fulfilled for the 0.025 mg/kg fortification level for glyphosate and AMPA in honey. The limit of detection (LOD) was set at 0.0075 mg/kg for glyphosate and AMPA in honey (equivalent to 30 % LOQ).

Interference
Control samples did not reveal any peaks ≥30 % LOQ in the chromatogram, which would interfere with the determination of glyphosate and AMPA in honey.

Matrix effects
Matrix effects were tested by comparing the peak area of glyphosate and AMPA prepared in matrix to those prepared in neat solvent. This determination was made at the LOQ (0.025 mg/kg) for each variety. Matrix effects

ranged from 8.7 to 18.3 % for glyphosate (all transitions), and -21.8 to 14.9 % for AMPA (all transitions). In addition, stable isotope enriched internal standards were used in this study and the use of the response ratio of analyte to internal standard generally corrects for matrix effects on detection. Therefore, solvent standards were used for quantification.

Stability in sample extracts
Glyphosate and AMPA were tested to be stable in final extracts of honey after 5 days of being stored at < 10 °C in the dark.

Conclusion
The analytical method was successfully validated for the determination of glyphosate and AMPA in honey at a limit of quantification (LOQ) of 0.025 mg/kg. The method fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate and AMPA in honey was not previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev 4.). No deviations to the applied test guidelines were reported. The method is suitable to be used for monitoring/ enforcement purposes.

Assessment and conclusion by RMS The method ME-2220 for the determination of glyphosate and AMPA in honey is validated with an LOQ of 0.025mg/kg for each analyte. Nevertheless, the extraction efficiency of the method is not demonstrated. **Data required.**

Data point	CA 4.2/010
Report author	[redacted]
Report year	2020
Report title	ILV of method ME-2220-01 and short term storage stability of glyphosate and its metabolite AMPA in honey
Report No	S19-04663
Document No	M-681330-01-1
Guidelines followed in study	Regulation (EC) No. 1107/2009 SANCO/825/00 rev. 8.1 OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance document on residue analytical methods, 2007 OECD GLP
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Eurofins Agroscience Services EcoChem GmbHEutinger Straße 24 75223 Niefern-Öschelbronn Germany

The analytical method ME-2220-01 was independently validated for the determination of glyphosate and AMPA in honey. Only minor modifications to the original method were made. Glyphosate and AMPA were determined by HPLC-MS/MS using at least two mass transitions and were quantitated by the use of internal standards. The LOQ for both analytes was established at 0.025 mg/kg, defined as the lowest validated fortification level.

Principle of the method
For analysis of glyphosate and AMPA honey samples (2.0 ± 0.1 g) were diluted in 0.1 % formic acid in water and stable isotope labeled internal standard solution was added. Samples were mixed using a high speed shaker and centrifuged. After filtration samples were submitted to HPLC-MS/MS detection operating in negative multiple reaction monitoring (MRM) mode.

Details to the HPLC using MS/MS detection and chromatographic parameters are summarised below.

HPLC – MS/MS:	Shimadzu HPLC pump LC-30 AD Sciex QTRAP 5500
Column:	Bio – Rad Cation – H Guard Column, 30 mm x 4.6 mm
Gradient:	Time (min) / %A / %B / Flow rate (mL/min) 0.00 / 80 / 20 / 0.5 0.50 / 80 / 20 / ↓ 1.00 / 80 / 20 / gradient increase 2.50 / 100 / 0 / ↓ 4.00 / 100 / 0 / 1.0 6.90 / 100 / 0 / 1.0 7.00 / 100 / 0 / 0.5 7.10 / 80 / 20 / 0.5 9.00 / 80 / 20 / 0.5
	Valve: 0 – 0.5 min to Waste, 0.5 – 5.5 min to MS/MS, 5.5 – 9.0 min to Waste
Column oven temperature:	40 °C
Injection volume:	5 µL
Mobile phase:	A: 0.1 % formic acid in H_2O B: Acetonitrile
Retention time:	Glyphosate: ~ 1.5 min Glyphosate IS: ~ 1.5 min AMPA: ~ 4.3 min AMPA IS: ~ 4.3 min
Scan type:	Negative Ion MRM
Ion source:	ESI

Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	350 °C
Curtain gas (CUR):	20 (arbitrary units)	Gas flow 1 (GS1):	40 (arbitrary units)
Collision Gas (CAD):	12 (arbitrary units)	Gas flow 2 (GS2):	60 (arbitrary units)

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-35	-25	-10
Glyphosate IS	172	63	-35	-30	-10

AMPA	110	63	-35	-26	-10
AMPA IS	114	63	-35	-26	-10
Confirmatory ions					
Glyphosate	168	79	-45	-45	-10
Glyphosate IS	172	79	-40	-50	-10
AMPA	110	79	-35	-40	-10
AMPA IS	114	79	-35	-38	-10
Confirmatory ions					
Glyphosate	168	150	-35	-15	-10
Glyphosate IS	172	154	-35	-15	-10

Findings

Recoveries

The samples were fortified with glyphosate and AMPA at fortification levels of 0.025 mg/kg, 0.25 mg/kg and 20 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤ 20 %. The detailed results are given in the table below.

Table 5.2-12: Results of the determination of glyphosate and AMPA in honey

Matrix	**Analyte**	**Target ion**	**Fortification level (mg/kg)**	**Recovery**			
				Range (%)	**Mean (%)**	**Relative standard deviation (%)**	**Number analyses (n)**
Honey	Glyphosate	168→63	0.025	102 – 109	107	3	5
			0.25	90 – 102	97	5	5
			20	95 – 111	106	6	5
	Glyphosate	168→79	0.025	104 – 105	105	1	5
			0.25	90 – 101	96	5	5
			20	92 – 109	103	7	5
	Glyphosate	168→150	0.025	100 – 105	103	2	5
			0.25	86 – 94	90	4	5
			20	94 – 103	97	4	5
	AMPA	110→63	0.025	104 – 109	106	2	5
			0.25	97 – 106	100	3	5
			20	82 – 99	92	9	5
	AMPA	110→79	0.025	99 – 106	103	3	5
			0.25	96 – 99	98	1	5
			20	94 – 103	99	4	5

Specificity

The concentration of the analytes in prepared samples was determined by liquid chromatography with MS/MS detection. The specificity of the detection is provided by monitoring three mass transitions for glyphosate and two mass transitions for AMPA. For each transition and each analyte, chromatograms of standards solution, of blank,

of fortified samples at the LOQ are provided. No interference (below 30% of the LOQ) is observed at the retention time of each analyte.

Linearity
Linearity of detector response was tested for glyphosate and AMPA using at least 10 matrix equivalent calibration standards covering the range of 0.75 ng/L to 600 ng/L (corresponding to 0.0075 to 6.0 mg/kg). Linear regression was performed with 1/x weighting and correlation coefficients > 0.99 for glyphosate and AMPA were observed.

Repeatability (Precision)
The relative standard deviations (RSDs) of recovery values at each fortification level were <20 %. Therefore the method complies with EU guideline document SANCO/825/00 rev.8.1 and SANCO/3029/99 rev 4.

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the matrix honey. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4.

Limit of Quantification and Detection
The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 120 % at a relative standard deviation (RSD) of ≤ 20 %. These criteria were fulfilled for the 0.025 mg/kg fortification level for glyphosate and AMPA in honey. The limit of detection (LOD) was set as 0.0075 mg/kg for glyphosate and AMPA in honey (equivalent to 30 % LOQ).

Interference
Control samples did not reveal any peaks ≥30 % LOQ in the chromatogram, which would interfere with the determination of glyphosate and AMPA in honey.

Matrix effects
Matrix effects on detection were generally corrected by the use of response ratio of analyte to internal standard compensating for any difference in response between sample and standard.

Stability in sample extracts
Extract stability was previously demonstrated during the primary validation of method ME-2220 and was not part of this study.

Conclusion
The analytical method was successfully validated for the determination of glyphosate and AMPA in honey at a limit of quantification (LOQ) of 0.025 mg/kg. The method fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

Assessment and conclusion by applicant:
The validation of the method for analysis of glyphosate and AMPA in honey was not previously evaluated at EU level. It was performed under GLP and meets current requirements (EU guideline SANCO/825/00 rev.8.1 and SANCO/3029/99 rev 4.). No deviations to the applied test guidelines were reported. The method is suitable to be used for monitoring/ enforcement purposes.

Assessment and conclusion by RMS The analytical method ME-2220 is independently validated for the determination of glyphosate and AMPA in honey with an LOQ of 0.025mg/kg.

Extraction efficiency of methods for animal matrices

The analytical methods for monitoring in animal commodities are using 0.1 % formic acid in water + dichloromethane in 50/50 proportion. as extraction solvent. The applicant indicated that the use of organic solvents (e.g. methylene chloride) during the sample preparation for glyphosate, AMPA, or *N*-acetyl glyphosate from animal matrices is for clean-up purposes only and is not necessary for extraction. However, the addition of organic solvent is done at the same time of 0.1% aqueous formic acid water, the organic solvent should be take into account in the extraction solvent.
In the submitted metabolism studies water with HCl or water/chloroform were used as extraction solvents. In the studies, that used water/chloroform extraction, the majority of the analytes were found in the aqueous phase,

verifying that an aqueous extraction is preferable for glyphosate and its metabolites. If glyphosate and /or AMPA, or *N*-acetyl-glyphosate are administered to laying hens or lactating goats, relevant amounts of residues were found in all matrices (milk, eggs, meat, fat, liver and kidney). Details on the efficiency of different solvents to extract incurred residues from animal matrices measured as extracted part of the total radioactive residue (TRR) are outlined in sections CA 6.2.2 and CA 6.2.3 and summarised in the table below.

Table 5.2-13: Extractability of the total radioactive residue of animal matrices with different solvents

Matrix	Extractable residue [% TRR (mg/kg)]	Extraction solvent	Reference
Poultry			
Kidney	Glyphosate : 87.9 – 95.2% TRR AMPA : 4.3 -10.3 %TRR [1]	Chloroform and water (1/1, v/v)	CA 6.2.2/003: [redacted] 1988, Metabolism of $^{14}C/^{13}C$-labeled glyphosate and aminomethylphosphonic acid in laying hens. Part II., Report No. [redacted]-7420 Extracted residue quantity was not clearly reported in the study report
Liver	Glyphosate : 42-70.1%TRR AMPA : 27.3 – 54.5%TRR [1]		
Fat	Glyphosate : 65.8 – 86.5%TRR AMPA : 11.5 -33%TRR [1]		
Thigh muscle	Glyphosate : 58.8 -75.8%TRR AMPA : 13 – 33.1%TRR [1]		
Breast muscle	Glyphosate : 43.7- 72%TRR AMPA : 11.7 – 44.9%TRR [1]		
Egg yolk	Glyphosate : 80.6 -84.3 %TRR AMPA : 15.5 – 19.1 %TRR [1]		
Liver	Glyposate 60.97%TRR AMPA : 22.53%TRR Extracted residue : 0.4402 ppm	For eggs Yolk : 150 mL 0.1N HCl/70 mL chloroform Egg white : 100 mL 0.1 N HCL/70 mL chloroform Thigh muscle : 200 mL 0.1N HCl/80 mL Chloroform Breast muscle : 0.1N HCl/Chloroform 200mL/80mL ; 140mL/70mL 150mL/70mL Liver : 0.1N HCl/chloroform (1/1)	CA 6.2.2/004: [redacted] 1994, [^{14}C-PMG] Glyphosate-trimesium: Nature of the residue in tissues and eggs of laying hens, Report No. RR-93-064B
Thigh muscle	Glyphosate : 61 % TRR AMPA : 4.06% TRR Extracted residue : 0.0401 ppm		
Breast muscle	Glyphosate : 39.05%TRR AMPA : 5 % TRR Extracted residue : 0.0292 ppm		
Fat	Glyphosate 40.66%TRR AMPA 3.31% TRR Extracted residue : 0.0293ppm		
Egg white	Glyphosate : 21.48% TRR AMPA : 2.07% TRR Extracted residue : 0.0189 ppm		
Egg yolk	Glyphosate : 59.54% TRR AMPA : 2.28% TRR Extracted residue : 0.24 ppm		
Egg white	Glyphosate : 10.9 % TRR N-acetyl AMPA : 4.34%TRR N-acetyl glyphosate : 41.48% TRR	Egg white, Tissue : 0.2 N HCl in water	CA 6.2.2/005: [redacted] 2007, The metabolism of [^{14}C]-*N*-acetylglyphosate (IN-MCX20) in

Table 5.2-13: Extractability of the total radioactive residue of animal matrices with different solvents

Matrix	Extractable residue [% TRR (mg/kg)]	Extraction solvent	Reference
	Extracted rsidue : 0.009µg/g	Egg Yolk : 0.2N HCL/ methanol (1/1)	laying hens, Report No. [redacted] 19795
Egg yolk	Glyphosate : 5.69% TRR N-acetyl AMPA : 1.1%TRR N-acetyl glyphosate : 68.40% TRR AMPA : 0.91%TRR Extracted residue : 0.187µg/g		
Liver	Glyphosate : 16.34% TRR N-acetyl AMPA : 4.04%TRR N-acetyl glyphosate : 63.82% TRR AMPA : 6.74%TRR Extracted residue : 0.483µg/g		
Muscle	Glyphosate : 7.19% TRR N-acetyl AMPA : 1.89%TRR N-acetyl glyphosate : 25.22% TRR AMPA : 16.6%TRR Extracted residue : 0.029µg/g		
Abdominal fat	Glyphosate : 39.43% TRR N-acetyl AMPA : 10.18%TRR N-acetyl glyphosate : 23.45% TRR AMPA : 11.29%TRR Extracted residue : 0.053µg/g		
Ruminants			
Liver	Glyphosate : 59.4%TRR AMPA : 21.4% TRR Extracted residue : 0.19 ppm eq PMG	kidney, liver and muscle : 0.1N HCl Fat : water and chloroform Milk : aqueous acetic acid 0.6% and chloroform	CA 6.2.3/002: [redacted] 1994, The nature of residues of orally administered [Phosphonomethylene-^{14}C] glyphosate-trimesium in goat tissues and milk, Report No. RR 93-062B
Kidney	Glyphosate : 86.3% TRR AMPA : 7.5% TRR Extracted residue : 5.23 ppm eq PMG		
Fat	Glyphosate : 91.3% TRR AMPA : 4.7% TRR Extracted residue : 0.03 ppm eq PMG		
Muscle	95Glyphosate : 87.1% TRR AMPA : 6.3% TRR.6 (0.024) Extracted residue : 0.02 ppm eq PMG		
Milk	Glyphosate : 22.3% TRR AMPA : 2.4% TRR Extracted residue : 0.02 ppm eq PMG		

Table 5.2-13: Extractability of the total radioactive residue of animal matrices with different solvents

Matrix	Extractable residue [% TRR (mg/kg)]	Extraction solvent	Reference
Kidney	Glyphosate : 84.2 – 94.7% TRR AMPA : 3.9 – 13.8% TRR [1] Extracted residue quantity was not clearly reported in the study report	Chloroform/ water (1/1, v/v)	CA 6.2.3/004: [redacted] 1988, Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphos-phonic acid in lactating goats. Part II, Report No. [redacted]-7458 Extracted residue quantity was not clearly reported in the study report
Liver	Glyphosate : 64.8 – 82.4% TRR AMPA : 11.4 -32.4% TRR [1]		
Muscle	Glyphosate : 70.7 – 90.8% TRR AMPA : 4.7 -14.2% TRR [1]		
Fat	Glyphosate : 87.1 – 90.3% TRR AMPA : 9 – 11.4% TRR [1]		
Milk [2]	Glyphosate : 53.7 - 64% TRR AMPA : 5.4 – 9.4% TRR [1]		
Liver	Glyphosate : 14.71% TRR (0.118 µg/g) N-acetyl glyphosate : 55.51% TRR (0.446 µg/g) AMPA : 8.45%TRR (0.068 µg/g) Extracted residue (0.669 µg/g)	0.2 N HCl in water	CA 6.2.3/005: [redacted] 2007, Metabolism of [^{14}C]-*N*-Acetylglyphosate (IN-MCX20) in the lactating goat, Report No. [redacted] 19796
Kidney	Glyphosate : 39.43% TRR (0.242µg/g) N-acetyl glyphosate : 23.45% TRR (3.742 µg/g) Extracted residue : 4.708 µg/g		
Milk	Glyphosate : 4.98% TRR (0.001 µg/g) N-acetyl glyphosate : 77.72% TRR (0.011µg/g) Extracted residue : 0.021 µg/g		
Muscle	N-acetyl glyphosate : 16.70% TRR (0.014 µg/g) Extracted residue : 0.036 µg/g		
Omental fat	Glyphosate : 6.03% TRR N-acetyl AMPA : 4.31%TRR N-acetyl glyphosate : 21.43% TRR AMPA : 0.5%TRR Extracted residue (mean of omental, renal and subcutaneous fat : 0.098 µg/g		
Renal fat	Glyphosate : 5.02% TRR		

Table 5.2-13: Extractability of the total radioactive residue of animal matrices with different solvents

Matrix	Extractable residue [% TRR (mg/kg)]	Extraction solvent	Reference
	N-acetyl AMPA : 0.59%TRR N-acetyl glyphosate : 73.19% TRR AMPA : 1.20%TRR		
Subcutaneous fat	Glyphosate : 2.65% TRR (N-acetyl AMPA : 14.86%TRR N-acetyl glyphosate : 64.73% TRR AMPA : 4.77%TRR		

1 Given ranges represent different experiments.

The study CA 6.2.2/001 and CA 6.2.3/001 are considered as supportive only in residue section, therefore they do not take into account for the assessment of the extraction efficiency.

Poultry:. Glyphosate was identified as major metabolite in liver and fat. In addition AMPA and *N*-acetyl AMPA were determined in egg white (only *N*-acetyl AMPA), egg yolk, liver, muscle and fat (see chapter 6.2.2).

Ruminants: Glyphosate was identified as major metabolite in liver and kidney, while AMPA and *N*-acetyl AMPA were major metabolites in liver and fat, respectively (see chapter 6.2.3).

By consideration of all available metabolism studies for poultry and ruminants, which reflect the proposed residues definition (sum of glyphosate, AMPA and *N*-acetyl glyphosate expressed as glyphosate), all relevant matrix groups (according to SANCO/825/00/rev. 8.1: milk, eggs, meat, fat, liver and kidney) are covered. It is considered that acidic water extraction is sufficient to extract the majority of the TRR..

Assessment and conclusion by RMS: RMS agrees that all available metabolism studies reflect the proposed residues definition (sum of glyphosate, AMPA and *N*-acetyl glyphosate expressed as glyphosate). The metabolism studies available used 0.2N HCl in water for extraction solvent. According to the SANTE/2017/10632 rev3, solvent mixtures are considered as being identical if their composition varies by not more than 20 %. However, as the extraction solvent used for monitoring methods is 100 mL 0.1% formic acid in water + 100 mL methylene chloride, it cannot be considered identical.
However, based on knowledge of behavior of glyphosate in solution by enforcement laboratories and the low solubility of glyphosate, AMPA and N-acetyl glyphosate in dichloromethane. It is not expected that dichloromethane modified the extraction efficiency in comparison to the solvent used in metabolism studies for plant. Moreover, the pH of the extraction solvent is not the same in the extraction solvent used for metabolism studies (0.2 N HCl, pH around 0.8) and the pH of the extraction solvent used for monitoring method (0.1% formic acid, pH around 2.8). This difference of pH in extraction solvent is not covered by the guidance document. Nevertheless, as the pH has no influence on the water solubility and log kow of glyphosate, AMPA and N-acetyl glyphosate, it is not expected that pH has an influence on extraction efficiency. In order to confirm this hypothesis, a strong argumentation to demonstrate that pH does not affect the extraction eeficiency should be provided during the peer review as no new studies on vertebrate can be requested to solve this point.

B.5.2.2 Methods for the determination of residues in soil

Residue definition: The residue definition in soil is glyphosate and AMPA

Glyphosate:
NOEC nitrogen transformation = 33.1 mg a.e./kg dw soil (highest tested dose)
AMPA:

NOEC Eisenia fetida = 131.9 mg AMPA/kg dw soil

Data point	CA 4.2/011
Report author	[redacted]
Report year	2015
Report title	Validation of an analytical method for the determination of Glyphosate and AMPA in soil using LC/MS/MS
Report No	S15-01216
Document No	Not available
Guidelines followed in study	Regulation (EC) No. 1107/2009 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

An analytical method for the determination of residues of glyphosate and AMPA in soil was validated. The analytes were determined by HPLC-MS/MS using two mass transitions and was quantitated by the use of internal standards. The LOQ was established at 0.05 mg/kg, defined as the lowest validated fortification level.

Principle of the method
Homogenized specimens of soil (10.0 g ± 0.1 g) were weighed into a 250 mL polypropylene bottle. Extraction was done by intensive shaking (30 seconds by hand and 20 minutes using a flatbed shaker) with 100 mL of 1 N NaOH.
Following extraction, an aliquot is acidified and mixed with stable isotope labelled standards prior to SPE clean-up procedures and analysed by LC/MS/MS with mass selective detection.
Glyphosate and AMPA residues were determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) was 0.05 mg/kg for both analytes for soil.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Agilent Series 1260 HPLC (Agilent Technologies) AB-Sciex API 5500 tandem mass spectrometer
Column:	Bio-Rad Fast Acid 100 x 7.8 mm, 9 µm
Column oven temperature:	25 °C
Injection volume:	40 µL
Mobile phase:	0.1 % formic acid in water (isocratic)
Flow rate:	1.5 mL/min
Evaporation solvent (post column):	Methanol at 0.70 mL/min
Split ratio:	The flow of 0.1 % formic acid in water + methanol (1.50 mL/min + 0.70 mL/min) is split 1:1 prior to entering the mass spectrometer
Retention time:	Glyphosate: ~ 2.7 min Glyphosate IS: ~ 2.7 min AMPA: ~ 13.2 min AMPA IS: ~ 13.2 min

Scan type:	Negative Ion MRM				
Ion source:	ESI				
Ion Spray Voltage (IS):	-4500 V		Ion Spray turbo heater (TEM):	400 °C	
Curtain gas (CUR):	30 (arbitrary units)		Gas flow 1 (GS1):	40 (arbitrary units)	
Collision Gas (CAD):	7 (arbitrary units)		Gas flow 2 (GS2):	60 (arbitrary units)	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	168	63	-60	-32	-19
Glyphosate IS	171	63	-85	-30	-5
AMPA	110	63	-25	-26	-21
AMPA IS	114	63	-75	-28	-23
Confirmatory ions					
Glyphosate	168	79	-60	-54	-9
Glyphosate IS	171	79	-85	-50	-15
AMPA	110	79	-25	-34	-15
AMPA IS	114	79	-75	-36	-7

Findings

Recoveries (accuracy)

Standard soil type no. 2.2 were fortified with glyphosate and AMPA at fortification levels 0.05 mg/kg and 0.5 mg/kg. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-14: Results of method validation for the determination of glyphosate and AMPA in soil

Matrix	**Analyte**	**Target ion**	**Fortification level (mg/kg)**	**Recovery**			
				Range (%)	**Mean (%)**	**Relative standard deviation (%)**	**Number of analyses (n)**
Soil type no. 2.2 (Germany – batc No F2.201.11)	Glyphosate	168→63	0.05	89 – 101	95	5.5	5
			0.50	80 – 109	93	12	5
		168→79	0.05	87 – 96	92	3.5	5
			0.50	78 – 100	88	10	5
	AMPA	110→63	0.05	78 – 98	88	8.6	5
			0.50	78 – 101	88	9.3	5
		110→79	0.05	86 – 95	91	3.8	5
			0.50	82 – 106	92	9.6	5

Specificity
For both transition and for glyphosate and AMPA, chromatograms of standards solutions, of samples, of untreated control and fortified samples at LOQ and 10xLOQ are provided. No interference is observed at the retention of glyphosate and AMPA. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity
The linearity of the detector response was confirmed by making single measurements of eight concentrations covering the ranges 1.0 to 200 ng/mL (equivalent to 0.01 to 2.0 mg/kg). The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20 %.

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for N-acetyl glyphosate were found for the analysed matrices.
Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.05 mg/kg was established for all matrices investigated. The limit of detection (LOD) was set as ≤ 30 % of the LOQ, which is 0.015 mg/kg.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate or AMPA.

Matrix effects
Matrix effect were not determined in this study. However, the use of internal standards compensates for any difference in response between samples and standards.

Stability of glyphosate and AMPA in sample extracts
Extract stability was investigated by re-injection of a processed set of fortification samples, that had been stored at +1 to +10 °C. The test indicated no decline of the recoveries during a storage period of at least 8 days under cool conditions. Glyphosate and AMPA are stable in soil extracts for at least 8 days when stored at +1 to +10 °C.

Conclusion
The method was successfully validated for the analysis of residues of glyphosate and AMPA in soil at 0.05 mg/kg (LOQ) and higher fortification levels and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in soil.

Assessment and conclusion by RMS: The method is considered valid to be used for monitoring glyphosate and AMPA residues in soil with an LOQ of 0.05mg/kg.

B.5.2.2 Methods for the determination of residues in water

Residue definition
The residue definition in water is “glyphosate and AMPA”.

Glyphosate:
NOEC = 1 mg a.e./L

AMPA:
NOEC daphnia magna = 12 mg AMPA/L

An overview on the proposed monitoring methods for analysis of glyphosate in water is given in the table below.

Matrix	Analyte(s)	Method	LOQ	Reference	Data point
Surface water	Glyphosate and AMPA	**Primary method:** LC-MS/MS quantitation after derivatisation with FMOC-Cl	0.03 µg/L	[redacted] (2010) Report no.: IF-10/01618859	CA 4.2/012
		ILV: LC-MS/MS quantitation after derivatisation with FMOC-Cl	0.03 µg/L	[redacted] (2011) Report no.: S10-02882	CA 4.2/013
Ground water	Glyphosate and AMPA	**Primary method:** LC-MS/MS quantitation after derivatisation with FMOC-Cl	0.03 µg/L	[redacted] (2010) Report no.: IF-10/01618859	CA 4.2/012
		ILV: LC-MS/MS quantitation after derivatisation with FMOC-Cl	0.03 µg/L	[redacted] (2011) Report no.: S10-02882	CA 4.2/013
Drinking water	Glyphosate and AMPA	**Primary method:** LC-MS/MS quantitation after derivatisation with FMOC-Cl	0.03 µg/L	[redacted] (2010) Report no.: IF-10/01618859	CA 4.2/012
		ILV: LC-MS/MS quantitation after derivatisation with FMOC-Cl	0.03 µg/L	[redacted] (2011) Report no.: S10-02882	CA 4.2/013

Primary method for the determination of glyphosate and AMPA in water

Data point	CA 4.2/012
Report author	[redacted]
Report year	2010
Report title	Validation of an analytical method: Determination of glyphosate and AMPA in water matrices using FMOC derivatization, manual SPE cleanup and LC-MS/MS quantitation.
Report No	IF-10/01618859
Document No	Not available
Guidelines followed in study	SANCO/825/00 rev. 7
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid

Category study in AIR 5 dossier (L docs)	Category 2a
Test facility	SGS Institut Fresenius GmbH, Im Maisel 14, 65232 Taumusstein, Germany

Principle of the method

The water specimens were buffered to approximately pH 8.5 with borate containing EDTA, followed by addition of FMOC-Cl in acetonitrile solvent. Following this, the water specimens were placed in a heated oven at 40 °C for at least 60 minutes to effect the derivatisation. The specimens were quenched with phosphoric acid, diluted to volume and transferred to the SPE cleanup.
Stable labelled $^{13}C_2$, ^{15}N-glyphosate and ^{13}C, ^{15}N-AMPA were used as internal standards to compensate for any matrix effects.
The processed specimen extracts were analysed by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (glyphosate: quantifier: 390→168, qualifier: 390→150; AMPA: quantifier: 332→110, qualifier: 332→136). The LOQ was 0.03 µg/L for both analytes in water.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Agilent Series 1200 HPLC (Agilent Technologies) AB-Sciex API 4000 tandem mass spectrometer			
Column:	Synergi Fusion – RP 150 x 4.6 mm, 4 µm (Phenomenex)			
Column oven temperature:	30 °C			
Injection volume:	5 – 10 µL			
Mobile phase:	A: 11.35 mM ammonium acetate (in purified water), pH 7.4 B: Methanol			
Flow rate:	Time (min)	%A	%B	Flow (mL/min)
	0.0	90	10	0.5
	3.0	90	10	0.5
	9.0	70	30	0.5
	16.0	50	50	0.5
	16.1	10	90	0.5
	18.0	10	90	0.5
	18.1	90	10	0.5
	25.0	90	10	0.5
	To waste	0 to 12 min		
	To MS	12 to 22 min		
	To waste	22 to 25 min		
Retention time:	Glyphosate: ~ 13 min Glyphosate IS: ~ 13 min AMPA: ~ 19 min AMPA IS: ~ 19 min			
Scan type:	Negative Ion MRM			
Ion source:	ESI			
Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	500 °C	

Curtain gas (CUR):	20 (arbitrary units)		Gas flow 1 (GS1):	80 (arbitrary units)	
Collision Gas (CAD):	7 (arbitrary units)		Gas flow 2 (GS2):	80 (arbitrary units)	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
Primary ions					
Glyphosate	390	168	-55	-18	-9
Glyphosate IS	393	171	-40	-18	-9
AMPA	332	110	-55	-12	-17
AMPA IS	334	112	-55	-12	-17
Confirmatory ions					
Glyphosate	390	150	-55	-34	-7
Glyphosate IS	393	153	-40	-36	-7
AMPA	332	136	-55	-22	-9
AMPA IS	334	138	-55	-22	-9

The characterization of water (i.e. pH, TOC and conductivity) is reported below:

water	pH	TOC	Conductivity
drinking water 100346607	7.43	1.33 mg/L	579 µs/cm
surface water 100343137	7.66	2.18 mg/L	94.2 µs/cm
ground water 100146269	6.58	0.30 mg/L	28.1 µs/cm

Validation

Recoveries (accuracy)

Surface water samples were fortified with glyphosate and AMPA at fortification levels 0.03 µg/L and 0.3 µg/L. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-15: Results of method validation for the determination of glyphosate and AMPA in water specimen

Matrix	Analyte	Target ion	Fortification level (µg/L)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Surface water	Glyphosate	390→168	0.03	97 – 107	100.2	4.1	5
			0.3	96 – 102	98.7	2.1	5
		390→150	0.03	97 – 108	102.5	5.0	5
			0.3	92 – 99	96.1	2.5	5
	AMPA	332→110	0.03	89 – 112	102.2	8.4	5
			0.3	94 – 113	99.8	8.3	5
		332→136	0.03	88 – 102	95.7	5.3	5
			0.3	94 – 103	98.6	3.9	5
Drinking water	Glyphosate	390→168	0.03	96 – 102	98.3	2.3	5
			0.3	95 – 97	96.6	0.8	5
		390→150	0.03	96 – 103	97.7	2.8	5
			0.3	92 – 100	96.5	2.9	5
	AMPA	332→110	0.03	100 – 108	104.1	3.6	5
			0.3	95 – 101	97.4	2.5	5
		332→136	0.03	99 – 104	101.4	2.2	5
			0.3	94 – 101	98.2	3.2	5
Ground water	Glyphosate	390→168	0.03	95 – 100	98.2	2.2	5
			0.3	96 – 102	97.9	2.3	5
		390→150	0.03	90 – 99	94.9	3.8	5
			0.3	94 – 101	96.9	3.2	5
	AMPA	332→110	0.03	94 – 109	101.0	5.0	5
			0.3	91 – 103	95.6	5.4	5
		332→136	0.03	95 – 101	97.7	3.0	5
			0.3	96 – 104	98.8	3.4	5

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

Specificity

Chromatograms from derivatised standard solutions, samples and blank materials (untreated drinking water, untreated surface water and untreated ground water) are provided. Extracts of control samples showed that no signals above 30 % of the LOQ indicating that no significant interferences were present. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity
For both analytes (glyphosate and AMPA), the linearity of the detector response was confirmed by making single measurements of eight concentrations covering the ranges 0.2 to 10 ng/mL. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)
The relative standard deviations (RSDs) of overall recoveries were below 20 %. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Accuracy
Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1. However, the recovery at 0.03 µg/L (0.03 ng/mL) seems to be determined outside the linear range of the calibration curve (0.2 – 10 ng/mL). This point should be clarified.

Limit of Quantification and Detection
The limit of quantification (LOQ) of 0.03 µg/L was established for water specimen investigated. The limit of detection (LOD) was not addressed.

Interference
No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate or AMPA.

Matrix effectsThe use of stable labelled $^{13}C_2$, ^{15}N-glyphosate and ^{13}C, ^{15}N-AMPA as internal standards compensates for any difference in response between samples and standards.
Stability of working solutions
The stability of working solutions used during the analytical phase of the study was investigated. The solutions of derivatised glyphosate and AMPA were found to be stable during the analytical phase (June 25, 2010 – July 14, 2010) when stored at 4 to 8 °C in the dark. The results of the stability testing indicate also the correctness of analyte weightings and the robustness of the analytical procedure.

Conclusion
The method was successfully validated for the analysis of residues of glyphosate and AMPA in surface, ground and drinking water at a LOQ of 0.03 µg/L and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010), SANCO/3029/99 rev. 4 (11/July/2000) and OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17 (13/Aug/2007).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring **glyphosate and AMPA** residues in surface water, drinking water and ground water.

Assessment and conclusion by RMS: The study was previously evaluated at EU level. The analytical method (ES-ME-0945-1) using LC-MS/MS is considered validated with a LOQ of 0.03 µg/L for the determination of residues of glyphosate and AMPA in surface, ground and drinking water. As the method is validated on 2 mass transitions, a confirmatory method is not necessary. 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.
However, the recovery at 0.03 µg/L (0.03 ng/mL) seems to be determined outside the linear range of the calibration curve (0.2 – 10 ng/mL). This point should be clarified.

Independent laboratory validation for the determination of glyphosate and AMPA in water

Data point	CA 4.2/013
Report author	[redacted]
Report year	2011
Report title	Independent laboratory validation of an analytical method for determination of residues of glyphosate and AMPA in drinking water
Report No	S10-02882
Document No	Not available
Guidelines followed in study	SANCO/825/00 rev. 7
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Test facility	Eurofins Agroscience Services GmbH Eutinger Str. 24, 75223 Niefern-Oschelbronn, Germany

Principle of the method

Drinking water samples were fortified and buffered to approximately pH 8.5 with borate containing EDTA, followed by addition of FMOC-Cl in acetonitrile solvent. Following this, the water specimens were placed in a heated oven at 40 °C for at least 60 minutes to effect the derivatisation. The specimens were quenched with phosphoric acid, diluted to volume and transferred to the SPE cleanup.
Stable labelled $^{13}C_2$, ^{15}N-glyphosate and ^{13}C, ^{15}N-AMPA were used as internal standards to compensate for any matrix effects.
The processed specimen extracts were analysed by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (glyphosate: quantifier: 390→168, qualifier: 390→150; AMPA: quantifier: 332→110, qualifier: 332→136). The LOQ was 0.03 µg/L for both analytes in water.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS:	Thermo Surveyor MS pump Plus with autosampler ThermoFinnigan TSQ Quantum Discovery Max triple quadrupole system
Column:	Synergi Fusion – RP 150 x 3.0 mm, 4 µm (Phenomenex)
Column oven temperature:	40 °C
Injection volume:	50 µL
Mobile phase:	A: Water B: Methanol C: 11 mM ammonium acetate

Flow rate:

Time (min)	%A	%B	%C	Flow (mL/min)
0.0	80	15	5	0.5
5.0	0	95	5	0.5
8.0	0	95	5	0.5
8.01	80	15	5	0.5
10.0	80	15	5	0.5

Retention time:			Glyphosate: ~ 3.7 min Glyphosate IS: ~ 3.7 min AMPA: ~ 4.9 min AMPA IS: ~ 4.9 min		
Scan type:			Negative Ion MRM		
Ion source:			ESI		
Ion Spray Voltage (IS):	-3300 V		Capillary temperature:	280 °C	
Sheath gas:	20 (arbitrary units)		Auxillary gas:	10 (arbitrary units)	
Collision Gas:	1.2 mTorr		Ion sweep gas:	off	
Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Collision Energy (CE) (V)	Quadrupole 1 width (amu)	Quadrupole 2 width (amu)
Primary ions					
Glyphosate	390	168	-11	1.20	1.20
Glyphosate IS	393	171	-11	1.20	1.20
AMPA	332	110	-19	1.20	1.20
AMPA IS	334	112	-11	1.20	1.20
Confirmatory ions					
Glyphosate	390	150	-19	1.20	1.20
Glyphosate IS	393	153	-19	1.20	1.20
AMPA	332	136	-11	1.20	1.20
AMPA IS	334	138	-19	1.20	1.20

Physico-chemical parameters of drinking water are reported below:

Parameter	Value	Unit
pH (at 17.5°C)	7.62	–
Specific electric conductivity (at 20°C)	754	µS/cm
Total hardness	4.1	mmol/L
Spectral absorption coefficient (at 436 nm)	< 0.1	m^{-1}
Dissolved organic carbon	0.44	mg/L

Validation

Recoveries (accuracy)

Drinking water samples were fortified with glyphosate and AMPA at fortification levels 0.03 µg/L and 0.3 µg/L. All average recoveries were between 70 % and 110 % with RSD ≤ 20 %. The detailed results are given in the table below.

Table 5.2-16: Results of method validation for the determination of glyphosate and AMPA in water specimen

Matrix	Analyte	Target ion	Fortification level (µg/L)	Recovery[1]			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Drinking water	Glyphosate	390→168	0.03	97 – 116	108	7	5
			0.3	94 – 103	99	3	5
		390→150	0.03	74 – 102	87	14	5
			0.3	94 – 102	97	3	5
	AMPA	332→110	0.03	95 – 111	102	7	5
			0.3	99 – 112	105	5	5
		332→136	0.03	91 – 113	102	9	5
			0.3	93 – 114	105	8	5

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

Specificity

Chromatograms from derivatised standard solutions, samples and blank materials (drinking water control samples) are provided. Extracts of control samples showed that no signals above 30 % of the LOQ indicating that no significant interferences were present. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. Further confirmatory techniques are not required.

Linearity

The linearity of the detector response was confirmed by making single measurements of seven concentrations covering the ranges 0.2 to 10 ng/mL (equivalent to 0.2 to 10 µg/L). The coefficient of determination (r^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Accuracy

Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1. However, the recovery at 0.03 µg/L (0.03 ng/mL) seems to be determined outside the linear range of the calibration curve (0.2 – 10 ng/mL). Data gap.

Limit of Quantification and Detection

The limit of quantification (LOQ) of 0.03 µg/L was established for water specimen investigated. The limit of detection (LOD) was set as 30 % of LOQ, which is 0.009 µg/L.

Interference

No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate or AMPA.

Matrix effects

The use of stable labelled $^{13}C_2$, ^{15}N-glyphosate and ^{13}C, ^{15}N-AMPA as internal standards compensates for any difference in response between samples and standards

Stability of working solutions

Not analysed as extraction and analysis were done within one day.

Conclusion
The analytical method was successfully and independently validated for the determination of glyphosate and AMPA in drinking water at a LOQ of 0.03 µg/L and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010), SANCO/3029/99 rev. 4 (11/July/2000) and OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17 (13/Aug/2007).

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring **glyphosate and AMPA** residues in drinking water.

Assessment and conclusion by RMS: The study was previously evaluated at EU level. The analytical method using LC-MS/MS for the determination of residues of glyphosate and AMPA is considered independently validated in drinking water with a LOQ of 0.03 µg/L.

B.5.2.2 Methods for the determination of residues in body fluids and tissues

Residue definition

The residue definition in tissues is "sum of glyphosate, AMPA and N-acetyl glyphosate expressed as glyphosate". The residue definition in body fluids is "glyphosate and AMPA".

An overview on the proposed monitoring method for analysis of glyphosate in body fluids is given in the table below.

Matrix	Analyte(s)	Method	LOQ	Reference; GLP	Data point
Body tissues	Please refer to CA 4.2 (a) animal matrices				
Body fluids					
Urine	Glyphosate and AMPA	**Primary method:** LC-MS/MS	0.01 mg/L	[redacted].,2016 Report no.: MSL0028163	CA 4.2/015

Primary method for the determination of glyphosate and AMPA in urine

Data point	CA 4.2/015
Report author	[redacted]
Report year	2016
Report title	Analytical method for Determination of glyphosate and AMPA in Urine
Report No	MSL0028163
Document No	Not available
Guidelines followed in study	US EPA OCSPP 860.1340 OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance document on residue analytical methods, 2007 SANCO/825/00 rev. 8.1
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)

Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (According to Guidance Document 7109/VI/94-Rev. 6.c1 the development and validation of an analytical method for monitoring purposes and post-registration control is not subject to GLP)
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1
Test facility	Monsanto Company, Environmental sciences, 800 N. Lindbergh Blvd. St. Louis, MO 63167, USA

Principle of the method

Glyphosate and AMPA were extracted from urine using formic acid in a final concentration of 0.1 %. After mixture with isotopically enriched glyphosate and AMPA internal standards the samples were analysed by LC-MS/MS. Glyphosate and AMPA were determined by LC-MS/MS in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) was 0.010 mg/L for both analytes in urine.

Instrumentation and Chromatographic Conditions:

HPLC – MS/MS: Shimadzu Prominence 20A
AB Sciex API 5000/5500

Column: Bio-Rad Cation – H Guard Column, 30 x 4.6 mm

Mobile phase: A: 0.1% formic acid in H_2O
B: Acetonitrile
C: 0.2 % phosphoric acid in water

Gradient:

Time (min)	%A	%B	A+B Flow (mL/min)	C Flow (mL/min)	Divert
0.0	80	20	0.5	0.0	To waste
1.0	80	20	0.625	0.0	To MS
2.5	100	0	0.8125	0.0	To MS
4.0	100	0	1.0	0.0	To MS
6.9	100	0	1.0	0.0	To waste
7.0	100	0	0.5	0.5	To waste
10.0	100	0	0.5	0.5	To waste
10.1	80	20	0.5	0.0	To waste
12.6			Controller stop		

Column oven temperature: 40 °C

Injection volume: 5 µL

Retention time: Glyphosate: ~ 1.5 min
Glyphosate IS: ~ 1.5 min
AMPA: ~ 4.3 min
AMPA IS: ~ 4.3 min

Scan type: Negative Ion MRM

Ion source: ESI

Period 1

Ion Spray Voltage (IS): -4500 V

Ion Spray turbo heater (TEM): 600 °C

Curtain gas (CUR):	20 (arbitrary units)	Entrance Potential:	-10 V
Collision Gas (CAD):	8 (arbitrary units)	Interface heater:	On
Gas flow 1 (GS1):	80 (arbitrary units)	Scan Time:	150 ms
Gas flow 2 (GS2):	60 (arbitrary units)		

Analyte:	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering Potential (DP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)
			Primary ion		
Glyphosate	168	63	-70	-33	-23
Glyphosate IS	172	63	-70	-33	-23
			Confirmatory ion		
Glyphosate	168	79	-65	-53	-31
Glyphosate IS	172	79	-65	-53	-31

Period 2

Ion Spray Voltage (IS):	-4500 V	Ion Spray turbo heater (TEM):	600 °C
Curtain gas (CUR):	20 (arbitrary units)	Entrance Potential:	-10 V
Collision Gas (CAD):	8 (arbitrary units)	Interface heater:	On
Gas flow 1 (GS1):	80 (arbitrary units)	Scan Time:	150 ms
Gas flow 2 (GS2):	60 (arbitrary units)		

			Primary ion		
AMPA	110	63	-100	-28	-24
AMPA IS	114	63	-100	-28	-24
			Confirmatory ion		
AMPA	110	79	-90	-40	-15
AMPA IS	114	79	-90	-40	-15

Validation

Recoveries (accuracy)

Porcine urine samples were fortified with glyphosate and AMPA at fortification levels 0.010 mg/L and 0.10 mg/L. All average recoveries were between 70 % and 110 % with RSD ≤20 %. The detailed results are given in the table below.

Table 5.2-17: Results of method validation for the determination of glyphosate and AMPA in urine specimen

Matrix	Analyte	Target ion	Fortification level (mg/L)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
Urine	Glyphosate	168→63	0.010	96 – 100	98	1.8	7
			0.10	98 – 103	100	2.0	6
		168→79	0.010	88 – 97	93	3.7	7

Table 5.2-17: Results of method validation for the determination of glyphosate and AMPA in urine specimen

Matrix	Analyte	Target ion	Fortification level (mg/L)	Recovery			
				Range (%)	Mean (%)	Relative standard deviation (%)	Number of analyses (n)
			0.10	90 – 99	94	2.9	6
	AMPA	110→63	0.010	93 – 106	100	4.3	7
			0.10	97 – 102	100	1.6	6
		110→79	0.010	93 – 97	94	1.8	7
			0.10	92 – 101	97	3.3	6

Specificity

Extracts of control samples showed that no signals above 30 % of the LOQ indicating that no significant interferences were present. LC-MS/MS with a quantifier ion and one qualifier ion is considered to be highly specific as a detection technique. For both AMPA and glyphosate, representative chromatograms of a matrix control sample, corresponding LOQ fortification, and calibration standard at the LOQ have been provided to show acceptable specificity and lack of interference. Further confirmatory techniques are not required.

Linearity

For both glyphosate and AMPA, the linearity of the detector response was confirmed by making single measurements of eight concentrations covering the ranges 0.06 to 12 µg/mL (equivalent to 0.0025 to 0.5 mg/L). The coefficient of determination (r^2) was ≥ 0.99 for all analytical determinations. A linear fit with 1/x weighting was used.

Repeatability (Precision)

The relative standard deviations (RSDs) of overall recoveries were below 20 %. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Accuracy

Acceptable mean recovery values over all fortification levels between 70 % and 110 % for glyphosate and AMPA were found for the analysed matrices. Therefore the method complies with EU guideline documents SANCO/825/00 rev. 8.1.

Limit of Quantification and Detection

The limit of quantification (LOQ) of 0.010 mg/L was established for urine. The limit of detection (LOD) was set to 30 % of the LOQ, which is 0.003 mg/L.

Interference

No significant interferences from the specimen matrix were detected at the retention times corresponding to glyphosate or AMPA.

Matrix effects

Ionization effects were assessed by comparing the instrument response ratio of the analyte and its associated stable-label internal standard in fortifications of diluent (no matrix) to fortifications of control matrix extract. The urine control matrix fortifications were within 9% of the fortified diluent for both ion transitions of glyphosate and within 14% for both ion transitions of AMPA (acceptance criterion: within ±15%). This indicates the procedures for urine do not contain significant bias from ionization effects.

Stability of glyphosate and AMPA in sample extracts
Extract stability was investigated for urine by re-injection of a processed set of fortification samples, that had been stored at approximately 4 °C. The test indicated no decline of the recoveries during a storage period of 4 days for the two analytes under cool conditions. Glyphosate and AMPA are stable in urine at approximately 4 °C for at least 4 days.

Conclusion
The method was successfully validated for the analysis of residues of glyphosate and AMPA in urine at a LOQ of 0.010 mg/L and fulfils the European requirements for enforcement residue analytical methods as outlined in Guidance Documents SANCO/825/00 rev. 8.1 (2010), SANCO/3029/99 rev. 4 (11/July/2000) and OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17 (13/Aug/2007).

Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level and it was not performed under GLP (in line with Guidance Document 7109/VI/94-Rev. 6.c1 for analytical method for monitoring purposes). It meets current requirements (EU guideline SANCO/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in urine.

Assessment and conclusion by RMS: The method is considered validated for the determination of residues of glyphosate and AMPA in urine with a LOQ of 0.010 mg/L.

B.5.2.2 Analytical methods for the determination of residues in air

Residue definition: The residue definition in air is glyphosate.

Data point	CA 4.2/014
Report author	[redacted]
Report year	2001
Report title	Validation of an analytical method for the determination of Glyphosate in air
Report No	PR01/007
Document No	Not available
Guidelines followed in study	SANCO/825/00 Rev. 6
Deviations from current test guideline	None (SANCO/825/00 rev. 8.1)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

An analytical method was validated for the determination of glyphosate in air.
The analytes were determined by GC-MS after derivatisation with trifluoroacetic acid, trifluoroacetic acid anhydride and trifluoroethanol. The limit of quantification (LOQ) was established at 0.5 µg/sample or 5 µg/m^3, defined as the lowest validated fortification level. The detection limit (LOD) was 0.1 µg/sample or 1 µg/m^3.

Principle of method
The air sample was enriched on SAX material (500 mg SAX-cartridge). The enriched glyphosate was eluted with 1 N HCL. Before the elution ^{13}C-Glyphosate was added as internal standard. After concentration the samples were derivatised with trifluoroacetic acid, trifluoroacetic acid anhydride and trifluoroethanol at 70 °C followed by liquid-liquid extraction as clean-up. The extract was analysed using capillary gas chromatography with mass selective detection in the select ion monitoring (SIM) mode using 3 typical fragment ions (target ion 411 m/z and

qualifiers ions 384 and 238 m/z for the glyphosate derivative, respectively target ion 412 m/z and qualifier ions 385 and 239 m/z for the derivative of the internal standard ^{13}C-glyphosate). The limit of quantification has been set at 5 µg/m³.

Instrumentation and Chromatographic Conditions:

GC – MS:	Dani 86.10 with autosampler LS 32 HP 5970 MSD with ChemStation
Column:	50 m CP-SIL 19 c.b. (corr OV1701), ID 0.25 mm, df = 0.4 µm, (Varian)
Injection volume:	3 µL
Carrier gas:	Helium 4.6 bar
Injector:	PTV, total, Split open after 8 min (25 mL/min) 70 °C (10 sec) with 250 °C/min to 280 °C (20 min)
Temperature program:	120 °C (3 min) with 10 °C/min to 140 °C (10 min) with 5 °C/min to 260 °C (15 min)
Fragment ions:	Glyphosate: *m/z* 411, 384, 238 ^{13}C – Glyphosate: *m/z* 412, 385, 239

Scan mode:	SIM	Dwell time:	100 msec
Multiplier voltage:	200 V	Run time:	25 min
Scans per second:	1.44	Solvent delay:	12 min

Findings

Recoveries (accuracy)

The fortification experiments were performed under enrichment conditions. The test substance dissolved in a small amount of water was given directly onto the absorber material. A second absorber cartridge (without substance) was placed downstream from the first. A constant air stream of 200 mL/min was sucked through both cartridges for at least 6 hours. The enrichment conditions were chosen to be worst case, with a temperature of 35°C and a relative humidity of 80 %. The validation data are shown in the table below.

Table 5.2-18: Recovery results of glyphosate in air

Commodity	Analyte	Fortification level (µg/cartridge)	Recovery Range (%)	Mean Recovery (%)	SD (%)	RSD (%)	Number of analyses (n)
Air	Glyphosate	0.5	73.6 – 78.3	76.2	1.8	2.4	5
		5[2]	79.1 – 81.8	80.7	1.2	1.5	5

[1] Corresponds to approx. 5 µg/m³ considering an enriched volume of approx. 100 L over the 6 – 7 h enrichment period

[2] Corresponds to approx. 50 µg/m³ considering an enriched volume of approx. 100 L over the 6 – 7 h enrichment period

Specificity

Mass selective detection in the select ion monitoring (SIM) mode with target ion 411 *m/z* and qualifiers ions 384 and 238 *m/z* for the glyphosate derivative, respectively target ion 412 *m/z* and qualifier ions 385 and 239 m/z for the derivative of the internal standard ^{13}C-glyphosate was performed. For each detected peak a SIM mass spectrum was recorded demonstrating the identity based on relative intensities of the ions. The method is considered to be highly specific and no confirmatory analytical method is required. Chromatograms of standards solution, of fortified sample of blank are provided. No interference is observed at the retention time of the glyphosate.

Linearity

The linearity of the detector response was confirmed by five calibration solutions covering the working range of 0.39 µg to 5.96 µg glyphosate. By second order curve adaption a correlation coefficients of r = 0.9999 for

glyphosate was obtained. Infact, according to the study report, due to the C13 isotope, the contribution of the isotope increase with the glyphosate concentration and results in the second order curve.

Accuracy
Mean recovery values for glyphosate for both fortification levels (LOQ and 10 x LOQ) in air were in the range of 70 to 110 %. The accuracy of the method is within the limits specified by current guidance.

Repeatability
Coefficients of variation (relative standard deviation) of recoveries obtained at each level of fortification and overall for each matrix were less than 20 %. The repeatability of the method is within the limits specified by current guidance.

Limit of Quantification
It is possible to determine glyphosate in air with a limit of quantification of 5 µg/m³.

Determination of retention capacity
For the highest concentration level (5 µg/cartridge) the second cartridge, which was placed downstream from the first, was analysed for residues of glyphosate. No breakthrough of glyphosate onto the second cartridge was observed.

Sorbent characteristics
The SAX sorbent material consisted of particles with mean particle size of 56 µm (with 10 % each being < 31 µm or > 80 µm). The sorbent material is fixed by porous polypropylene frits of 20 µm pore size to the cartridge. Thus the cartridges are suitable to retain particle associated residues as well.

Storage stability
The storage stability was tested in three enrichment experiments under the same conditions as described above. These enriched samples were stored in a refrigerator for 8 days, at >1 °C and <10 °C.
The measured recoveries show that the samples are stable for 8 days under the described conditions.

Conclusion
The method for the determination of glyphosate in air was successfully validated at an LOQ of 5 µg/m³. It is proposed that the method is suitable for use in support of post-registration data requirements for glyphosate in the EU.

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/825/00 rev. 8.1). The method is considered valid to be used for monitoring glyphosate and AMPA residues in air.

Assessment and conclusion by RMS: The method is validated for the determination of glyphosate in air with an LOQ of 5 µg/m³.

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.

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?
KCA 4.2-001	[redacted]	2016	Analytical method for the determination of Glyphosate and AMPA in matrices of plant origin Report No.: MSL0027298 Document No.: - Monsanto Company GLP/GEP: N Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-002	[redacted]	2015	Independent laboratory validation of an analytical method for determination of glyphosate and AMPA in different matrices of plant origin Report No.: S14-05172 (GAB-1434V) Document No.: - Eurofins Agroscience Services Chem GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-004	[redacted]	2016	Analytical method for the determination of N-Acetyl glyphosate in matrices of plant origin Report No.: MSL0027300 Document No.: - Monsanto Company GLP/GEP: N Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-005	[redacted]	2016	Independent Laboratory Validation of an	N	Y	First submission in EU	GRG	N

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?
			Analytical Method for the Determination of N-Acetyl glyphosate in Matrices of Plant Origin Report No.: S15-04467 Document No.: MSL0027695 Eurofins Agroscience Services Chem GmbH GLP/GEP: y Published: N					
KCA 4.2-006	[redacted]	2016	Analytical Method for the Determination of Glyphosate and AMPA in Matrices of Animal Origin Report No.: MSL0027299 Document No.: - Monsanto Company GLP/GEP: N Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-007	[redacted]	2016	Analytical Method for the Determination of N-Acetyl Glyphosate in Matrices of Animal Origin Report No.: MSL0027301 Document No.: - Monsanto Company GLP/GEP: N Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-008	[redacted]	2016	Independent Laboratory Validation of Analytical Methods for the determination of Glyphosate and its Metabolites N-Acetyl	N	Y	First submission in EU	GRG	N

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?
			Glyphosate and AMPA in matrices of animal origin Report No.: S15-04468 Document No.: MSL0027696 Eurofins Agroscience Services Chem GmbH GLP/GEP: Y Published: N					
KCA 4.2-009	[redacted]	2019	Validation of Monsanto ME-2220 analytical method for the determination of glyphosate and AMPA residues in honey Report No.: SGS-19-01-01 Document No.: MSL 0030583 SGS North America, Inc. GLP Laboratory GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-010	[redacted]	2020	ILV of method ME-2220-01 and short term storage stability of glyphosate and its metabolite AMPA in honey Report No.: S19-04663 Document No.: M-681330-01-1 Eurofins Agroscience Services Chem GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-011	[redacted]	2015	Validation of an analytical method for the determination of Glyphosate and	N	Y	First submission in EU	GRG	N

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?
			AMPA in soil using LC-MS-MS Report No.: S15-01216 Document No.: - Eurofins Agroscience Services Chem GmbH GLP/GEP: Y Published: N					
KCA 4.2-014	[redacted]	2001	Validation of an analytical method for the determination of Glyphosate in air Report No.: PR01/007 Document No.: UCL GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 4.7 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 4.2-015	[redacted]	2016	Analytical method for Determination of glyphosate and AMPA in Urine Report No.: MSL0028163 Document No.: Monsanto Company GLP/GEP: N Published: N	N	Y	First submission in EU	GRG	N
KCA 4.2-012	[redacted]	2010	Validation of an analytical method: Determination of Glyphosate and AMPA in water matrices using FMOC derivatization, manual SPE cleanup and LC-MS-MS quantitation Report No.: IF-10/01618859 Document No.: - SGS Institut	N	N	-	GTF	Y RAR 2017: KIIA 4.5 (OECD) Monograph 1998: - Monograph Trimesium: -

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?
			Fresenius GmbH GLP/GEP: Y Published: N					
KCA 4.2-013	[redacted]	2011	Independent laboratory validation of an analytical method for determination of residues of Glyphosate and AMPA in drinking water Report No.: S10-02882 Document No.: - Eurofins Agroscience Services Chem GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 4.5 (OECD) Monograph 1998: - Monograph Trimesium: -

[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).