

REGISTRATION REPORT
Part B
Section 5
Analytical Methods
Detailed summary of the risk assessment

Product code: HBZ10
Product name: Wizard
Chemical active substances:
Phenmedipham, 125 g/L
Ethofumesate, 125 g/L

Central Zone
Zonal Rapporteur Member State: Poland

CORE ASSESSMENT
(Authorisation - Art. 33 application)

Applicant: UPL Holdings Coöperatief U.A.
Submission date: October 2021, updated December 2022
Finalisation date: December 2022 (initial Core Assessment)
September 2023 (final Core Assessment)

Version history

When	What
October 2021	Applicant submission
December 2022	Additional information/data on analytical methods for phenmedipham for monitoring purposes received from Applicant at the request of the zRMS.
December 2022	<p>Initial assessment by the zRMS</p> <p>The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency.</p>
September 2023	<p>Final report (Core Assessment updated following the commenting period)</p> <p>Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant is struck through and shaded.</p>

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances ~~and relevant impurities~~ in the plant protection product.

Noticed data gaps are: Analytical methods for the relevant impurities EMS and iBMS in the formulation should be provided, as this is required according to Reg. (EU) 284/2013.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are: none

Commodity/crop	Supported / Not supported
Plant: high water, high acid, high oil, high protein/high starch content (dry) and difficult matrices	Supported
Animal: Muscle, milk, eggs, fat, liver, kidney	Supported
Soil	Supported
Water	Supported
Air	Supported
Body fluids and tissues	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham and Ethofumesate in plant protection product is provided as follows:

Comments of zRMS:	The analytical method was successfully validated for the determination of Phenmedipham and Ethofumesate in plant protection product according to the requirements laid down by SANCO3030/99 rev.5.
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Reference:	KCP 5.1.1/01
Report	Validation of the Methods of Determination of Ethofumesate and Phenmedipham and specified impurities in an EC Formulation, in Compliance with Good Laboratory Practice, Norris, D. (2021), Study Number: DNA6255.
Guideline(s):	SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Ethofumesate and Phenmedipham Analysis:

The assay of Ethofumesate and Phenmedipham was performed using approximately 0.1 g of Formulation. The mass of the Formulation was accurately recorded, transferred to a 100 mL volumetric flask, and made to partial volume with Methanol. The samples were sonicated for 5 minutes and left to cool to

ambient temperature. These solutions were then made to volume with Methanol and used for assay by injecting each solution once into the HPLC-PDA under the following conditions:

HPLC-PDA Conditions – Active Ingredients:

Instrument: Shimadzu HPLC-PDA
Mode: Gradient Reverse Phase
Column: Inertsil ODS-3V, (250 mm x 4.6 mm)
Packing: ODS-3V
Eluent: A: Methanol
B: Deionised Water adjusted to pH 3 with Phosphoric Acid
Wavelength: 225 nm
Injection Volume: 10 µL
Flow Rate: 1.0 mL/minute
Column Temperature: 25°C
Data Collection: LabSolutions
Retention Times: Ethofumesate: Approximately 22.1 to 22.9 minutes
Phenmedipham: Approximately 18.2 to 18.9 minutes

HPLC-PDA Gradient Conditions:

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	61	39
25.00	61	39
25.10	80	20
35.00	80	20
35.10	61	39
45.00	61	39

LC-QTOF Conditions – MS Spectral Analysis for Ethofumesate and Phenmedipham

LC Conditions:

Instrument: Agilent 1200 Series HPLC-DAD
Mode: Gradient Reverse Phase
Column: Inertsil ODS-3V, (250 mm x 4.6 mm)
Packing: ODS-3V
Eluent: A: Methanol
B: Deionised Water adjusted to pH 3 with Formic Acid
Wavelength: 225 nm
Injection Volume: 10 µL
Flow Rate: 1.0 mL/minute
Column Temperature: 25°C

LC Gradient Conditions:

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	61	39
25.00	61	39
25.10	80	20
35.00	80	20
35.10	61	39
45.00	61	39

MS Conditions:

Instrument:	Agilent 6500 Series Q-TOF Mass Spectrometer		
Mode/Source:	Agilent Jetstream ESI		
Ionisation:	Positive		
MS Scan Range:	30 m/z to 1000 m/z		
MSMS Scan Range:	30 m/z to 500 m/z		
Extracted Ions:	n/a (Full Scan)		
Acquisition Rate:	1 Spectra/Second		
Acquisition Time:	1000 ms/Spectra		
Retention Times:	Ethofumesate: Approximately 20.4 minutes Phenmedipham: Approximately 17.4 minutes		
Gas Temperature:	250°C	VCap:	3000V
Drying Gas Flow:	6 L/minute	Nozzle Voltage:	2000V
Nebulizer:	30 psig	Fragmentor:	100V
Sheath Gas:	250°C	Skimmer:	65V
Sheath Gas Flow:	6 L/minute	OCT 1 RF Vpp:	750V
Collision Energy:	0V to 30V		
Data Acquisition:	MassHunter		

RESULTS AND DISCUSSIONS

Linearity of Ethofumesate:

The linearity was determined from sixteen injections of eight concentrations of standard ranging from a Blank to 1 mg/mL. The samples were prepared for analysis at a sample concentration of 1 mg/mL. Sample DNA6253/1 has a declared Ethofumesate content of 125g/L, this therefore equates to a concentration 0.125 mg/mL, which falls within the limits of the linearity range. The plot possesses a correlation coefficient of 1.0000, based on individual values.

Sample Precision of Ethofumesate:

To show the Sample Precision, six samples of approximately 0.1 g of sample DNA6253/1 were prepared in 100 mL volumetric flasks and made to partial volume with Methanol. The samples were sonicated for 5 minutes and left to cool to ambient temperature. These solutions were then made to volume with Methanol and injected into the HPLC-PDA. The values ranged from 119.8 g/L to 120.8 g/L with a mean of 120.4 g/L, a standard deviation of 0.389 and a percentage relative standard deviation of 0.323.

Recovery of Ethofumesate:

It is known that the sample DNA6253/1 contains approximately 125 g/L Ethofumesate. This equates to 0.125 mg/mL as the samples were made at 1.0 mg/mL concentration.

Therefore, the recovery samples were prepared for analysis by weighing the Formulation Blank sample DNA6255/1 at 1.0 mg/mL and spiking at 0.125 mg/mL using a certified Ethofumesate reference standard. To achieve this, approximately 0.01 g of Formulation Blank Sample DNA6255/1 was weighed into a 10 mL volumetric flask, spiked with 1.25 mL of 1.0 mg/mL Ethofumesate Reference Standard Solution, and made to partial volume with Methanol. The sample was sonicated for 5 minutes and cooled to room temperature, before making to final volume with Methanol. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results indicate a percentage recovery range of 100.2% to 101.6%, with a mean of 101.0%, a standard deviation of 0.594 and a percentage relative standard deviation of 0.589

LOQ Recovery of Ethofumesate:

The LOQ and LOD are set at the same level and are defined as the lowest point on the linearity, which for Ethofumesate is 0.005 mg/mL. This equates to 5.0 g/L, as the samples were prepared at 1.0 mg/mL concentration.

Therefore, the LOQ Recovery samples were prepared for analysis by spiking samples of the Formulation Blank DNA6255/1 at 0.005 mg/mL using the certified Ethofumesate reference standard material.

To achieve this, approximately 0.1 g of the Formulation Blank sample DNA6255/1 was weighed into a 100 mL volumetric flask, spiked with 0.5 mL of 1.0 mg/mL Ethofumesate reference standard solution, and made to partial volume with Methanol. The sample was sonicated for 5 minutes, allowed to cool to room temperature and made to final volume with Methanol. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results indicate a percentage recovery range of 99.06% to 101.9% with a mean of 99.94%, a standard deviation of 1.021 and a relative standard deviation of 1.021.

Specificity of Ethofumesate:

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the specificity chromatograms Ethofumesate eluted at 22.8 minutes and other possible significant peaks were accounted for by assaying a solvent blank, the Formulation Blank DNA6255/1, and standards of Phenmedipham, Impurity 1, Impurity 2, and Impurity 3.

There were no peaks present in these chromatograms at the same elution time as Ethofumesate. This demonstrates that there were no analyte interferences.

Linearity of Phenmedipham:

The linearity was determined from sixteen injections of eight concentrations of standard ranging from a Blank to 1 mg/mL. The samples were prepared for analysis at a sample concentration of 1 mg/mL. Sample DNA6253/1 has a declared Phenmedipham content of 125 g/L, this therefore equates to a concentration 0.125 mg/mL, which falls within the limits of the linearity range. The plot possesses a correlation coefficient of 0.9999, based on individual values.

Sample Precision of Phenmedipham:

To show the Sample Precision, six samples of approximately 0.1 g of sample DNA6253/1 were prepared in 100 mL volumetric flasks and made to partial volume with Methanol. The samples were sonicated for 5 minutes and left to cool to ambient temperature. These solutions were then made to volume with Methanol and injected into the HPLC-PDA. The values ranged from 125.9 g/L to 128.6 g/L with a mean of 127.7 g/L, a standard deviation of 1.154 and a percentage relative standard deviation of 0.904.

Recovery of Phenmedipham:

It is known that the sample DNA6253/1 contains approximately 125 g/L Phenmedipham. This equates to 0.125 mg/mL as the samples were made at 1.0 mg/mL concentration.

Therefore, the recovery samples were prepared for analysis by weighing the Formulation Blank sample DNA6255/1 at 1.0 mg/mL and spiking at 0.125 mg/mL using a certified Phenmedipham reference standard.

To achieve this, approximately 0.01 g of Formulation Blank Sample DNA6255/1 was weighed into a 10 mL volumetric flask, spiked with 1.25 mL of 1.0 mg/mL Phenmedipham Reference Standard Solution, and made to partial volume with Methanol. The sample was sonicated for 5 minutes and cooled to room temperature, before making to final volume with Methanol. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results indicate a percentage recovery range of 97.33% to 98.69%, with a mean of 98.15%, a standard deviation of 0.599 and a percentage relative standard deviation of 0.610.

LOQ Recovery of Phenmedipham:

The LOQ and LOD are set at the same level and are defined as the lowest point on the linearity, which for Phenmedipham is 0.001 mg/mL. This equates to 1.0 g/L, as the samples were prepared at 1.0 mg/mL concentration.

Therefore, the LOQ Recovery samples were prepared for analysis by spiking samples of the Formulation Blank DNA6255/1 at 0.001 mg/mL using the certified Phenmedipham reference standard material.

To achieve this, approximately 0.1 g of the Formulation Blank sample DNA6255/1 was weighed into a 100 mL volumetric flask, spiked with 0.1 mL of 1.0 mg/mL Phenmedipham reference standard solution, and made to partial volume with Methanol. The sample was sonicated for 5 minutes, allowed to cool to room temperature and made to final volume with Methanol. Six separate solutions were prepared in this way and then injected into the HPLC-PDA. The results indicate a percentage recovery range of 93.16% to 98.94% with a mean of 95.85%, a standard deviation of 2.039 and a relative standard deviation of 2.127.

Specificity of Phenmedipham:

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the specificity chromatograms Phenmedipham eluted at 18.9 minutes and other possible significant peaks were accounted for by assaying a solvent blank, the Formulation Blank DNA6255/1, and standards of Ethofumesate, Impurity 1, Impurity 2, and Impurity 3.

There were no peaks present in these chromatograms at the same elution time as Phenmedipham. This demonstrates that there were no analyte interferences.

Table 5.2.1.1-1 Methods suitable for the determination of active substances Phenmedipham and Ethofumesate in plant protection product HBZ10

	Phenmedipham	Ethofumesate
Author(s), year	Norris, 2021	Norris, 2021
Principle of method	LC-MS/MS	LC-MS/MS
Linearity	linear between 0 – 1 mg/mL equivalent to 0 (blank) – 1000 g/L concentration in formulation $y=0.000000023x - 0.000686$ $R^2 = 0.9999$ $n = 8$ (duplicate injection)	linear between 0 – 1 mg/mL mL (equivalent to 0 (blank) – 1000 g/L concentration in formulation) $y = 0.000000080x - 0.00111$ $R^2 = 1.0000$ $n = 8$ (duplicate injection)
Precision – Repeatability Mean n = 6 (% RSD)	%RSD = 0.904 Hr = 0.495	%RSD = 0.323 Hr = 0.175
Recovery at 125 g/L n = 6 (% Recovery)	Mean Recovery = 98.15% %RSD = 0.610 Hr = 0.332	Mean Recovery = 101.0% %RSD = 0.589 Hr = 0.322
LOQ Recovery at 5 g/L (Ethofumesate) LOQ Recovery at 1 g/L (Phenmedipham) n = 6 (% Recovery)	Mean Recovery = 95.85% %RSD = 2.127 Hr = 0.558	Mean Recovery = 99.94% %RSD = 1.021 Hr = 0.343
Interference/ Specificity	The UV and MS spectra for Phenmedipham confirmed the species identification. Phenmedipham eluted at 18.9 minutes and there were no other peaks present at the same elution time as Phenmedipham	The UV and MS spectra for Ethofumesate confirmed the species identification. Ethofumesate eluted at 22.8 minutes and there were no other peaks present at the same elution time as Ethofumesate

CONCLUSION

This method of determination of Ethofumesate and Phenmedipham in plant protection product has been successfully validated in accordance with the SANCO/3030/99 rev.5 guidelines and is considered as fully acceptable.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

As per COMMISSION IMPLEMENTING REGULATION (EU) 540/2011 (and according to FAO Specification Code 77/1/(s)/7)

Phenmedipham Minimum purity 970 g/kg

No relevant impurities are present in technical Phenmedipham. The information on significant impurities is confidential. Please refer to Part C. The active substance source used in HBZ10 is approved on the EU level, please refer to Part C of the present dossier for further details.

No other relevant impurities are formed during manufacture of the plant protection product or from degradation of the product during storage.

As per COMMISSION IMPLEMENTING REGULATION (EU) 2016/1426 (and according to FAO Specification 233/TC)

Ethofumesate Minimum purity 970 g/kg

Relevant impurities:

The following impurities are of toxicological concern and must not exceed the following levels in the technical material:

- EMS; ethyl methane sulfonate: maximum of 0.1 mg/kg
- iBMS; iso-butyl methane sulfonate: maximum of 0.1 mg/kg

RMS: Analytical methods for the relevant impurities EMS and iBMS in the formulation should be provided, as this is required according to Reg. (EU) 284/2013.

Details of significant impurities are provided in Part C. The active substance source used in HBZ10 is approved on the EU level, please refer to Part C of the present dossier for further details.

~~As those impurities are results of manufacturing process of the active substance Ethofumesate only, an analytical method for the determination of these impurities in the formulation has not been provided.~~

However, an Analytical method for the determination of the impurities in the formulated product is available and is provided below in Part C.

Comments of zRMS:	The analytical method was successfully validated for the determination of the impurities:
	<ul style="list-style-type: none"> - Impurity 1 (3-Methylaniline (3MA)) - Impurity 2 (Toluene) - Impurity 3 (3-Aminophenol)
	in plant protection product according to the requirements laid down by SANCO3030/99 rev. 5.

Reference: KCP 5.1.1/02

Report Validation of the Methods of Determination of Ethofumesate and Phenmedipham and specified impurities in an EC Formulation, in Compliance with Good Laboratory Practice, Norris, D. (2021), Study Number: DNA6255.

Guideline: SANCO/3030/99 rev.5

Deviations: No

GLP: Yes

Acceptability: Yes

MATERIALS AND METHODS

Impurity 1 (3-Methylaniline (3-MA)) and Impurity 2 (Toluene) Analysis:

The assay of Impurity 1 and Impurity 2 was performed using approximately 0.5 g of Formulation. The mass of the Formulation was accurately recorded, transferred to a 100 mL volumetric flask, and made to volume with Acetonitrile. These solutions were then used for assay by injecting each solution once into the HPLC-DAD.

The chromatographic conditions until 25 minutes into the assay are identical for both methods and therefore identical for the Impurity 1 and Impurity 2 analysis. For the sample assays an additional gradient is employed to ensure that any residual coformulants have been flushed from the column. This ensures that there is no potential carry-over between the sample assays and the following injections.

HPLC-DAD Conditions – Impurity 1 and Impurity 2 Standards Analysis:

Instrument:	Agilent 1100/1200 Series HPLC-DAD
Mode:	Isocratic Reverse Phase
Column:	Grace Alltima C8, (250 mm x 4.6 mm)
Packing:	C8, 5 µm
Eluent:	37% Acetonitrile 63% Deionised Water adjusted to pH 3 with Phosphoric Acid
Wavelength:	210 nm
Injection Volume:	10 µL
Flow Rate:	1.0 mL/minute
Column Temperature:	30°C
Data Collection:	LabSolutions
Retention Times:	Impurity 1: Approximately 3.8 to 3.9 minutes

Impurity 2: Approximately 21.5 to 21.8 minutes

HPLC-DAD Gradient Conditions:

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	37	63
25.00	37	63
26.00	65	35
39.00	65	35
40.00	37	63
60.00	37	63

GC-MSD with Headspace Sampler Conditions – MS Spectral Analysis for Impurity 1 and Impurity 2 conditions

Instrument: Shimadzu GC-MSD with HS-20 Headspace Sampler
 Column: RTX-1 (30 m x 0.32 mm x 5.0 µm)
 Temperatures:
 Column: 40°C held for 3 minutes, then 10°C/minute to 260°C held for 5 minutes
 Injector: 33°C
 SIM: 65 m/z, 77 m/z, 91 m/z, 92 m/z, 106 m/z and 107 m/z
 Carrier Gas: Helium
 Data Collection: GCMS Solutions
 Retention Time: Impurity 1: Approximately 17.0 minutes
 Impurity 2: Approximately 10.8 minutes

Headspace Conditions:
 Cycle Time: 32 minutes
 Shake Strength: 4/5

Oven Temperature: 80°C
 Loop Temperature: 150°C
 Transfer Line: 180°C

Impurity 3 (3-Aminophenol) Analysis:

The assay of Impurity 3 was performed using approximately 0.1 g of the Formulation. The mass of the Formulation was accurately recorded, transferred to a 100 mL volumetric flask, and made to volume with Acetonitrile. The solutions were diluted 1:10 in Acetonitrile and used for assay by injecting each solution once into the LC-QQQ under the following conditions:

LC-QQQ Conditions - Impurity 3:

Instrument: Agilent Ultivo LC-QQQ Mass Spectrometer
 Mode: Isocratic Reverse Phase
 Column: Waters Sunfire C18 (150 mm x 4.6 mm)
 Packing: C18, 3.5 µm
 Eluent: 80% Acetonitrile
 20% Deionised Water adjusted to pH 3 with Formic Acid
 Flow Rate: 0.5 mL/minute
 Injection Volume: 3 µL
 Column Temperature: 25°C
 Retention Time: Approximately 2.8 minutes
 Data Acquisition: MassHunter

Ionisation:	Positive	Sheath Gas Temperature:	250°C
Gas Temperature:	200°C	Sheath Gas flow:	8 L/minute
Gas Flow:	6 L/minute	Capillary:	3500V
Nebulizer:	30 psi	Nozzle Voltage:	2000V

RESULTS AND DISCUSSIONS

Linearity of Impurity 1 (3-Methyl aniline (3MA)):

The linearity was determined from sixteen injections of eight concentrations of standard ranging from a blank to 100 mg/L. The samples were prepared for analysis at a sample concentration of 5 mg/mL. From the Sample Precision it is known that sample DNA6253/1 contains no detectable Impurity 1 above the LOQ Level of 0.1 g/kg. Recovery Precision was performed at 1.00 g/kg, this therefore equates to a concentration of 5.00 mg/L, which falls within the limits of the linearity range. The plot possesses a correlation coefficient of 1.0000, based on individual values.

Sample Precision of Impurity 1 (3-Methyl aniline (3MA)):

To show the Sample Precision, six samples of approximately 0.5 g of sample DNA6253/1 were prepared in 100 mL volumetric flasks and made to volume with Acetonitrile and injected once into the HPLC-DAD.

Impurity 1 (3-Methyl aniline (3MA)) was not detected above the LOQ level of 0.10 g/kg.

Recovery Precision of Impurity 1 (3-Methyl aniline (3MA)):

From the Sample Precision it is known that sample DNA6253/1 contains no detectable Impurity 1 above the LOQ Level of 0.1 g/kg. Therefore, a Recovery Precision was performed at 1.0 g/kg. This equates to 5 mg/L as the samples were made at 5 mg/mL concentration.

Therefore, the Recovery Precision samples were prepared for analysis by spiking samples of DNA6253/1 at 5 mg/L using the certified reference standard material. This was achieved by weighing approximately 0.5 g of sample DNA6253/1 into a 100 mL volumetric flask, spiked with 500 µL of 1000 mg/L Impurity 1 reference standard solution and making to volume with Acetonitrile. Six separate solutions were prepared in this way and injected into the HPLC-DAD. The results indicate a percentage recovery range of 99.24% to 100.4%, with a mean of 99.85%, a standard deviation of 0.392 and a percentage relative standard deviation of 0.393.

LOQ Recovery of Impurity 1 (3-Methyl aniline (3MA)):

The LOQ and LOD are set at the same level and are defined as the lowest point on the linearity, which for Impurity 1 is 0.50 mg/L. This equates to 0.1 g/kg as the samples were prepared at 5 mg/mL concentration.

There is no detectable Impurity 1 present in the sample DNA6253/1 above the LOQ Level of 0.1 g/kg. Therefore, the LOQ Recovery was performed by spiking Impurity 1 onto sample DNA6253/1.

Therefore, the LOQ Recovery samples were prepared for analysis at 0.50 mg/L using the certified reference standard material. This was achieved by weighing approximately 0.5 g of sample DNA6253/1 into a 100 mL volumetric flask, spiked with 50 µL of 1000 mg/L Impurity 1 reference standard solution and making to volume with Acetonitrile. Six separate solutions were prepared in this way and injected into the HPLC-DAD. The results indicate a percentage recovery range of 94.66% to 98.33% with a mean of 96.22%, a standard deviation of 1.263 and a percentage relative standard deviation of 1.312.

Specificity of Impurity 1 (3-Methyl aniline (3MA)):

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the specificity chromatograms Impurity 1 eluted at 3.8 minutes and other possible significant peaks were accounted for by assaying a solvent blank, the Formulation Blank DNA6255/1, and standards of Ethofumesate, Phenmedipham, Impurity 2, and Impurity 3.

There were no peaks present in these chromatograms at the same elution time as Impurity 1. This demonstrates that there were no analyte interferences.

Linearity of Impurity 2 (Toluene):

The linearity was determined from sixteen injections of eight concentrations of standard ranging from a blank to 100 mg/L. The samples were prepared for analysis at a sample concentration of 5.0 mg/mL. From the Sample Precision it is known that sample DNA6253/1 contains no detectable Impurity 2 above the LOQ Level of 0.1 g/kg. Recovery Precision was performed at 1.00 g/kg, this therefore equates to a concentration of 5.00 mg/L, which falls within the limits of the linearity range. The plot possesses a correlation coefficient of 1.0000, based on individual values.

Sample Precision of Impurity 2 (Toluene):

To show the Sample Precision, six samples of approximately 0.5 g of sample DNA6253/1 were prepared in 100 mL volumetric flasks and made to volume with Acetonitrile and injected once into the HPLC-DAD.

Impurity 2 (Toluene) was not detected above the LOQ level of 0.10 g/kg.

Recovery Precision of Impurity 2 (Toluene):

From the Sample Precision it is known that sample DNA6253/1 contains no detectable Impurity 2 above the LOQ Level of 0.1 g/kg. Therefore, a Recovery Precision was performed at 1.0 g/kg. This equates to 5 mg/L as the samples were made at 5 mg/mL concentration.

Therefore, the Recovery Precision samples were prepared for analysis by spiking samples of DNA6253/1 at 5 mg/L using the certified reference standard material. This was achieved by weighing approximately 0.5 g of sample DNA6253/1 into a 100 mL volumetric flask, spiked with 500 µL of 1000 mg/L Impurity 2 reference standard solution and making to volume with Acetonitrile. Six separate solutions were prepared in this way and injected into the HPLC-DAD. The results indicate a percentage recovery range of 99.42% to 100.4%, with a mean of 99.80%, a standard deviation of 0.333 and a percentage relative standard deviation of 0.333.

LOQ Recovery of Impurity 2 (Toluene):

The LOQ and LOD are set at the same level and are defined as the lowest point on the linearity, which for Impurity 2 is 0.50 mg/L. This equates to 0.1 g/kg as the samples were prepared at 5 mg/mL concentration.

There is no detectable Impurity 2 present in the sample DNA6253/1 above the LOQ Level of 0.1 g/kg. Therefore, the LOQ Recovery was performed by spiking Impurity 2 onto sample DNA6253/1.

Therefore, the LOQ Recovery samples were prepared for analysis at 0.50 mg/L using the certified reference standard material. This was achieved by weighing approximately 0.5 g of sample DNA6253/1 into a 100 mL volumetric flask, spiked with 50 µL of 1000 mg/L Impurity 2 reference standard solution and making to volume with Acetonitrile. Six separate solutions were prepared in this way and injected into the HPLC-DAD. The results indicate a percentage recovery range of 96.17% to 102.7% with a mean of 99.82%, a standard deviation of 2.553 and a percentage relative standard deviation of 2.557.

Specificity of Impurity 2 (Toluene):

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the specificity chromatograms Impurity 2 eluted at 21.6 minutes and other possible significant peaks were accounted for by assaying a solvent blank, the Formulation Blank DNA6255/1, and standards of Ethofumesate, Phenmedipham, Impurity 1, and Impurity 3.

There were no peaks present in these chromatograms at the same elution time as Impurity 2. This demonstrates that there were no analyte interferences.

Linearity of Impurity 3 (3-Aminophenol):

The linearity was determined from sixteen injections of eight concentrations of standard ranging from a blank to 1.0 mg/L. The samples were prepared for analysis at a sample concentration of 1 mg/mL and diluted 1:10. From the Sample Precision it is known that the sample DNA6253/1 contains no detectable Impurity 3 above the LOQ Level of 0.1 g/kg. Recovery Precision was performed at 1 g/kg, this therefore equates to a concentration of 0.10 mg/L, which falls within the limits of the linearity range. The plot possesses a correlation coefficient of 0.9995, based on individual values.

Sample Precision of Impurity 3 (3-Aminophenol):

To show the Sample Precision, six samples of approximately 0.1 g of sample DNA6253/1 were prepared in 100 mL volumetric flasks and made to volume with Acetonitrile. The samples were diluted 1:10 in Acetonitrile and injected once into the LC-QQQ.

Impurity 3 (3-Aminophenol) was not detected above the LOQ level of 0.10 g/kg.

Recovery Precision of Impurity 3 (3-Aminophenol):

From the Sample Precision it is known that sample DNA6253/1 contains no detectable Impurity 3 above the LOQ Level of 0.1 g/kg. Therefore, a Recovery Precision was performed at 1.0 g/kg. This equates to 0.1 mg/L as the samples were made at 1 mg/mL concentration and diluted 1:10.

Therefore, the Recovery Precision samples were prepared for analysis by spiking samples of DNA6253/1 at 0.1 mg/L using the certified reference standard material. This was achieved by weighing approximately 0.1 g of sample DNA6253/1 into a 100 mL volumetric flask, spiked with 1.00 mL of 100 mg/L Impurity 3 reference standard solution and making to volume with Acetonitrile. Six separate solutions were prepared in this way, diluted 1:10 in Acetonitrile, and injected into the LC-QQQ. The results indicate a percentage recovery range of 100.0% to 100.5%, with a mean of 100.2%, a standard deviation of 0.171 and a percentage relative standard deviation of 0.171.

LOQ Recovery of Impurity 3 (3-Aminophenol):

The LOQ and LOD are set at the same level and are defined as the lowest point on the linearity, which for Impurity 3 is 0.01 mg/L. This equates to 0.1 g/kg as the samples were prepared at 1 mg/mL concentration and diluted 1:10.

There is no detectable Impurity 3 present in the sample DNA6253/1 above the LOQ Level of 0.1 g/kg. Therefore, the LOQ Recovery was performed by spiking Impurity 3 onto sample DNA6253/1.

Therefore, the LOQ Recovery samples were prepared for analysis at 0.01 mg/L using the certified reference standard material. This was achieved by weighing approximately 0.1 g of sample DNA6253/1 into a 100 mL volumetric flask, spiked with 100 µL of 100 mg/L Impurity 3 reference standard solution and making to volume with Acetonitrile. Six separate solutions were prepared in this way, diluted 1:10 in Acetonitrile, and injected into the LC-QQQ. The results indicate a percentage recovery range of 98.86% to 100.8% with a mean of 99.52%, a standard deviation of 0.702 and a percentage relative standard deviation of 0.706.

Specificity of Impurity 3 (3-Aminophenol):

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the specificity chromatograms Impurity 3 eluted at 2.8 minutes and other possible significant peaks were accounted for by assaying a solvent blank, the Formulation Blank DNA6255/1, and standards of Ethofumesate, Phenmedipham, Impurity 1, and Impurity 2.

There were no peaks present in these chromatograms at the same elution time as Impurity 3. This demonstrates that there were no analyte interferences.

Table 5.2.1.2-1 Methods suitable for the determination of impurities in plant protection product HBZ10

	Impurity 1 (3-Methylaniline (3MA))	Impurity 2 (Toluene)	Impurity 3 (3-Aminophenol)
Author(s), year	Norris, 2021	Norris, 2021	Norris, 2021
Principle of method	LC-MS/MS	LC-MS/MS	LC-MS/MS
Linearity	linear between 0 – 100 mg/L $y = 0.000024x - 0.0216$ $R^2 = 1.000$ $n = 8$ (duplicate injection)	linear between 0 – 100 mg/L $y = 0.000020x + 0.00876$ $R^2 = 1.0000$ $n = 8$ (duplicate injection)	linear between 0 – 1 mg/L $y = 0.000000349x - 0.000557$ $R^2 = 0.9995$ $n = 8$ (duplicate injection)
Precision $n = 6$ (% RSD)	No detectable Impurity 1 above the LOQ Level of 0.1 g/kg	No detectable Impurity 2 above the LOQ Level of 0.1 g/kg	No detectable Impurity 3 above the LOQ Level of 0.1 g/kg
Recovery Precision at 1 g/kg $n = 6$ (% Recovery)	Mean Recovery = 99.85% %RSD = 0.393 Hr = 0.104	Mean Recovery = 99.80% %RSD = 0.333 Hr = 0.0879	Mean Recovery = 100.2% %RSD = 0.171 Hr = 0.0451
LOQ Recovery at 0.1 g/kg $n = 6$ (% Recovery)	Mean Recovery = 96.22% %RSD = 1.312 Hr = 0.243	Mean Recovery = 99.82% %RSD = 2.557 Hr = 0.477	Mean Recovery = 99.52% %RSD = 0.706 Hr = 0.132
Interference/ Specificity	The UV and MS spectra for Impurity 1 confirmed the species identification. Impurity 1 eluted at 3.8 minutes and there were no other peaks present at the same elution time as Impurity 1.	The UV and MS spectra for Impurity 2 confirmed the species identification. Impurity 2 eluted at 21.6 minutes and there were no other peaks present at the same elution time as Impurity 2.	The MS spectra for Impurity 3 confirmed the species identification. Impurity 3 eluted at 2.8 minutes and there were no other peaks present at the same elution time as Impurity 3.

CONCLUSION

This method of determination of Impurities 1, 2, and 3 in plant protection product has been successfully validated in accordance with the SANCO/3030/99 rev.5 guidelines and is considered as fully acceptable.

In addition, applicant committed development of an analytical method for the determination of the Ethofumesate impurities EMS and iBMS in the HBZ10 formulation to comply with Reg. (EU) 284/2013 data requirements. This study was conducted in two parts; the first one considered as the development part was intended to develop the method for EMS and iBMS determination in the formulation at an LOQ of < 0.013 mg/kg (non-GLP), while the second part referred to the SANCO/3030/99 rev. 5 GLP analysis of iBMS and EMS in the formulation occurring after method development.

Comments of zRMS:	Analytical methods for the relevant impurities EMS and iBMS in the formulation should be provided, as this is required according to Reg. (EU) 284/2013.
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	Since the data could not be prepared and supplemented during the commenting period, a data gap was identified.
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A summary of the first part of the study is provided below.

Reference:

KCP 5.1.1/03

Report

Method development for the analysis of EMS and iBMS in an EC formulation containing 125g/L Ethofumesate and Phenmedipham, Pomeroy, D. (2023), Study Number: DNA7245.

Guideline(s):

-

Deviations:

No

GLP:

No

Acceptability:

Yes

Method development was attempted using both GC-MSD with Headspace analyser and liquid injection GC-MSD. As well as experimenting with the use of alternative solvents.

Method development Step 1:

First method development was conducted using the Gas Chromatograph with Headspace Analyser with the VF-624 ms column (30m x 0.32mm x 1.8µm).

Approximately 2.0g of EC Formulation Sample DNA7245/1 was accurately transferred to a 10mL volumetric flask and made to 10mL Volume with a solution of 40g/L Sodium Chloride in Deionised Water. Initially, this was analysed on full scan under the conditions below to determine where the EMS and iBMS eluted. Once retention time for these peaks obtained, the method was tailored using SIM ions, in order to blind the instrument to other compounds, as well as increasing the sensitivity of the targeted compounds.

Using GC-MSD with Headspace analyser, the method suffered from interference and had poor sensitivity therefore this method could not be used.

Method development Step 2:

Second method development was conducted using liquid injection GC-MSD, applying laboratory's internal standard Technical Material method for EMS and iBMS to ascertain if this methodology could be used with the HBZ10 formulation.

Approximately 2.0g of EC Formulation Sample DNA7245/1 was accurately transferred to a 10mL volumetric flask and made to partial volume with Dichloromethane. The samples were sonicated for 5 minutes before being left to cool to ambient temperature. The sample were made to total volume with Dichloromethane and injected into the GC-MSD.

When assaying the sample, a large number of peaks eluting through the region of the EMS and iBMS were found. High concentration recovery spikes could be seen but had a large amount of interference. It would not be possible to see recovery spikes anywhere close to the required limit of detection of the specification through this interference.

Method development Step 3:

Third method development has been conducted using another commonly used GC solvent, Methanol, which is a moderate extraction solvent.

A 2g sample of DNA7245/1 was prepared in 10mL Methanol, as was a sample of DNA7245/1 spiked at 20mg/L EMS and iBMS. These provided a different profile to the samples prepared in Dichloromethane,

whereby the EMS and iBMS peaks were sharper and seemed to suffer with less interference throughout the region of the EMS and iBMS.

A calibration was prepared for both EMS and iBMS in Methanol down to 0.01mg/L.

100mg/mL and 200mg/mL concentration samples were also prepared by weighing approximately 1.0g and 2.0g of EC Formulation Sample DNA7245/1 accurately transferred to a 10mL volumetric flask and made to partial volume with Methanol. The samples were sonicated for 5 minutes before being left to cool to ambient temperature. The sample were made to total volume with Methanol.

Spiked samples of 200mg/mL concentration sample were also prepared at 0.20mg/L EMS and iBMS and 0.02mg/L EMS and iBMS. As well as spiked samples of 100mg/mL concentration at 0.10mg/L EMS and iBMS and 0.01mg/L EMS and iBMS.

It was found that these lower-level spikes were unable to be seen through the interference from the sample eluting through the region of the EMS and iBMS. Therefore, it would not be possible to meet the required limit of detection of the specification with this method. The lowest reproducible recovery for EMS and iBMS was achieved at 50mg/kg, equating to 400mg/kg relative to the Ethofumesate Active Ingredient.

Method development Step 4:

Fourth method development has been conducted using Hexane and Pentane as solvents, which are both weak non-polar solvents.

Method using Hexane as solvent:

Approximately 10g of EC Formulation Sample DNA7245/1 was accurately transferred to a 50mL volumetric flask and made to partial volume with Hexane. The samples were sonicated for 5 minutes before being left to cool to ambient temperature. The samples were made to total volume with Hexane. The samples were allowed to settle for 30 minutes, and the supernatant fluid pipetted off the top and used for analysis. Spiked samples at 2mg/L EMS and iBMS and 0.20mg/L EMS and iBMS were also prepared in this way.

Using Hexane as a solvent there were peaks eluting through the region of the EMS and iBMS causing interference to the recovery spikes, and the lower 0.20mg/L iBMS spike was completely lost within the interference.

Method using Pentane as solvent:

Approximately 10g of EC Formulation Sample DNA7245/1 was accurately transferred to a 50mL volumetric flask and made to partial volume with Pentane. The samples were sonicated for 5 minutes before being left to cool to ambient temperature. The samples were made to total volume with Pentane. The samples were allowed to settle for 30 minutes, and the supernatant fluid pipetted off the top and used for analysis. Spiked samples at 2mg/L EMS and iBMS and 0.20mg/L EMS and iBMS were also prepared in this way.

Using Pentane as a solvent there were peaks eluting through the region of the EMS and iBMS causing interference to the recovery spikes, and the lower 0.20mg/L iBMS spike was completely lost within the interference.

Conclusion:

Method development was attempted using both GC-MSD with Headspace analyser and liquid injection GC-MSD, also by experimenting with the use of alternative solvents.

Using GC-MSD with Headspace analyser the method suffered from interference and had poor sensitivity therefore this method could not be used.

Using GC-MSD with Dichloromethane, Hexane and Pentane as solvents, large amounts of interference could be seen throughout the region where EMS and iBMS peaks eluted.

Using GC-MSD in Methanol the lowest reproducible recovery for EMS and iBMS was achieved at 50mg/kg, equating to 400mg/kg relative to the Ethofumesate active substance. It was not possible to

achieve a recovery at lower concentrations due to interference within the HBZ10 formulation sample DNA7245/1.

In addition, and according to laboratory's previous experience working with EMS and iBMS, the use of LC-QQQ is not possible. For liquid chromatography with triple quadrupole, it is necessary to scan the compounds to generate a method with specific ion transitions for the molecules. For EMS and iBMS no viable transitions are able to be detected in either Positive or Negative electrospray, and as such LC-QQQ is not able to be used to analyse EMS and iBMS.

Due to the small Ethofumesate Active Ingredient Content meaning very low specifications for EMS and iBMS, and the amount of interference within the EC Formulation, no viable method has been achieved which would be able to be validated and meet SANCO 3030/99 rev 5 criteria.

5.2.1.3 Description of analytical methods for the determination of co-formulants (KCP 5.1.1)

Not required since no relevant formulants are present in HBZ10.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Phenmedipham: The CIPAC method available for the determination of Phenmedipham in technical grade active substance can be found in the FAO specification 77/1/(m)/1.3 (CIPAC P80, CIPAC 1C, p. 2181).

Ethofumesate: The CIPAC method available for the determination of Ethofumesate in technical grade active substance can be found in the FAO specification 233/TC/M/3 (CIPAC Handbook J, p.44, 2000)

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Phenmedipham:

According to Reg. (EU) 2015/2075 and EFSA Journal 2018;16(1):5151 (not currently in force) the residue definitions of Phenmedipham have been concluded as described below.

Table 5.2.2-1 Residue definition for active substance Phenmedipham

Matri	Residue definition for monitoring according to Reg. (EU) 2015/2075 (current)	Residue definition for monitoring according to EFSA Journal 2018;16(1):5151 (not currently in force)
Food of plant origin:	Phenmedipham	RAC: Phenmedipham (roots and fruit crops, only) Processed: Phenmedipham and MHPC expressed as Phenmedipham.
Food of animal origin:	Phenmedipham	MHPC expressed as Phenmedipham (ruminants only)
Soil:	Phenmedipham, MHPC	Phenmedipham, MHPC
Sediment	-	Phenmedipham, MHPC
Surface water:	Phenmedipham, MHPC	Phenmedipham, MHPC
Drinking/ground water:	Phenmedipham, MHPC	Phenmedipham, MHPC
Air:	Phenmedipham	Phenmedipham
Body fluids and tissues	Phenmedipham	Phenmedipham, MHPC

Several highly specific methods were performed for all regulatory relevant matrices and are summarized in the respective points.

Ethofumesate:

According to EFSA Journal 2016;14(1):4374 the residue definitions of Ethofumesate have been concluded as described below.

Table 5.2.2-2 Residue definition for active substance Ethofumesate

Matrix	Residue definition
Food of plant origin:	Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as ethofumesate)
Food of animal origin:	Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) (their sum expressed as ethofumesate)
Soil:	Ethofumesate
Sediment	Ethofumesate
Water surface:	Ethofumesate
Drinking/ground:	Ethofumesate
Air:	Ethofumesate
Body fluids and tissues:	Ethofumesate

For relevant data included for determination of residues, we refer to the studies submitted in the Annex II dossier for the inclusion of Phenmedipham and Ethofumesate into Annex I. No supplementary studies are required.

An overview on the acceptable methods and possible data gaps for analysis of residues of Phenmedipham and Ethofumesate for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies, it is referred to Appendix 2.

Table 5.2.2-1 Validated methods for the generation of pre-authorization data

Component of residue definition: Phenmedipham (or metabolite MHPC, m-Toluidine)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Sugar beet - leaf and roots (Residues)	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	LC-MS/MS	Wrede, A. (1999), C004350 EU agreed 1st Annex inclusion
Sugar beet - foliage and root (Residues)	Primary	Phenmedipham LOQ 0.05 mg/kg	GLC/ECD	Kossmann, K. and Jenny, N. (1973), R8 EU agreed 1st Annex inclusion
Sugar beet - leaf and roots (Residues)	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC/UV	Straszewski, A. and Wrede-Rücker, A. (1993), R171 EU agreed 1st Annex inclusion
Sugar beet – leaf and roots (Residues)	Primary	Phenmedipham LOQ 0.05 mg/kg	GLC/ECD	Kossmann, K. (1974), R9 EU agreed 1st Annex inclusion
Sugar beet – leaf and roots (Residues)	Primary	Phenmedipham LOQ 0.01 mg/kg MHPC 0.01 mg/kg	LC/MS/MS	Stouvenot, C. (2021), R C0327 New method Please refer to Appendix 2
Sugar beet - root (Residues)	Primary	Phenmedipham LOQ 0.05 mg/kg	GLC/ECD	Williamson, P. (1995), R506 EU agreed 1st Annex inclusion
	Primary	Phenmedipham LOQ 0.02 mg/kg	GLC/MSD	Specht, W. (1988a), R137 EU agreed 1st Annex inclusion
	Primary	Phenmedipham LOQ 0.02 mg/kg	GLC/thermionic AFID capillary column	Specht, W. (1988b), R149 EU agreed 1st Annex inclusion
Animal products, food of animal origin (Residues)	Primary	MHPC LOQ 0.05 mg/kg	GLC/ECD (liver, fat, muscle, kidney and milk)	Wrede-Rucker, A. (1992), R166 EU agreed 1st Annex inclusion
	Primary	Phenmedipham; LOQ 0.05 mg/kg (tissues), 0.02 mg/kg (milk), 0.05 mg/kg (egg) MHPC LOQ 0.05 mg/kg (tissues), 0.02 mg/kg (milk), 0.05 mg/kg (egg)	HPLC-UV (tissue, milk and egg)	Wrede, A. (1998), A64037 EU agreed 1st Annex inclusion
Soil, water, sediment (Environmental fate)	Soil - Primary	Phenmedipham LOQ 0.02 mg/kg	GLC/MSD	Specht, W (1988a), R137 EU agreed 1st Annex inclusion
	Soil - Primary	Phenmedipham LOQ 0.02 mg/kg	GLC/thermionic AFID capillary column	Specht, W. (1988b), R149 EU agreed 1st Annex inclusion
	Soil (type 2.1, 2.2, 2.3) – Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC/UV	Moede, J. (1989), W171/2 EU agreed 1st Annex inclusion
	Soil (type 2.1, 2.2, 2.3) – Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC/UV	Offizorz, P. (1992a), C547 EU agreed 1st Annex inclusion
	Soil (type 2.1, and	Phenmedipham	GLC/ECD	Scheuermann, H.-J. (1986), W133

Component of residue definition: Phenmedipham (or metabolite MHPC, m-Toluidine)				
	2.3) – Primary	LOQ 0.05 mg/kg		EU agreed 1st Annex inclusion
	Water - Primary	Phenmedipham LOQ 0.05 µg/L	HPLC/UV	Offizorz, P. (1992b), C548 EU agreed 1st Annex inclusion
	Water - Primary	Phenmedipham LOQ 5 µg/L MHPC LOQ 5 µg/L	HPLC/UV	Straszewski, A. (1990), W210 EU agreed 1st Annex inclusion
	Water – Primary	Phenmedipham LOQ 0.1 µg/L	HPLC/UV	Moede, J. (1988), W148 EU agreed 1st Annex inclusion
Air (Exposure)	Air – Primary	Phenmedipham LOQ 28 µg/m ³	HPLC/UV	Wrede – Rücker, A. (1993b), W265/2 EU agreed 1st Annex inclusion
	Air - Primary	Phenmedipham LOQ 10 µg/m ³	HPLC/UV	Chambers, J. and Everitt, S. (1998), A64017 EU agreed 1st Annex inclusion
Water (Ecotoxicology)	Primary	Phenmedipham LOQ 0.100 mg test item/L MHPC and m- Toluidine LOQ 0.00200 mg standard/L	LC-MS/MS	Scheerbaum, D. (2021), SO20127 / DAI18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	Phenmedipham LOQ 0.0100 mg test item/L MHPC and m- Toluidine LOQ 0.00200 mg standard/L	LC-MS/MS	Scheerbaum, D. (2021), SO20126 / SPO18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	Phenmedipham LOQ 0.0100 mg test item/L MHPC and m- Toluidine LOQ 0.00200 mg standard/L	LC-MS/MS	Scheerbaum, D. (2021), SO20128 / SLG18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	Phenmedipham LOQ 0.0100 mg test item/L MHPC and m- Toluidine LOQ 0.00200 mg standard/L	LC-MS/MS	Scheerbaum, D. (2021), SO20129 / SMS18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	MHPC LOQ 0.0100 mg standard/L	LC-MS/MS	Scheerbaum, D. (2021), SO20407 / DRE19098 New method Please refer to Appendix 2
Dechlorinated tap water (test item stock solution) 50% sucrose solution (feeding solution) (Ecotoxicology)	Primary	Phenmedipham LOQ 0.3 g test item/L (Dechlorinated tap water) LOQ 0.6 g test item/L (Feeding solution)	LC-MS/MS	Klix, V. (2021), SO20046 / IUO18743 New method Please refer to Appendix 2
Dechlorinated tap water (Ecotoxicology)	Primary	Phenmedipham LOQ 40.0 g test item/L	LC-MS/MS	Klix, V. (2021), SO20045 / IUT18743 New method Please refer to Appendix 2
Dechlorinated tap water (test item stock solution) 50% sucrose solution (feeding solution)	Primary	Phenmedipham LOQ 0.300 g test item/L	LC-MS/MS	Klix, V. (2021), SO20047 / IBC18743 New method Please refer to Appendix 2

Component of residue definition: Phenmedipham (or metabolite MHPC, m-Toluidine)				
(Ecotoxicology)				
Dechlorinated tap water (test item stock solution) (Ecotoxicology)	Primary	Phenmedipham LOQ 0.300 g test item/L	LC-MS/MS	Klix, V. (2021), SO20048 / IBL18743 New method Please refer to Appendix 2
Tap water (Ecotoxicology)	Primary	Phenmedipham LOQ 5 g test item/L	LC-MS/MS	Winkelmann, G. (2021), SO20031 / TNK18743 New method Please refer to Appendix 2
	Primary	Phenmedipham LOQ 5 g test item/L	LC-MS/MS	Winkelmann, G. (2021), SO20032 / TNW18743 New method Please refer to Appendix 2

Table 5.2.2-2 Validated methods for the generation of pre-authorization data

Component of residue definition: Ethofumesate (and metabolites NC 9607, NC 20645)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Sugar beet - roots (Residues)	Primary	Ethofumesate LOQ 0.05 mg/kg NC 9607 LOQ 0.05 mg/kg NC 20645 LOQ 0.05 mg/kg	GC-FPD	Cole, M. G. (2000), M-187353-01 EU agreed Method
Sugar beet - tops (Residues)	Primary	Ethofumesate LOQ 0.05 mg/kg NC 20645 LOQ 0.05 mg/kg		
Sugar beet - roots (Residues)	Primary	Ethofumesate LOQ 0.05 mg/kg NC 8493 LOQ 0.05 mg/kg NC 9607 LOQ 0.05 mg/kg NC 8493 (addition of NC 8493) LOQ 0.05 mg/kg NC 20645 (addition of NC 9607) LOQ 0.05 mg/kg	GC-FPD	Whiteoak, R. J.; Crofts, M.; Harris, R. J.; (1973), M-155727-01 Whiteoak, R. J.; Crofts, M.; Harris, R. J.;(1976), M-155728-01 EU agreed Method
Sugar beet - tops (Residues)	Primary	Ethofumesate LOQ 0.02 mg/kg NC 8493 LOQ 0.05 mg/kg NC 9607 LOQ 0.02 mg/kg NC 8493 (addition of NC 8493) LOQ 0.10 mg/kg NC 20645 (addition of NC 9607) LOQ 0.05 mg/kg		
Lettuce (residues)	Primary	Ethofumesate 0.01 mg/kg NC 9607 0.01 mg/kg NC 20645 (free and conjugated) 0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2013a), R B3016 EU agreed Method
Rape – seed (Residues)	Primary	NC 20645 LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. (2013), M-459806-01 and MR-12/056/M-448288-01 EU agreed Method
Rape – seed (residues)	Primary	Ethofumesate 0.01mg/kg NC 9607 0.01mg/kg NC 20645 (free and conjugated) 0.01mg/kg	LC-MS/MS	Schlewitz, P. (2013a), R B3016 EU agreed Method
Sugar beet - leaf (Residues)	Primary	NC 20645 LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. (2013), M-459806-01 and MR-12/056/M-448288-01 EU agreed Method
Sugar beet - roots (Residues)	Primary	NC 20645 LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. (2013), M-459806-01 and MR-12/056/M-448288-01 EU agreed Method
Bean - pod (Residues)	Primary	NC 20645 LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. (2013), M-459806-01 and MR-12/056/M-448288-01 EU agreed Method
Orange - fruit (Residues)	Primary	NC 20645 LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. (2013), M-459806-01 and MR-12/056/M-448288-01

Component of residue definition: Ethofumesate (and metabolites NC 9607, NC 20645)				
				EU agreed Method
	Primary	Ethofumesate 0.01mg/kg NC 9607 0.01mg/kg NC 20645 (free and conjugated) 0.01mg/kg	LC-MS/MS	Schlewitz, P. (2013a), R B3016 EU agreed Method
Sugar beet - roots and tops (Residues)	Primary	Ethofumesate LOQ 0.05 mg/kg NC 9607 LOQ 0.05 mg/kg	GC-MS (TIC)	Helgers, A. (1997), M-165366-02-1 and Godfrey, T. L. (1996), M-165212-01-1 EU agreed Methods
Sugar beet - leaf and body (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	GC-MS	Schulte, G. (2013), M-444836-02 and Konrad S. (2012), M-438402-01-1 EU agreed Methods
Orange (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg		
Sugar beet - whole plant with roots (early growth stage) Sugar beet - leaves with tops Sugar beet - roots (Residues)	Primary	Ethofumesate LOQ 0.1 mg/kg NC20645 (detected as NC 9607) LOQ 0.1 mg/kg	GC-MS	Hamberger, R. (2013), 12A04042-01-SSSB EU agreed Method
Sugar beet - leaves and roots (Residues)	Primary	Ethofumesate LOQ 0.05 mg/kg NC20645 (extraction for free analyte) LOQ 0.05 mg/kg NC20645 (extraction for conjugated analyte) LOQ 0.05 mg/kg	GC-MS	Schlewitz P. (2014), R B1312, Tandy, R. (2012a), S09-01656 and Perny A. (2002), A0019 EU agreed Methods
Sugar beet - whole plant with roots (early growth stage) Sugar beet - leaves with tops Sugar beet - roots (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 and NC20645 (detected as NC 9607) LOQ 0.05 mg/kg NC8493 LOQ 0.01 mg/kg	GC-MS	Huauilmé, J.-M. (2013a), BPL12/436/GC and Hamberger, R. (2013), 12A04042-01-SSSB EU agreed Methods
Sugar beet - whole plant with roots (early growth stage) Sugar beet - leaves with tops Sugar beet - roots (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC20645 (detected as NC 9607) LOQ 0.005 mg/kg NC8493 LOQ 0.01 mg/kg	GC-MS	Chevallier, E. (2012), BPL11/380/GC and Hamberger, R. (2012), 11A04042-01-VMSB EU agreed Methods
Sugar beet - whole plant with roots (early growth stage) Sugar beet - leaves with tops Sugar beet - roots (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 and NC20645 (detected as NC 9607) LOQ 0.005 mg/kg NC8493 LOQ 0.01 mg/kg	GC-MS	Huauilmé, J.-M. (2013b), BPL12/435/GC and Hamberger, R. (2013), 12A04042-01-SSSB EU agreed Methods
Carrot - roots and leaves (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 and NC20645 (detected as	GC-MS	Spence, Ch. (2014), 34890 and Hamberger, R. (2014) (validation in Hamberger, R. (2012) 11A04042-01-VMSB)

Component of residue definition: Ethofumesate (and metabolites NC 9607, NC 20645)				
		NC 9607) LOQ 0.005 mg/kg		EU agreed Methods
Spinach - mature leaves (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 and NC20645 (detected as NC 9607) LOQ 0.005 mg/kg		
Radish - roots and leaves (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 and NC20645 (detected as NC 9607) LOQ 0.005 mg/kg		
Cereal - grain, forage, hay, and straw (Residues)	Primary	Ethofumesate LOQ 0.05 mg/kg NC 9607 and NC20645 (detected as NC 9607) LOQ 0.025 mg/kg		
Rice (residues)	Primary	Ethofumesate 0.01mg/kg NC 9607 0.01mg/kg NC 20645 (free and conjugated) 0.01mg/kg	LC-MS/MS	Schlewitz, P. (2013a), R B3016 EU agreed Method
Tea (residues)	Primary	Ethofumesate 0.01mg/kg NC 9607 0.01mg/kg NC 20645 (free and conjugated) 0.01mg/kg	LC-MS/MS	Schlewitz, P. (2013a), R B3016 EU agreed Method
Whole milk (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg NC 20645 LOQ 0.05 mg/kg	GC-FPD	Castro, L. E. (1994), M-237976-01 and Cole, M. G. (2000), M-187353-01 EU agreed Methods
Cream Whey Milk Muscle Fat Liver Kidney (Residues)	Primary	Ethofumesate LOQ 0.01 mg/kg NC8493 LOQ 0.01 mg/kg NC 9607 (analysed as NC 20645) LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg	LC-MS/MS	Perez, R., Schmitt, J. L., Patel, D. (2013), M-467206-01 and Gould, T. J. (2010), M- 388797-01-1 EU agreed Methods
Soil - Soil 1 AX, Soil 2 HH, Soil 3 DD, Soil 4 WW (Environmental fate)	Primary	NC 8493* LOQ 0.012 mg/kg	LC-MS/MS	Traub, M. (2011), M-431094-01 EU agreed Method
Soil - Soil 1 AX, Soil 2 HH, Soil 3 DD, Soil 4 WW (Environmental fate)	Primary	NC 8493* LOQ 0.016 mg/kg LOQ 0.019 mg/kg for Soil 3 DD	LC-MS/MS	Traub, M. (2012), M-431784-01 EU agreed Method
Soil - Soil 1 AX, Soil 2 HH, Soil 3 DD, Soil 4 WW (Environmental fate)	Primary	AE C639175 (i.e. NC 20645-potassium) LOQ 0.319 mg/kg	LC-MS/MS	Traub, M. (2012), M-432551-01-1 EU agreed Method

Component of residue definition: Ethofumesate (and metabolites NC 9607, NC 20645)				
Soil, water (Efficacy)	No analytical method provided			
Water (Ecotoxicology)	Primary	Ethofumesate LOQ 0.100 mg test item/L	LC-MS/MS	Scheerbaum, D. (2020), SO20127 / DAI18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	Ethofumesate LOQ 0.0100 mg test item/L	LC-MS/MS	Scheerbaum, D. (2021), SO20126 / SPO18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	Ethofumesate LOQ 0.0100 mg test item/L	LC-MS/MS	Scheerbaum, D. (2021), SO20128 / SPO18743 New method Please refer to Appendix 2
Water (Ecotoxicology)	Primary	Ethofumesate LOQ 0.0100 mg test item/L	LC-MS/MS	Scheerbaum, D. (2021), SO20129 / SMS18743 New method Please refer to Appendix 2
Dechlorinated tap water (test item stock solution) 50% sucrose solution (feeding solution) (Ecotoxicology)	Primary	Ethofumesate LOQ 0.3 g test item/L (Dechlorinated tap water) 0.6 g test item/L (Feeding solution)	LC-MS/MS	Scheerbaum, D. (2021), SO20046 / IUO18743 New method Please refer to Appendix 2
Dechlorinated tap water (Ecotoxicology)	Primary	Ethofumesate LOQ 40.0 g test item/L	LC-MS/MS	Klix, V. (2021), SO20045 / IUT18743 New method Please refer to Appendix 2
Dechlorinated tap water (test item stock solution) 50% sucrose solution (feeding solution) (Ecotoxicology)	Primary	Ethofumesate LOQ 0.300 g test item/L	LC-MS/MS	Klix, V. (2021), SO20047 / IBC18743 New method Please refer to Appendix 2
Dechlorinated tap water (test item stock solution) (Ecotoxicology)	Primary	Ethofumesate LOQ 0.300 g test item/L	LC-MS/MS	Klix, V. (2021), SO20048 / IBL18743 New method Please refer to Appendix 2
Tap water (Ecotoxicology)	Primary	Ethofumesate LOQ 5 g test item/L	LC-MS/MS	Winkelmann, G. (2021), SO20031 / TNK18743 New method Please refer to Appendix 2
	Primary	Ethofumesate LOQ 5 g test item/L	LC-MS/MS	Winkelmann, G. (2021), SO20032 / TNW18743 New method Please refer to Appendix 2

* The metabolite NC 8493 was a major metabolite in soil photolysis. Therefore, the degradation rate was investigated

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

For analytical methods for the determination of the active substance and relevant impurities in the plant protection product please refer to Point 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of Phenmedipham (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3.2.1-1 Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	RAC: Phenmedipham (roots and fruit crops, only) Processed: Phenmedipham and MHPC expressed as Phenmedipham	0.01 – 7.0 mg/kg	Reg. (EU) 2015/2075
Plant, high acid content		0.01 – 0.3 mg/kg	Reg. (EU) 2015/2075
Plant, high protein/high starch content (dry commodities)		0.01 – 0.10 mg/kg	Reg. (EU) 2015/2075
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2015/2075
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2015/2075
Muscle	MHPC expressed as Phenmedipham (ruminants only)	0.05 mg/kg	Reg. (EU) 2015/2075
Milk		0.05 mg/kg	Reg. (EU) 2015/2075
Eggs		0.05 mg/kg	Reg. (EU) 2015/2075
Fat		0.05 mg/kg	Reg. (EU) 2015/2075
Liver, kidney		0.05 mg/kg	Reg. (EU) 2015/2075
Soil (Ecotoxicology)	Phenmedipham, MHPC	0.02 mg/kg	Annex I inclusion ¹
Drinking water (Human toxicology)	Phenmedipham, MHPC	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Phenmedipham, MHPC	0.05 µg/L	Annex I inclusion
Air	Phenmedipham	10 µg/m ³	Annex I inclusion
Tissue (meat or liver)	Not required as the active substance is not toxic or very toxic		
Body fluids			

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham in plant matrices is given in the following table. Since Annex I inclusion no new study on the active substance has been evaluated. For the detailed evaluation of new studies, please refer to Appendix 2.

¹ Draft Assessment Report on Phenmedipham

Table 5.3.2.2-1 Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Phenmedipham (and metabolite MHPC)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content (Sugar beet leaves)	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	LC/MS/MS	Wrede, A. (1999), C004350 EU agreed method
	Primary	Phenmedipham LOQ 0.05 mg/kg	GLC/ECD	Williamson, P. (1995), R506 EU agreed 1st Annex inclusion
	Primary	Phenmedipham LOQ 0.01 mg/kg MHPC 0.01 mg/kg	LC/MS/MS	Stouvenot, C. (2021), R C0327 New method Please refer to Appendix 2
	ILV	-	-	-
	Confirmatory	Phenmedipham LOQ 0.01 mg/kg MHPC 0.01 mg/kg	LC/MS/MS	Stouvenot, C. (2021), R C0327 New method Please refer to Appendix 2
High acid content (strawberry)	Primary	Phenmedipham LOQ 0.05 mg/kg	GLC/ECD	Kossmann, K. and Jenny, N. (1973), R8 EU agreed method
	ILV	-	-	-
	Confirmatory (if required)	Not required	-	-
High oil content	No method provided			
High protein/high starch content (Sugar beet roots)	Primary	Phenmedipham LOQ 0.05mg/kg MHPC LOQ 0.05mg/kg	LC/MS/MS	Wrede, A. (1999), C004350 EU agreed method
	Primary	Phenmedipham LOQ 0.05 mg/kg	GLC/ECD	Williamson, P. (1995), R506 EU agreed 1st Annex inclusion
	Primary	Phenmedipham LOQ 0.02 mg/kg	GLC/MSD	Specht, W. (1988a), R137 EU agreed 1st Annex inclusion
	Primary	Phenmedipham LOQ 0.01 mg/kg MHPC 0.01 mg/kg	LC/MS/MS	Stouvenot, C. (2021), R C0327 New method Please refer to Appendix 2
	ILV	Phenmedipham LOQ 0.02 mg/kg	GLC/MSD	Wrede, A. (2002), C020746 EU agreed method
	Confirmatory	Phenmedipham LOQ 0.01 mg/kg MHPC 0.01 mg/kg	LC/MS/MS	Stouvenot, C. (2021), R C0327 New method Please refer to Appendix 2

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3.2.2-2 Statement on extraction efficiency

	Method for products of plant origin
	<p>Extraction efficiency was not assessed for the purposes of the present submission.</p> <p>For the purposes of the present submission, the risk assessment for Phenmedipham is not performed in line with the provisions of the Guideline Document SANCO/2010/13170 rev. 14 of 7th October 2016. As the expiration date of Phenmedipham Annex I inclusion is 31st of July 2022 and no new end points have been agreed on EU level. The comprehensive risk assessment for Phenmedipham will be performed following the renewal of the active substance taking into account the current end points and guidance documents.</p>

zRMS comments:

In EFSA Journal 2014;12(8):3807 it is stated that *During the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS was evaluated and validated for the determination of phenmedipham in plant matrices with an LOQ of 0.05 mg/kg in high water content commodities (sugar beet) (Finland, 1999). However, this method is validated for only one mass transition and therefore cannot be considered highly specific according to the current guidance document (EC, 2010b). Moreover no ILV was available.*

An analytical method using GC-MS was also evaluated and validated for the determination of phenmedipham in plant matrices with an LOQ of 0.02 mg/kg in high water content commodities (sugar beet) (Finland, 2002). However, this method is not validated on three ion fragments and therefore cannot be considered highly specific according to the current guidance document (EC, 2010b). Moreover no ILV was available.

In addition, after Annex I inclusion, the RMS also evaluated a HPLC-MS/MS method and its ILV, which was validated for the determination of phenmedipham with an LOQ of 0.01 mg/kg in high water content (sugar beet), high fat content (oil seed rape), acidic (orange) and dry (wheat grain) commodities (Finland, 2010). This method is validated for only one mass transition and cannot be considered highly specific according to the current guidance document (EC, 2010b). Validation data concerning the second mass transition is missing. According to the RMS, validation data concerning the second transition will be submitted in the framework of the renewal of the approval of the active substance under Regulation (EC) No 1107/2009 (January 2015).

According to Reg. (EU) 2015/2075 for the substance Phenmedipham, the lowest MRL values for matrices with a high content of water, fat, acidic matrix, and dry matrix are 0.01 mg/kg.

The validated LOQ of 0.05 mg/kg of the EU agreed methods by Wrede, A. (1999), Williamson, P. (1995), Kossmann, K. and Jenny, N. (1973) is not sufficient to monitor these lowered MRLs for food of plant origin. According to the guide SANTE/2020/12830, Rev.1, 24. February 2021, appropriate analytical methods (for monitoring purposes) and their ILV for the determination of Phenmedipham should be provided with a LOQ equal to the lowest value of the currently applicable MRL values for the matrix (LOQ=0.01 mg/kg).

The Applicant provided an analytical method for the determination of phenmedipham in a high water and protein/starch matrix (dry matrix) with a LOQ of 0.01 mg/kg (Stouvenot, C. (2021), R C0327) which was accepted by the evaluator (see point A 2.1.1.3.1), however, the ILV of this method is missing.

The Applicant submitted additional data requested by zRMS and the following answer:

“As being part of the task force Phenmedipham, the data requested by zRMS have already been generated and submitted under renewal process of Phenmedipham.

*The following study reports are provided hereby to Polish authorities. Studies summary as well as the conclusions of their evaluation by RMS Finland during Phenmedipham renewal are available in **RAR version May 2022.***

Analytical methods in plant matrices:

Analytical method for the determination of Phenmedipham residues (Phenmedipham) in plants in sugar beet roots (commodities with high water content), orange (commodities with high acid content), wheat grain (dry commodities) and oil seed rape (commodities with high oil content) with a LOQ = 0.01 mg/kg:

KCA 4.2/26: Klimmek, S.; Gizler, A. (2014) report AVE-0201V 1st amendment of C028889 (“Validation of DFG method S 19 (extended revision) for the determination of residues of desmedipham, phenmedipham and their metabolites EHPC and MHPC in/on plant material by means of liquid chromatography with Tandem mass spectrometric detection (LC-MS/MS)”)

KCA 4.2/27: Anspach, T. (2002) report C028890 1st addendum of C028889

KCA 4.2/28: Freitag, Th. (2014) report P612051807 amendment to report MR-146/05 (“Independent laboratory validation of the DFG Method S19 (extended revision) for the determination of residues of desmedipham, Phenmedipham, and their Metabolites EHPC and MHPC in/on plant material”).”

Conclusions:

The method (Klimmek, S.; Gizler, A.; 2014) is acceptably validated and suitable for the determination of phenmedipham in sugar beet roots (commodities with high water content), orange (commodities with high acid

content), wheat grain (dry commodities) and oil seed rape (commodities with high oil content) with LOQ=0.01 mg/kg.

The ILV (Freitag, Th. (2014) report P612051807 amendment to report MR-146/05) is adequately validated for the determination of phenmedipham in sugar beet root (commodities with high water content), orange fruit (commodities with high acid content), wheat grain (dry commodities) and canola oil seed (commodities with high oil content) with LOQ of 0.01 mg/kg.

No additional data are required.

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham in animal matrices is given in the following tables. Since Annex I inclusion no new study on the active substance has been evaluated. No new methods have been submitted under this application.

Table 5.3.2.3-1 Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Phenmedipham (and metabolite MHPC)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Billian, P. (2003b), MR-004/03 EU agreed method
	ILV	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Brumhard, B. (2003), MR-041/03 EU agreed method
	Confirmatory (if required)	Not required	-	-
Eggs	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Billian, P. (2003b), MR-004/03 EU agreed method
	ILV	-	-	-
	Confirmatory (if required)	Not required	-	-
Meat	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Billian, P. (2003b), MR-004/03 EU agreed method
	ILV	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Brumhard, B. (2003), MR-041/03 EU agreed method
	Confirmatory (if required)	Not required	-	-
Fat	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Billian, P. (2003a), MR-538/03 EU agreed method
	ILV	-	-	-
	Confirmatory (if required)	Not required	-	-
Kidney, liver	Primary	Phenmedipham LOQ 0.05 mg/kg MHPC LOQ 0.05 mg/kg	HPLC-MS/MS	Billian, P. (2003a), MR-538/03 EU agreed method
	ILV	-	-	-
	Confirmatory (if required)	Not required	-	-

zRMS comments:

In the EFSA Journal 2014;12(8):3807 it is stated that *during the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS and its ILV were evaluated and validated for the determination of phenmedipham and its metabolite MHPC in food of animal origin with an LOQ of 0.05 mg/kg for each compound in milk, meat, fat, liver, kidney and eggs (Finland, 2003). This method can be confirmed by an HPLC-UV method validated for the determination of phenmedipham and its metabolite MHPC with LOQs for each compound of 0.05 mg/kg in meat and eggs and 0.02 mg/kg in milk. However, a confirmatory method is missing for the determination of phenmedipham and MHPC in fat, liver and kidney.*

In addition, after Annex I inclusion, France also evaluated a HPLC-MS/MS method and its ILV, which was validated for the determination of phenmedipham and its metabolite MHPC with LOQs of 0.01 mg/kg for each compound in eggs, fat and muscle and of 0.02 mg/kg for each compound in liver and kidney (France, 2014). This method can be used as a confirmatory method for the determination of phenmedipham and MHPC in fat, liver and kidney.

Hence it is concluded that phenmedipham and its metabolite MHPC can be enforced in food of animal origin with

an LOQ of 0.05 mg/kg for each compound in milk, eggs, fat, muscle, liver and kidney.

No additional data are required.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham in soil is given in the following tables. Since Annex I inclusion no new study on the active substance has been evaluated. No new methods have been submitted under this application.

Table 5.3.2.4-1 Validated methods for soil (if appropriate)

Component of residue definition: Phenmedipham (and metabolite MHPC)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary (LUFA Standard soil 2.2)	Phenmedipham LOQ 0.01 mg/kg MHPC LOQ 0.01 mg/kg	HPLC-MS/MS	Anspach, T. (2003a), BAY-0223V EU agreed method
Confirmatory	Not required	-	-

zRMS comments:

Residues of phenmedipham and MHPC in soil can be monitored by HPLC-MS/MS with a LOQ 0.01 mg/kg.
No additional data are required.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham in surface and drinking water is given in the following tables. Since Annex I inclusion no new study on the active substance has been evaluated. No new methods have been submitted under this application.

Table 5.3.2.5-1 Validated methods for water (if appropriate)

Component of residue definition: Phenmedipham (and metabolite MHPC)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	Phenmedipham LOQ 0.1 µg/L MHPC LOQ 0.1 µg/L	HPLC/UV	Wrede, A. (2000), C007532 EU agreed method
	Primary	Phenmedipham LOQ 0.01 µg/L MHPC LOQ 0.01 µg/L	HPLC-MS/MS	Anspach, T. (2003b), BAY-0225V EU agreed method
	ILV	-	-	-
	Confirmatory	Not required	-	-
Surface water	Primary	Phenmedipham LOQ 0.1 µg/L MHPC LOQ 0.1 µg/L	HPLC/UV	Wrede, A. (2000), C007532 EU agreed method
	Primary	Phenmedipham LOQ 0.01 µg/L MHPC LOQ 0.01 µg/L	HPLC-MS/MS	Anspach, T. (2003b), BAY-0225V EU agreed method
	ILV	-	-	-
	Confirmatory	Not required	-	-

zRMS comments:

Residues of phenmedipham and MHPC in water can be monitored by HPLC-MS/MS with a LOQ 0.01 µg/L.
An independent laboratory validation (ILV) for the method for the determination of residues of phenmedipham and MHPC in drinking water is missing. Based on the indication of the SANTE/2020/12830, Rev.1 24. February 2021, the ILV for drinking water should be submitted.

The Applicant submitted additional data requested by zRMS and the following answer:

Analytical methods in water:

During the evaluation process of Phenmedipham and the metabolite MHPC for Annex I inclusion of directive 91/414/EC, the water analytical method for monitoring purposes (Anspach, T. (2003), BAY-0225V) was modified in order to follow the state of the art in conduct of analytical methods in water. The modified method has not been taken into account for a corresponding update of the DAR and thus has been provided as part of the renewal process of Phenmedipham. This updated analytical method and its ILV are those provided in this response dossier.

Analytical method and its ILV for the determination of Phenmedipham residues (Phenmedipham and MHPC) in water with a LOQ = 0.05 µg/L:

KCA 4.2 /32: Krebber, R.; Braume, M. (2013) report MR-13/085 (“Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS”)

KCA 4.2 /33: Stanislawski, T. (2013) report P3117 G (“Independent laboratory validation of BCS methods 01333 and 01387 for the determination of various pesticides in surface water by DI-HPLC-MS/MS”).

Conclusions:

The method (Krebber, R.; Braume, M. (2013) report MR-13/085) and its ILV (Stanislawski, T. (2013) report P3117 G) is acceptably validated for the determination of phenmedipham and its metabolite MHPC in surface water with a LOQ of 0.05 µg/L.

No additional data are required.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham in air is given in the following tables. Since Annex I inclusion no new study on the active substance has been evaluated. No new methods have been submitted under this application.

Table 5.3.2.6-1 Validated methods for air (if appropriate)

Component of residue definition: Phenmedipham			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Phenmedipham LOQ 10 µg/m ³	HPLC/UV	Chambers, J. and Everitt, S. (1998), A64017 EU agreed method
Confirmatory	Not required	-	-

zRMS comments:

Phenmedipham residues in air can be monitored by reversed phase high performance liquid chromatography with UV detector (RP/HPLC-UV) with a LOQ of 10 µg/m³.

No additional data are required.

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Phenmedipham in body fluids and tissues is given in the following table. Since Annex I inclusion no new study on the active substance has been evaluated. No new methods have been submitted under this application.

Table 5.3.2.7-1 Methods for body fluids and tissues (if appropriate)

Component of residue definition: Phenmedipham (and metabolite MHPC)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Phenmedipham LOQ 0.05 mg/kg (tissues), 0.02 mg/kg (milk), 0.05 mg/kg (egg) MHPC LOQ 0.05 mg/kg (tissues), 0.02 mg/kg (milk), 0.05 mg/kg (egg)	HPLC/UV (tissue, milk, and egg)	Wrede, A. (1998), A64037 EU agreed 1 st Annex inclusion
Primary	MHPC LOQ 0.05 mg/kg	GLC/ECD (liver, fat, muscle, kidney, and milk)	Wrede-Rucker, A. (1992), R166 EU agreed 1 st Annex inclusion
Confirmatory	Not required	-	-

zRMS comments:

The active substance phenmedipham was evaluated at the EU level according to the old data requirements. The Commission Regulation (EU) No 284/2013 is applicable now.

In Regulation (EU) No 283/2013 it is stated that “...methods, with a full description, shall be submitted for the analysis in body fluids and tissues for the active substance and relevant metabolites” and this is a new requirement of SANTE/2020/12830. According to the SANTE/2020/12830: “Analytical methods for monitoring residues in body fluids and tissues are required for detection of active substances and/or metabolites in humans and animals after possible intoxications or for biomonitoring purposes, regardless of their toxicological classification.”

Therefore, an analytical method for the residues of phenmedipham (and metabolite MHPC) in body fluids and tissues is required.

The Applicant submitted additional data requested by zRMS:

Analytical methods in body fluids:

Analytical method for the determination of Phenmedipham residues (Phenmedipham and MHPC) in body fluids (plasma) with a LOQ = 50 µg/L:

KCA 4.2/26: Kaussmann, M. (2016) report P683166504 (“Analytical Method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS”)

Conclusions:

According to the EFSA Journal 2018;16(1):5151 the method for monitoring in animal products can be used for determination of phenmedipham and MHPC in body tissues.

The method 01486 permits the determination of residues of phenmedipham and MHPC in plasma with the LOQ of 50 µg/L for each compound.

No additional data are required.

5.3.2.8 Other studies/ information

No additional studies/ information is submitted.

5.3.3 Description of analytical methods for the determination of residues of Ethofumesate (KCP 5.2)

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition changed for plant and animal matrices (inclusion of metabolite ethofumesate carboxylic acid (NC 20645) and its conjugate). The current residues definitions are listed in the table below.²

² EFSA Journal 2016;14(1):4374

Table 5.3.3.1-1 Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as Ethofumesate)	0.03 – 1.5 mg/kg	Reg. (EU) 2017/1016
Plant, high acid content		0.03 mg/kg	Reg. (EU) 2017/1016
Plant, high protein/high starch content (dry commodities)		0.03 – 0.1 mg/kg	Reg. (EU) 2017/1016
Plant, high oil content		0.03 mg/kg	Reg. (EU) 2017/1016
Plant, difficult matrices (hops, spices, tea)		0.1 – 15 mg/kg	Reg. (EU) 2017/1016
Muscle	Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) (their sum expressed as Ethofumesate)	0.03 mg/kg	Reg. (EU) 2017/1016
Milk		0.03 mg/kg	Reg. (EU) 2017/1016
Eggs		0.03 mg/kg	Reg. (EU) 2017/1016
Fat		0.03 mg/kg	Reg. (EU) 2017/1016
Liver, kidney		0.03 mg/kg	Reg. (EU) 2017/1016
Soil (Ecotoxicology)	Ethofumesate	0.05 mg/kg	EU agreed endpoint ³
Drinking water (Human toxicology)	Ethofumesate	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Ethofumesate	0.05 µg/L	EU agreed endpoint
Air	Ethofumesate	0.1 µg/m ³	EU agreed endpoint
Tissue (meat or liver)	Ethofumesate	0.01 mg/kg	EU agreed endpoint
Body fluids		0.01 mg/kg	EU agreed endpoint

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Ethofumesate in plant matrices is given in the following tables. Since Annex I inclusion new study on the active substance has been evaluated in the renewal of active substance. However, no new methods have been submitted under this application.

³ Renewal Assessment Report for Ethofumesate

Table 5.3.3.2-1 Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Ethofumesate (and metabolite NC 9607, NC 20645)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content (Sugar beet leaf)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. and Diehl, P. (2014), M-479926-01 EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Betson, S. (2014), RL/SN/2014-001 EU agreed method
	Confirmatory (if required)	Not required	-	-
High acid content (Orange)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. and Diehl, P. (2014), M-479926-01 EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Betson, S. (2014), RL/SN/2014-001 EU agreed method
	Confirmatory (if required)	Not required	-	-
High oil content (Rape seed)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. and Diehl, P. (2014), M-479926-01 EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Betson, S. (2014), RL/SN/2014-001 EU agreed method
	Confirmatory (if required)	Not required	-	-
High protein/high starch content (dry) (Wheat - grain)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Schulte, G. and Diehl, P. (2014), M-479926-01 EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Betson, S. (2014), RL/SN/2014-001 EU agreed method
	Confirmatory (if required)	Not required	-	-
Difficult (if required, depends on intended use) (Hop – green cone)	Primary	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645*	LC-MS/MS	Schulte, G. and Diehl, P. (2014), M-479926-01 EU agreed method

Component of residue definition: Ethofumesate (and metabolite NC 9607, NC 20645)				
		LOQ 0.01 mg/kg		
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 9607* LOQ 0.01 mg/kg NC 20645* LOQ 0.01 mg/kg	LC-MS/MS	Betson, S. (2014), RL/SN/2014-001 EU agreed method
	Confirmatory (if required)	Not required	-	-

* determined as NC 20645 and calculated as ethofumesate

Table 5.3.3.2-2 Statement on extraction efficiency

Method for products of plant origin	
	Extraction efficiency was not assessed for the purposes of the present submission. For the purposes of the present submission, the risk assessment for Ethofumesate is not performed in line with the provisions of the Guideline Document SANCO/2010/13170 rev. 14 of 7 th October 2016. As the expiration date of Ethofumesate Annex I inclusion is 31 st of October 2031 and no new end points have been agreed on EU level. The comprehensive risk assessment for Ethofumesate will be performed following the renewal of the active substance taking into account the current end points and guidance documents.

zRMS comments:

Sufficient analytical method for the determination of ethofumesate (according to the residue definition) residues in crops (Schulte, G.; Diehl, P.; 2014; Method 01392) and its ILV (Betson, S.; 2014) is available (RAR, 2015). The method has been validated with LOQ=0.01mg/kg by LC-MS/MS for ethofumesate, NC 9607 as NC 20645 and NC 20645 separately in high water content, dry, fatty, acidic and no group (hop) commodities. As the method is highly specific (two mass transitions), confirmatory method is not required.
Extraction efficiency of the method and efficiency of the acidic hydrolysis have been demonstrated for high water content commodities in the RAR of ethofumesate (2015).
No additional data are required.

5.3.3.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Ethofumesate in animal matrices is given in the following table. Since Annex I inclusion new study on the active substance has been evaluated in the renewal of active substance. However, no new methods have been submitted under this application.

Table 5.3.3.3-1 Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Ethofumesate (and metabolite NC 20645, NC 9607)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Jooß, S. (2012), P 2371 G EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2013b), R B1218 EU agreed method
	Confirmatory (if required)	Not required	-	-
Eggs	Primary	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Jooß, S. (2012), P 2371 G EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2013b), R B1218 EU agreed method
	Confirmatory (if required)	Not required	-	-
Meat	Primary	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Jooß, S. (2012), P 2371 G EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2013b), R B1218 EU agreed method
	Confirmatory (if required)	Not required	-	-
Fat	Primary	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Jooß, S. (2012), P 2371 G EU agreed method
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2013b), R B1218 EU agreed method
	Confirmatory (if required)	Not required	-	-
Liver	Primary	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Jooß, S. (2012), P 2371 G EU agreed method

Component of residue definition: Ethofumesate (and metabolite NC 20645, NC 9607)				
	ILV	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2013b), R B1218 EU agreed method
	Confirmatory (if required)	Not required	-	-

zRMS comments:

Sufficient analytical method for the determination of ethofumesate and its two metabolites NC 9607 (2-ketoethofumesate) and NC 20645 (2-methylpropionic acid ethofumesate) in various animal matrices (Jooß, S. (2012), P 2371 G) and its ILV (Schlewitz, P. (2013b), R B1218) is available (RAR, 2015). The method has been validated with a limit of quantitation (LOQ) of 0.01 mg/kg per analyte, always expressed as Ethofumesate by LC-MS/MS. As the method is highly specific (two mass transitions), confirmatory method is not required. According to the Regulation 2017/1016 the residue definition for monitoring purposes for animal matrices: Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as ethofumesate).

This method (Jooß, S. (2012), P 2371 G) does not include a hydrolysis step, so conjugates are not quantified in this method. However, as it is stated in RAR (2015), “no residues (according to the residue definition) above the LOQ are expected in food of animal commodities regarding the representative use: sugar beets”. Therefore, no further data is required for the registration of HBZ10.

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Ethofumesate in soil is given in the following table. Since Annex I inclusion new study on the active substance has been evaluated in the renewal of active substance. However, no new methods have been submitted under this application.

Table 5.3.3.4-1 Validated methods for soil (if appropriate)

Component of residue definition: Ethofumesate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary (Soil Höfchen and Soil Laacher Hof)	Ethofumesate LOQ 0.05 mg/kg	LC-MS/MS	Brumhard, B. (2003), M- 122176-01-1 EU agreed method
Primary (Standard soil BBA 2.3)	Ethofumesate LOQ 0.005 mg/kg NC 8493 LOQ 0.02 mg/kg	GC-MS	Hamberger, R. (2012b), 12A04042-01-VMS EU agreed method
Confirmation (Standard soil 2.2)	Ethofumesate LOQ 0.05 mg/kg	GC-MS	Schneider, E. (2000), OFC00004917 EU agreed method

zRMS comments:

Sufficient analytical method is available for the determination of ethofumesate in soil (RAR, 2015). The GC-MS/MS method has been sufficiently validated in soil (LOQ = 0.005 mg/kg for ethofumesate and LOQ = 0.02 mg/kg for NC 8493).
No additional data are required.

5.3.3.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Ethofumesate in surface and drinking water is given in the following table. Since Annex I inclusion new study on the active

substance has been evaluated in the renewal of active substance. However, new methods have been submitted under this application.

Table 5.3.3.5-1 Validated methods for water (if appropriate)

Component of residue definition: Ethofumesate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	Ethofumesate LOQ 0.05 µg/L	LC-MS/MS	Jooß, S. (2011), P 2368 G EU agreed method
	ILV	-	-	-
	Confirmatory	Not required	-	-
Surface water	Primary	Ethofumesate LOQ 0.05 µg/L	LC-MS/MS	Jooß, S. (2011), P 2368 G EU agreed method
	Primary	Ethofumesate LOQ 0.05 µg/L	LC-MS/MS	Krebber, R. and Braune, M. (2013), MR-13/085 EU agreed method
	ILV	Ethofumesate LOQ 0.05 µg/L	LC-MS/MS	Stanislowski, T. (2013), P3117 G EU agreed method
	Primary	Ethofumesate LOQ 0.1 µg/L NC 9607 LOQ 0.1 µg/L NC 20645 (determined as NC 9606) LOQ 0.1 µg/L	GC-MS	Hamberger, R. (2012c), 12A04042-01-VMWA EU agreed method
	ILV	Ethofumesate LOQ 0.1 µg/L NC 9607 LOQ 0.1 µg/L NC 20645 (determined as NC 9606) LOQ 0.1 µg/L	GC-MS	Brown D. (2014), S13-04250 EU agreed method
	Confirmatory	Not required	-	-

zRMS comments:

Sufficient analytical method (Krebber, R.; Braune, M., 2013, MR-13/085) and its ILV (Class, T., Stanislowski T., 2013, P3117 G) is available for the determination of ethofumesate in surface water (RAR, 2015). The LC-MS/MS method 01387 has been sufficiently validated in surface water with a limit of quantitation (LOQ) of 0.05 µg/L. As the method is highly specific (two mass transitions), confirmatory method is not required.
No additional data are required.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Ethofumesate in air is given in the following table. Since Annex I inclusion no new study on the active substance has been evaluated. No new methods have been submitted under this application.

Table 5.3.2.6-1 Validated methods for air (if appropriate)

Component of residue definition: Ethofumesate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	LOQ 1.6 µg/m ³	GC-FPD	Wrede-Rucker, A. (1993), W139 Annex I Inclusion
Primary	LOQ 0.5 µg/m ³	GC-FPD	Reichert, N. (1994), C506 Annex I Inclusion
Primary	LOQ 0.1 µg/m ³	GC-MS	Schneider, E. (1994a), W174 Annex I Inclusion
Primary	LOQ 0.02 µg/m ³	GC-MS	Schneider, E. (1994b), PR93/016 Annex I Inclusion
Primary	LOQ 0.1 µg/m ³	GC-MS	Heintze, A. (2003), 20021050/01-CMLU Annex I Inclusion
Primary	LOQ 0.021 µg/m ³	GC-MS	Rooseboom-Reimers, A. (2003), V4799/02 Annex I Inclusion

zRMS comments:

Sufficient analytical methods (Heintze, A.; 2003; Rooseboom-Reimers, A.; 2003) are available for the determination of ethofumesate in air (RAR, 2015).

The GC-MS method has been sufficiently validated in air with a limit of quantitation (LOQ) of 0.1 µg/m³ (Heintze, A.; 2003) and of 0.021 µg/m³ (Rooseboom-Reimers, A.; 2003). As the methods are highly specific (two mass transitions), confirmatory method is not required.

No additional data are required.

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Ethofumesate in body fluids and tissues is given in the following table. Since Annex I inclusion new study on the active substance has been evaluated in the renewal of active substance. However, no new methods have been submitted under this application.

Table 5.3.2.7-1 Methods for body fluids and tissues (if appropriate)

Component of residue definition: Ethofumesate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary (Milk)	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg	LC-MS/MS	Jooß, S. (2012), P 2371 G EU agreed method
Primary (Meat)	Ethofumesate LOQ 0.01 mg/kg NC 20645 LOQ 0.01 mg/kg NC 9607 LOQ 0.01 mg/kg		
Primary (Dog plasma)	Ethofumesate LOQ 0.1 mg/L	HPLC-UV	McKenzie, J. (1994), C507 Annex I inclusion

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

zRMS comments:

According to the RAR (2015) analytical methods are available for animal matrices including tissues (meat) and fluids (milk) in this DRAR (Jooß S., 2012) and is as well addressed for dog plasma (McKenzie 1994) in the original

DAR (1998).

Analytical method (McKenzie, 1994) for the determination of ethofumesate residues in body fluids is available and has been validated by HPLC-UV with LOQ=0.1mg/L for ethofumesate in dog plasma (RAR, 2015).

Analytical method Jooß, S. (2012) is available and has been validated by LC-MS/MS with LOQ=0.01mg/kg for ethofumesate, NC 9607 and NC20645 (free) separately in meat, egg, fat, milk, liver, kidney. As the method is highly specific confirmatory method is not required.

No additional data are required.

5.3.2.8 Other studies/ information

No additional studies/ information is submitted.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01 KCP 5.1.1/02	Norris, D.	2021	Validation Of The Methods Of Determination Of Ethofumesate And Phenmedipham And Specified Impurities In An EC Formulation, In Compliance With Good Laboratory Practice Report No. DNA6255 David Norris Analytical Laboratories Ltd, UK GLP Unpublished	N	UPL
KCP 5.1.1/03	Pomeroy, D.	2023	Certificate of analysis for method development for the analysis of EMS and iBMS in an EC formulation containing 125g/L Ethofumesate and Phenmedipham Report No. DNA7245 David Norris Analytical Laboratories Ltd, UK Non GLP Unpublished	N	UPL
KCP 10.2.1/01	Scheerbaum, D.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Acute immobilization test to <i>Daphnia magna</i> , semi-static, 48 hours Report No. SO20127 / DAI18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.2.1/02	Scheerbaum, D.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Alga, Growth Inhibition Test with <i>Pseudokirchneriella subcapitata</i> , 72 hours Report No. SO20126 / SPO18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.2.1/03	Scheerbaum, D.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Aquatic Plant Toxicity Test, <i>Lemna gibba</i> , semi-static, 7 days Report No. SO20128 / SLG18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP	Scheerbaum, D.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Water-Sediment <i>Myriophyllum spicatum</i> Toxicity Test	N	UPL

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
10.2.1/04			semi-static, 14 d Report No. SO20129 / SMS18743 Noack Laboratorien GmbH GLP Unpublished		
KCP 10.2.2/01	Scheerbaum, D.	2021	MHPC: <i>Daphnia magna</i> Reproduction Test, Semi-static, 21 days Report No. SO20407 / DRE19098 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.3.1.1.1/02	Klix, V.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Acute Oral Toxicity test on the Bumblebee <i>Bombus terrestris</i> Report No. SO20046 / IUO18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.3.1.1.2/02	Klix, V.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Acute Contact Toxicity Test on the Bumblebee <i>Bombus terrestris</i> Report No. SO20045 / IUT18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.3.1.2/01	Klix, V.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Chronic oral toxicity test on the Honeybee <i>Apis mellifera</i> (Hymenoptera, Apidae) Report No. SO20047 / IBC18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.3.1.3/01	Klix, V.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Honeybee (<i>Apis mellifera</i>) larval toxicity test, repeated exposure Report No. SO20048 / IBL18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.6.2/01	Winkelmann, G.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Report No. SO20031 / TNK18743 Noack Laboratorien GmbH GLP Unpublished	N	UPL
KCP 10.6.2/02	Winkelmann, G.	2021	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Terrestrial Plant Test: Vegetative Vigour Test Report No. SO20032 / TNW18743 and its 1 st amendment Noack Laboratorien GmbH GLP Unpublished	N	UPL

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review for the active substance phenmedipham and ethofumesate

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1	Wrede, A.	1999	DATA GENERATION METHOD WITH VALIDATION FOR SUGAR BEETS BY LC-MS/MS PHENMEDIPHAM (AE B038584), DESMEDIPHAM (AE B038107), AE B038210, AE F132319 Report No. C004350 not available GLP Unpublished	N	TFP
KCP 5.1	Kossmann, K., Jenny, N.A.	1973	PHENMEDIPHAM Analytical methods for pesticides and plant growth regulators, 7, 1973, 611-623. Report No. A61343 not available Not GLP Unpublished	N	AGE
KCP 5.1	Kossmann, K	1974	RESIDUE ANALYSIS OF PLANT PROTECTION PRODUCTS: PHENMEDIPHAM (RUECKSTANDSANALYTIK VON PFLANZENSCHUTZMETTELN: PHENMEDIPHAM). Report No. A61863 Rueckstandsanalytik von Pflanzenschutzmitteln. Verlag Chemie, Weinheim, deerfied beach (Florida, USA), Basel, 233-B-1 Not GLP Published	N	-
KCP 5.1	Williamson, P.F.	1995	KEMIFAM: DETERMINATION OF PHENMEDIPHAM RESIDUES IN SUGAR BEET AT HARVEST AND TO PREPARE DECLINE CURVES Report No. A62782 not available Not GLP Unpublished	N	TFP
KCP 5.1	Specht, W.	1988a	UEBERPRUEFUNG DER ANWENDBARKEIT DER DFGMULTIMETHODE S 19 ZUR QUANTITATIVEN BESTIIVEVIUNG VON RUECKSTAENDEN VON PHENMEDIPHAM IN BODEN, WASSER UND RUEBENKOERPERN Report No. A62003 Chemische Laboratorien GmbH Not GLP Unpublished	N	TFP
KCP 5.1	Specht, W.	1988b	UEBERPRUEFUNG DER ANWENDBARKEIT EINER MODIFIZIERTEN DFG-MULTIMETHODE S 6-A ZUR QUANTITATIVEN BESTIMIVIUNG VON RUECKSTAENDEN VON PHENMEDIPHAM IN BODEN, SER UND RUEBENKOERPERN	N	TFP

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report No. A62015 Chemische Laboratorien GmbH Not GLP Unpublished		
KCP 5.1	Wrede-Rucker, A.	1992	ANALYTICAL METHOD FOR. THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM IN TISSUE AND MILK BY GLC Report No. R166 Schering AG, Berlin, Germany Not GLP Unpublished	N	TFP
KCP 5.1	Wrede, A.	1998	ANALYTICAL METHOD AND VALIDATION FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM AND ITS METABOLITE MI-IPC IN TISSUE, MILK AND EGG BY HPLC CODE: AE B038584 Report No. A64037 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Not GLP Unpublished	N	TFP
KCP 5.1	Moede, J.	1989	ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF PHENMEDPHAM AND A MAJOR METABOLITE IN SOIL BY HPLC Report No. A62523 Schering AG, Berlin, Germany Not GLP Unpublished	N	TFP
KCP 5.1	Offizorz, P.	1992a	METHOD VALIDATION: TOP PURE PHENMEDEPHAM AND METABOLITE METHYLHYDROXYPHENYLCARBAMATE (MHPC) IN/ON SOIL Report No. A62750, C547 Bayer Crop Science AG Not GLP Unpublished	N	TFP
KCP 5.1	Scheuermann, H.J.	1986	ANALYTICAL METHOD FOR. THE DETERMINATION OF TOTAL RESIDUES OF PHENMEDIPHAM IN SOIL (38 584/8) Report No. A62471, W133 Schering AG, Berlin, Germany Not GLP Unpublished	N	TFP

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1	Offizorz, P.	1992b	METHOD VALIDATION: TOP2 PURE PHENMEDIPHAM IN WATER Report No. A62751, C548 Bayer Crop Science AG Not GLP Unpublished	N	TFP
KCP 5.1	Straszewski, A.	1990	ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM AND MAJOR METABOLITES IN WATER BY HPLC Report No. A62609, W210 Schering AG, Berlin, Germany Not GLP Unpublished	N	TFP
KCP 5.1	Moede, J.	1988	ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM IN WATER (38 584/3) Report No. A62486, W148 Schering AG, Berlin, Germany Not GLP Unpublished	N	TFP
KCP 5.1	Wrede-Rücker, A.	1993a	ANALYTICAL METHOD FOR THE DETERMINATION OF PHENMEDIPHAM IN AIR Report No. A62667, W265/2 SCC Scientific Consulting Company Not GLP Unpublished	N	TFP
KCP 5.1	Chambers, J., Everitt, S.	1998	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF PHENMEDIPHAM IN AIR, 1998 PHENMEDIPHAM ACTIVE SUBSTANCE CODE: AE B038584 Report No. A64017 AgrEvo UK Ltd. Not GLP Unpublished	N	TFP
KCP 5.1	Cole, M.G.	2000	VALIDATION OF AN ANALYTICAL METHOD FOR THE RESIDUES OF NC 20645 IN SUGAR BEET ROOTS AND WHOLE MILK, USA, 1998 CODE: AE C639175 00 1B97 0001 Report No. C004116 not available Not GLP Unpublished	N	TFP

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1	Schulte, G.	2013a	ANALYTICAL METHOD 01343 FOR THE DETERMINATION OF RESIDUES OF OPEN-RING-2-KETO ETHOFUMESATE (AE C520645) IN/ON PLANT MATRICES BY HPLC-MS/MS - METHOD FOR STORAGE STABILITY Report No. MR-12/056 not available Not GLP Unpublished	N	TFE
KCP 5.1	Schulte, G.	2013b	STORAGE STABILITY OF OPEN-RING-2-KETO ETHOFUMESATE (AE C520645) IN PLANT MATRICES FOR 24 MONTHS - PHASE REPORT AFTER 6 MONTHS Report No. M-459806-01 not available GLP Unpublished	N	TFE
KCP 5.1	Helgers, A.	1997	ETHOFUMESATE AND LENACIL SUSPENSION CONCENTRATE 300 + 120 G/L AE B049913 02 SC 37 A101 AND AE B049913 02 WP42 A101 ETHOFUMESATE AND LENACIL SC COMPARED WITH A WP FORMULATION IN SUGAR BEET; DETERMINATION OF RESIDUES IN SUGAR BEET ROOTS AND AND TOPS FOLLOWING ONE PRE-EMERGENCE APPLICATION; ITALY, 1995 Report No. M-165366-02-1 not available GLP Unpublished	N	TFE
KCP 5.1	Godfrey, T.L.	1996	ETHOFUMESATE AND METABOLITE ANALYTICAL GRADES AE B049913 AND AE C509607 (NC 8438 AND NC 9607) ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE AND MAJOR METABOLITE IN SUGAR BEET (ROOTS AND TOPS) BY GC/MSD Report No. A89687 not available GLP Unpublished	N	TFE
KCP 5.1	Schulte, G.	2013c	AMENDMENT NO. 1 TO REPORT NO: 10-2109 - DETERMINATION OF THE RESIDUES OF ETHOFUMESATE IN/ON SUGAR BEET AFTER SPRAY APPLICATION OF ETHOFUMESATE SC 500 IN THE FIELD IN SPAIN, ITALY AND GREECE Report No. 10-2109 not available Not GLP Unpublished	N	TFE

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1	Konrad, S.	2012	ANALYTICAL METHOD 00955/M002 FOR THE DETERMINATION OF ETHOFUMESATE AND ITS METABOLITE AE C509607 IN THREE DIFFERENT PLANT GROUPS (SUGAR BEET, LEAF AND BODY AND ORANGE) Report No. M-438402-01-1 not available Not GLP Unpublished	N	TFE
KCP 5.1	Hamberger, R.	2013	DETERMINATION OF THE STORAGE STABILITY OF ETHOFUMESATE AND ITS METABOLITE NC20645 IN SUGAR BEET MATRICES DURING STORAGE AT < OR = TO -18°C FOR A PERIOD OF 12 MONTHS not available not available GLP Unpublished	N	ACM
KCP 5.1	Schlewitz, P.	2014	FROZEN STORAGE STABILITY OF RESIDUES OF ETHOFUMESATE METABOLITE NC 20645 IN SUGAR BEET (ROOTS AND TOPS WITH LEAVES) Report No. B1312 Anadiag S.A., Haguenau, France GLP Unpublished	N	UPL
KCP 5.1	Tandy, R.	2012	DETERMINATION OF RESIDUES OF ETHOFUMESATE, PHENMEDIPHAM AND DESMEDIPHAM AFTER ONE APPLICATION OF ETHOFOL 500SC OR THREE APPLICATIONS OF BETASANA TRIO SC IN SUGAR BEET (OUTDOOR) AT 4 SITES IN NORTHERN EUROPE 2009 Report No. S09-01656 Eurofins Agroscience Services LTD, UK GLP Unpublished	N	UPL
KCP 5.1	Perny, A.	2002	VALIDATION OF THE METHOD OF ANALYSIS OF THE RESIDUES OF ETHOFUMESATE AND ITS METABOLITE 2-KETO ETHOFUMESATE (FREE AND CONJUGATED FORM) IN SUGAR BEETS Report No. A0019 Anadiag S.A., Haguenau, France GLP Unpublished	N	ACM
KCP 5.1	Huaultmé, J.-M.	2013a	MAGNITUDE OF RESIDUE OF ETHOFUMESATE AND METABOLITES IN SUGAR BEET RAW AGRICULTURAL COMMODITIES AFTER ONE FOLIAR APPLICATION OF ETHOFUMESATE 500 G/L SC	N	ACM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			- 4 TRIALS (2 HARVEST TRIALS AND 2 DECLINE CURVE TRIALS) NORTHERN EUROPE (THE NETHERLANDS, BELGIUM) - 2012 Report No. BPL12/436/GC BIOTEK Agriculture GLP Unpublished		
KCP 5.1	Chevallier, E.	2012	MAGNITUDE OF RESIDUE OF ETHOFUMESATE AND METABOLITES IN SUGAR BEET RAW AGRICULTURAL COMMODITIES AFTER ONE FOLIAR APPLICATION OF ETHOFUMESATE 500 G/L SC - 4 TRIALS (2 HARVEST TRIALS AND 2 DECLINE CURVE TRIALS) NORTHERN EUROPE (THE NETHERLANDS, BELGIUM) - 2011 Report No. BPL11/380/GC BIOTEK Agriculture GLP Unpublished	N	ACM
KCP 5.1	Hamberger, R.	2012a	ANALYTICAL PHASE REPORT - MAGNITUDE OF RESIDUE OF ETHOFUMESATE AND METABOLITES IN SUGAR BEET RAW AGRICULTURAL COMMODITIES AFTER ONE FOLIAR APPLICATION OF ETHOFUMESATE 500 G/L SC - 4 TRIALS (2 HARVEST TRIALS AND 2 DECLINE CURVE TRIALS) NORTHERN EUROPE (THE NETHERLANDS, BELGIUM) - 2011 not stated not available Not GLP Unpublished	N	TFE
KCP 5.1	Huauilmé, J.-M.	2013b	MAGNITUDE OF RESIDUE OF ETHOFUMESATE AND METABOLITES IN SUGAR BEET RAW AGRICULTURAL COMMODITIES AFTER ONE FOLIAR APPLICATION OF ETHOFUMESATE 500 G/L SC - 4 TRIALS (2 HARVEST TRIALS AND 2 DECLINE CURVE TRIALS) SOUTHERN EUROPE (ITALY, SPAIN)-2012 Report No. BPL12/435/GC BIOTEK Agriculture GLP Unpublished	N	ACM
KCP 5.1	Spence, Ch.	2014	EVALUATION OF ETHOFUMESATE HERBICIDE RESIDUES CROP ROTATION STUDY, CEREAL, ROOT AND LEAFY VEGETABLE CROPS FOLLOWING SUGAR BEET - ONE APPLICATION TO TWO TRIALS INITIATED IN 2012 - NEU (THE UNITED KINGDOM) AND SEU (ITALY) Report No. 34890 Charles River Laboratories, Edinburgh, UK GLP	N	ACM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.1	Hamberger, R.	2014	ANALYTICAL PHASE REPORT - EVALUATION OF ETHOFUMESATE HERBICIDE RESIDUES CROP ROTATION STUDY, CEREAL, ROOT AND LEAFY VEGETABLE CROPS FOLLOWING SUGAR BEET - ONE APPLICATION TO TWO TRIALS INITIATED IN 2012 - NEU (THE UNITED KINGDOM) AND SEU (ITALY). not stated not available Not GLP Unpublished	N	TFE
KCP 5.1	██████	1994	ETHOFUMESATE-DERIVED RESIDUES IN THE MEAT AND MILK OF DAIRY COWS; RESULTING FROM ORAL INGESTION OF ETHOFUMESATE ██████ Not GLP Unpublished	Y	BCS
KCP 5.1	██████	2013	FREEZER STORAGE STABILITY OF ETHOFUMESATE IN ANIMAL MATRIX SAMPLES - INTERIM REPORT ██████ GLP Unpublished	N Y	TFE
KCP 5.1	██████	2010	ETHOFUMESATE - MAGNITUDE OF THE RESIDUE IN DAIRY COW ██████ GLP Unpublished	Y	BCS
KCP 5.1	Traub, M.	2011	AE C508493 (ETHOFUMESATE-2-HYDROXY): AEROBIC DEGRADATION IN FOUR EUROPEAN SOILS Report No. S11-00957 not available Not GLP Unpublished	N	TFE
KCP 5.1	Traub, M.	2012a	AE C509607: AEROBIC DEGRADATION IN FOUR EUROPEAN SOILS Report No. S11-009558 not available	N	TFE

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Not GLP Unpublished		
KCP 5.1	Traub, M.	2012b	ETHOFUMESATE-CARBOXYLIC ACID (AS POTASSIUM SALT: AE C639175): AEROBIC DEGRADATION IN FOUR EUROPEAN SOILS Report No. S11-03264 not available Not GLP Unpublished	N	TFE
KCP 5.1	Whiteoak, R.J., Crofts, M., Harris, R.J.	1973	ANALYTICAL METHOD FOR RESIDUE IN SUGAR BEET TREATED WITH NORTRON Report No. A83491/ M-155727-01 not available Not GLP Unpublished	N	TFE
KCP 5.1	Whiteoak, R.J., Crofts, M., Harris, R.J.	1976	ANALYTICAL METHOD FOR RESIDUES IN SUGARBEET TREATED WITH NORTRON Report No. A83492/ M-155728-01 not available Not GLP Unpublished	N	TFE
KCP 5.2	Straszewski, A., Wrede-Rücker, A.	1993	ANALYTICAL METHOD FOR. THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM AND A MAJOR METABOLITE IN SUGAR BEETS (LEAVES/ROOTS) BY HPLC Report No. A62037 Schering AG, Berlin, Germany Not GLP Unpublished	N	TFP
KCP 5.2	Wrede-Rucker, A.	1992	ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM IN TISSUE AND MILK BY GLC Report No. R166 Schering AG, Berlin, Germany Not GLP Unpublished Submitted in: KCP 5.1/07	N	TFP
KCP 5.2	Chambers, J., Everitt, S.	1998	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF PHENMEDIPHAM IN AIR, 1998 PHENMEDIPHAM ACTIVE SUBSTANCE CODE: AE B038584 Report No. A64017	N	TFP

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			AgrEvo UK Ltd. Not GLP Unpublished Submitted in: KCP 5.1/16		
KCP 5.2	Billian, P.	2003	SUPPLEMENT E001 OF THE ANALYTICAL METHOD 00802 FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM, DESMEDIPHAM AND THEIR METABOLITES MHPC AND EHPC IN/ON MILK, MEAT AND EGG BY HPLC-MS/MS Report No. C030876 not available Not GLP Unpublished	N	TFP
KCP 5.2	Brumhard, B.	2003a	INDEPENDENT LABORATORY VALIDATION OF ENFORCEMENT METHOD 00802/E001 FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM, DESMEDIPHAM AND THEIR METABOLITES MHPC AND EHPC IN/ON SAMPLE MATERIALS OF ANIMAL ORIGIN BY HPLC-MS/MS Report No. C031372 Bayer Crop Science AG Not GLP Unpublished	N	TFP
KCP 5.2	Wrede, A.	2000	ENFORCEMENT METHOD AND VALIDATION OF SURFACE AND DRINKING WATER BY HPLC/UV PHENMEDIPHAM, AE B038210 CODE: AE B038584, AE B038210 Report No. C007532 Aventis Cropscience GmbH, Frankfurt am Main, Germany Not GLP Unpublished	N	TFP
KCP 5.2	Anspach, T.	2003	ENFORCEMENT METHOD (INCLUDING VALIDATION) FOR THE DETERMINATION OF RESIDUES OF PHENMEDIPHAM AND ITS METABOLITE MHPC IN DRINKING AND SURFACE WATER Report No. C029326 Chemische Laboratorien GmbH Not GLP Unpublished	N	TFP
KCP 5.2	Schulte, G., Diehl, P.	2014	VALIDATION OF THE ANALYTICAL METHOD 01392 FOR THE DETERMINATION OF THE RELEVANT ETHOFUMESATE METABOLITES IN PLANT MATRICES BY HPLC-MS/MS Report No. M-479926-01 not available GLP	N	TFE

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.2	Betson, S.	2014	INDEPENDENT LABORATORY VALIDATION (ILV) OF THE ANALYTICAL METHOD 01392 FOR THE DETERMINATION OF THE RELEVANT ETHOFUMESATE METABOLITES IN PLANT MATRICES BY HPLC-MS/MS Report No. M-497682-01-1 not available GLP Unpublished	N	TFE
KCP 5.2	Schlewitz, P.	2013a	validation of the analytical method for the determination of Ethofumesate (free form) and NC 20645 (free and conjugated form) in high protein/starch content, high water content, high oil content, high acid content and difficult commodities Report No. R B3016 Anadiag S.A., Haguenau, France GLP Published: no	N	UPL
KCP 5.2	Jooß, S.	2012	ETHOFUMESATE - VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF THE ETHOFUMESATE AND ITS TWO METABOLITES NC 9607 AND NC 20645 IN FOODSTUFFS OF ANIMAL ORIGIN Report No. P 2371 G PTRL Europe, Ulm, Germany GLP Unpublished	N	UPL
KCP 5.2	Schlewitz, P.	2013	INDEPENDENT LABORATORY VALIDATION OF AN ANALYTICAL METHOD FOR THE ANALYSIS OF ETHOFUMESATE AND ITS TWO METABOLITES NC 9607 AND NC 20645 IN FOODSTUFFS OF ANIMAL ORIGIN Report No. R B1218 Anadiag S.A., Haguenau, France GLP Unpublished	N	UPL
KCP 5.2	Brumhard, B.	2003b	METHOD 00806 FOR THE DETERMINATION OF RESIDUES OF ETHOFUMESATE IN SOIL BY HPLCMS/MS Report No. 00806 not available Not GLP Unpublished	N	TFP

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	Schneider, E.	2000	CONFIRMATION METHOD FOR THE DETERMINATION OF RESIDUES OF ETHOFUMESATE IN SOIL Report No. PR00/003 UCL Umwelt Control Labor, Köln, Germany GLP Unpublished	N	FCS
KCP 5.2	Hamberger, R.	2012b	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF ETHOFUMESATE AND ITS METABOLITES NC8493 IN SOIL Report No. 12A04042-01-VMS CIP Chemisches Institut Pforzheim GmbH, Germany GLP Unpublished	N	ACM
KCP 5.2	Jooß, S.	2011	ETHOFUMESATE - VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE IN WATER Report No. P 2368 G PTRL Europa, Ulm, Germany GLP Unpublished	N	UPL
KCP 5.2	Krebber, R., Braune, M.	2013	ANALYTICAL METHOD 01387 FOR THE DETERMINATION OF VARIOUS PESTICIDES IN DRINKING AND SURFACE WATER BY HPLC-MS/MS Report No. MR-13/085 not available Not GLP Unpublished	N	TFE
KCP 5.2	Stanislawski, T.	2013	INDEPENDENT LABORATORY VALIDATION OF BCS ANALYTICAL METHODS 01333 AND 01387 FOR DETERMINATION OF VARIOUS PESTICIDES IN SURFACE WATER BY DI-HPLC-MS/MS Report No. P3117 G not available Not GLP Unpublished	N	TFP
KCP 5.2	Hamberger, R.	2012c	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF ETHOFUMESATE AND ITS METABOLITES NC9607 AND NC20645 IN SURFACE WATER Report No. 12A04042-01-VMWA CIP Chemisches Institut Pforzheim GmbH, Germany GLP Unpublished	N	ACM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	Brown, D.	2014	ETHOFUMESATE - INDEPENDENT LABORATORY VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE AND ITS METABOLITES NC 20645 AND NC 9607 IN SURFACE WATER Report No. S13-04250 Eurofins Agrosience Services GmbH GLP Unpublished	N	UPL
KCP 5.2	Wrede-Rücker, A.	1993b	ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE IN AIR Report No. W139 not stated Not GLP Unpublished	N	AGE
KCP 5.2	Reichert, N.	1994	DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF ETHOFUMESATE AND OXO-METABOLITE OF ETHOFUMESATE IN AIR Report No. C506 not available GLP Unpublished	N	AGE
KCP 5.2	Schneider, E.	1994a	ETHOFUMESATE: VALIDATION OF AN ANALYTICAL METHOD FOR DETERMINATION IN AIR (INCLUSIVE ETHOFUMESATE-2-KETO) Report No. NC 8438 / W174 not available GLP Unpublished	N	AGE
KCP 5.2	Schneider, E.	1994b	DETERMINATION OF ETHOFUMESATE IN AIR Report No. PR93/016, method DrK078 not available GLP Unpublished	N	FCS
KCP 5.2	McKenzie, J.	1994	VALIDATION OF A PLASMA ASSAY, ETHOFUMESATE IN DOG PLASMA Report No. C507 not available GLP Unpublished	N	AGE

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.2/26	Klimmek, S.; Gizler, A.	2014	Validation of DFG method S 19 (extended revision) for the determination of residues of desmedipham, phenmedipham and their metabolites EHPC and MHPC in/on plant material by means of liquid chromatography with Tandem mass spectrometric detection (LC-MS/MS) Eurofins Agrosience Services Chem, Hamburg, Germany Amendment No. 1 to Report No. AVE-0201V Edition Number: M-216103-02-1 Date: 2014-11-27 GLP/GEP: yes, unpublished	N	Task Force on Phenmedipham
KCA 4.2/27	Anspach, T.	2002	Validation of DFG method S 19 (extended revision) for the determination of residues of desmedipham, phenmedipham and their metabolites EHPC and MHPC in/on plant material by means of liquid chromatography with Tandem mass spectrometric detection (LC-MS/MS) Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Report No.: C028890 Date: 2002-12-13 GLP/GEP: no, unpublished	N	Task Force on Phenmedipham
KCA 4.2/28	Freitag, Th.	2014	Independent laboratory validation of the DFG Method S19 (extended revision) for the determination of residues of medipham, Phenmedipham, and their Metabolites EHPC and MHPC in/on plant material Bayer CropScience, Monheim , Germany Report No.: P612051807 (amendment to Report No. MR-146/05) Edition Number: M-261837-02-1 Date: 2014-08-14; amended 12.5.2016 GLP/GEP: yes, unpublished	N	Task Force on Phenmedipham
KCA 4.2 /32	Krebber, R.; Braune, M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Bayer CropScience AG, Monheim, Germany Report No.: MR-13/085, Edition Number: M-466732-01-1 Report No.: MR-13/085 Date: 2013-10-09 GLP/GEP: yes, unpublished	N	Task Force on Phenmedipham
KCA 4.2 /33	Stanislawski, T.	2013	Independent laboratory validation of BCS methods 01333 and 01387 for the determination of various pesticides in surface water by DI-HPLC-MS/MS PTRL Europe, Ulm, Germany Report No.: P3117 G, Edition Number: M-470714-02-1 Date: 2013-12-13	N	Task Force on Phenmedipham

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP/GEP: yes, unpublished		
KCA 4.2 /34	Kaussmann, M.	2016	Analytical Method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS Bayer Report No.: 01486 Edition Number: M-556577-01-1 Method Report No.: P683166504 Date: 2016-06-06 GLP/GEP: Yes, unpublished	N	Task Force on Phenmedipham

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Phenmedipham

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1 Methods for risk assessments of physical and chemical properties tests

No new or additional studies have been submitted.

A 2.1.1.2 Methods for risk assessments of toxicological studies

No new or additional studies have been submitted.

A 2.1.1.3 Methods for risk assessments of residues studies

A 2.1.1.3.1 Analytical method 1

Comments of zRMS:	<p>The analytical method has been successfully validated for the analysis of phenmedipham (free and conjugated forms), MHPC (free and conjugated forms) and 3-methylaniline in sugar beet (roots and leaves with tops).</p> <p>The method was validated at 0.01 mg/kg for each matrix and each analyte.</p> <p>Recoveries and precision data comply with the requirements of SANCO/3029/99 rev.4 as mean recoveries are within the range 70-110% and RSD are less than 20% for both matrices.</p> <p>Recoveries and precision data comply with the requirements of the new SANTE/2020/12830, Rev.1 guideline as mean recoveries are within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.</p> <p>The method is acceptable.</p>
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Reference:	KCP 5.1.2/01
Report	Validation of the Analytical Method for the Analysis of Phenmedipham (Free and Conjugated Forms), MHPC (Free and Conjugated Forms) and 3-Methylaniline in Sugar Beet (Leaves with Tops and Roots), Stouvenot, C., 2021, report No R C0327
Guideline(s):	SANCO/3029/99 rev.4 SANTE/2020/12830, Rev.1
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Analyte	Phenmedipham, MHPC (free and conjugated forms) and m-toluidine	
Matrix	Sugar beet (leaves with tops, and roots)	
Quantification; Specificity	<p>LC-MS/MS</p> <p><u>Phenmedipham</u>: quantifier [m/z]: 301.1 > 168.0 qualifier [m/z]: 301.1 > 135.8</p> <p><u>MHPC</u>: quantifier [m/z]: 168.1 > 136.0 qualifier [m/z]: 168.1 > 107.9</p> <p><u>m-Toluidine</u>: quantifier [m/z]: 108.0 > 91.1 qualifier [m/z]: 108.0 > 65.0</p>	
Dissolution/extraction	Acetonitrile : ultra-pure water (80 : 20) containing 1% formic acid	
Partition/clean-up	Not applicable, samples were diluted with acetonitrile containing 1% formic acid	
Accuracy (fortified)	<u>STEP No. 1</u>	<u>STEP No. 1</u>

samples)	<p><u>Phenmedipham free (leaves with tops):</u> 1 × LOQ: 90.7% (n = 5) 10 × LOQ: 94% (n = 5)</p> <p><u>Phenmedipham free (roots):</u> 1 × LOQ: 95.1% (n = 5) 10 × LOQ: 98.5% (n = 5)</p> <p><u>STEP No. 2</u> <u>Phenmedipham conj. as MHPC (leaves with tops):</u> 1 × LOQ: 68.9% (n = 5) 10 × LOQ: 72.8% (n = 5)</p> <p><u>Phenmedipham conj. as MHPC (roots):</u> 1 × LOQ: 82.2% (n = 5) 10 × LOQ: 94.6% (n = 5)</p>	<p><u>MHPC free (leaves with tops):</u> 1 × LOQ: 84.9% (n = 5) 10 × LOQ: 79.4% (n = 5)</p> <p><u>MHPC free (roots):</u> 1 × LOQ: 93.9% (n = 5) 10 × LOQ: 92.4% (n = 5)</p> <p><u>STEP No. 2</u> <u>MHPC conj. as MHPC (leaves with tops):</u> 1 × LOQ: 87.3% (n = 5) 10 × LOQ: 89.6% (n = 5)</p> <p><u>MHPC conj. as MHPC (roots):</u> 1 × LOQ: 78.8% (n = 5) 10 × LOQ: 81.2% (n = 5)</p> <p><u>m-Toluidine (leaves with tops):</u> 1 × LOQ: 78.8% (n = 5) 10 × LOQ: 82.5% (n = 5)</p> <p><u>m-Toluidine (roots):</u> 1 × LOQ: 94.4% (n = 5) 10 × LOQ: 88.2% (n = 5)</p>
Precision	<p><u>STEP No. 1</u> <u>Phenmedipham free (leaves with tops):</u> 1 × LOQ: 2.3% 10 × LOQ: 4.0%</p> <p><u>Phenmedipham free (roots):</u> 1 × LOQ: 0.8% 10 × LOQ: 1.8%</p> <p><u>STEP No. 2</u> <u>Phenmedipham conj. as MHPC (leaves with tops):</u> 1 × LOQ: 7.5% 10 × LOQ: 3.5%</p> <p><u>Phenmedipham conj. as MHPC (roots):</u> 1 × LOQ: 9.0% 10 × LOQ: 3.8%</p>	<p><u>STEP No. 1</u> <u>MHPC free (leaves with tops):</u> 1 × LOQ: 6.7% 10 × LOQ: 5.6%</p> <p><u>MHPC free (roots):</u> 1 × LOQ: 1.2% 10 × LOQ: 2.7%</p> <p><u>STEP No. 2</u> <u>MHPC conj. as MHPC (leaves with tops):</u> 1 × LOQ: 2.9% 10 × LOQ: 3.3%</p> <p><u>MHPC conj. as MHPC (roots):</u> 1 × LOQ: 9.8% 10 × LOQ: 3.6%</p> <p><u>m-Toluidine (leaves with tops):</u> 1 × LOQ: 1.4% 10 × LOQ: 1.3%</p> <p><u>m-Toluidine (roots):</u> 1 × LOQ: 0.4% 10 × LOQ: 2.0%</p>
Linearity of response	<p><u>STEP No. 1</u> <u>Phenmedipham free (leaves with tops):</u> $y = 1.0857E-05x - 0.30$ $r^2 = 0.99992$</p> <p><u>Phenmedipham free (roots):</u> $y = 6.4557E-06x - 0.45$ $r^2 = 0.99978$</p>	<p><u>STEP No. 1</u> <u>MHPC free (leaves with tops):</u> $y = 6.8910E-05x - 0.16$ $r^2 = 0.99976$</p> <p><u>MHPC free (roots):</u> $y = 2.0218E-05 + 0.04$ $r^2 = 0.99996$</p> <p><u>STEP No. 2</u> <u>MHPC conj. as MHPC (leaves with tops):</u> $y = 2.6035E-05 - 0.09$ $r^2 = 0.99994$</p> <p><u>MHPC conj. as MHPC (roots):</u> $y = 1.1483E-05 + 0.10$ $r^2 = 0.99999$</p> <p><u>m-Toluidine (leaves with tops):</u> $y = 2.2640E-06x - 0.002$ $r^2 = 0.99983$</p> <p><u>m-Toluidine (roots):</u> $y = 1.3895E-06x + 0.007$ $r^2 = 0.99999$</p>
Calibration range	<p>Phenmedipham and MHPC (free forms – step 1): 1.5 – 60 ng/mL Phenmedipham and MHPC (free and conj. forms as MHPC – step 2): 0.5 – 60 ng/mL m-Toluidine: 0.15 – 6 ng/mL</p>	

Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	<u>For each analyte (Phenmedipham free, MHPC free, Phenmedipham conj. and MHPC conj.) and each matrix:</u> 0.01 mg analyte/kg (1 × LOQ) 0.10 mg analyte/kg (100 × LOQ)

CONCLUSION

Method was properly validated in line with the provisions of both SANCO/3029/99 rev. 4 and SANTE/2020/12830 Rev.1 and is deemed to be acceptable.

A 2.1.1.4 Methods for risk assessments of environmental fate studies

No new or additional studies have been submitted.

A 2.1.1.5 Methods for risk assessments of ecotoxicology studies

A 2.1.1.5.1 Analytical method 1

Comments of zRMS:	An analytical method for the determination of the active ingredients phenmedipham and ethofumesate, as well as the metabolites MHPC and m-Toluidine (phenmedipham) of the test item Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10) in water media was validated according to the guideline SANCO/3029/99 rev. 4 The limit of quantification (LOQ) of the analytical method was 0.100 mg test item/L for phenmedipham and ethofumesate and 0.002 mg standard/L for MHPC and m-Toluidine. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference:	KCP 10.2.1/01
Report	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Acute immobilization test to <i>Daphnia magna</i> , semi-static, 48 hours, Scheerbaum, D., 2021, report No SO20127 / DAI18743
Guideline(s):	OECD 202 (2004)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	Ethofumesate and Phenmedipham, as well as the Phenmedipham metabolites MHPC and m-Toluidine
Matrix	Water
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> : quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.18 > 259.02 qualifier [m/z]: 287.18 > 161.17 <u>MHPC</u> : quantifier [m/z]: 168.03 > 92.99

	qualifier [m/z]: 168.03 > 135.97 <u>m-Toluidine</u> : quantifier [m/z]: 107.98 > 64.97 qualifier [m/z]: 107.98 > 93.27	
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid	
Partition/clean-up	Not applicable, samples were diluted with acetonitrile containing 0.2% formic acid and dilution medium	
Accuracy (fortified samples)	<u>Phenmedipham</u> : 1 × LOQ: 93% (n = 5) 100 × LOQ: 100% (n = 5) <u>Ethofumesate</u> : 1 × LOQ: 101% (n = 5) 100 × LOQ: 102% (n = 5)	<u>MHPC</u> : 1 × LOQ: 104% (n = 5) 500 × LOQ: 101% (n = 5) <u>m-Toluidine</u> : 1 × LOQ: 104% (n = 5) 500 × LOQ: 96% (n = 5)
Precision	<u>Phenmedipham</u> : 1 × LOQ: 1.1% 100 × LOQ: 5.0% <u>Ethofumesate</u> : 1 × LOQ: 1.2% 100 × LOQ: 1.4%	<u>MHPC</u> : 1 × LOQ: 1.5% 500 × LOQ: 1.6% <u>m-Toluidine</u> : 1 × LOQ: 7.1% 500 × LOQ: 4.4%
Linearity of response	<u>Phenmedipham</u> : $y = 77265.8x + 6366.19$ $r^2 = 0.999179$ <u>Ethofumesate</u> : $y = 58705.8x + 5831.37$ $r^2 = 0.996867$	<u>MHPC</u> : $y = 50094.3x - 1312.21$ $r^2 = 0.999214$ <u>m-Toluidine</u> : $y = 8045.57x + 105.606$ $r^2 = 0.997242$
Calibration range	0.5 – 10.0 µg/L	
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)	
Assessment of matrix effects presented?	Yes	
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes	
Example chromatograms included in the report?	Yes	
LOQ	<u>Phenmedipham and Ethofumesate</u> : 0.100 mg test item/L (1 × LOQ) 10.0 mg test item/L (100 × LOQ) <u>MHPC and m-Toluidine</u> : 0.00200 mg standard/L (1 × LOQ) 1.00 mg standard/L (500 × LOQ)	

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.2 Analytical method 2

Comments of zRMS:	An analytical method for the determination of the active ingredients phenmedipham and ethofumesate, as well as the metabolites MHPC and m-Toluidine (<u>phenmedipham</u>) of the test item Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10) in water media was validated according to the guideline SANCO/3029/99 rev. 4 The limit of quantification (LOQ) of the analytical method was 0.0100 mg test item/L for phenmedipham and ethofumesate and 0.002 mg standard/L for MHPC and m-Toluidine. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference: KCP 10.2.1/02
Report Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours, Scheerbaum, D., 2021, report No SO20126 / SPO18743
Guideline(s): OECD 201 (2011)
Deviations: No deviation with impact on quality and integrity of the study.
GLP: Yes
Acceptability: Yes

Analyte	Ethofumesate and Phenmedipham, as well as the Phenmedipham metabolites MHPC and m-Toluidine	
Matrix	Water	
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> : quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.18 > 259.02 qualifier [m/z]: 287.18 > 161.17 <u>MHPC</u> : quantifier [m/z]: 168.03 > 92.99 qualifier [m/z]: 168.03 > 135.97 <u>m-Toluidine</u> : quantifier [m/z]: 107.98 > 64.97 qualifier [m/z]: 107.98 > 93.27	
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid	
Partition/clean-up	Not applicable, samples were diluted with acetonitrile containing 0.2% formic acid and dilution medium	
Accuracy (fortified samples)	<u>Phenmedipham</u> : 1 × LOQ: 108% (n = 5) 600 × LOQ: 106% (n = 5) <u>Ethofumesate</u> : 1 × LOQ: 107% (n = 5) 600 × LOQ: 105% (n = 5)	<u>MHPC</u> : 1 × LOQ: 98% (n = 5) 500 × LOQ: 100% (n = 5) <u>m-Toluidine</u> : 1 × LOQ: 99% (n = 5) 500 × LOQ: 94% (n = 5)
Precision	<u>Phenmedipham</u> : 1 × LOQ: 5.9% 600 × LOQ: 2.3% <u>Ethofumesate</u> : 1 × LOQ: 6.3% 600 × LOQ: 0.78%	<u>MHPC</u> : 1 × LOQ: 3.4% 500 × LOQ: 1.5% <u>m-Toluidine</u> : 1 × LOQ: 9.1% 500 × LOQ: 2.5%
Linearity of response	<u>Phenmedipham</u> : $y = 74533.2x + 1990.88$ $r^2 = 0.999487$ <u>Ethofumesate</u> : $y = 58813.1x - 904.97$ $r^2 = 0.998552$	<u>MHPC</u> : $y = 49602.1x - 1881.84$ $r^2 = 0.999465$ <u>m-Toluidine</u> : $y = 7380.14x + 4030.27$ $r^2 = 0.991338$
Calibration range	0.5 – 10.0 µg/L	
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)	
Assessment of matrix effects presented?	Yes	
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes	
Example chromatograms included in the report?	Yes	
LOQ	<u>Phenmedipham and Ethofumesate</u> : 0.0100 mg test item/L (1 × LOQ) 6.00 mg test item/L (600 × LOQ) <u>MHPC and m-Toluidine</u> : 0.00200 mg standard/L (1 × LOQ) 1.00 mg standard/L (500 × LOQ)	

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.3 Analytical method 3

Comments of zRMS:	<p>An analytical method for the determination of the active ingredients phenmedipham and ethofumesate, as well as the metabolites MHPC and m-Toluidine (phenmedipham) of the test item Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10) in water media was validated according to the guideline SANCO/3029/99 rev. 4</p> <p>The limit of quantification (LOQ) of the analytical method was 0.0100 mg test item/L for phenmedipham and ethofumesate and 0.002 mg standard/L for MHPC and m-Toluidine.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.</p>
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Reference:	KCP 10.2.1/03
Report	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Aquatic Plant Toxicity Test, <i>Lemna gibba</i> , semi-static, 7 days, Scheerbaum, D., 2021, report No SO20128 / SLG18743
Guideline(s):	OECD 221 (2006)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	Ethofumesate and Phenmedipham, as well as the Phenmedipham metabolites MHPC and m-Toluidine
Matrix	Water
Quantification; Specificity	<p>LC-MS/MS</p> <p><u>Phenmedipham</u>:</p> <p>quantifier [m/z]: 301.14 > 168.07</p> <p>qualifier [m/z]: 301.14 > 108.04</p> <p><u>Ethofumesate</u>:</p> <p>quantifier [m/z]: 287.18 > 259.02</p> <p>qualifier [m/z]: 287.18 > 161.17</p> <p><u>MHPC</u>:</p> <p>quantifier [m/z]: 168.03 > 92.99</p> <p>qualifier [m/z]: 168.03 > 135.97</p> <p><u>m-Toluidine</u>:</p> <p>quantifier [m/z]: 107.98 > 64.97</p> <p>qualifier [m/z]: 107.98 > 93.27</p>
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted with acetonitrile containing 0.2% formic acid and dilution medium

Accuracy (fortified samples)	<u>Phenmedipham:</u> 1 × LOQ: 103% (n = 5) 600 × LOQ: 104% (n = 5) <u>Ethofumesate:</u> 1 × LOQ: 105% (n = 5) 600 × LOQ: 104% (n = 5)	<u>MHPC:</u> 1 × LOQ: 104% (n = 5) 500 × LOQ: 101% (n = 5) <u>m-Toluidine:</u> 1 × LOQ: 102% (n = 5) 500 × LOQ: 96% (n = 5)
Precision	<u>Phenmedipham:</u> 1 × LOQ: 4.1% 600 × LOQ: 2.2% <u>Ethofumesate:</u> 1 × LOQ: 2.6% 600 × LOQ: 2.8%	<u>MHPC:</u> 1 × LOQ: 2.2% 500 × LOQ: 1.4% <u>m-Toluidine:</u> 1 × LOQ: 3.5% 500 × LOQ: 5.2%
Linearity of response	<u>Phenmedipham:</u> $y = 70191.5x + 15084.6$ $r^2 = 0.998544$ <u>Ethofumesate:</u> $y = 54607.2x + 2960.89$ $r^2 = 0.999044$	<u>MHPC:</u> $y = 52452.6x - 3422.07$ $r^2 = 0.998946$ <u>m-Toluidine:</u> $y = 8324.99x + 979.667$ $r^2 = 0.997939$
Calibration range	0.5 – 10.0 µg/L	
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)	
Assessment of matrix effects presented?	Yes	
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes	
Example chromatograms included in the report?	Yes	
LOQ	<u>Phenmedipham and Ethofumesate:</u> 0.0100 mg test item/L (1 × LOQ) 6.00 mg test item/L (600 × LOQ) <u>MHPC and m-Toluidine:</u> 0.00200 mg standard/L (1 × LOQ) 1.00 mg standard/L (500 × LOQ)	

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposed.

A 2.1.1.5.4 Analytical method 4

Comments of zRMS:	<p>An analytical method for the determination of the active ingredients phenmedipham and ethofumesate, as well as the metabolites MHPC and m-Toluidine (phenmedipham) of the test item Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10) in water and sediment media was validated according to the guideline SANCO/3029/99 rev. 4.</p> <p>The limit of quantification (LOQ) of the analytical method was:</p> <ul style="list-style-type: none"> - Aqueous Layer - 0.0100 mg test item/L for phenmedipham and ethofumesate and 0.002 mg standard/kg for MHPC and m-Toluidine; - Sediment - 0.100 mg test item/L for phenmedipham and ethofumesate and 0.02 mg standard/kg for MHPC and m-Toluidine. <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.</p>
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Reference: KCP 10.2.1/04
Report Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Water-Sediment *Myriophyllum spicatum* Toxicity Test semi-static, 14 d, Scheerbaum, D., 2021, report No SO20129 / SMS18743
Guideline(s): OECD 239 (2014)
Deviations: No deviation with impact on quality and integrity of the study.
GLP: Yes
Acceptability: Yes

Analyte	Ethofumesate and Phenmedipham, as well as the Phenmedipham metabolites MHPC and m-Toluidine	
Matrix	Water	
Quantification; Specificity	LC-MS/MS <u>Phenmedipham:</u> quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate:</u> quantifier [m/z]: 287.12 > 259.02 qualifier [m/z]: 287.12 > 121.07 <u>MHPC:</u> quantifier [m/z]: 168.03 > 92.99 qualifier [m/z]: 168.03 > 135.97 <u>m-Toluidine:</u> quantifier [m/z]: 107.98 > 64.97 qualifier [m/z]: 107.98 > 93.27	
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid	
Partition/clean-up	Not applicable, samples were diluted with acetonitrile containing 0.2% formic acid and dilution medium	
Accuracy (fortified samples)	<u>Aqueous layer</u> <u>Phenmedipham:</u> 1 × LOQ: 91% (n = 5) 1000 × LOQ: 105% (n = 5) <u>Ethofumesate:</u> 1 × LOQ: 96% (n = 5) 1000 × LOQ: 108% (n = 5) <u>MHPC:</u> 1 × LOQ: 92% (n = 5) 500 × LOQ: 106% (n = 5) <u>m-Toluidine:</u> 1 × LOQ: 72% (n = 5) 500 × LOQ: 106% (n = 5)	<u>Sediment</u> <u>Phenmedipham:</u> 1 × LOQ: 99% (n = 4) 100 × LOQ: 98% (n = 5) <u>Ethofumesate:</u> 1 × LOQ: 99% (n = 5) 100 × LOQ: 104% (n = 5) <u>MHPC:</u> 1 × LOQ: 95% (n = 5) 50 × LOQ: 99% (n = 5) <u>m-Toluidine:</u> 1 × LOQ: 82% (n = 5) 50 × LOQ: 94% (n = 5)
Precision	<u>Aqueous layer</u> <u>Phenmedipham:</u> 1 × LOQ: 7.9% 1000 × LOQ: 6.1% <u>Ethofumesate:</u> 1 × LOQ: 3.5% 1000 × LOQ: 4.3% <u>MHPC:</u> 1 × LOQ: 4.2% 500 × LOQ: 1.5% <u>m-Toluidine:</u> 1 × LOQ: 4.8% 500 × LOQ: 2.6%	<u>Sediment</u> <u>Phenmedipham:</u> 1 × LOQ: 4.1% 100 × LOQ: 2.0% <u>Ethofumesate:</u> 1 × LOQ: 2.7% 100 × LOQ: 2.8% <u>MHPC:</u> 1 × LOQ: 6.8% 50 × LOQ: 0.86% <u>m-Toluidine:</u> 1 × LOQ: 4.7% 50 × LOQ: 1.0%
Linearity of response	<u>Phenmedipham:</u> $y = 156440x - 19347$ $r^2 = 0.999009$ <u>Ethofumesate:</u> $y = 30683.9x + 3637.85$ $r^2 = 0.996319$	<u>MHPC:</u> $y = 46995x + 6145.96$ $r^2 = 0.999111$ <u>m-Toluidine:</u> $y = 6371.68x + 3915.21$ $r^2 = 0.992570$
Calibration range	0.5 – 10.0 µg/L	
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single	Yes (n = 7)	

points)?	
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ (aqueous layer)	<u>Phenmedipham and Ethofumesate:</u> 0.0100 mg test item/L (1 × LOQ) 10.00 mg test item/L (1000 × LOQ) <u>MHPC and m-Toluidine:</u> 0.00200 mg standard/L (1 × LOQ) 1.00 mg standard/L (500 × LOQ)
LOQ (sediment)	<u>Phenmedipham and Ethofumesate:</u> 0.100 mg test item/L (1 × LOQ) 10.00 mg test item/L (100 × LOQ) <u>MHPC and m-Toluidine:</u> 0.0200 mg standard/L (1 × LOQ) 1.00 mg standard/L (50 × LOQ)

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.5 Analytical method 5

Comments of zRMS:	An analytical method for the determination of MHPC in water media was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.0101 mg test item/L corresponding to 0.00961 mg MHPC/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference:	KCP 10.2.2/01
Report	MHPC: <i>Daphnia magna</i> Reproduction Test, Semi-static, 21 days, Scheerbaum, D., 2021, report No SO20407 / DRE19098
Guideline(s):	OECD 211 (2012)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	MHPC
Matrix	Water
Quantification; Specificity	LC-MS/MS <u>MHPC:</u> quantifier [m/z]: 168.03 > 92.99 qualifier [m/z]: 168.03 > 135.97
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted with acetonitrile containing 0.2% formic acid and dilution medium
Accuracy (fortified samples)	1 × LOQ: 99% (n = 5) 6000 × LOQ: 98% (n = 5)
Precision	1 × LOQ: 1.3% 6000 × LOQ: 0.67%
Linearity of response	y = 18163.6x + 49.4174 r ² = 0.993616
Calibration range	0.5 – 10.0 µg/L
Does the calibration consist of at least 3 levels	Yes (n = 7)

(duplicated points) or 5 levels (single points)?	
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	0.0100 mg test item/L (1 × LOQ) 60.00 mg test item/L (6000 × LOQ)

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.6 Analytical method 6

Comments of zRMS:	An analytical method for the determination of ethofumesate and phenmedipham in dechlorinated tap water (test item stock solution) and in 50% sucrose solution (feeding solution) was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was: - in dechlorinated tap water - 0.3 g test item/L - in feeding solution - 0.6 g test item/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference:	KCP 10.3.1.1.1/02
Report	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Acute Oral Toxicity test on the Bumblebee <i>Bombus terrestris</i> , Klix, V., 2021, report No SO20046 / IUO18743
Guideline(s):	OECD 247 (2017)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	Ethofumesate and Phenmedipham	
Matrix	Dechlorinated tap water (test item stock solution) 50% sucrose solution (feeding solution)	
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> : quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.12 > 259.02 qualifier [m/z]: 287.12 > 161.17	
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid	
Partition/clean-up	Not applicable, samples were diluted	
Accuracy (fortified samples)	<u>Dechlorinated tap water</u> <u>Phenmedipham</u> 1 × LOQ: 104% (n = 5) 1250 × LOQ: 107% (n = 5) <u>Ethofumesate</u> 1 × LOQ: 100% (n = 5) 1250 × LOQ: 108% (n = 5)	<u>Feeding solution</u> <u>Phenmedipham</u> 1 × LOQ: 98% (n = 5) 80 × LOQ: 102% (n = 5) <u>Ethofumesate</u> 1 × LOQ: 101% (n = 5) 80 × LOQ: 106% (n = 5)
Precision	<u>Dechlorinated tap water</u> <u>Phenmedipham</u> 1 × LOQ: 2.6% 1250 × LOQ: 3.0% <u>Ethofumesate</u>	<u>Feeding solution</u> <u>Phenmedipham</u> 1 × LOQ: 0.90% 80 × LOQ: 1.1% <u>Ethofumesate</u>

	1 × LOQ: 13% 1250 × LOQ: 2.8%	1 × LOQ: 3.3% 80 × LOQ: 1.7%
Linearity of response	<u>Phenmedipham</u> : $y = 92778x - 4889.06$ $r^2 = 0.998966$ <u>Ethofumesate</u> : $y = 89483.7x + 24342.1$ $r^2 = 0.999746$	
Calibration range	0.5 – 10.0 µg/L	
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)	
Assessment of matrix effects presented?	Yes	
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes	
Example chromatograms included in the report?	Yes	
LOQ	<u>Dechlorinated tap water</u> 0.3 g test item/L (1 × LOQ) 375 g test item/L (1250 × LOQ) <u>Feeding solution</u> 0.6 g test item/L (1 × LOQ) 48.0 g test item/L (80 × LOQ)	

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.7 Analytical method 7

Comments of zRMS:	An analytical method for the determination of ethofumesate and phenmedipham in dechlorinated tap water (test item stock solution) was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 40.0 g test item/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference:	KCP 10.3.1.1.2/02
Report	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Acute Contact Toxicity Test on the Bumblebee <i>Bombus terrestris</i> , Klix, V., 2021, report No SO20045 / IUT18743
Guideline(s):	OECD 246 (2017)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	Ethofumesate and Phenmedipham
Matrix	Dechlorinated tap water (test item stock solution)
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> : quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.12 > 259.02 qualifier [m/z]: 287.12 > 161.17
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted

Accuracy (fortified samples)	Phenmedipham 1 × LOQ: 99% (n = 5) 10 × LOQ: 97% (n = 5) Ethofumesate 1 × LOQ: 102% (n = 5) 10 × LOQ: 103% (n = 5)
Precision	Phenmedipham 1 × LOQ: 3.4% 10 × LOQ: 2.2% Ethofumesate 1 × LOQ: 4.8% 10 × LOQ: 3.5%
Linearity of response	Phenmedipham: $y = 140036x + 6922.59$ $r^2 = 0.999802$ Ethofumesate: $y = 93572.3x + 13872.6$ $r^2 = 0.999779$
Calibration range	0.5 – 10.0 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	40.0 g test item/L (1 × LOQ) 400 g test item/L (10 × LOQ)

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.8 Analytical method 8

Comments of zRMS:	An analytical method for the determination of ethofumesate and phenmedipham in dechlorinated tap water (test item stock solution) and in 50% sucrose solution (feeding solution) was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.300 g test item/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference: KCP 10.3.1.2/01
Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Chronic oral toxicity test on the Honeybee *Apis mellifera* (Hymenoptera, Apidae), Klix, V., 2021, report No SO20047 / IBC18743
Report
Guideline(s): OECD 245 (2017)
Deviations: No deviation with impact on quality and integrity of the study.
GLP: Yes
Acceptability: Yes

Analyte	Ethofumesate and Phenmedipham
Matrix	Dechlorinated tap water (test item stock solution) 50% sucrose solution (feeding solution)
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> :

	quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.18 > 259.02 qualifier [m/z]: 287.18 > 161.17
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted
Accuracy (fortified samples)	<u>Phenmedipham</u> 1 × LOQ: 104% (n = 5) 1250 × LOQ: 107% (n = 5) <u>Ethofumesate</u> 1 × LOQ: 100% (n = 5) 1250 × LOQ: 108% (n = 5)
Precision	<u>Phenmedipham</u> 1 × LOQ: 2.6% 1250 × LOQ: 3.0% <u>Ethofumesate</u> 1 × LOQ: 13% 1250 × LOQ: 3.1%
Linearity of response	<u>Phenmedipham</u> : $y = 98779.1x + 10882.7$ $r^2 = 0.999647$ <u>Ethofumesate</u> : $y = 74804.4x + 14595.1$ $r^2 = 0.996545$
Calibration range	0.5 – 10.0 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	0.300 g test item/L (1 × LOQ) 375 g test item/L (1250 × LOQ)

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.9 Analytical method 9

Comments of zRMS:	An analytical method for the determination of ethofumesate and phenmedipham in dechlorinated tap water (test item stock solution) was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.300 g test item/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference: KCP 10.3.1.3/01

Report Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Honeybee (*Apis mellifera*) larval toxicity test, repeated exposure, Klix, V., 2021, report No SO20048 / IBL18743

Guideline(s): OECD 239 (2016) (ENV/JM/MONO(2016)34)

Deviations: Yes

The study was modified from the guidance as follows, due to new research results (see Pollinator Research Task Force, LLC and Schmehl et. al, section 14). These modifications increased the survival of the larva.

- No dental rolls and no sterilising solution were filled into the wells underneath the grafting cells in the 48-well plate.
- The water content of diet A and B was increased.
- Between day 7 to 8 the larvae were transferred to the pupal plates.
- The adult emergence was determined daily from day 18 to 20. The adult bees were removed from the plates.

GLP: Yes

Acceptability: Yes

Analyte	Ethofumesate and Phenmedipham
Matrix	Dechlorinated tap water (test item stock solution)
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> : quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.18 > 259.02 qualifier [m/z]: 287.18 > 161.17
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted
Accuracy (fortified samples)	<u>Phenmedipham</u> 1 × LOQ: 104% (n = 5) 200 × LOQ: 107% (n = 4)* <u>Ethofumesate</u> 1 × LOQ: 100% (n = 5) 200 × LOQ: 105% (n = 5)
Precision	<u>Phenmedipham</u> 1 × LOQ: 2.6% 200 × LOQ: 4.4% <u>Ethofumesate</u> 1 × LOQ: 13% 200 × LOQ: 12%
Linearity of response	<u>Phenmedipham</u> : y = 70977.1x + 8559.93 r ² = 0.999215 <u>Ethofumesate</u> : y = 54414.8x + 6173.72 r ² = 0.998352
Calibration range	0.5 – 10.0 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 7)
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	0.300 g test item/L (1 × LOQ) 60.0 g test item/L (200 × LOQ)

* Outlier determined by Grubb's Test for Outliers

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.10 Analytical method 10

Comments of zRMS:	An analytical method for the determination of ethofumesate and phenmedipham, active ingredients of the test item, in tap water was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 5 g test item/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference:	KCP 10.6.2/01
Report	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Winkelmann, G., 2021, report No SO20031 / TNK18743
Guideline(s):	OECD 208 (2006)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	Ethofumesate and Phenmedipham
Matrix	Tap water
Quantification; Specificity	LC-MS/MS <u>Phenmedipham</u> : quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate</u> : quantifier [m/z]: 287.12 > 121.07 qualifier [m/z]: 287.12 > 259.02
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted
Accuracy (fortified samples)	Phenmedipham 1 × LOQ: 96% (n = 5) 15 × LOQ: 101% (n = 5) Ethofumesate 1 × LOQ: 101% (n = 5) 15 × LOQ: 99% (n = 5)
Precision	Phenmedipham 1 × LOQ: 2.1% 15 × LOQ: 1.7% Ethofumesate 1 × LOQ: 5.9% 15 × LOQ: 3.0%
Linearity of response	Phenmedipham: $y = 293.373x + 196.064$ $r^2 = 0.999770$ Ethofumesate: $y = 119.784x - 41.2084$ $r^2 = 0.999032$
Calibration range	5.0 – 50.0 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 6)
Assessment of matrix effects presented?	Yes

Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	5 g test item/L (1 x LOQ) 75 g test item/L (15 x LOQ)

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.5.11 Analytical method 11

Comments of zRMS:	An analytical method for the determination of ethofumesate and phenmedipham, active ingredients of the test item, in tap water was validated according to the guideline SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 5 g test item/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.
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Reference:	KCP 10.6.2/02
Report	Ethofumesate/Phenmedipham 125/125 g/L EC (HBZ10): Terrestrial Plant Test: Vegetative Vigour Test, Winkelmann, G., 2021, report No SO20032 / TNW18743
Guideline(s):	OECD 227 (2006)
Deviations:	Yes Inadvertently, the environmental conditions from 2020-07-06 to 2020-07-14 were deleted before saving. Therefore, no data are available for this period. For <i>Brassica napus</i> , at 0.141 L product/ha: One replicate (replicate No 5) was identified as outlier, determined by Grubb's test, only 7 replicates were used for calculations. These deviations were considered to have no impact on integrity or validity of the study.
GLP:	Yes
Acceptability:	Yes

Analyte	Ethofumesate and Phenmedipham
Matrix	Tap water
Quantification; Specificity	LC-MS/MS <u>Phenmedipham:</u> quantifier [m/z]: 301.14 > 168.07 qualifier [m/z]: 301.14 > 108.04 <u>Ethofumesate:</u> quantifier [m/z]: 287.12 > 121.07 qualifier [m/z]: 287.12 > 259.02
Dissolution/extraction	Acetonitrile : ultra-pure water (50 : 50) containing 0.1% formic acid
Partition/clean-up	Not applicable, samples were diluted
Accuracy (fortified samples)	Phenmedipham 1 x LOQ: 96% (n = 5) 15 x LOQ: 101% (n = 5) Ethofumesate 1 x LOQ: 101% (n = 5) 15 x LOQ: 99% (n = 5)
Precision	Phenmedipham 1 x LOQ: 2.1% 15 x LOQ: 1.7% Ethofumesate 1 x LOQ: 5.9%

	15 × LOQ: 3.0%
Linearity of response	Phenmedipham: $y = 325.052x + 217.583$ $r^2 = 0.997469$ Ethofumesate: $y = 157.119x - 16.7069$ $r^2 = 0.998778$
Calibration range	5.0 – 50.0 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (n = 6)
Assessment of matrix effects presented?	Yes
Absence of interference >30% of LOQ in blank sample demonstrated?	Yes
Example chromatograms included in the report?	Yes
LOQ	5 g test item/L (1 x LOQ) 75 g test item/L (15 x LOQ)

CONCLUSION

Method was properly validated in line with the provisions of SANCO/3029/99 rev. 4 and is deemed to be acceptable for pre-registration purposes.

A 2.1.1.6 Methods for risk assessments of efficacy studies

No new or additional studies have been submitted.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

~~No new or~~ Additional studies have been submitted.

Comments of zRMS:	An analytical method for the determination of phemnedipham and MHPC in plasma has been provided by Applicant at the request of the zRMS. This method has already been generated and submitted under renewal process of Phenmedipham. Studies summary as well as the conclusions of their evaluation by RMS Finland during Phenmedipham renewal are available in RAR version May 2022. The method has been accepted.
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Report:	KCA 4.2 /34; Kaussmann, M.; 2016; M-556577-01-1
Title:	Analytical Method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS
Report No:	P683166504
Document No:	M-556577-01-1
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
GLP/GEP:	yes

Summary of analytical method 01486 (as given in Phenmedipham RAR, version May 2022):

Principle of the method

The analytical method 01486 was developed for the determination of residues of phemnedipham and MHPC (as well as other analytes) in plasma. Only results relevant to phemnedipham and MHPC are reported here.

Plasma samples were deproteinized by mixing with a solution of acetonitrile/water (6/1, v/v) containing 56 mg/L ammonium acetate and 0.14 mL/L formic acid and subsequent centrifugation. An aliquot of the supernatant was subjected to HPLC-MS/MS operating in the positive ion mode. Quantification was done using matrix matched standards. Recovery samples were prepared by fortifying control samples of cattle plasma with diluted stock solutions of phemnedipham and its metabolite MHPC in acetonitrile+10 mL/L formic acid. The fortification levels were 50 and 500 µg/L for both analytes.

Specificity

Analysis of control specimens of plasma by HPLC-MS/MS yielded residues of phemnedipham and MHPC below 30% of the LOQ for both mass transitions. For determination of both analytes two mass transitions, one for quantification and the other for confirmation, were used as listed below.

Quantification (amu): 318 ◊ 168 (phemnedipham)
168 ◊ 136 (MHPC)

Confirmation (amu) : 318 ◊ 136 (phemnedipham)
168 ◊ 108 (MHPC)

Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Mean recovery rates of the confirmatory mass transition for both fortification levels were in the range of 98-100% for phemnedipham and in the range of 102-104% for MHPC. RSDs of the confirmatory mass transition were well below 20% at fortification levels of 50 and 500 µg/L for both analytes. The recovery results on the confirmatory transitions are summarised in Table 4.2-33 (phemnedipham) and Table 4.2-34 (MHPC).

Table 4.2-33: Recovery results from validation of the method 01486 - Recoveries and relative standard deviations (RSDs) for phemnedipham (confirmatory mass transition)

Matrix	Fortification level (FL) µg/L	Recoveries % (Single values)					Per FL		Overall	
							Mean [%]	RSD [%]	Mean [%]	RSD [%]
Plasma	50	93	109	109	95	92	100	8.7	99	8.2
	500	97	99	98	86	110	98	8.7		

FL: Fortification Level. RSD: Relative Standard Deviation

Table 4.2-34: Recovery results from validation of the method 01486 - Recoveries and relative standard deviations (RSDs) for MHPC (confirmatory mass transition)

Matrix	Fortification level (FL)* µg/L	Recoveries % (Single values)					Per FL		Overall	
							Mean [%]	RSD [%]	Mean [%]	RSD [%]
Plasma	50	106	106	111	94	91	102	8.5	103	7.6
	500	98	104	110	94	112	104	7.4		

FL: Fortification Level. RSD: Relative Standard Deviation

* Fortification level expressed as MHPC

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with phemnedipham and MHPC at two different concentrations. Mean recoveries per fortification level for both analytes and for all sample matrices were clearly in the range of 70-120% referring to the quantifier transitions. The recovery results for the quantifier transitions are summarised in Table 4.2-35 (phemnedipham) and Table 4.2-36 (MHPC).

Table 4.2-35: Recovery results from validation of the method 01486 - Recoveries and relative standard deviations (RSDs) for phemnedipham (quantifier mass transition)

Matrix	Fortification level (FL) µg/L	Recoveries % (Single values)					Per FL		Overall	
							Mean [%]	RSD [%]	Mean [%]	RSD [%]
Plasma	50	97	103	114	95	93	100	8.4	99	7.7
	500	93	99	100	87	105	97	7.2		

FL: Fortification Level. RSD: Relative Standard Deviation

Table 4.2-36: Recovery results from validation of the method 01486 - Recoveries and relative standard deviations (RSDs) for MHPC (quantifier mass transition)

Matrix	Fortification level (FL)* µg/L	Recoveries % (Single values)					Per FL		Overall	
							Mean [%]	RSD [%]	Mean [%]	RSD [%]
Plasma	50	104	107	109	95	92	101	7.4	102	7.2
	500	96	107	108	94	112	103	7.7		

FL: Fortification Level. RSD: Relative Standard Deviation

* Fortification level expressed as MHPC

Linearity

The linearity of the detector response was confirmed by injecting at least 5 matrix-matched external standard solutions covering the working range of 1.5-75 µg/L for phemnedipham and MHPC (corresponding to 15 µg/L to 750 µg/L in plasma) with correlation coefficients (r) of ≥ 0.998 (1/x weighted regression). The linear range has well covered all fortification levels.

Limit of quantification

The limit of quantification (LOQ) was defined as the lowest fortification level with a mean recovery between 70% and 110%, with a relative standard deviation not exceeding 20% and blanks not exceeding 30% for quantification transitions. These criteria are considered as fulfilled for the 50 µg/L fortification level for plasma. The limit of detection (LOD) for phemnedipham and MHPC was defined as the lowest measured standard concentration in the matrix matched standard linearity (15 µg/L in plasma). Residues in the untreated sample used for recovery experiments were not detectable (<30% of LOQ).

Precision (repeatability)

The precision and repeatability of the method can be assessed on the basis of the determined relative standard deviations (RSD) for the mean values of the recovery rates. The relative standard deviation (RSD) over both fortification levels ranged from 7.2% to 8.7% for phemnedipham and MHPC and for both mass transitions. These values are within guideline requirements of RSD (<20%).

Matrix effects

Matrix matched standards were used for the evaluation of all analytes to compensate for matrix effects.

Stability of analytes

Storage stability in sample extracts (i.e. supernatant after centrifugation of plasma proteins): The stability in final extracts was examined for plasma over a period of 6 days at $\leq +6^{\circ}\text{C}$. Three control plasma extracts were reanalysed using freshly prepared calibration curves. Phemnedipham and MHPC were found to be stable in final extracts for at least six days in cattle plasma (Table 4.2-37 and 4.2-38).

According to SANCO/825/00 rev. 8.1 (16/11/2010), the recoveries in the fortified samples stored for 6 days at $\leq +6^{\circ}\text{C}$ are within the acceptable range of 70-120%. Therefore, stability of phemnedipham and its metabolite MHPC is sufficiently proven in extracts stored at refrigerator conditions.

Table 4.2-37: Stability of phemnedipham in fortified plasma (m/z 318 \diamond 168) at $<+6^{\circ}\text{C}$

Matrix	Fortification level (FL) $\mu\text{g/L}$	Storage period	Recovery rates %			Mean value [%]	RSD [%]
Plasma	500	Initial analysis	115	87	103	102	13.8
		6 days	98	99	101	99	1.5

FL: Fortification Level. RSD: Relative Standard Deviation

Table 4.2-38: Stability of MHPC in fortified plasma (m/z 168 \diamond 136) at $<+6^{\circ}\text{C}$

Matrix	Fortification level (FL)* $\mu\text{g/L}$	Storage period	Recovery rates %			Mean value [%]	RSD [%]
Plasma	500	Initial analysis	106	101	107	105	3.1
		6 days	102	103	104	103	1.0

FL: Fortification Level. RSD: Relative Standard Deviation

* Fortification level expressed as MHPC

Storage stability in plasma

Control plasma samples were fortified with 500 $\mu\text{g/L}$ phemnedipham and 500 $\mu\text{g/L}$ MHPC and stored in a freezer at $\leq -18^{\circ}\text{C}$. A set of three samples was analysed with a freshly prepared calibration curve after 3 days of storage. The results show that under freezer conditions all analytes were stable for a storage period of at least 3 days (Table 4.2-39 and 4.2-40).

According to SANCO/825/00 rev. 8.1 (16/11/2010), the recoveries in the fortified samples stored for 3 days at $\leq -18^{\circ}\text{C}$ are within the acceptable range of 70-120%. Therefore, stability of phemnedipham and its metabolite MHPC is sufficiently proven at freezer conditions.

Table 4.2-39: Stability of phemnedipham in fortified plasma (m/z 318 \diamond 168) at $<-18^{\circ}\text{C}$

Matrix	Fortification level (FL) $\mu\text{g/L}$	Recovery rates %			Mean value [%]	RSD [%]
Plasma	500	106	103	96	102	5.0

FL: Fortification Level. RSD: Relative Standard Deviation

Table 4.2-40: Stability of MHPC in fortified plasma (m/z 168 \diamond 136) at $\leq -18^{\circ}\text{C}$

Matrix	Fortification level (FL) $\mu\text{g/L}$	Recovery rates %			Mean value [%]	RSD [%]
Plasma	500	104	106	97	102	4.6

FL: Fortification Level. RSD: Relative Standard Deviation

* Fortification level expressed as MHPC

Conclusion

The data presented demonstrate that using method 01486 permits the determination of residues of phemnedipham and MHPC in plasma with satisfactory accuracy, precision and repeatability. The method is therefore considered to be valid as monitoring/ enforcement method with parent compound and MHPC metabolite. The LOQ for the analytical targets phemnedipham and MHPC in plasma is 50 µg/L for each compound.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for Ethofumesate

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.2.1.1 Methods for risk assessments of physical and chemical properties tests

Please refer to point A 2.1.1.1.

A 2.2.1.2 Methods for risk assessments of toxicological studies

No new or additional studies have been submitted.

A 2.2.1.3 Methods for risk assessments of residues studies

No new or additional studies have been submitted.

A 2.2.1.4 Methods for risk assessments of environmental fate studies

No new or additional studies have been submitted.

A 2.2.1.5 Methods for risk assessments of ecotoxicology studies

Please refer to point A 2.1.1.5.

A 2.2.1.6 Methods for risk assessments of efficacy studies

No new or additional studies have been submitted.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted.