

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.01851.H.1.A

Product name(s): see Part A

Chemical active substances:

Ethofumesate, 500 g/L

Clopyralid, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Country organisation / representative of ADAMA,
as given in Part A

Submission date: July 2024

MS Finalisation date: March 2025 (initial Core Assessment)

June 2025 (final Core Assessment)

Version history

When	What
July 2024	dRR version 1 submitted by the Applicant.
March 2025	Initial zRMS assessment (with regard to the proposed composition change). 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.
June 2025	Final report (Core Assessment updated following the commenting period) 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. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

DATA PROTECTION CLAIM

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A

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5 Analytical methods

This document summarizes the analytical methods on the plant protection product ADM.01851.H.1.A, a suspension concentrate (SC) containing 500 g/L ethofumesate and 75 g/L clopyralid, for use in sugar beet and fodder beet in Central Zone according to Article 33 of the Regulation 1107/2009.

This application follows the data requirements for the active substance laid down in Regulation (EC) No. 283/2013 for the active substance ethofumesate and clopyralid, and Regulation (EC) No. 284/2013 for the plant protection product ADM.01851.H.1.A.

5.1 Conclusion and summary of assessment

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

Noticed data gaps are:

- None

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

Noticed data gaps are:

- A method for clopyralid in body fluids with an LOQ of 0.01 mg/L;
- Verification of the efficiency of the extraction procedures used in monitoring methods for clopyralid for animal products (EFSA, 2018);
- Analytical method for clopyralid used in the developmental toxicity study in rats (EFSA, 2018).

The above methods for clopyralid are considered a data gap and should be addressed at the active substance and product renewal.

Commodity/crop	Supported/ Not supported
Sugar beet	supported
Fodder beet	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 the active substances in the plant protection product ADM.01851.H.1.A (containing 500 g/L ethofumesate and 75 g/L clopyralid) is provided as follows.

Comments of zRMS:	Study acceptable. The analytical method for the determination of clopyralid and ethofumesate in plant protection product was fully validated according to SANCO/3030/99 rev. 5.
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Reference:	KCP 5.1.1/01
Report	ADM.01851.H.1.A: Validation of analytical method for active ingredients and content determination Massardi E. (2024) Report no. CPU-024-23, ADAMA Reference No. 000116317
Guideline(s):	Yes, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analysis of clopyralid and ethofumesate in ADM.01851.H.1.A (Clopyralid 75 + Ethofumesate 500 SC) was done by high performance liquid chromatography (HPLC) coupled with UV-DAD detection using external standard technique.

This analytical method was used for the content determination of clopyralid and ethofumesate during storage stability testing.

Study period:

Experimental start date: 26. July, 2023

Experimental completion date: 05. October, 2023

Test material:

ADM.01851.H.1.A: Batch no. U3301P1, Content: clopyralid 74.3 g/L and ethofumesate: 513.3 g/L

Matrix Blank, Batch no. BLU3301

Clopyralid (reference material): Batch no. 264-3598, Purity: 97.3%

Ethofumesate (reference material): Batch no. 290-3859, Purity: 99.2%.

Sample preparation:

Preparation of the test item solutions for Precision (Repeatability)

Five solutions of the test item (labelled AI_A to AI_E) were prepared by weighing about 500 mg of the test item into a 100.00 mL volumetric flask. The test item was dispersed by adding 10.00 mL of water and then made up to volume with acetonitrile. The solution was placed into an ultrasonic bath for 5 minutes and an aliquot was filtered on syringe filter PTFE at 0.20 µm. This solution was used for the determination of Clopyralid. For the determination of Ethofumesate, the filtered solution was diluted 1:5 in acetonitrile by transferring 4.00 mL into a 20.00 mL volumetric flask. The solutions were finally analysed by the HPLC-UV/DAD technique.

Preparation of the fortified sample solutions Recovery (Accuracy)

Three fortified solutions, at about 80%, 100 % and 120% of nominal active substance content in the test item, were prepared in duplicate by weighing an amount of blank formulation and each analytical standard, taking into account their purity, into a 50.00 mL volumetric flask, as reported in the following table. The substances were dispersed by adding 5.00 mL of water and then made up to volume with acetonitrile. The solution was placed into an ultrasonic bath for 5 minutes and an aliquot was filtered on syringe filter PTFE at 0.20 µm. This solution was used for the determination of Clopyralid. For the determination of Ethofumesate, the filtered solution was diluted 1:5 in acetonitrile by transferring 4.00 mL into a 20.00 mL volumetric flask. The solutions were finally analysed by the HPLC-UV/DAD technique.

HPLC-DAD Conditions:

HPLC	HPLC series 1260 Infinity II with Diode Array Detector / AGILENT		
Column	Kinetex 5µm XB-C18, 100 Å, 150 mm x 4.6 mm / Phenomenex		
Column temperature	30°C		
Mobile phase	A: water+ 0.1% v/v phosphoric acid B: acetonitrile		
Gradient	Time (min)	Eluent A (%)	Eluent B (%)
	0	80	20
	20	0	100
Injection Volume	5 µL		
Flow	1.0 mL/min		
Detector	280 nm		
Retention time:	Clopyralid: about 4.1 minutes Ethofumesate: about 12.1 minutes		

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances clopyralid and ethofumesate in plant protection product ADM.01851.H.1.A

	Clopyralid	Ethofumesate
Author(s), year	Massardi E. (2024)	
Principle of method	High performance liquid chromatograph (HPLC) with UV-DAD detection	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Six levels (injected in duplicate) in the range of 200.36 µg/mL to 451.51 µg/mL for Clopyralid analyte, corresponding to an active ingredient content ranging from 46.24 g/L to 104.21 g/L in the test item (about ±40% of nominal concentration of target analyte). Y = 5.4728x -30.0516, r = 0.9999	Six levels (injected in duplicate) in the range of 250.23 µg/mL to 601.15 µg/mL for Ethofumesate analyte, corresponding to an active ingredient content ranging from 288.77 g/L to 693.73 g/L in the test item (about ±40% of nominal concentration of target analyte). Y =2.5814x – 17.1782, r = 0.9998
Precision – Repeatability Mean n = 5 samples (%RSD)	For the precision (repeatability) test the comparison between the relative standard deviation (%RSD) and the modified Horwitz equation (%RSDr) gave an Horrat (Hr) value for each active substance in compliance with the acceptability criterion (Hr ≤ 1).	
	From the data and results obtained the precision assessed is: 75.6 g/L ± 0.2 g/L %RSD = 0.23 Modified Horwith equation: 2.02 Horrat (Hr): 0.11 Precision: ± 0.2	From the data and results obtained the precision assessed is 517.4 g/L ± 3.5 g/L RSD = 0.68 %Modified Horwith equation: 1.51 Horrat (Hr): 0.45 Precision: ± 3.5
Accuracy n = 2, two samples for each level (% Recovery)	Spiking of six aliquots of blank formulation with a known amount of each analytical standard at three fortification levels in duplicate, corresponding to about 80 %, 100 % and 120 % of nominal active substances concentration in the test item solution.	
	Recovery (%)	
	80 %	100 %
	101.1	101.9
	101.1	102.1
	101.7	101.5
	Mean recovery: 101.56	
	Mean Recovery (%)	
	80 %	100 %
	102.4	102.6
	101.9	101.4
	101.5	102.2
	Mean recovery: 101.99	

	Clopyralid	Ethofumesate
Interference/ Specificity	From the comparison of chromatograms, each peak of active substances is well separated from the other peaks, therefore following the chromatographic conditions of method, interferences can be avoided and each active substance content can be reliably determined in the test item. Interferences were <3% of the total peak area target analyte	

Conclusion

The analytical method for the active ingredients clopyralid and ethofumesate determination in ADM.01851.H.1.A was fully validated in regard of linearity, precision, accuracy and specificity. Validation acceptance criteria are based on guidance for generation and reporting methods of analysis in support of data requirements of SANCO/3030/99 rev. 5. The method is acceptable.

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

According to Commission Implementing Regulation (EC) 2016/1426 the following impurities are of toxicological concern and must not exceed the following levels in the technical material:

Ethofumesate:

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

Clopyralid:

There are no relevant impurities in clopyralid therefore no analytical methods are required.

Comments of zRMS:	Study acceptable. The analytical method for the determination of ethyl methanesulfonate (EMS) and isobutyl methanesulfonate (IBMS) in plant protection product was fully validated according to SANCO/3030/99 rev. 5.
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Reference:	KCP 5.1.1/02 filed under KCP 2.1/01
Report	ADM.01851.H.1.A: physical-chemical characterization, accelerated storage stability at +54 °C for 14 days and low temperature stability at 0 °C for 7 days Massardi E. (2024a) Report no. CPU-006-24, ADAMA Reference No. 000118842
Guideline(s):	Yes, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analysis of impurities content of ethyl methanesulfonate (EMS) and isobutyl methanesulfonate (IBMS) in ADM.01851.H.1.A (Clopyralid 75 + Ethofumesate 500 SC) was assessed by GC-MS detection using external standard technique.

This analytical method was used for the content determination of EMS and IBMS during storage stability testing.

Study period:

Experimental start date: 15. April, 2024

Experimental completion date: 14. June, 2024

Test material:

ADM.01851.H.1.A: Batch no. U3311B, Content: clopyralid 78.01 g/L and ethofumesate: 516.24 g/L

Matrix Blank, Batch no. BLU3301

Ethyl methanesulfonate (EMS) reference material: Batch no. 319-4148-01, Purity: 98.8%

Isobutyl methanesulfonate (IBMS) reference material: Batch no. 319-4147-01, Purity: 97.0%.

Sample preparation:

Preparation of reference materials solution for fortification

The Stock_MIX_A reference material solution prepared for the linearity test was diluted in methanol by transferring 1.25 mL into a 25.00 mL volumetric flask, obtaining FS_MIX solution

Preparation of the fortified sample solutions

Spike Low_I:

A fortified solution was prepared in quintuple by adding 0.080 mL of FS_MIX solution to about 1 g of test item, accurately weighed into vials for the GC-MS analysis.

Spike Medium_I:

A fortified solution was prepared in duplicate by adding 0.200 mL of FS_MIX solution to about 1 g of test item, accurately weighed into vials for the GC-MS analysis.

Spike High_I:

A fortified solution was prepared in duplicate by adding 0.400 mL of FS_MIX solution to about 1 g of test item, accurately weighed into vials for the GC-MS analysis.

Preparation of the test item solutions

Using the analytical balance, five replicates of the test item (labelled I_A to I_E) were prepared by weighing about 1 g of the test item into a vial and analysed by GC-MS.

The impurities content in the test item at the end of the accelerated storage stability was determined by analysing three independent solutions of the test item (labelled from I_A to I_C) prepared in the same way.

GC-MS Conditions:

GC-MS	GC series Trace 1310 with Single Quadrupole Mass Detector / THERMO
Column	ZB-624plus - 30 m x 0.25 mm x 1.4 µm / Phenomenex
Oven temperature	Initial temperature: 90 °C, hold for 15 minutes 1st ramp: 30°C/min till T = 280 °C, hold for 5 minutes
Injection	Temperature: 200°C Mode: Split 1:5 with flow 10.0 mL/min Injection Volume: 1 mL
Carrier Gas	Helium, flow 2.0 mL/min
MS	Ionization mode: EI Polarity: Positive Ion source temperature: 280 °C Transfer line temperature: 280 °C Scan time: 0.02 s
Analyte: EMS SIM 1st time segment (from 0 to 14 minutes)	Quantifier: 79 1st Qualifier: 97 2nd Qualifier: 109
Analyte: IBMS SIM 2nd time segment (from 14 minutes to end run)	Quantifier: 79
Retention time:	EMS: about 10.3 minutes IBMS: about 17.5 minutes

Validation - Results and discussions

Table 5.2-2: Recovery and repeatability results from method validation of EMS and IBMS in in plant protection product ADM.01851.H.1.A

Sample Code	Concentration of the analyte added (mg/L)	Total recovery (%)	Mean recovery per level (%)	Mean recovery (%)
	EMS			
Spike Low_I_A	0.04	71.0	70.6	79.57
Spike Low_I_B	0.04	70.5		

Spike Low_I_C	0.04	70.3		
Spike Low_I_D	0.04	70.4		
Spike Low_I_E	0.04	70.5		
Spike Med_I_A	0.10	85.4	85.5	
Spike Med_I_B	0.10	85.6		
Spike High_I_A	0.2	96.6	96.1	
Spike High_I_B	0.2	95.7		
IBMS				
Spike Low_I_A	0.04	80.7	79.5	82.25
Spike Low_I_B	0.04	82.8		
Spike Low_I_C	0.04	78.4		
Spike Low_I_D	0.04	78.4		
Spike Low_I_E	0.04	77.0		
Spike Med_I_A	0.10	80.1	80.1	
Spike Med_I_B	0.10	80.2		
Spike High_I_A	0.2	92.6	91.3	
Spike High_I_B	0.2	89.9		

Confidence interval (%): 70 – 13%

Table 5.2-3: Methods suitable for the determination of relevant impurities (EMS and IBMS) in plant protection product ADM.01851.H.1.A

	EMS	IBMS
Author(s), year	Massardi E. (2024a)	
Principle of method	Gas Chromatography with mass detection (GC-MS)	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Six levels (injected in duplicate) in the range of 0.02 µg/mL to 0.5 µg/mL for each impurity, corresponding to an impurity content ranging from 0.02 mg/L to 0.5 mg/L in the test item and from 0.04 mg/kg to 1.0 mg/kg in the active substance. The calibration plots were obtained using the least squares method and each correlation coefficient (r) was calculated using regression analysis. The acceptability criterion was $r \geq 0.99$ (or $R^2 \geq 0.98$).	
	y = 141951.7767x - 203.8296, r = 0.9954	y = 58090.3157 - 202.4494, r = 0.9958
Precision – Repeatability Mean n = 5 samples (%RSD)	For the precision (repeatability) test the comparison between the relative standard deviation (%RSD) and the modified Horwitz equation (%RSD _r) for the EMS and IBMS impurity gives an Horrat (Hr) value $Hr \leq 1$, therefore the precision was in compliance with the acceptability criterion.	
	Repeatability calculated on the five low fortification level replicates on the results expressed as (mg/L): Mean value: 0.0277 mg/L %RSD = 0.41 Modified Horwith equation: 18.39 Horrat (Hr): 0.02 Precision: ± 0.0001	Repeatability calculated on the five low fortification level replicates on the results expressed as (mg/L): Mean value: 0.032 mg/L %RSD = 2.87 Modified Horwith equation: 17.97 Horrat (Hr): 0.16 Precision: ± 0.001
Accuracy n = 2 resp. 5, samples for each level (% Recovery)	Spiking of in total nine aliquots of blank formulation with a known amount of each analytical standard at three fortification. The results obtained in the recovery test for the EMS and IBMS impurity complies with the acceptability criterion (Confidence interval 70-130%). For details see table above.	
LOQ / LOD	Data from linearity test were used to calculate the LOD, whereas data from recovery test were used to calculate the LOQ. The limit of detection (LOD), defined as half of the lowest calibration level, was 0.01 µg/mL, corresponding to an impurity content of 0.01 mg/L in the test item and of 0.02 mg/kg on active substance for each impurity. The limit of quantification (LOQ), defined as lowest fortification level, was 0.04 mg/L in the test item and 0.08 mg/kg on active substance, corresponding to a final injected solution of 0.04 µg/mL	
Interference/ Specificity	The comparison of chromatograms shows that the peak of each impurity is well separated from interferences. Therefore, following the adjusted chromatographic conditions of method interferences can be avoided and each impurity content can be reliably determined in the test item. Representative chromatograms are shown in the report. Confirmatory - For the ethyl methanesulfonate impurity, the identity of impurity chromatographic peak is confirmed since the method used is highly specific (mass spectrometry with three ions followed). The	

	EMS	IBMS
	followed ions are 79 m/z (quantifier), 97 m/z and 109 m/z (qualifiers). For the isobutyl methanesulfonate impurity, the identity of impurity chromatographic peak was investigated and confirmed by comparing the MS spectra from GC-MS analysis as reported in the following paragraph. The mass spectra are given in the report.	

Conclusion

The analytical method for the impurity determination ethyl methanesulfonate (EMS) and isobutyl methanesulfonate (IBMS) determination in ADM.01851.H.1.A was fully validated in regard of linearity, precision, accuracy, LOQ and specificity. Validation acceptance criteria are based on guidance for generation and reporting methods of analysis in support of data requirements of SANCO/3030/99 rev. 5. The method is acceptable.

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

None of the formulants or their constituents of the plant protection product ADM.01851.H.1.A are considered by the applicant to represent compounds of particular toxicological, ecotoxicological or environmental concern. The submission of analytical methods for such is therefore not considered to be required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

For the active substance Ethofumesate Technical the CIPAC method no. 233/TC/M/- in Handbook J page 44 is available. An ethofumesate suspension concentrate can be analysed using CIPAC method no. 233/SC/M/- and an ethofumesate emulsion concentrate using CIPAC method no. 233/EC/M/- as described in CIPAC Handbook J page 48 - 50.

For the active substance Clopyralid, there is currently no CIPAC method available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Ethofumesate

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

Table 5.2-4: Validated methods for the generation of pre-authorization data for ethofumesate in plant and animal products

Plant residue definition for risk assessment Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate, expressed as ethofumesate) (EFSA, 2016) Animal residue definition for risk assessment Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate, expressed as ethofumesate) (EFSA, 2016)				
Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Sugar beet tops Sugar beet roots (Residues)	Primary method Method RESID/73/18/1 and RESID/73/18/2 (with some modifications)		GC-FPD	Whiteoak, R. J.; Crofts, M.; Harris, R. J.; 1973 Report: M-155727-01 and
	Ethofumesate	0.05 mg/kg 0.02 mg/kg		Whiteoak, R. J.; Crofts, M.; Harris, R. J. 1976 Report: M-155728-01
	NC 8493	0.05 mg/kg 0.05 mg/kg		
	NC 9607	0.05 mg/kg 0.02 mg/kg		<i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	NC 8493	0.05 mg/kg 0.10 mg/kg		
	NC 20645 (addition of NC 9607)	0.05 mg/kg 0.05 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-FPD)	
Sugar beet (immature plant) Sugar beet (body) Sugar beet (leaves) (Residues)	Primary method Method RESID/84/42		GC-FPD	Manley, J. D., Snowdon, P.J., 1984 Report M-155729-01-1
	Ethofumesate NC 8493 (free form) NC 8493 (conjugated form) NC 9607 (free form) NC 20645 (conjugated form)	No LOQ For all analytes		<i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-FPD)	
Peas and sugar beet roots (Residues)	Primary method Method AL 081/96-0		GC-MS	Wrede, A., 2000 Document no: M-199547-01
	Ethofumesate	No LOQ		<i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	

Plant residue definition for risk assessment Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate, expressed as ethofumesate) (EFSA, 2016) Animal residue definition for risk assessment Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate, expressed as ethofumesate) (EFSA, 2016)				
Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Sugar beets (Residues)	Primary method Method A0019 Ethofumesate, 2-keto ethofumesate	0.05 mg/kg	GC-MS	Perny, A.,2002 Document No. A0019 <i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	
Sugar beet, leaf and body (Residues)	Primary method Method 00955/M002* Ethofumesate, 2-keto ethofumesate	0.01 mg/kg	GC-MS	Konrad S., 2012 Document no: M-438402-01-1 Schulte, G.; 2013; Document no: M-459805-01 <i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	
Sugar beet leaf, sugar beet root (Residues)	Primary method Method 01343 open-ring-2-ketoethofumesate (AE C520645, NC 20645)	0.01 mg/kg	LC-MS/MS	Schulte, G.; 2013 Document no: M-459806-01 <i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Wheat, sunflower seed (Residues)	Primary method Method PR00/001 Ethofumesate, NC 9607 (free form), NC 20645 (free and conjugated form)	0.02 mg/kg	GC-MS	Thom, M.; 2005 Document no: M-351876-01-1 <i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	
Lettuce, Cereal grain (barley) (Residues)	Primary method RES-00278 Ethofumesate and ethofumesate-2-keto	0.01 mg/kg	GC-MS	KCP 5.1.2/01 filed under KCP 8/02 (KCA 6.1/03) Watson G., 2021 Report No: RES-00278 Study ID: 000106576 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	
KCA 4.1.2 /25; Ross, D. B.; Roberts, N. L.;1977; M-155301-01 KCA 4.1.2 /26; Whiteoak, R. J.;1990; Document no: M-155384-01 KCA 4.1.2 /27; Harris, R. J.;1975; Document no: M-155288-01 KCA 4.1.2 /28; Peatman, M. H.; 1999; Document no: M-185949-01				<i>EU agreed</i> <i>First Annex I inclusion, Monograph and base line dossier (D-008920)</i>

Plant residue definition for risk assessment Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate, expressed as ethofumesate) (EFSA, 2016) Animal residue definition for risk assessment Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate, expressed as ethofumesate) (EFSA, 2016)				
Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Whole milk (Residues)	Primary method Method XB/01/01		GC-FPD	Castro, L. E.; 1994; Document no: M-237976-01 Cole, M. G.; 2000 Document no: M187353-01 <i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Ethofumesate, NC 9607	0.01 mg/kg		
	NC 20645	0.05 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-FPD)	
Cattle matrices (Cream, Whey, Milk, Muscle, Fat, Liver, Kidney) (Residues)	Primary method Method AD-001-A10-02		LC-MS/MS	Author(s), 2010 Document no: M-388797-01-1 Perez, R.; Schmitt, J. L.; Patel, D., 2014 Document no: M-467206-01 <i>EU agreed</i> <i>RAR Ethofumesate, 2015</i>
	Ethofumesate, NC 9607–measured as NC 20645 – and NC 20645 itself and NC 8493	0.01 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Soil, water, sediment,... (Environmental fate)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water,... (Efficacy)	No additional data.			
	Confirmatory (if required)	--	--	--
Feed, body fluids (Toxicology)	No additional data.			
	Confirmatory (if required)	--	--	--
Body fluids, air,.... (Exposure)	Primary			
	Confirmatory (if required)			

* Residue analytical method 00955/M002 was developed as a data collection method for the determination of the residues of ethofumesate (parent compound) and the common moiety NC 9607 (which comprises the metabolites NC 9607 and NC 20645 and conjugates of NC 20645) in/on plant matrices.

Table 5.2-5: Validated methods for the generation of pre-authorization data for ethofumesate in soil, water and air

Component of residue definition: Soil: Ethofumesate and NC 8493 Ground water, surface water and sediment: Ethofumesate, 2-hydroxy-ethofumesate (NC 8493) and openring-2-keto-ethofumesate (NC 20645) Air: Ethofumesate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Reconstituted water according to ISO 6341). <i>(Fish - Oncorhynchus mykiss</i> Acute toxicity) OECD 203 (Ecotoxicology)	Primary method	0.5525 mg/L Ethofumesate	LC-MS/MS	KCP 5.1.2/02 filed under KCP 10.2/01 Renner P. 2024 Study No: 23 48 AFA 0006 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
ISO Standard water 6341 (<i>Daphnia magna</i> – Acute toxicity) OECD 202 (Ecotoxicology)	Primary method	1.981 mg/L Ethofumesate	LC-MS/MS	KCP 5.1.2/03 filed under KCP 10.2/02 Renner P. 2024a Study No: 23 48 ADL 0020 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
OECD medium (<i>Raphidocelis subcapitata</i> - algal growth inhibition test) OECD 201 (Ecotoxicology)	Primary method	0.6104 mg/L Ethofumesate	LC-MS/MS	KCP 5.1.2/04 filed under KCP 10.2/03 Renner P. 2024b Study No: 23 48 AAL 0014 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Smart and Barko medium / sediment (<i>Myriophyllum spicatum</i> L.) OECD 239 (Ecotoxicology)	Primary method	4.841 µg/L Ethofumesate	LC-MS/MS	KCP 5.1.2/05 filed under KCP 10.2/04 Warning D., 2022 Study No: 22 48 AMS 0001 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
50% aqueous sucrose solution, dose verification - Chronic Honey Bee study (<i>Apis mellifera</i> L.) OECD 245 (Ecotoxicology)	Primary method	110 mg/kg Ethofumesate	LC-MS/MS	KCP 5.1.2/06 filed under KCP 10.3.1.2/01 Ruhland S. 2023 Study No: 22 48 BAC 0024 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Larval diet C, dose verification – Honey Bee (<i>Apis mellifera</i> L.) OECD 239 (Ecotoxicology)	Primary method	4.94 mg/kg Ethofumesate	LC-MS/MS	KCP 5.1.2/07 filed under KCP 10.3.1.3/01 Kleebaum K., 2022 Study No: 22 48 BLC 0025 See Appendix 2
	Confirmatory	--	Not required, highly specific detection system was used (LC-	

Component of residue definition: Soil: Ethofumesate and NC 8493 Ground water, surface water and sediment: Ethofumesate, 2-hydroxy-ethofumesate (NC 8493) and openring-2-keto-ethofumesate (NC 20645) Air: Ethofumesate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	(if required)		MS/MS)	
Seedling Emergence and Seedling Growth – aqueous solution (Ecotoxicology)	Primary method	2424 mg/L	HPLC-DAD	KCP 5.1.2/08 filed under KCP 10.6.2/01 Friedemann, 2024 Study No: 23 46 PSE 0012 See Appendix 2
	Confirmatory (if required)	--	Not required, specific detection system was used (LC-UV)	
Vegetative Vigour – aqueous solution (Ecotoxicology)	Primary method	2424 mg/L	HPLC-DAD	KCP 5.1.2/09 filed under KCP 10.6.2/02 Friedemann, 2024a Study No: 23 46 PVV 0011 See Appendix 2
	Confirmatory (if required)	--	Not required, specific detection system was used (LC-UV)	
Water, buffer solutions,... (Properties)	No additional data.			
	Confirmatory (if required)	--	--	--

Clopyralid

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

Table 5.2-6: Validated methods for the generation of pre-authorization data for clopyralid in plant and animal products

Plant residue definition for risk assessment Clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) – pending the outstanding clarification on the nature of “polar clopyralid” (EFSA, 2018) Animal residue definition for risk assessment Clopyralid common moiety (sum of clopyralid, its salts and glycine conjugates expressed as clopyralid) (EFSA 2018)				
Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Barley, Wheat: Forage, grain, straw Grass: forage, hay, seed Oilseed rape: grain, straw (Residues)	Primary method Method GRM 01.16	0.01 mg/kg	GC/MSD	Hastings, 2002a GRM 01.16 <i>EU agreed</i> <i>DAR Clopyralid, 2005</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	
Wheat: grain, straw, sugar beet (Residues)	ILV of GRM 01.16	0.01 mg/kg	GC/MSD	Rawle 2002 GHE-P-9567 <i>EU agreed</i> <i>DAR Clopyralid, 2005</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-MS)	
Grass (Residues)	Primary method Method ERC 94.8	0.05 mg/kg	GC-ECD	Wood, 1994 ERC 94.8 <i>EU agreed</i> <i>DAR Clopyralid, 2005</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-ECD)	
Sugar Beets, Molasses, pulp, sugar (Residues)	Primary method Method GRM 94.04	0.05 mg/kg	GC-MSD	Kubitschek & Kyle, 1994 ARC 79.5 <i>EU agreed</i> <i>DAR Clopyralid, 2005</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-ECD)	
Sugar Beets, refined sugar (Residues)	Primary method Method ERC 88.2	0.005 mg/kg	GC-ECD	Anon, 1989 ERC 88.2 <i>EU agreed</i> <i>DAR Clopyralid, 2005</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-ECD)	
Sugar Beets, Molasses sugar (Residues)	Primary method Method ERC 88.1	0.05 mg/kg	GC-ECD	Anon, 1988 ERC 88.1 <i>EU agreed</i> <i>DAR Clopyralid, 2005</i>

Plant residue definition for risk assessment Clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) – pending the outstanding clarification on the nature of “polar clopyralid” (EFSA, 2018) Animal residue definition for risk assessment Clopyralid common moiety (sum of clopyralid, its salts and glycine conjugates expressed as clopyralid) (EFSA 2018)				
Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	--	Not required, highly specific detection system was used (GC-ECD)	
Strawberries (Residues)	n.a.	n.a.	n.a	KCP 5.1.2/10 filed under KCA 6.1/02 Teassdale, R.,1996 LOA available, no study summary included
Dried Beans (Residues)	n.a	n.a	n.a	KCP 5.1.2/11 filed under KCA 6.1/01 Skaggs, C.S., Penning, B.N. 2021 LOA available, no study summary included
Bovine and Poultry Tissues (Residues)	Primary method Method 120483	0.01 mg/kg	LC-MS/MS	Anon, Shaffer, S., 2012 120483 <i>EU agreed</i> <i>RAR Clopyralid, 2017</i>
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Soil, water, sediment,... (Environmental fate)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water,... (Efficacy)	No additional data.			
	Confirmatory (if required)	--	--	--
Feed, body fluids (Toxicology)	No additional data.			
	Confirmatory (if required)	--	--	--
Body fluids, air,... (Exposure)	No additional data.			
	Confirmatory (if required)	--	--	--

n.a. study not shared, LOA from owner available.

Table 5.2-7: Validated methods for the generation of pre-authorization data for clopyralid in soil, water and air

Component of residue definition: Clopyralid Soil: Clopyralid Ground water, surface water and sediment: Clopyralid Air: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Reconstituted water according to ISO 6341). (<i>Fish - Oncorhynchus mykiss</i> Acute toxicity) OECD 203 (Ecotoxicology)	Primary method	LOQ Clopyralid = 0.08073 mg/L	LC-MS/MS	KCP 5.1.2/02 filed under KCP 10.2/01 Renner P. 2024 Study No: 23 48 AFA 0006 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
ISO Standard water 6341 (<i>Daphnia magna</i> – Acute toxicity) OECD 202 (Ecotoxicology)	Primary method	LOQ Clopyralid = 0.2894 mg/L	LC-MS/MS	KCP 5.1.2/03 filed under KCP 10.2/02 Renner P. 2024a Study No: 23 48 ADL 0020 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
OECD medium (<i>Raphidocelis subcapitata</i> - algal growth inhibition test) OECD 201 (Ecotoxicology)	Primary method	LOQ Clopyralid = 0.2894 mg/L	LC-MS/MS	KCP 5.1.2/04 filed under KCP 10.2/03 Renner P. 2024b Study No: 23 48 AAL 0014 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Smart and Barko medium / sediment (<i>Myriophyllum spicatum</i> L.) OECD 239 (Ecotoxicology)	Primary method	0.6887 µg/L Clopyralid	LC-MS/MS	KCP 5.1.2/05 filed under KCP 10.2/04 Warning D., 2022 Study No: 22 48 AMS 0001 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
50% aqueous sucrose solution, dose verification - Chronic Honey Bee study (<i>Apis mellifera</i> L.) OECD 245 (Ecotoxicology)	Primary method	15.7 mg/kg Clopyralid	LC-MS/MS	KCP 5.1.2/06 filed under KCP 10.3.1.2/01 Ruhland S. 2023 Study No: 22 48 BAC 0024 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Larval diet C, dose verification – Honey Bee (<i>Apis mellifera</i> L.) OECD 239 (Ecotoxicology)	Primary method	0.702 mg/kg Clopyralid	LC-MS/MS	KCP 5.1.2/07 filed under KCP 10.3.1.3/01 Kleebaum K., 2022 Study No: 22 48 BLC 0025 See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

Component of residue definition: Clopyralid Soil: Clopyralid Ground water, surface water and sediment: Clopyralid Air: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Seedling Emergence and Seedling Growth – aqueous solution (Ecotoxicology)	Primary method	354.2 mg/L	HPLC-DAD	KCP 5.1.2/08 filed under KCP 10.6.2/01 Friedemann, 2024 Study No: 23 46 PSE 0012 See Appendix 2
	Confirmatory (if required)	--	Not required, specific detection system was used (LC-UV)	
Vegetative Vigour – aqueous solution (Ecotoxicology)	Primary method	354.2 mg/L	HPLC-DAD	KCP 5.1.2/09 filed under KCP 10.6.2/02 Friedemann, 2024a Study No: 23 46 PVV 0011 See Appendix 2
	Confirmatory (if required)	--	Not required, specific detection system was used (LC-UV)	
Water, buffer solutions,... (Properties)	No additional data.			
	Confirmatory (if required)	--	--	--

zRMS comments:

The applicant provided several analytical methods for risk assessment. The validity criteria for the analytical methods according to SANTE/2020/12830 have been met. All new methods are acceptable.
More details can be found in Appendix 2.

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)

Analytical methods for the determination of the active substances and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of active substance 1: Ethofumesate (KCP 5.2)

For this application, it is referred to the following EU concluded residue definitions:

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 5.3-1: 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) (EFSA, 2016)	0.03* mg/kg	Commission Regulation (EU) 2017/1016 of 14 June 2017 and EFSA Journal 2016;14(1):4374
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)			
Plant, high oil content			
Plant, difficult matrices (tea, coffee beans, hops, spices)	Ethofumesate (Sum of ethofumesate, 2-keto-ethofumesate, open-ring-2-keto-ethofumesate and its conjugate, expressed as ethofumesate) (Reg. (EU) 2017/1016)	0.1* mg/kg	
Muscle	Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) (their sum expressed as ethofumesate) (EFSA, 2016) and Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as ethofumesate) (Reg. (EU) 2017/1016)	0.03* mg/kg	According to the EFSA Journal 2016;14(1):4374
Milk			
Eggs			
Fat			
Liver, kidney			
Honey		0.05* mg/kg	According to Commission Regulation (EU) 2017/1016
Soil (Ecotoxicology)	Ethofumesate	0.05 mg/kg	Common limit according to SANTE/2020/12830-rev.2 and EFSA Journal 2016;14(1):4374
Drinking water (Human toxicology)	Ethofumesate	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Ethofumesate	Chronic (flow-through), FFLC Growth, NOEC 0.156 mg a.s./L (mm)	EFSA Journal 2016;14(1):4374

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Air	Ethofumesate	0.5 µg/m ³	EFSA Journal 2016;14(1):4374 AOEL of 2.5 mg/kg body weight per day (dog 90 days)
Tissue (meat or liver)	Ethofumesate	0.01 mg/kg	EFSA Journal 2016;14(1):4374
Body fluids		0.01 mg/L	EFSA Journal 2016;14(1):4374 Not classified as T / T+ The common limits for body fluids according to SANTE/2020/12830-rev.2 .

The value of MRLs (Regulation 2017/1016): 0.2 mg/kg (sugar beet roots, ~~beet roots~~)

* Indicates lower limit of analytical determination

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 for analysis of ethofumesate in plant matrices is given in the following tables. No new or additional studies were submitted.

Table 5.3-2: Validated methods for ethofumesate in food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as ethofumesate)				
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 High acid content High oil content High protein/high starch content (dry)	Primary (Method 01392)	0.01 mg/kg Ethofumesate NC 9607* NC 20645*	LC-MS/MS	Schulte, G., Diehl, P., 2014 MR-13/101 <i>EU agreed (EFSA Journal 2016;14(1):4374)</i>
	ILV	0.01 mg/kg Ethofumesate NC 9607* NC 20645*	LC-MS/MS	Betson, S., 2014, Report No. M-475932-01-1 <i>EU agreed (EFSA Journal 2016;14(1):4374)</i>
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	

* determined as NC 20645 and calculated as ethofumesate

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 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	No residues (according to the residue definition; refer to chapter B.7) above the LOQ are present in food commodities (representative use: sugar beets). Therefore, there is no need to address extraction efficiency. Reference to guidance document SANTE/2017/10632 (see RAR of Ethofumesate Volume 3 – B.5 (AS)) Common moiety method was used by Schulte, G., Diehl, P.; 2014, MR-13/101. Expected Residues: > LOQ Sugar beet tops < LOQ for Sugar beet roots Furthermore, 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).

zRMS comments:

Proposed uses for ADM.01851.H.1.A: sugar beet, fodder beet.

Residues definition for plants:

According to the EFSA Journal 2016;14(1):4374 and Regulation 2017/1016: Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as ethofumesate)

The value of MRL (Regulation 2017/1016):

- 0.2 mg/kg for sugar beet roots

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.

5.3.2.3 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 ethofumesate in animal matrices is given in the following tables. No new or additional studies were submitted.

Table 5.3-4: Validated methods for food and feed of animal origin

Component of residue definition: Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) (their sum expressed as ethofumesate)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk, Eggs, Muscle, Fat, Kidney, Liver	Primary	0.01 mg/kg	LC-MS/MS	Jooß S., 2012, P 2371 G EU agreed (EFSA Journal 2016;14(1):4374)
	ILV	0.01 mg/kg	LC-MS/MS	Schlewitz, P. 2013b, R B1218 EU agreed (EFSA Journal 2016;14(1):4374)
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	No residues (according to the residue definition; refer to chapter B.7) above the LOQ are expected in food of animal commodities regarding the representative use: sugar beets. Therefore, there is no need to address extraction efficiency. Reference to guidance document SANTE/2017/10632. (see RAR of Ethofumesate Volume 3 – B.5 (AS))

zRMS comments:

Residues definition for animal origin:

According to the EFSA Journal 2016;14(1):4374: Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) (their sum expressed as ethofumesate)

According to the Regulation 2017/1016: Ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate (their sum expressed as ethofumesate)

The value of MRLs (Regulation 2017/1016):

- 0.03 mg/kg* for foodstuff of animal origin
- - 0.05* mg/kg for honey

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 ADM.01851.H.1.A.

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

An overview on the acceptable methods for analysis of ethofumesate in soil is given in the following tables. No new or additional studies were submitted.

Table 5.3-6: Validated methods for soil

Component of residue definition: Ethofumesate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary Method 00806	0.05 mg/kg	LC-MS/MS one transitions	Brumhard, B; 2003, 00806 EU agreed (EFSA Journal 2016;14(1):4374)
Confirmatory	0.05 mg/kg	GC-MS	Schneider, E.; 2000, OFC00004917 EU agreed (EFSA Journal 2016;14(1):4374)

zRMS comments:

Sufficient analytical method is available for the determination of ethofumesate in soil (RAR, 2015). The LC-MS/MS method 00806 has been sufficiently validated in soil (LOQ = 50 µg/kg or 0.05 mg/kg). Since only one transition has been reported for method 00806, an additional GC-MS method (PR00/003) has been provided for first Annex I inclusion and submitted for confirmation.
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 for analysis of ethofumesate in surface and drinking water is given in the following tables. No new or additional studies were submitted.

Table 5.3-7: Validated methods for water

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	-	-	Not required, covered with surface water validation data*
	ILV	-	-	
	Confirmatory	-	-	
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Krebber, R.; Braune, M., 2013, MR-13/085

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
				EU agreed (EFSA Journal 2016;14(1):4374)
	ILV	0.05 µg/L	HPLC-MS/MS	Class, T., Stanislawski T., 2013, P3117 G EU agreed (EFSA Journal 2016;14(1):4374)
	Confirmatory (if required)	-	Not required, highly specific detection system was used (HPLC-MS/MS).	

* As noted within the guideline-SANTE/2020/12830 “Provided that a method has been successfully validated for surface water at the LOQ required for drinking water (≤ 0.1 µg/L), no separate validation in drinking water is required” – this applies here, therefore the method can be applied for monitoring purposes for surface water and ground (drinking) water, for the quantification of Ethofumesate.

zRMS comments:

Sufficient analytical method (Krebber, R.; Braune, M., 2013, MR-13/085) and its ILV (Class, T., Stanislawski 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 tables. No new or additional studies were submitted.

Table 5.3-8: Validated methods for air

Component of residue definition: Ethofumesate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.5 µg/m ³	GC-MS	Schneider, E.; 2000; OFC00004919 EU agreed (EFSA Journal 2016;14(1):4374)
Confirmatory (if required)	-	Not required, highly specific detection system was used (GC-MS)	

zRMS comments:

Sufficient analytical method (Schneider, E.; 2000; OFC00004919) is 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 0.5 µg/m³. As the method is 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. No new or additional studies were submitted.

Table 5.3-9: Methods for body fluids and tissues

Component of residue definition: Ethofumesate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary (dog plasma)	0.1 mg/L	HPLC-UV	McKenzie 1998, A87557 EU agreed (EFSA Journal 2016;14(1):4374)
Confirmatory (if required)	-	Not required, specific detection system was used (HPLC-UV).	

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 of 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.

5.3.3 Description of analytical methods for the determination of residues of active substance 2: Clopyralid (KCP 5.2)

For this application, it is referred to the following EU concluded residue definitions:

5.3.3.1 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 5.3-10: 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	Clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) – pending the outstanding clarification on the nature of “polar clopyralid” (EFSA, 2018)	~~0.03~~ 0.05 mg/kg	Commission Regulation (EU) ~~2021/1807~~ 2024/2612
Plant, high acid content		0.5 mg/kg	
Plant, high protein/high starch content (dry commodities)		~~≥~~ 0.05 mg/kg	
Plant, high oil content		0.5 mg/kg	
Plant, difficult matrices (tea, coffee beans, hops, spices)		0.5 mg/kg	
	Clopyralid (Regulation (EU) 2024/2612)		
Muscle	Clopyralid and its salts OR for milk: clopyralid common moiety (sum of clopyralid, its salts and glycine conjugates expressed as clopyralid) (EFSA, 2018)	0.05* mg/kg	Commission Regulation (EU) ~~2021/1807~~ 2024/2612
Milk			
Eggs			
Fat			
Liver, kidney			
Honey			
	Clopyralid (Regulation (EU) 2024/2612)	0.15 mg/kg	
Soil (Ecotoxicology)	Clopyralid	~~0.05~~ 0.5 µg/kg	Acc to EFSA Journal 2018;16(7):5389

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Drinking water (Human toxicology)	Clopyralid	0.05 µg/L	Acc to EFSA Journal 2018;16(7):5389
Surface water (Ecotoxicology)	Clopyralid	0.05 µg/L	Acc to EFSA Journal 2018;16(7):5389
Air	Clopyralid	4.5 µg/m³	Acc to EFSA Journal 2018;16(7):5389
Tissue (meat or liver)	Clopyralid	0.01 mg/kg 0.01 mg/L	The common limits for body tissues and body fluids according to SANTE/2020/12830-rev.2
Body fluids (blood and urine)			

* Indicates lower limit of analytical determination

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

An overview on the acceptable methods for analysis of clopyralid in plant matrices is given in the following tables. No new or additional studies were submitted.

Table 5.3-11: Validated methods for clopyralid in food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) – pending the outstanding clarification on the nature of “polar clopyralid” (EFSA, 2018)				
Clopyralid (Regulation (EU) n° ~~2021/1807~~ 2024/2612)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Wet Crops –Wheat Forage Dry Crops – Wheat Grain Acidic Crops – Orange Oily Crops – Canola Seed	Primary (Method 120610)	0.01 mg/kg	LC/MS/MS	CA 4.2 (a)/1, Vogl, E. (2012), 120610 <i>EU agreed (RAR 2017)</i>
Wet Crop – Wheat Whole Plant Oily Crop – Oilseed Rape Seed	ILV	0.01 mg/kg	LC-MS/MS	Austin R., 2012 120614 <i>EU agreed (RAR 2017)</i>
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	
Dry crop – Rye Grain Wet crop - Lettuce Oily crop - Oilseed Rape Seed Acidic crop - Lemon	Primary (Method 130729)	0.01 mg/kg	LC-MS/MS (QuEChERS)	Lindner, M., Giesau, A., 2013 130729 <i>EU agreed (RAR 2017)</i>
Wet Crop - Lettuce Acidic Crop - Lemon	ILV	0.01 mg/kg	LC-MS/MS (QuEChERS)	Austin, R., Turner, R., 2014 130728 <i>EU agreed (RAR 2017)</i>
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	For clopyralid, no new extraction efficiency studies were conducted in conjunction with the validation of the new residue analytical method for the determination of residues of clopyralid. The same alkaline extraction solution and extraction procedure used in previous methods and nature of residue studies for the determination of residues of clopyralid in agricultural commodities were used in the method (Vogl, E. (2012)). Agreed in RAR of clopyralid (2017).

zRMS comments:

Proposed uses for ADM.01851.H.1.A: sugar beet, fodder beet.

Residues definition for plant origin:

- Clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) – pending the outstanding clarification on the nature of “polar clopyralid” (EFSA, 2018);
- Clopyralid (Regulation (EU) 2024/2612)

The value of MRL (Regulation 2024/2612):

- 1 mg/kg for sugar beet roots

An analytical method for enforcement analysing for clopyralid, its salts and conjugates was assessed during the EU pesticides peer review (EFSA, 2018). The method (based on LC–MS/MS) is sufficiently validated for the determination of residues of clopyralid, its salts and conjugates in the four main plant matrices. The enforcement method quantifies residues at or above the LOQ of 0.01 mg/kg for the total residue (sum of clopyralid, its salts and conjugates) in the four main plant matrices.

According to the EFSA Journal 2018;16(8):5389 – “Peer review of the pesticide risk assessment of the active substance clopyralid”: *Clopyralid, its salts and conjugates can be monitored in food and feed of plant origin by liquid chromatography with tandem mass spectrometry (LC–MS/MS) with a limit of quantification (LOQ) of 0.01 mg/kg in each commodity group.*

(...) In addition, there is a validated multiresidue quick, easy, cheap, effective and safe (QuEChERS) method using LC–MS/MS which can be used for monitoring of the clopyralid residues in high water and high acid content plant commodities and fat and milk with a LOQ of 0.01 mg/kg.

According to the EFSA Journal 2021;19(1):6389:

The method (based on LC-MS/MS) is sufficiently validated for the determination of residues of clopyralid, its salts and conjugates in the crops under consideration. It allows quantifying residues at or above the LOQ of 0.01 mg/kg for the total residue (sum of clopyralid, its salts and conjugates) in crops with dry/high starch, high water, high acid and high oil content.

Note: This method involves a hydrolysis step that releases free clopyralid from its conjugates.

EFSA stated (DOI: 10.2903/j.efsa.2024.8546) that *The assessment of extraction efficiency in plant matrices could be further investigated for the plant matrices of concern under the future MRL review. It is recommended to verify whether the sample preparation conditions are expected to release free clopyralid from its various conjugated forms and to confirm the extraction efficiency according to the requirements of the extraction efficiency Guidance, SANTE 2017/10632 GD (European Commission, 2023).*

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 clopyralid in animal matrices is given in the following tables. No new or additional studies were submitted.

Table 5.3-13: Validated methods for food and feed of animal origin

Component of residue definition: Clopyralid and its salts OR for milk: clopyralid common moiety (sum of clopyralid, its salts and glycine conjugates expressed as clopyralid) (EFSA, 2018) Clopyralid (Regulation (EU) n° ~~2021/1807~~ 2024/2612)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Bovine: muscle, fat, kidney, liver and milk Poultry: eggs, fat, muscle and liver	Primary 120483	0.01 mg/kg	LC/MS/MS	Shaffer, S., 2012, 120483 EU agreed (RAR 2017)
Bovine: milk and muscle Poultry: liver and egg	ILV	0.01 mg/kg	LC-MS/MS	Gemrot, F., 2012, 120484 EU agreed (RAR 2017)
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	
Bovine: milk, meat, fat, liver, Poultry: eggs	Primary (Method 130729)	0.01 mg/kg	LC-MS/MS (QuEChERS)	Lindner, M., Giesau, A., 2013 Report No. 130729 EU agreed (RAR 2017)
Bovine: milk, fat	ILV	0.01 mg/kg	LC-MS/MS (QuEChERS)	Austin, R., Turner, R., 2014 Report No. 130728 EU agreed (RAR 2017)
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	No new extraction efficiency studies were conducted in conjunction with the validation of these residue analytical methods. The extraction efficiency for clopyralid using heated alkaline extraction was previously determined and deemed acceptable using in-grown poultry and bovine tissues (Poultry Tissue DAS Report ID: GH-C 752, 1974 and Bovine Tissue DAS Report ID: GH-C 811, 1975). Agreed in RAR of clopyralid (2017).

zRMS comments:

Residues definition for food and feed of animal origin:

- Clopyralid and its salts, or
- for milk: Clopyralid common moiety (sum of clopyralid, its salts and glycine conjugates expressed as clopyralid) (EFSA, 2018);
- Clopyralid (Regulation (EU) 2024/2612)

An analytical method for enforcement analysing for clopyralid and its salts in food of animal origin was assessed during the EU pesticides peer review (EFSA, 2018).

According to the EFSA Journal 2018;16(8):5389 – “Peer review of the pesticide risk assessment of the active substance clopyralid”: *Residues of clopyralid and its salts in food of animal origin can be determined by LC–MS/MS with a LOQ of 0.01 mg/kg in all animal matrices. However, it should be noted that the efficiency of the extraction procedures used in this method was not verified. In addition, there is a validated multiresidue quick, easy, cheap, effective and safe (QuEChERS) method using LC–MS/MS which can be used for monitoring of the clopyralid residues in high water and high acid content plant commodities and fat and milk with a LOQ of 0.01 mg/kg. It should be noted that also for this method efficiency of the extraction procedures used for animal products was not verified, therefore a data gap has been identified.*

Concluded, the method based on QuEChERS for milk and fat, both with LOQ of 0.01 mg/kg was not fully validated since the extraction efficiency was not demonstrated and a data gap was identified in the peer review.

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

An overview on the acceptable methods for analysis of clopyralid in soil is given in the following table. No new or additional studies were submitted.

Table 5.3-15: Validated methods for soil

Component of residue definition: Clopyralid				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Loamy Sand Sandy Clay Loam Loam Silt Loam	Primary Method 120612	0.50 µg/kg	LC-MS/MS	Vincent T. P., 2013 120612 <i>EU agreed</i> <i>RAR Clopyralid, 2017</i>
Sandy Loam	ILV	0.50 µg/kg	LC-MS/MS	Austin, R., Turner, R. (2014) <i>EU agreed</i> <i>RAR Clopyralid, 2017</i>
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS).	

zRMS comments:

Clopyralid residues in environmental matrices can be monitored by LC–MS/MS with LOQ of 0.5 µg/kg in soil. 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 for analysis of clopyralid in drinking, ground and surface water is given in the following table. No new or additional studies were submitted.

Table 5.3-16: Validated methods for water

Component of residue definition: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking Water, Ground Water, and Surface Water	Primary	0.05 µg/L	LC-MS/MS	Shaffer S., 2012, 120611 <i>EU agreed (RAR 2017)</i>
	ILV	0.05 µg/L	LC-MS/MS	Austin R., Turner R., 2013 120613 <i>EU agreed (RAR 2017)</i>
	Confirmatory (if required)	-	Not required, highly specific detection system was used (HPLC-MS/MS).	

zRMS comments:

Clopyralid residues in environmental matrices can be monitored by LC–MS/MS with LOQ of 0.05 µg/L in water. No additional data are required.

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

An overview on the acceptable methods and possible data gaps for analysis of clopyralid in air is given in the following table. No new or additional studies were submitted.

Table 5.3-17: Validated methods for air

Component of residue definition: Clopyralid				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Ambient Air	Primary	4.5 µg/m ³	LC-MS/MS	Bacher R.,2012, 120601
Warm Humid Air	XAD tube	4.5 µg/m ³		EU agreed (RAR 2017)
	Confirmatory (if required)	-	Not required, highly specific detection system was used (LC-MS/MS)	

zRMS comments:

Clopyralid residues in environmental matrices can be monitored by LC–MS/MS with LOQ of 4.5 µg/m³ in air. No additional data are required.

5.3.3.7 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 clopyralid in body fluids and tissues is given in the following table. No new or additional studies were submitted.

Table 5.3-18: Methods for body fluids and tissues

Component of residue definition: Clopyralid				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Human Blood	Primary	0.05 mg/L	LC-MS/MS	Senciuc, M., 2014, 130727
Human Urine				EU agreed (RAR 2017)
	Confirmatory (if required)	-	Not required, specific detection system was used (LC-MS/MS).	

zRMS comments:

According to the current requirement of SANTE/2020/12830, Rev.2, 14. February 2023 *The LOQ shall meet 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues. Higher LOQs are acceptable for analytically challenging analytes, if justified.*

It should be noted that according to the SANCO/825/00 rev. 8.1, which was in place at the time of the active substance clopyralid submission, the LOQ in body fluids should be 0.05 mg/L, as in available method for body fluids (Senciuc, M., 2014).

Since introduction of the new SANTE/2020/12830, Rev.2, 14. February 2023, the LOQ in body fluids should be 0.01 mg/L. A method for clopyralid in body fluids with an LOQ of 0.01 mg/L is therefore considered a data gap relating to the active substance and as such this should be addressed at the active substance renewal.

5.3.3.8 Other studies/ information

No new or additional studies were 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	Massardi E.	2024	ADM.01851.H.1.A: Validation of analytical method for active ingredients and content determination Research Center BioSphereS by Biotecnologie BT, Italy Report No. CPU-024-23, ADAMA reference no. 000116317 GLP, Unpublished	N	ADAMA
KCP 5.1.2/01	Watson, G.	2022	Ethofumesate: Storage Stability of Residues of Ethofumesate and its Metabolite in Lettuce and Cereal Grain Stored Frozen for up to Two Years Report No. RES-00278 Study ID 000106576 GLP, Unpublished Filed under KCA 6.1/02 of ethofumesate	N	ADAMA
KCP 5.1.2/02	██████	2024	Acute toxicity of ADM.01851.H.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour static test Report no. 23 48 AFA 0006, ADAMA Reference No. 000116374 ██████ GLP, Unpublished Filed under KCP 10.2/01	Y	ADAMA
KCP 5.1.2/03	Renner P.	2024a	Acute toxicity of ADM.01851.H.1.A to <i>Daphnia magna</i> in a 48-hour static test Report no. 23 48 ADL 0020, ADAMA Reference No. 000116373 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.2/03	N	ADAMA
KCP 5.1.2/04	Renner P.	2024b	Effects of ADM.01851.H.1.A on <i>Raphidocelis subcapitata</i> in an algal growth inhibition test Report no. 23 48 AAL 0014, ADAMA Reference No. 000116375 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.2/03	N	ADAMA
KCP 5.1.2/05	Warning D.	2022	Effects of ADM.01851.H.1.A on <i>Myriophyllum spicatum</i> in a static water-sediment system 22 48 AMS 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.2/04	N	ADAMA

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 5.1.2/06	Ruhland S.	2023	Chronic toxicity of ADM.01851.H.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions ~~18 48 BAC 0007~~ 22 48 BAC 0024 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.3.1.2/01	N	ADAMA
KCP 5.1.2/07	Kleebaum K.	2022	ADM.01851.H.1.A – Repeated Exposure of Honey Bee (<i>Apis mellifera</i> L.) Larvae under Laboratory Conditions 22 48 BLC 0025 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.3.1.3/01	N	ADAMA
KCP 5.1.2/08	Friedemann A.	2024	Effects of ADM.01851.H.1.A on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions 23 46 PSE 0012 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.6.2/01	N	ADAMA
KCP 5.1.2/09	Friedemann A.	2024a	Effects of ADM.01851.H.1.A on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions 23 46 PVV 0011 BioChem agrar, Machern OT Gerichshain, Germany GLP, Unpublished Filed under KCP 10.6.2/02	N	ADAMA
KCP 5.1.2/10	Teasdale, R.	1996	Frozen Storage Stability of Clopyralid Residues in Strawberries Corteva Report No. GHE-P-4832 Study No. CEMS-235 CEM Analytical Services Ltd. GLP Unpublished Filed under KCA 6.1/02 of clopyralid	N	Corteva Agriscience
KCP 5.1.2/11	Skaggs, C.S., Penning, B.N.	2021	Storage Stability of Clopyralid for One Year in Dried Beans Corteva Report No. 191728 Study No. SGS-19-01-08 SGS North America, Inc GLP	N	Corteva Agriscience

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 Filed under KCA 6.1/01 of clopyralid		

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

The list below was not validated by the zRMS since majority of the active substance data have been taken from the EU review of Ethofumesate and the complete list of studies evaluated at the EU level is provided in Vol. 2 of the RAR (2015).

Ethofumesate: For data protected studies owned by Bayer the applicant has a Letter of Co-ownership. For studies owned by the TFE (TaskForce Ethofumesate), ADAMA Agricultural Solutions and all its affiliates has also access as ADAMA Deutschland GmbH is member of the TFE. For studies owned by UPL the TFE has either equivalent studies or a Letter of Access.

Clopyralid: For studies owned by Corteva (previously Dow AgroSciences S.A.S.) and submitted for the renewal of clopyralid the applicant has a Letter of Access. Studies from the initial clopyralid approval (DAR, 2005) are not data protected.

List of information, tests and studies which are considered as relied upon by the RMS for the evaluation with a view to the approval or renewal of the active substance Clopyralid.

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	Previous evaluation (DAR) or Renewal
CP 5.1.2	Whiteoak R. J., Crofts M., Harris, R.J.	1973	ANALYTICAL METHOD FOR RESIDUE IN SUGAR BEET TREATED WITH NORTRON Fisons plc, United Kingdom Bayer CropScience, Report No.: A83491/ M-155727-01, Non GLP, unpublished	N	Bayer CropScience	Original DAR 1998, KCA 4.1.2/28
CP 5.1.2	Whiteoak, R. J.; Crofts, M.; Harris, R. J.	1976	ANALYTICAL METHOD FOR RESIDUES IN SUGAR BEET TREATED WITH NORTRON Fisons plc, United Kingdom Bayer CropScience, EPA MRID No.: 00084997 Report number: A83492/ M-155728-01 Non GLP, unpublished	N	Bayer CropScience	Original DAR 1998, KCA 4.1.2/29
CP 5.1.2	Manley, J. D.,	1984	ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR	N	Bayer CropScience	Original DAR

	Snowdon, P.J.,		METABOLITES IN SUGAR BEET (IMPROVED METHOD) FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report number: A83493/M-155729-01-1 Non GLP, unpublished			1998, KCA 4.1.2/30
CP 5.1.2	Wrede, A.;	2000	Validation of the method AL 081/96-0 in peas and sugar beet roots by GC-MSD - ethofumesate - Code: AE B049913 Report No: C009934, Document no: M-199547-01-1 Aventis CropScience GmbH, Germany GLP, unpublished	N	Bayer Crop Science	KCA 4.1.2/18, KCA 4.2/04
CP 5.1.2	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 Beet Report number: A0019 GLP	N	UPL	RAR 2015, Volume 3, B5
CP 5.1.2	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) Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 00955/M002 / M-438402-01-1 GLP, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3, B5
CP 5.1.2	Schulte G.	2013	Formation of 2-keto-ethofumesate (AE C509607) by acidic extraction of plant matrices containing open-ring-2-keto-ethofumesate (AE C520645) - (sugar beet (leaf), sugar beet (body), orange (fruit), wheat (grain)) Bayer CropScience, Report No.: MR-13/061/ M-459805-01 GLP, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3, B5
CP 5.1.2	Schulte G.	2013	STORAGE STABILITY OF OPEN-RING-2-KETO ETHOFUMESATE (AE C520645) IN PLANT MATRICES FOR 24 MONTHS - PHASE REPORT AFTER 6 MONTHS Bayer CropScience Report No: MR-13/086, M-459806-01 GLP, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3, KCA 6.1
CP 5.1.2	Thom M.	2005	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in various plant commodities Report No.: OFC00004832, M-351876-01-1 GAB Analytik GmbH, Germany GLP, unpublished	N	ADAMA	RAR 2015, Volume 3, KCA 4.2/22
CP 5.1.2	Ross, D. B.; Roberts, N. L	1977	RESIDUES IN MILK AND TISSUES FOLLOWING A 28-DAY FEEDING STUDY WITH ETHOFUMESATE IN DAIRY COWS - PART 1 Huntingdon Research Centre Ltd., Huntingdon, United Kingdom	N	Bayer CropScience	Original DAR 1998, KCA 4.1.2/25

			Bayer CropScience, Report number: A83024, M-155301-01-1 Non GLP, unpublished			
CP 5.1.2	Whiteoak, R. J	1990	GAS LIQUID CHROMATOGRAPHIC DETERMINATION OF RESIDUES OF ETHOFUMESATE AND ITS METABOLITES IN MILK AND CATTLE TISSUES Schering AG, Berlin, Germany Bayer CropScience Report no: A83109, M-155384-01-1 GLP, unpublished	N	Bayer CropScience	Original DAR 1998, KCA 4.1.2/26
CP 5.1.2	Harris, R. J.	1975	INVESTIGATION OF TISSUE AND EGG RESIDUES FROM HENS FOLLOWING DIETARY INTAKE OF NC 8438 FOR 21 DAYS Fisons plc, United Kingdom Bayer CropScience, Report no: A83011, M-155288-01-1 Non GLP, unpublished	N	Bayer CropScience	Original DAR 1998, KCA 4.1.2/27
CP 5.1.2	Peatman, M. H.	1999	Review of analytical methodology for residues in edible animal products (dairy, tissues, fat and offal) Ethofumesate AE B049913 AgrEvo UK Crop Protection Ltd., Chesterford Park, United Kingdom Bayer CropScience, Report no: C003328, M-185949-01-1 Non GLP, unpublished	N	Bayer CropScience	Original DAR 1998, KCA 4.1.2/28
CP 5.1.2	Castro, L. E.	1994	Ethofumesate-derived residues in the meat and milk of dairy cows: resulting from oral ingestion of ethofumesate AgrEvo USA Company, Residue Chemistry, Pikeville, NC, USA Bayer CropScience, Report No: B002201/M-237976-01 Non GLP, unpublished	N	Bayer CropScience	Original DAR 1998
CP 5.1.2	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 Aventis CropScience USA LP, Residue Chemistry, Pikeville, NC, USA Bayer CropScience Report No: C004116/M-187353-01 Non GLP	N	Bayer CropScience	Original DAR 1998
CP 5.1.2	Author(s)	2010	Ethofumesate - Magnitude of the residue in dairy cow Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience Report No.: RAADP014/M-388797-01-1 GLP, unpublished	N	Bayer CropScience	Original DAR 1998
CP 5.1.2	Perez R.,	2014	FREEZER STORAGE STABILITY OF ETHOFUMESATE IN ANIMAL MATRIX SAMPLES -	N	Task Force	RAR 2015,

	Schmitt J.L., Patel D.		INTERIM REPORT Bayer Crop Science Report No.: M-467206-01, RAADP031 GLP, unpublished		Ethofumesate	Volume 3, KCA 6.1
CP 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 Bayer CropScience, Report. number: MR-13/101, M-479926-01 GLP, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3
CP 5.2	Ingham R.	2014	Letter of access - Regulation (EC) No. 1107/2009 - Active substance - Ethofumesate post Annex I inclusion - Letter of access from UPL to protected data United Phosphorus Limited, Cheshire, United Kingdom TF- Ethofumesate, Report No.: M-475932-01-1, GLP: n.a., unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3
CP 5.2	Spiegel K.	2014	Ethofumesate - Discussion on the usability of plant enforcement method 01392 for metabolite AE C520645 in matrices with high oil content Report No.: M-497717-01 Non GLP, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3
CP 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 GPL, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3, KCA 4.2/03
CP 5.2	Schlewitz P.	2013b	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	Task Force Ethofumesate	RAR 2015, Volume 3, KCA 4.2/04
CP 5.2	Brumhard B.	2003	Method 00806 for the determination of residues of Ethofumesate in soil by HPLC-MS/MS Report No.: 00806, M-122176-01-1 Bayer CropScience GLP, unpublished	N	Bayer CropScience	RAR 2015, Volume 3, KCA 4.2/26
CP 5.2	Schneider E.	2000	PR00/003 - Confirmation method for the determination of residues of ethofumesate in soil Report No. OFC00004917, M-351953-01-1 Dr Krebs Analytik, Koeln, Germany GLP, unpublished	N	ADAMA	RAR 2015, Volume 3, KCA 4.2/15
CP 5.2	Krebber R.,	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water	N	Task Force	RAR 2015,

	Braune M.		by HPLC-MS/MS Report No.: MR-13/085, M-466732-01-1 Bayer CropScience GLP, unpublished		Ethofumesate	Volume 3, KCA 4.2/27
CP 5.2	Class T. 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 PTRL Europe, Ulm, Germany GPL, unpublished	N	Task Force Ethofumesate	RAR 2015, Volume 3, KCA 4.2/28
CP 5.2	Schneider E.	2000	PR00/02 – Validation of an analytical method for the determination of residues of ethofumesate in air – Monitoring method Report No.: OFC00004919 UCL GmbH, Koeln, Germany GLP, unpublished	N	ADAMA	RAR 2015, Volume 3, KCA 4.2/16
CP 5.2	██████	1994	Ethofumesate: Oral (Capsule/Gavage) maximum tolerated dose (MTD) and 28 day repeat dose range finding study in dog ██████ Report No.: A87557 Bayer CropScience GLP, unpublished	Y	Task Force Ethofumesate	Original DAR 1998, KCA 5.3.1
Clopyralid						
IIA 4.1.2/01	Hastings M.J.	2002a	Determination of residues of clopyralid on agricultural crops by gas chromatography with negative-ion chemical ionization mass spectrometry. Dow AgroSciences LLC, Indianapolis, Indiana, USA Report No.: GH-C 5439, Date:19.04.2002, GLP, Non Published DAS No. O97	N	DAS	In DAR 2005
IIA 4.1.2/02	Rawle, N.	2002	Independent laboratory validation of a method for the determination of clopyralid residues in crops. CEM Analytical Services Ltd. North Ascot, UK Report No.: CEMS-1671, Date: 12.03.2002, GLP, Non Published DAS No. O95	N	DAS	In DAR 2005
IIA 4.1.2/03	Wood, S.J	1994	Determination of residues of clopyralid in grass. Dow Elanco Europe, Letcombe Laboratory, Wantage, Oxon, UK	N	DAS	In DAR 2005

			Report No.: RV94.08, Date: 30.08.1994 GLP, Non Published DAS No. O47			
IIA 4.1.2/04	Kubitschek, C.E. and Kyle, J.A.	1994	Determination of clopyralid in sugar beets and sugar beet processed products by capillary gas chromatography/mass spectrometry. North American Environmental Chemistry Laboratory, Dow Elanco, Indianapolis, Indiana, USA Report No.: GRM 94.04 Date: 16.06.1996 Non GLP, Non Published DAS No. O102	N	DAS	In DAR 2005
IIA 4.1.2/05	Anon	1989	Analytical method: Determination of clopyralid residues in refined sugar. Dow Elanco Europe, Letcombe Laboratory, Wantage, Oxon, UK Report No.: ERC 88.2, Date: 25.10.1989 Non GLP, Non Published DAS No. O34	N	DAS	In DAR 2005
IIA 4.1.2/06	Anon	1988	Analytical method: Determination of clopyralid residues in sugar beet molasses. Dow Elanco Europe, Letcombe Laboratory, Wantage, Oxon, UK Report No.: ERC 88.1, Date: 25.10.1988 Non GLP, Non Published DAS No. O31	N	DAS	In DAR 2005
CA 4.2 (a)/1	Vogl E.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS ABC Laboratories, Inc., Columbia, Missouri, USA DAS Report No. 120610 21.9.2012 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal
CA 4.2 (a)/2	Austin R.	2012	Independent Laboratory Validation of Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS" Battelle UK Ltd, Ongar, Essex, United Kingdom DAS Report No. 120614 12.10.2012 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal

CA 4.2	Clements, B, Bolton, A	1996	Determination of the Stability of Clopyralid Residues in Pasture under Frozen Storage Conditions DAS Study No. GHE-P-5350 CEM Analytical Services (CEMAS), North Ascot, Berkshire, UK GLP Unpublished	N	Corteva Agriscience	LOA
CA 4.2	Foster, D.R., Blakeslee, B.A., Rutherford, B.S.	1996	Frozen Storage Stability of Clopyralid, 2,4-D in Corn Grain, Straw and Fodder DAS Study No. GHE-C-3779 / RES93050.01 DowElanco, Indianapolis, Indiana, US GLP Unpublished	N	Corteva Agriscience	LOA
CA 4.2	Phillips, A. M.	1994	Determination of residues of clopyralid in sugar beet processed fractions DAS Report No. GH-C 3305 North American Environmental Chemistry Laboratory GLP Unpublished	N	Corteva Agriscience	LOA
CA 4.2	Dial, G.E., Lindsay, D.A.	2006	Frozen Storage Stability of Clopyralid in Oilseed Rape DAS Report No. 120939 / 020122.02 Dow AgroSciences, LLC, Indianapolis, Indiana USA GLP Unpublished	N	Corteva Agriscience	LOA
CA 4.2 (a)/3	████	2012	Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection DAS Report No. 120483 17.9.2012 GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	Submitted for the purpose of renewal
CA 4.2 (a)/4	████	2012	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices DAS Report No. 120484 11.9.2012 GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	Submitted for the purpose of renewal
CA 4.2 (a)/5	Lindner, M., Giesau, A.	2013	Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin 7.10.2013 DAS Report No. 130729 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal
CA 4.2 (a)/6	Austin, R.,	2014	Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample	N	DAS	Submitted for

	Turner, R.		Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin DAS Report No. 130728 25.2.2014 GLP/GEP (Y/N): Yes Published (Y/N): No			the purpose of renewal
CA 4.2 (b)/1	Vincent, T. P.	2013	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS ABC Laboratories, Inc., Columbia, Missouri, USA DAS Report No. 120612 20.2.2013 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal
CA 4.2 (b)/2	Austin, R., Turner, R.	2014	Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS Battelle UK Ltd, Chelmsford, Essex, United Kingdom DAS Report No. 140079 19.5.2014 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal
CA 4.2 (b)/3	Shaffer S.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS ABC Laboratories, Inc., Columbia, Missouri, USA DAS Report No. 120611 4.12.2012 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal
CA 4.2 (b)/4	Austin, R., Turner, R.	2014	Independent Laboratory Validation of Dow AgroSciences Method 120611, “Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS” Battelle UK Ltd, Ongar, Essex, United Kingdom DAS Report No. 120613 5.4.2013 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal
CA 4.2 (c)/1	Bacher R.	2012	The Development and Validation of a Method for the Analysis of Clopyralid in Air PTRL Europe GmbH, D-89081 Ulm, Germany DAS Report No. 120601 4.10.2012 GLP/GEP (Y/N): Yes	N	DAS	Submitted for the purpose of renewal

			Published (Y/N): No			
CA 4.2 (d)/1	Senciuc, M.	2014	Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s) DAS Report No. 130727 9.7.2014 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Submitted for the purpose of renewal

LOA from Corteva available.

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 the active substance 1: Clopyralid

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

New studies have been submitted where necessary.

For validations of analytical methods used for data generation for risk assessment, please refer to A 2.2.1.

A 2.1.2 Methods for post-authorization control and monitoring purposes for the active substance of clopyralid (KCP 5.2)

In relation to residues in the commodities for which use of this product is proposed (sugar beet and fodder beet) and in appropriate animal matrices and environmental samples, the applicant has full access to the appropriate monitoring methods found acceptable in the RARs (or to studies which have been deemed equivalent) and the conclusions made on the basis of those studies.

No further consideration is therefore necessary in this evaluation.

A 2.2 Analytical methods for the active substance 2: Ethofumesate

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

New studies have been submitted where necessary.

All method validation for pre-registration methods are filed under this data point.

Statement on the analytical method for the determination of ethofumesate and its metabolites in residue studies:

1. Definition of residues in plants for monitoring and risk assessment

The residue definition for ethofumesate in plant commodities for monitoring and risk assessment has been proposed in the EFSA conclusion published 19 January 2016 (doi:10.2903/j.efsa.2016.4374) as following: Sum of ethofumesate, ethofumesate-lactone (NC 9607), ethofumesate-carboxylic acid (NC 20645) and its conjugate, expressed as ethofumesate.

(or: Sum of ethofumesate, 2-keto-ethofumesate, open-ring-2-keto-ethofumesate and its conjugate, expressed as ethofumesate - as expressed in SANCO/11739/2013 and Commission Regulation (EU) 2017/1016).

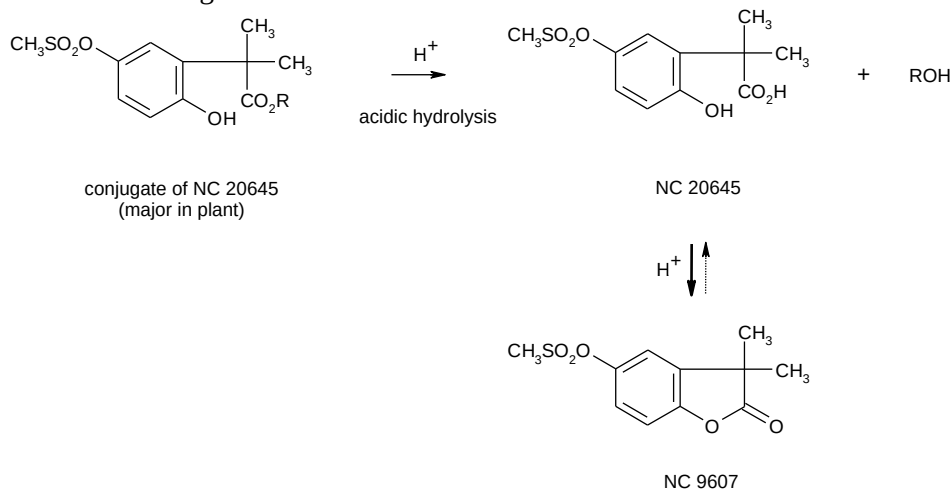
2. Suitability of Analytical Methods for Analysis of Ethofumesate and its Metabolites according to the proposed residue definition

With reference to the RAR, point B.7.3.1, Tandy, R. (2012b; KCA 6.3.1/15) and of Weir, A. (2014; KCA 6.3.1/16), it was concluded that residue trials can be used to support the residue definition if they were done using acidic conditions and GC/MS analysis.

The rationale behind this conclusion is as follows:

Under acidic conditions conjugated NC20645 (= conjugated open-ring-2-keto, ethofumesate-carboxylic acid) is transformed into free NC20645 (= open ring 2-keto-ethofumesate). This open-ring 2-keto-ethofumesate however always is in chemical equilibrium with its tautomer, which is the closed form, the 2-keto-Ethofumesate (which chemically is a lactone). Under acidic conditions the open-ring 2-keto-ethofumesate is completely and immediately transformed into the closed form, the 2-keto-ethofumesate, which then is the analysed form.

See the following scheme:



NC 9607 is formed nearly quantitatively by acidic hydrolysis of the major plant metabolite, the conjugate of NC 20645. So by using these specific method conditions, the open ring 2-keto-ethofumesate and its conjugate are analysed intrinsically by analysing the 2-keto-ethofumesate.

The analytical method used in the following residue studies are performed under acid conditions. Therefore, the residues of ethofumesate and ethofumesate-2-keto (NC 9607) as determined in the below cited residue studies also cover the residues of the open-ring-2-keto-ethofumesate and its conjugate.

The residue studies and the corresponding methods are in line with the proposed residue definition.

For further information about the method, please refer to the RAR, point B.5, studies Schulte, G.; Diehl, P. (2014), Betson, S. (2014) and Spiegel, K. (2014).

Residue analytical methods

Comments of zRMS:	The analytical method has been evaluated and accepted by zRMS-PL in RR – Part B5 for AG-E1-500 SC1/ Ethosat 500 SC (January 2022). This study has not been reassessed in the framework of this application. The conclusions of the assessment are presented below: <i>Analytical method RES-00278 has been successfully validated for the determination of residues of ethofumesate and ethofumesate-2-keto residues in lettuce and cereal grain at a LOQ of 0.01 mg/kg.</i> <i>Acceptable mean recoveries in the range 70 – 110% and a relative standard deviation (RSD) of less than 20% were found for ethofumesate and ethofumesate-2-keto at each fortification level and for both primary and confirmatory transitions in for each matrix type.</i> <i>No residues of ethofumesate or ethofumesate-2-keto greater than 30% of the LOQ were detected in any of the control samples.</i> <i>This method meets the requirements of SANCO/3029/99 rev. 4.</i> <i>The study is acceptable.</i>
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Reference:	KCP 5.1.2/01 (filed under KCP 8/02 (KCA 6.1/02)
Report	Watson G., 2021: Ethofumesate: Storage Stability of Residues of Ethofumesate and its Metabolite in Lettuce and Cereal Grain Stored Frozen for up to Two Years, Report No: RES-00278, Study ID: 000106576
Guideline(s):	SANCO/3029/99 rev. 4 and SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

The analytical method RES-00278 for the determination of ethofumesate and ethofumesate-2-keto residues in lettuce and cereal grain was validated. The method involves extraction by reflux using ethyl acetate / n-hexane (1/1, v/v). Extracts are concentrated and the retained matrix is then refluxed under acidic conditions before filtration and concentration. Extracts from both reflux steps are combined and concentrated to dryness. Extracts are re-dissolved in n-hexane and passed through a silica gel and C18 cartridge before reducing to dryness and re-dissolving in ethyl acetate. Quantification of ethofumesate and ethofumesate-2-keto is achieved by GC-MS monitoring of three fragment ions for each analyte. The limit of quantification (LOQ) of the method was 0.01 mg/kg.

Test substance:

Test substance	Ethofumesate reference material
Batch No.	226119
Purity	98.6%
CAS No.	26225-79-6
Reference substance:	Ethofumesate-2-keto, reference material
Batch No.	1095584
Purity	98.6%
CAS No.	26244-33-7

Control lettuce was purchased from a local supermarket and was homogenised using a Robot Coupe R10 cutter mixer to produce a composite sample for method validation.

Control cereal grain (barley grain) was purchased from an online supplier and was homogenised using a Fritsch Pulverisette 19 cutting mill to produce a composite sample for method validation.

Homogenised sub-samples of each test commodity (10 g) were fortified with standard solutions of ethofumesate and ethofumesate-2-keto in ethyl-acetate. Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.01 mg/kg) and five at a higher level (10x LOQ; 0.1 mg/kg). Matrices used were lettuce and cereal grain. The fortified samples were analysed alongside untreated control samples and reagent blank.

Analytical method:

Method type	GC-MS		
Equipment	Agilent Technologies 6890N Gas Chromatograph coupled to an Agilent 5973 inert Mass Spectrometer with an Agilent 7683 autosampler		
Column	30 m x 0.25 mm i.d, HP-5 (5% Phenyl Methyl Siloxane), 0.25µm df		
Injection Mode	Pulsed Splitless (30 psi/1 min)		
Carrier Gas	Helium, 1.0 ml/min constant flow		
Injection Volume	2.0 microlitres		
Temperatures	Injector Temperature 270 °C Transfer Line Temperature 300 °C Source temperature 230 °C Quad temperature 150 °C		
Expected retention times (approx.)	Ethofumesate 12.2 mins, ethofumesate-2-keto 11.5 mins		
Oven temperature program	Rate (°C/min)	Temperature (°C)	Time (min)
	-	100	1.0
	10	260	1.0
	30	300	5.0
Mass Spectrometric Parameters for Ethofumesate and Ethofumesate-2-keto			
Ionization mode	Electron impact (EI)		
Dwell	15 msec		
Ethofumesate	Primary ion 286 m/z 1 Confirmatory ion 1 207 m/z 1		

	Confirmatory ion 2 161 m/z
Ethofumesate-2-keto	Primary ion 256 m/z Confirmatory ion 1 177 m/z Confirmatory ion 2 121 m/z

Results and discussions

Recoveries of ethofumesate and ethofumesate-2-keto obtained from lettuce and cereal grain at each fortification level using method RES-00278 are presented in the tables below. Other validation parameters of the method are presented in the following table.

Table A 1: Recovery results from method validation of ethofumesate and ethofumesate-2-keto using the analytical method RES-00278 in lettuce and cereal grain

Matrix	Analyte	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)	Comments
Reagent blank		NOP**	-	-	-	-	-
Control		NOP**	-	-	-	-	-
Control		NOP**	-	-	-	-	-
Lettuce head	Ethofumesate	Primary ion 286 m/z					
		0.01*	119, 102, 96, 101, 95	96 – 119 (n = 5)	103	9.6	-
		0.1	92, 92, 91, 91, 92	91 – 92 (n = 5)	92	0.7	-
		Overall		91 - 119 (n = 10)	97	9.0	-
		Confirmatory ion 207 m/z					
		0.01*	111, 87, 85, 85, 92	85 - 111 (n = 5)	92	12.1	-
		0.1	102, 101, 99, 99, 101	99 - 102 (n = 5)	100	1.2	-
		Overall		85 - 111 (n = 10)	96	9.0	-
		Confirmatory ion 161 m/z					
		0.01*	120, 101, 100, 101, 96	96 - 120 (n = 5)	104	9.0	-
		0.1	89, 90, 88, 87, 89	97 - 90 (n = 5)	89	1.2	-
		Overall		96 - 120 (n = 10)	96	10.5	-
Cereal grain	Ethofumesate	Primary ion 286 m/z-					
		0.01*	98, 97, 95, 100, 100	95 - 100 (n = 5)	98	2.1	-
		0.1	92, 94, 92, 90, 90	90 - 94 (n = 5)	91	2.0	-
		Overall		90 - 100 (n = 10)	95	4.2	-
		Confirmatory ion 207 m/z					
		0.01*	98, 94, 94, 94, 91	91 - 98 (n = 5)	90.0	3.5	-

Matrix	Analyte	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)	Comments
		0.1	89, 91, 89, 87, 87	87 - 91 (n = 5)	93.1	7.9	-
		Overall		(n = 10)	91.5	6.1	-
		Confirmatory ion 161 m/z					
		0.01*	96, 93, 90, 90, 89	89 - 96 (n = 5)	92	3.2	-
		0.1	88, 93, 90, 88, 88	88 - 93 (n = 5)	89	2.2	-
		Overall		88 - 93 (n = 10)	91	2.9	-
Lettuce head	Ethofumesate-2-keto	Primary ion 256 m/z					
		0.01*	90, 91, 89, 88, 91	88 - 91 (n = 5)	90	1.5	-
		0.1	96, 98, 99, 97, 96	96 - 99 (n = 5)	97	1.3	-
		Overall		88 - 99 (n = 10)	93	4.2	-
		Confirmatory ion 177 m/z					
		0.01*	89, 92, 92, 90, 90	89 - 92 (n = 5)	91	1.5	-
		0.1	98, 100, 102, 99, 96	96 - 102 (n = 5)	99	2.1	-
		Overall		89 - 102 (n = 10)	91.5	6.1	-
		Confirmatory ion 121 m/z					
		0.01*	83, 78, 82, 86, 92	78 - 92 (n = 5)	84	6.1	-
		0.1	102, 94, 102, 99, 101	94 - 102 (n = 5)	100	3.4	-
		Overall		78 - 102 (n = 10)	92	9.8	-
Cereal grain	Ethofumesate-2-keto	Primary ion 256 m/z					
		0.01*	85, 88, 86, 93, 86	85 - 93 (n = 5)	88	3.3	-
		0.1	90, 91, 92, 92, 94	90 - 94 (n = 5)	92	1.6	-
		Overall		85 - 94 (n = 10)	90	3.3	-
		Confirmatory ion 177 m/z					
		0.01*	93, 93, 85, 95, 94	85 - 95 (n = 5)	90.0	3.5	-
		0.1	93, 91, 91, 92, 94	91 - 94 (n = 5)	93.1	7.9	-
		Overall		85- -94	91.5	6.1	-

Matrix	Analyte	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)	Comments
				(n = 10)			
		Confirmatory ion 121 m/z					
		0.01*	89, 87, 77, 101, 101	77 – 101 (n = 5)	91	11.3	-
		0.1	94, 94, 94, 92, 96	92 – 96 (n = 5)	94	1.4	-
		Overall		77 – 101 (n = 10)	93	7.6	-

* 0.01 mg/kg = limit of quantification, defined by the lowest validated fortification level. Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

**No Observable Peak

% Mean and % RSD calculated using rounded values.

Table A 2: Characteristics of the data collection analytical method used for the quantification of ethofumesate and ethofumesate-2-keto residues in lettuce and cereal grain

	Ethofumesate	Ethofumesate-2-keto
Selectivity	Final determination of ethofumesate and ethofumesate-2-keto was conducted by GC-MS monitoring 3 fragment ions per analyte; For each matrix a reagent blank and two control specimens were extracted and analysed to investigate the presence of ethofumesate / ethofumesate-2-keto and/or matrix interference at the retention time of ethofumesate / ethofumesate-2-keto. The selectivity of the method was demonstrated as no matrix interferences or residues of ethofumesate / ethofumesate-2-keto were observed at or above 30% of the LOQ in the reagent blank sample or the control samples. Representative chromatograms of a blank sample and control samples are presented in the report.	
Linearity/ calibration	The linearity of the detector was checked for each matrix by single injection of matrix-matched calibration standards at 6 concentration levels ranging from 0.0075 µg/mL (0.003 mg/kg) to 0.5 µg/mL (0.2 mg/kg). This calibration range is equivalent to 30% of the LOQ up to 200% of the upper fortification level. The calibration curves were linear with correlation coefficients (r) greater than 0.995. Detailed calibration data are presented in the report	
	Lettuce (286 m/z): y = 979014x + 5043.9, R = 0.9999 Lettuce (207 m/z): y = 2887905.5030x + 215.0197, R = 0.9999 Lettuce (161 m/z): y = 1795514.8434x + 13574.2348, R = 0.9999 Cereal grain (286 m/z): y = 679563x + 2660.5, R = 0.9996 Cereal grain (207 m/z): y = 2120950.6065x + 13546.4266, R = 0.9995 Cereal grain (161 m/z) y = 1382607.8686x + 11331.3604, R = 0.9996	Lettuce (256 m/z): y = 697917x + 308.97, R = 1.0000 Lettuce (177 m/z) y = 742185.2459x + 451.5430, R = 1.0000 Lettuce (121 m/z) y = 267102.5040x - 592.6808, R = 1.0000 Cereal grain (256 m/z) y = 513854x + 2107.9, R = 0.9998 Cereal grain (177 m/z) y = 533286.3852x + 1928.7410, R = 0.9997 Cereal grain (121 m/z) y = 177959.8951x + 1319.8996, R = 0.9990
Accuracy / Recovery	The accuracy and precision of the method was successfully demonstrated for each matrix type as the mean recovery values for ethofumesate and ethofumesate-2-keto at each fortification level were between 70 – 110% (see table above).	
Repeatability	The relative standard deviation (RSD) was ≤ 20% (see table above).	
Limit of quantification (LOQ)	The limit of quantitation (LOQ) was set to 0.01 mg/kg for all crops and each analyte, defined by the lowest validated fortification level.	
Limit of detection LOD	The limit of detection was confirmed to be less than 30 % of the LOQ for ethofumesate and ethofumesate-2-keto in each matrix as demonstrated by the response of the bottom calibration standard (equivalent to 30% of the LOQ) which was visually confirmed to be greater than three times signal to noise for all mass ions.	
Matrix effects	Matrix effects on detection were evaluated for ethofumesate / ethofumesate-2-keto were deemed to be significant (> 20 %) in some cases. Therefore, matrix-matched calibration standards were used for	

	Ethofumesate	Ethofumesate-2-keto
	quantification.	
Extract Stability	Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared matrix matched calibration standards after at least 7 days refrigerated storage. Mean recovery values were in the acceptable range of 70 – 110% with an RSD of less than 20% and were within $\pm 20\%$ of the original result. Final extracts were therefore shown to be stable when stored refrigerated for 7 days.	
Standard Stability	Solvent calibration standards were shown to be stable for 34 days when stored refrigerated which covers the period of time standards were used in the study. The peak area of a freshly prepared 0.025 µg/mL LOQ equivalent calibration standard (mean of three injections) was compared to the peak area of a stored standard of the same concentration (mean of three injections). The difference between the two was $\leq 10\%$.	

Conclusion

Analytical method RES-00278 has been successfully validated for the determination of residues of ethofumesate ethofumesate-2-keto in lettuce and cereal grain by GC-MS with a LOQ of 0.01 mg/kg.

Ecotoxicological analytical methods

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in the aquatic test matrix with an LOQ of 0.08073 mg/L for clopyralid and 0.5525 mg/L of ethofumesate. Recovery results were in a range of 70 – 110% with an RSD $\leq 20\%$. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 2 have been met. The method is acceptable.
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Reference:	KCP 5.1.2/02 (filed under KCP 10.2/01)
Report	Acute toxicity of ADM.01851.H.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour static test Report no. 23 48 AFA 0006, ADAMA Reference No. 000116374
Guideline(s):	SANTE/2020/12830 rev. 2
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for concentration determination of clopyralid and ethofumesate in test substance ADM.01851.H.1.A was validated in test solution (reconstituted water according to ISO 6341). The limit of quantification (LOQ) of the method was 0.08073 mg/L clopyralid and 0.5525 mg/L ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by LC-MS/MS.

Study period:

Experimental start date: 24. July, 2023

Experimental completion date: 02. August, 2023

Materials:

Clopyralid (reference material): Batch no. 802406, purity: 99.61 $\pm$ 0.36%, HPC Standards GmbH

Ethofumesate (reference material): Batch no. 792174, purity: 99.7 $\pm$ 0.5%, HPC Standards GmbH

Analytical method:

Method type	HPLC-MS	
Equipment	Shimadzu HPLC-MS/MS 8050 System with quadrupole mass spectrometric detector	
Column	ACE Excel 3 Super C18, 3 µm, 100 * 2.1 mm	
Column temperature	35°C	
Injection volume	50µL	
Flow rate	0.2 mL/min	
Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 40% B 1.5 min 50% B 2.0 min 80% B 3.0 min 100% B 4.0 min 100% B 4.1 min 40% B 8.0 min 40% B Run time: 8.00 min	
Retention time	Approx.2.3 – 2.6 min Clopyralid Approx.5.1 – 5.2 min Ethofumesate	
Detector	Shimadzu LCMS-8050, Triple quadrupole mass spectrometer	
Ions monitored	Clopyralid: ESI positive, [M+H] + MRM m/z 191.8 → 110.0 m/z 191.8 →145.9 (quantifier)	Ethofumesate: ESI positive, [M+H] + MRM m/z 287.0 → 259.1 (quantifier) m/z 287.0 → 161.1

Results and discussions

Recovery results were in a range of 70 – 120% % with an RSD ≤ 20 per level.

Table A 3: Recovery results from method validation of clopyralid in reconstituted water according to ISO 6341

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	0.08073	0.03886 ¹ 0.07161 0.08132 0.08581 0.08236	0.08028	48.81 88.7 100.7 106.3 102.0	99.4	7.6
High	4.037	4.691 4.204 4.106 4.189 4.427	4.323	116.2 104.1 101.7 103.8 109.7	107.1	5.5

n.d.= clopyralid was not detected, LOQ = 0.08073 mg/L

¹ = outlier according to Gubbs test

Table A 4: Recovery results from method validation of ethofumesate in reconstituted water according to ISO 6341

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.		-	-	-
Low (LOQ)	0.5525	0.5011 0.5694 0.5955 0.6165 0.6103	0.5786	90.7 103.0 107.8 111.6 110.4	104.7	8.1
High	27.63	30.33 29.16 27.75 28.98 28.93	29.03	109.8 105.5 100.4 104.9 104.7	105.1	3.2

n.d.= etofumesate was not detected, LOQ = 0.5525 mg/L

Table A 5: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in reconstituted water according to ISO 6341

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by LC-MS/MS detection and the ansence of interfering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	The detector response for clopyralid was quadratic in the range from 1.078 to 15.40 µg/L. The corresponding calibration range regarding analytical dilution was from 21.56µg/L to 1928 µg/L. $Y=-1771.06x^2+177585x+30512.6$, $r^2= 0.99905$	The detector response for ethofumesate was quadratic in the range from 7.447 to 106.4 µg/L. The corresponding calibration range regarding analytical dilution was from 148.9µg/L to 34042 µg/L. $Y=-255.225x^2+104774x+95532.1$, $r^2= 0.99984$
Accuracy Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	0.08073 mg/L clopyralid; corresponds to the lowest validated fortification level.	0.5525 mg/L ethofumesate; corresponds to the lowest validated fortification level.
LOD	0.02156 mg/l clopyralid; corresponds to the lowest calibrated level.	0.1489 mg/L ethofumesate; corresponds to the lowest calibrated level.
Matrix effects	Matrix matched calibrations with analytical reference material including seven calibration levels were used.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 1 and is suitable for the determination of clopyralid and ethofumesate in reconstituted water according to ISO 6341 with an LOQ of 0.08073 mg/L for clopyralid and 0.5525 mg/L of ethofumesate.

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in the test solution with an LOQ of 0.2894 mg/L clopyralid and 1.981 mg/L ethofumesate. Recovery results were in a range of 70 – 120% with an RSD ≤ 20%. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 2 have been met. The method is acceptable.
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Reference: KCP 5.1.2/03 (filed under KCP 10.2/02)

Report Acute toxicity of ADM.01851.H.1.A to *Daphnia magna* in a 48-hour static

test
Renner P., 2024
Report no. 23 48 ADL 0020, ADAMA Reference No. 000116373

Guideline(s): SANTE/2020/12830 rev. 2

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method for concentration determination of clopyralid and ethofumesate in test substance ADM.01851.H.1.A was validated in test solution (reconstituted water according to ISO 6341). The limit of quantification (LOQ) of the method was 0.2894 mg/L clopyralid and 1.981 mg/L ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by LC-MS/MS.

Study period:

Experimental start date: 11. July, 2023

Experimental completion date: 14. July, 2023

Materials:

Clopyralid (reference material): Batch no. 802406, purity: 99.61±0.36%, HPC Standards GmbH

Ethofumesate (reference material): Batch no. 792174, purity: 99.7 ±0.5%, HPC Standards GmbH

Analytical method:

Method type	HPLC-MS	
Equipment	Shimadzu HPLC-MS/MS 8050 System with quadrupole mass spectrometric detector	
Column	ACE Excel 3 Super C18, 3 µm, 100 * 2.1 mm	
Column temperature	35°C	
Injection volume	50µL	
Flow rate	0.2 mL/min	
Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 40% B 1.5 min 50% B 2.0 min 80% B 3.0 min 100% B 4.0 min 100% B 4.1 min 40% B 8.0 min 40% B Run time: 8.00 min	
Retention time	Approx.2.3 – 2.6 min Clopyralid Approx.5.1 – 5.2 min Ethofumesate	
Detector	Shimadzu LCMS-8050, Triple quadrupole mass spectrometer	
Ions monitored	Clopyralid: ESI positive, [M+H] +	Ethofumesate: ESI positive, [M+H] +
	MRM	MRM
	m/z 191.8 → 110.0 m/z 191.8 → 145.9 (quantifier)	m/z 287.0 → 259.1 (quantifier) m/z 287.0 → 161.1

Results and discussions

Recovery results were in a range of 70 – 120% % with an RSD ≤ 20 per level.

Table A 6: Recovery results from method validation of clopyralid in reconstituted water according to ISO 6341

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	0.2894	0.2876 0.3021 0.2921 0.2946 0.2947	0.2942	99.4 104.4 100.9 101.8 101.8	101.7	1.8
High	4.453	4.706 4.641 4.559 4.656 4.653	4.643	105.7 104.2 102.4 104.6 104.5	104.3	1.1

n.d.= clopyralid was not detected, LOQ = 0.2894 mg/L

Table A 7: Recovery results from method validation of ethofumesate in reconstituted water according to ISO 6341

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	1.981	2.048 2.019 2.047 2.065 2.059	2.047	103.4 101.9 103.3 104.2 103.9	103.4	0.86
High	30.47	31.48 30.98 31.08 30.77 30.58	30.98	103.3 101.7 102.0 101.0 100.3	101.7	1.1

n.d.= etofumesate was not detected, LOQ = 1.981 mg/L

Table A 8: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in reconstituted water according to ISO 6341

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by LC-MS/MS detection and the absence of interfering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	The detector response for clopyralid was quadratic in the range from 1.620 to 24.92 µg/L. The corresponding calibration range regarding analytical dilution was from 77.14 µg/L to 5538 µg/L. $Y = -13396.46x - 1357.6$, $r^2 = 0.99989$	The detector response for etofumesate was quadratic in the range from 11.19 to 172.1 µg/L. The corresponding calibration range regarding analytical dilution was from 532.8 µg/L to 38254 µg/L. $Y = -48.1514x^2 + 23903.5x + 51481.6$, $r^2 = 0.99943$
Accuracy / Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	0.2894 mg/L clopyralid; corresponds to the lowest validated fortification level.	1.981 mg/L ethofumesate; corresponds to the lowest validated fortification level.
LOD	0.07714 mg/L clopyralid; corresponds to the lowest calibrated level.	0.5328 mg/L ethofumesate; corresponds to the lowest calibrated level.
Matrix effects	Matrix matched calibrations with analytical reference material including six calibration levels were used.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 2 and is suitable for the determination of clopyralid and ethofumesate in reconstituted water according to ISO 6341 with an LOQ of 0.2894 mg/L for clopyralid and 1.981 mg/L of ethofumesate.

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in OECD algal medium with an LOQ of 0.08918 mg/L clopyralid and 0.6104 mg/L ethofumesate. Recovery results were in a range of 70 – 120% with an RSD ≤ 20%. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 2 have been met. The method is acceptable.
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Reference:	KCP 5.1.2/04 (filed under KCP 10.2/04)
Report	Effects of ADM.01851.H.1.A on <i>Raphidocelis subcapitata</i> in an algal growth inhibition test Renner P., 2024b Report no. 23 48 AAL 0014, ADAMA Reference No. 000116375
Guideline(s):	SANTE/2020/12830 rev. 2
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for concentration determination of clopyralid and ethofumesate in test substance ADM.01851.H.1.A was validated in test solution (OECD algal medium according to OECD 201). The limit of quantification (LOQ) of the method was 0.08918 mg/L clopyralid and 0.6104 mg/L ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by LC-MS/MS.

Study period:

Experimental start date: 10. July, 2023

Experimental completion date: 18. July, 2023

Materials:

Clopyralid (reference material): Batch no. 802406, purity: 99.61±0.36%, HPC Standards GmbH

Ethofumesate (reference material): Batch no. 792174, purity: 99.7 ±0.5%, HPC Standards GmbH

Analytical method:

Method type	LC-MS/MS	
Equipment	Shimadzu HPLC-MS/MS 8050 System with quadrupole mass spectrometric detector	
Column	ACE Excel 3 Super C18, 3 µm, 100 * 2.1 mm	
Column temperature	35°C	
Injection volume	35µL	
Flow rate	0.2 mL/min	
Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 40% B 1.5 min 50% B 2.0 min 80% B 3.0 min 100% B 4.0 min 100% B 4.1 min 40% B 8.0 min 40% B Run time: 8.00 min	
Retention time	Approx.2.3 – 2.5 min Clopyralid Approx.5.1 – 5.2 min Ethofumesate	
Detector	Shimadzu LCMS-8050, Triple quadrupole mass spectrometer	
Ions monitored	Clopyralid: ESI positive, [M+H] +	Ethofumesate: ESI positive, [M+H] +
	MRM m/z 191.8 → 110.0 m/z 191.8 → 145.9 (quantifier)	MRM m/z 287.0 → 259.1 (quantifier) m/z 287.0 → 161.1

Results and discussions

Recovery results were in a range of 70 – 120% % with an RSD ≤ 20 per level.

Table A 9: Recovery results from method validation of clopyralid in OECD algal medium according to OECD 201

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	0.08918	0.09105 0.08211 0.08789 0.09135 0.08577	0.08763	102.1 92.1 98.5 102.4 96.2	98.3	4.4
High	4.459	4.637 4.361 4.368 4.429 4.426	4.444	104.0 97.8 97.9 99.3 99.3	99.7	2.5

n.d.= clopyralid was not detected, LOQ = 0.08918 mg/L

Table A 10: Recovery results from method validation of ethofumesate in OECD algal medium according to OECD 201

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	0.6104	0.5450 0.5560 0.5923 0.5407 0.5610	0.5590	89.3 91.1 97.0 88.6 91.9	91.6	3.6
High	30.52	30.03 27.46 28.34 29.91 29.13	28.97	98.4 90.0 92.9 98.0 95.4	94.9	3.7

n.d.= etofumesate was not detected, LOQ = 0.6104 mg/L

Table A 11: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in OECD algal medium according to OECD 201

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by LC-MS/MS detection and the ansence of interfering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	The detector response for clopyralid was quadratic in the range from 2.912 to 44.80 µg/L. The corresponding calibration range regarding analytical dilution was from 23.30 µg/L to 5376 µg/L (7 levels). $Y=92.1267x^2-73094.3x+3083.86$, $r^2= 0.99991$	The detector response for etofumesate was quadratic in the range from 20.12 to 309.5 µg/L. The corresponding calibration range regarding analytical dilution was from 160.9 µg/L to 37136 µg/L (7 levels). $Y=-79.6269x^2+69598x+374232$, $r^2= 0.99943$
Accuracy Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	0.08918 mg/L clopyralid; corresponds to the lowest validated fortification level.	0.6104 mg/L ethofumesate; corresponds to the lowest validated fortification level.
LOD	0.02330 mg/l clopyralid; corresponds to the lowest calibrated level.	0.1609 mg/L ethofumesate; corresponds to the lowest calibrated level.
Matrix effects	Matrix matched calibrations with analytical reference material including seven calibration levels were used.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 2 and is suitable for the determination of clopyralid and ethofumesate in OECD algal medium according to OECD 201 with an LOQ of 0.08918 mg/L for clopyralid and 0.6104 mg/L of ethofumesate.

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in test solution with an LOQ of 0.6887 µg/L clopyralid and 4.841 µg/L ethofumesate. Recovery results were in a range of 70 – 120% with an RSD ≤ 20%. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 1 have been met. The method is acceptable.
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Reference: KCP 5.1.2/05 (filed under KCP 10.3.1.2/01)

Report Effects of ADM.01851.H.1.A on *Myriophyllum spicatum* in a static water-sediment system

Warning D., 2022
Report no. 22 48 AMS 0001, ADAMA Reference No. 000110709

Guideline(s): SANTE/2020/12830 rev. 1

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method for concentration determination of clopyralid and ethofumesate in test substance ADM.01851.H.1.A was validated in test solution (Smart Barko test medium). The limit of quantification (LOQ) of the method was 0.6887 µg/L clopyralid and 4.841 µg/L ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by LC-MS/MS.

Study period:

Experimental start date: 29. August, 2022

Experimental completion date: 15. September, 2022

Materials:

ADM.01851.H.1.A: Batch U3111P1, containing 6.43% (74.8g/L) Clopyralid, 45.2% (525.1 g/L Ethofumesate)

Clopyralid (reference material): Batch no. 264-3598, purity: 97.2%

Ethofumesate (reference material): Batch no. 290-3859, purity: 99.2%

Table A 12: Recovery results from method validation of clopyralid in OECD algal medium according to OECD 201 test solution

Matrix	Nominal concentration (µg/L)	Analysed concentration (µg/L)	Mean analysed concentration (µg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	0.6887	0.7160 0.6901 0.6890 0.6891 0.7035	0.6976	104.0 100.2 100.1 100.1 102.2	101.3	1.7
High	655.9	695.9 669.8 743.2 699.0 697.1	701.0	106.1 102.1 113.3 106.6 106.3	106.9	3.8

n.d.= clopyralid was not detected, LOQ = 0.6887 µg/L

Table A 13: Recovery results from method validation of ethofumesate in OECD algal medium according to OECD 201 test solution

Matrix	Nominal concentration (µg/L)	Analysed concentration (µg/L)	Mean analysed concentration (µg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	4.841	4.670 4.796 4.829 4.538 4.724	4.711	96.5 99.1 99.8 93.7 97.6	97.3	2.4
High	4610	4542 4598 4842 4452 4502	4587	98.5 99.7 105.0 96.6 97.6	99.5	3.3

n.d.= etofumesate was not detected, LOQ = 4.841 µg/L

Table A 14: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in Smart Barko test medium

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by LC-MS/MS detection and the ansence of interfering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	The detector response for clopyralid was quadratic in the range from 0.1981 to 8.255 µg/L (7 levels). $Y = -1054x^2 + 65795x + 557$, $r^2 = 0.99999$	The detector response for etofumesate was quadratic in the range from 1.391 to 57.94 µg/L (7 levels). $Y = 14423x + 2338$, $r^2 = 0.99991$
Accuracy Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	0.6887 µg/L clopyralid; corresponds to the lowest validated fortification level.	4.841 µg/L ethofumesate; corresponds to the lowest validated fortification level.
LOD	0.1981 µg/l clopyralid; corresponds to the lowest calibrated level.	1.391 µg/L ethofumesate; corresponds to the lowest calibrated level.
Matrix effects	Matrix matched calibrations with analytical reference material including seven calibration levels were used.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 1 and is suitable for the determination of clopyralid and ethofumesate in Smart Barko test medium with an LOQ of 0.6887 µg/L for clopyralid and 4.841 µg/L of ethofumesate.

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in feeding solution (sample matrix: sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan) with an LOQ of 15.7 mg/kg clopyralid and 110 mg/kg ethofumesate. Recovery results were in a range of 70 – 120% with an RSD ≤ 20%. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 1 have been met. The method is acceptable.
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Reference: KCP 5.1.2/06 (filed under KCP 10.3.1.2/01)

Report Ruhland S., 2023: Chronic toxicity of ADM.01851.H.1.A to the honey bee *Apis mellifera* L. under laboratory conditions, Report No: 22 48 BAC 0024,

ADAMA Reference No.: 000110706

Guideline(s): SANTE/2020/12830 rev. 1

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method for clopyralid and ethofumesate content determination was validated in feeding solution (sample matrix: sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan). The limit of quantification (LOQ) of the method was 15.7 mg/kg clopyralid and 110 mg/kg ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by LC-MS/MS.

Study period:

Experimental start date: 04. October, 2022

Experimental completion date: 08. November, 2022

Materials:

ADM.01851.H.1.A: Batch U3111P1, containing 6.43% (74.8g/L) Clopyralid, 45.2% (525.1 g/L Ethofumesate)

Clopyralid (reference material): Batch no. 264-3598, purity: 97.2%

Ethofumesate (reference material): Batch no. 290-3859, purity: 99.2%

Analytical method:

Method type	LC-MS/MS	
Equipment	LC-MS Agilent 1200 system	
Column	ACE C18, Amide 3 µm, 100 * 2.1 mm	
Column temperature	45°C	
Injection volume	10 µL	
Flow rate	0.4 mL/min	
Mobile phase	A: Water containing 0.1% acetic acid in water B: Acetonitrile 0.00 min 10% B 0.50 min 10% B 3.00 min 60% B 5.00 min 100% B Run time: 6.00 min	
Retention time	Approx.2.04 min Clopyralid Approx.5.02 min Ethofumesate	
Detector	Agilent G6460 Triple quadrupole mass spectrometer	
Ions monitored	Clopyralid: ESI positive, [M+H] +	Ethofumesate: ESI positive, [M+H] +
	MRM m/z 191.8 → 146 (quantifier) m/z 191.8 → 110 (qualifier)	MRM m/z 287.0 → 121.1 (quantifier) m/z 287.0 → 259 (qualifier)

Results and discussions

Recovery results were in a range of 70 – 120% % with an RSD ≤ 20 per level.

Table A 15: Recovery results from method validation of clopyralid in sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan

Matrix	Nominal concentration (mg/kg)	Analysed concentration (mg/kg)	Mean analysed concentration (mg/kg)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	15.7	13.7 13.4 13.7 14.3 14.6	13.9	87.3 85.4 87.5 91.4 93.4	89.0	3.7
High	1740	1639 1505 1617 1542 1643	1589	94.2 86.5 92.9 88.6 94.4	91.3	3.9

n.d.= clopyralid was not detected, LOQ = 15.7 mg/kg

Table A 16: Recovery results from method validation of ethofumesate in sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan

Matrix	Nominal concentration (mg/kg)	Analysed concentration (mg/kg)	Mean analysed concentration (mg/kg)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	110	96.1 95.9 95.0 94.3 93.6	95.0	87.3 97.1 86.3 85.6 85.0	86.3	1.1
High	12232	11527 10495 10449 10278 11033	10753	94.2 85.8 85.4 84.0 90.2	87.9	4.8

n.d.= etofumesate was not detected, LOQ = 110 mg/kg

Table A 17: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by LC-MS/MS detection and the ansence of interfering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	The detector response for clopyralid was quadratic in the range from 5.95 to 119 µg/L (at least 5 levels). $Y = 201.10323x - 15.513989$, $r^2 = 0.9994$	The detector response for etofumesate was quadratic in the range from 41.5 to 831 µg/L (at least 5 levels). $Y = -0.005931x^2 + 22.113868x + 94.357109$, $r^2 = 0.9999$
Accuracy Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	15.7 mg/kg (corresponding to 26.1 µg/L) clopyralid; corresponds to the lowest validated fortification level.	110 mg/kg ethofumesate (corresponding to 183 µg/L); corresponds to the lowest validated fortification level.
Matrix effects	Matrix effects are below ± 20% for all analytes and are therefore non-significant.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 1 and is suitable for the determination of clopyralid and ethofumesate in sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan with an LOQ of 15.7 mg/kg for clopyralid and 110 mg/kg of ethofumesate.

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in feeding diet for honey bee larvae (diet C) with an LOQ of 0.702 mg/kg for clopyralid and 4.94 mg/kg of ethofumesate. Recovery results were in a range of 70 – 120% with an RSD $\leq$ 20%. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 1 have been met. The method is acceptable.
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Reference:	KCP 5.1.2/07 (filed under KCP 10.3.1.3/01)
Report	Kleebaum K., 2022: ADM.01851.H.1.A – Repeated Exposure of Honey Bee (<i>Apis mellifera</i> L.) Larvae under Laboratory Conditions, Report No: 22 48 BLC 0025, ADAMA Reference No.: 000110708
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for clopyralid and ethofumesate content determination was validated in feeding diet for honey bee larvae (diet C). The limit of quantification (LOQ) of the method was 0.702 mg/kg clopyralid and 4.94 mg/kg ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by LC-MS/MS.

Study period:

Experimental start date: 05. October, 2022

Experimental completion date: 06. October, 2022

Materials:

ADM.01851.H.1.A: Batch U3111P1, containing 6.43% (74.8g/L) Clopyralid, 45.2% (525.1 g/L Ethofumesate)

Clopyralid (reference material): Batch no. 264-3598, purity: 97.2%

Ethofumesate (reference material): Batch no. 290-3859, purity: 99.2%

Analytical method:

Method type	LC-MS/MS
Equipment	LC-MS Agilent 1200 system
Column	ACE C18, Amide 3 μ m, 100 * 2.1 mm
Column temperature	45°C
Injection volume	10 μ L
Flow rate	0.4 mL/min
Mobile phase	A: Water containing 0.1% acetic acid in water B: Acetonitrile 0.00 min 10% B 0.50 min 10% B 3.00 min 60% B 5.00 min 100% B Run time: 6.00 min
Retention time	Approx.2.45 min Clopyralid Approx.5.02 min Ethofumesate

Detector	Agilent G6460 Triple quadrupole mass spectrometer	
Ions monitored	Clopyralid: ESI positive, [M+H] +	Ethofumesate: ESI positive, [M+H] +
	MRM m/z 191.8 → 146 (quantifier) m/z 191.8 → 110 (qualifier)	MRM m/z 287.0 → 121.1 (quantifier) m/z 287.0 → 259 (qualifier)

Results and discussions

Recovery results were in a range of 70 – 120% % with an RSD ≤ 20 per level.

Table A 18: Recovery results from method validation of clopyralid in feeding diet for honey bee larvae (diet C)

Matrix	Nominal concentration (mg/kg)	Analysed concentration (mg/kg)	Mean analysed concentration (mg/kg)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	0.702	0.706 0.665 0.690 0.721 0.718	0.700	101 94.7 98.3 103 102	99.7	3.3
High	163	161 159 158 161 160	159	98.7 95.8 96.6 98.8 97.9	97.5	1.4

n.d.= clopyralid was not detected, LOQ = 0.702 mg/kg

Table A 19: Recovery results from method validation of ethofumesate in feeding diet for honey bee larvae (diet C)

Matrix	Nominal concentration (mg/kg)	Analysed concentration (mg/kg)	Mean analysed concentration (mg/kg)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	4.95	4.50 4.71 4.71 4.66 4.72	4.66	91.1 95.4 95.3 94.4 95.6	94.4	2.0
High	1148	1087 1052 1065 1059 1039	1060	94.6 91.6 92.7 92.2 90.2	92.3	1.2

n.d.= etofumesate was not detected, LOQ = 4.94 mg/kg

Table A 20: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in feeding diet for honey bee larvae (diet C)

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by LC-MS/MS detection and the absence of interfering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	The detector response for clopyralid was quadratic in the range from 1.72 to 43.0 µg/L (at least 5 levels). $Y=0.068002x^2+32.962827x+11.153052$, $r^2=0.9997$	The detector response for etofumesate was quadratic in the range from 12.1 to 302 µg/L (at least 5 levels). $Y=-0.018539x^2+40.489586x+44.659067$, $r^2=0.9996$
Accuracy Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	0.702 mg/kg (corresponding to 8.43µg/L) clopyralid; corresponds to the lowest validated fortification level.	4.94 mg/kg ethofumesate (corresponding to 59.3µg/L); corresponds to the lowest validated fortification level.
Matrix effects	Matrix effects are below ± 20% for all analytes and are therefore non-significant.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 1 and is suitable for the determination of clopyralid and ethofumesate in feeding diet for honey bee larvae (diet C) with an LOQ of 0.702 mg/kg for clopyralid and 4.94 mg/kg of ethofumesate.

Comments of zRMS:	The method has been validated for determination of clopyralid and ethofumesate in aqueous spray solution with an LOQ of 354.9 mg/L clopyralid and 2429 mg/L ethofumesate. Recovery results were in a range of 70 – 120% with an RSD ≤ 20%. The validity criteria for the analytical method according to SANTE/2020/12830 rev. 2 have been met. The method is acceptable.
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Reference:	CP 5.1.2/08 (filed under KCP 10.6.2/01)
Report	Effects of ADM.01851.H.1.A on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions, Friedemann A., 2024, Report No: 23 46 PSE 0012, ADAMA Reference No.: 000116380
Guideline(s):	SANTE/2020/12830 rev. 2
Deviations:	None
GLP:	Yes
Acceptability:	Yes

And

Reference:	CP 5.1.2/09 (filed under KCP 10.6.2/02)
Report	Effects of ADM.01851.H.1.A on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions, Friedemann A., 2024a, Report No: 23 46 PVV 0011, ADAMA Reference No.: 000116381
Guideline(s):	SANTE/2020/12830 rev. 2
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for concentration determination of clopyralid and ethofumesate in test substance ADM.01851.H.1.A was validated in test solution (aqueous spray solution). The limit of quantification (LOQ) of the method was 354.9 mg/L clopyralid and 2429 mg/L ethofumesate. The quantitative measurements of clopyralid and ethofumesate were achieved by HPLC-DAD.

Study period:

Experimental start date: 06 December, 2023

Experimental completion date: 22 December, 2023

Materials:

ADM.01851.H.1.A: Batch U3111P1, containing 6.43% (74.8g/L) Clopyralid, 45.2% (525.1 g/L Ethofumesate)

Clopyralid (reference material): Batch no. 264-3598, purity: 97.2%

Ethofumesate (reference material): Batch no. 290-3859, purity: 99.2%

Analytical method:

Method type	LC-MS/MS
Equipment	Shimadzu HPLC system with Shimadzu Photo-array detector (PDA)
Column	Phenomenex Synergy Polar, 150 x 2.0 mm, 4 µm
Column temperature	40°C
Injection volume	11 µL
Flow rate	0.00 min 0.280 mL/min 5.20 min 0.400 mL/min 6.50 min 0.400 mL/min 8.51 min 0.280 mL/min
Mobile phase	A: water with 0.1% formic acid (v+v) B: acetonitrile with 0.1% formic acid (v+v) 0.00 min 30% B 3.1 min 30% B 7.00 min 60% B 8.50 min 60% B 8.51 min 95% B 9.50 min 95% B 9.51 min 30% Run time: 13.00 min
Retention time	Approx.3.15 – 3.18 min for clopyralid Approx.8.61 – 8.65 min for ethofumesate
Detector	The signal was recorded from 190 nm to 315 nm. The wavelengths λ clopyralid = 224 nm and λ = ethofumesate = 279 nm

Results and discussions

Recovery results were in a range of 70 – 120% % with an RSD $\leq$ 20 per level.

Table A 21: Recovery results from method validation of clopyralid in aqueous spray solution

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	354.9		359.3		101	0.18
High	969.6		983.3		101	0.37

n.d.= clopyralid was not detected, LOQ = 354.9 mg/L

Table A 22: Recovery results from method validation of ethofumesate in aqueous spray solution

Matrix	Nominal concentration (mg/L)	Analysed concentration (mg/L)	Mean analysed concentration (mg/L)	Recovery (%)	Mean recovery (%)	CV (%)
Blank	0.0000	n.d. n.d.	-	-	-	-
Low (LOQ)	2429		2421		100	1.0
High	6636		6693		101	1.4

n.d.= etofumesate was not detected, LOQ = 2429 mg/L

Table A 23: Characteristics for the analytical method used for validation of clopyralid and ethofumesate in aqueous spray solution

	Clopyralid	Ethofumesate
Specificity-interferences	The specificity of the method was assured by HPLC-DAD and the ansence of interffering peaks. Blank values did not exceed 30% of the method LOQ (<30% of LOQ).	
Linearity/calibration	External matrix matched calibtation in the range from 1.250 to 7.815 µg/L. The corresponding calibration range regarding analytical dilution was from 87.44 mg/L to 1202 mg/L (5 levels). $Y=104491x-271.437$, $r^2= 0.9999$	External matrix matched calibtation in the range from 8.643 to 54.02 mg/L. The corresponding calibration range regarding analytical dilution was from 604.4 mg/L to 8311 mg/L (5 levels). $Y=19246.4x-38073.41$, $r^2= 0.9999$
Accuracy Recovery	Mean recoveries at each level were in the range between 70% and 120%. (see table above).	
Repeatability	RSD was below 20% per level (see table above).	
LOQ	354.9 mg/L clopyralid; corresponds to the lowest validated fortification level.	2429 mg/L ethofumesate; corresponds to the lowest validated fortification level.
LOD	87.44 mg/l clopyralid; corresponds to 1.250 mg/L regarding overall dilution.	604.4 mg/L ethofumesate; corresponds to 8.643 mg/L regarding overall dilution
Matrix effects	Matrix matched calibrations with analytical reference material including five calibration levels injected in duplicate were used.	

Conclusion

The analytical method fulfils the requirements of SANTE/2020/12830 rev. 2 and is suitable for the determination of clopyralid and ethofumesate in aqueous spray solution with an LOQ of 354.8 mg/L for clopyralid and 2429 mg/L of ethofumesate.

A 2.2.2 Methods for post-authorization control and monitoring purposes for the active substance of ethofumesate (KCP 5.2)

For validations of analytical methods used for data generation for risk assessment, please refer to A 2.1.1.

In relation to residues in the commodities for which use of this product is proposed (sugar beet and fodder beet) and in appropriate animal matrices and environmental samples, the applicant has full access to the appropriate monitoring methods found acceptable in the RARs (or to studies which have been deemed equivalent) and the conclusions made on the basis of those studies. No further consideration is therefore necessary in this evaluation.