

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB1817H

Product name: **Eledura**

Chemical active substances:

Prosulfocarb, 667 g/L

Diflufenican, 14 g/L

Halauxifen-methyl, 1.33 g/L

Cloquintocet-mexyl, 1.33 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Globachem NV

Submission date: May 2021

MS Finalisation date: January 2022

Version history

When	What
May 2021	Initial submission by applicant for approval of new product.
January 2022	Initial zRMS RR

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5 Analytical methods

5.1 Conclusion and summary of assessment

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

Sufficiently sensitive and selective analytical methods are **not** available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are: **none**

- ~~data gap 1~~
- ~~data gap 2~~
- ~~data gap 3~~

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions. All analytical methods are active substance data and were provided in the EU review of prosulfocarb, diflufenican and halauxifen-methyl. Moreover, the applicant submitted a method for prosulfocarb residues determination validated in dry and oily matrices as well as diflufenican residues determination fully validated method for drinking water. In addition a group of analytical methods suitable in aquatic toxicity and soil ecotox studies were submitted for prosulfocarb, diflufenican and halauxifen-methyl. All submitted in the context of this authorization request, methods have been accepted.

The text of the applicant was not rewritten. The zRMS, evaluating residue methods, text is on grey background.

Noticed data gaps are: none, in the context of this authorisation request.

Commodity/crop	Supported/ Not supported
Cereals	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 prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl in plant protection product is provided as follows:

Comments of zRMS:	The method is validated and can be applied for analysing, prosulfocarb, diflufenican and halauxifen-methyl in the PPP. zRMS considers presented data on Cloquintocet-mexyl (safener) validation as supplementary one only. It is not an active substance accepted at EU level.
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Reference: KCP 5.1.1

Report	Validation of the methods of determination of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl in an EC formulation containing 667 g/L prosulfocarb, 14 g/L diflufenican, 1.33 g/L halauxifen-methyl and 1.33 g/L cloquintocet-mexyl, in compliance with good laboratory practice. XXXX D., 2020, DNA5656
Guideline(s):	Yes, SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A sample is diluted in acetonitrile and analysed by LC-QQQ.

LC-QQQ LC Conditions – MS Analysis

Instrument:	Agilent 1200 Series LC-QQQ
Mode:	Isocratic Reverse Phase
Column:	Grace Genesis C8, (250mm x 4.6mm)
Packing:	C8, 3µm
Eluent:	75% Acetonitrile + 25% Deionised Water with Trifluoro Acetic Acid at pH 2.6
Flow Rate:	1.0mL/min
Injection Volume:	2.0µL
Column Temperature:	25°C
Data Collection:	Mass Hunter
Retention Time:	Prosulfocarb: approximately 8.2 minutes Diflufenican: approximately 6.9 minutes Halauxifen-methyl: approximately 4.6 minutes Cloquintocet-mexyl: approximately 6.4 minutes

LC-QQQ MS Conditions – MS Analysis

Instrument:	Agilent 6470 Series QQQ Mass Spectrometer		
Ionisation:	Positive	Sheath Gas Temperature:	350°C
Gas Temperature:	250°C	Sheath Gas flow:	10L/min
Gas Flow:	8L/min	Capillary Voltage:	3500V
Nebulizer:	30psi	Nozzle Voltage:	500V

Prosulfocarb:

MRM Precursor Ion: 252.1m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
252.1	128.1	200	121	12	5
252.1	91.1	200	121	36	5
252.1	65.1	200	121	68	5

Diflufenican:

MRM Precursor Ion: 395.1m/z

MRM	MRM	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)

Precursor Ion (m/z)	Product Ion (m/z)				
395.1	266.0	200	179	28	5
395.1	246.0	200	179	44	5
395.1	238.0	200	179	48	5

Halauxifen-methyl:

MRM Precursor Ion: 345.0m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
345.0	284.8	200	88	20	5
345.0	249.8	200	88	36	5
345.0	206.8	200	88	64	5

Cloquintocet-mexyl:

MRM Precursor Ion: 336.1m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
336.1	191.9	200	88	32	5
336.1	161.9	200	88	60	5
336.1	237.8	200	88	16	5
336.1	178.9	200	88	40	5
336.1	150.9	200	88	64	5

The LC-QQQ is extremely sensitive to the Cloquintocet-Mexyl molecules which provides a very strong response. To keep the calibration within a linear scale two ion transitions were used for the quantitative analysis. Three additional spectral ions were added for the Mass Spectral analysis.

Data Acquisition: Mass Hunter

The standards are prepared in acetonitrile.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of the active substances prosulfocarb, diflufenican and halauxifen-methyl and the safener cloquintocet-mexyl in plant protection product GLOB1817H

	Prosulfocarb	Diflufenican	Halauxifen-methyl	Cloquintocet-mexyl
Author(s), year	XXXX D., 2020			
Principle of method	LC-QQQ			
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	0 - 10.0 mg/L r = 0.9983	0 - 10.0 mg/L r = 0.9997	0 - 1.0 mg/L r = 0.9984	0 - 0.05 mg/L r = 0.9983

	Prosulfocarb	Di flufenican	Halauxifen-methyl	Cloquintocet-mexyl
n=7				
Precision – Repeatability Mean n = 6 (%RSD)	Mean: 668.0 ± 2.956 g/L %RSD: 0.443 Horrat: 0.311	Mean: 13.82 ± 0.086 g/L %RSD: 0.622 Horrat: 0.244	Mean: 1.316 ± 0.012 g/L %RSD: 0.912 Horrat: 0.251	Mean: 1.335 ± 0.00913 g/L %RSD: 0.684
Accuracy n = 6 (% Recovery)	Mean: 100.7 ± 0.523% %RSD: 0.519 Horrat: 0.364	Mean: 99.56 ± 0.842% %RSD: 0.846 Horrat: 0.332	Mean: 96.88 ± 0.854% %RSD: 0.882 Horrat: 0.242	Mean: 99.12 ± 0.120% %RSD: 0.121
Interference/ Specificity	In the specificity chromatograms 1 mg/L prosulfocarb eluted at 8.2 min with a response of 292548. Other significant peaks were accounted for by assaying samples of the three other active ingredients, a solvent blank and a sample of the formulation blank. There were no other peaks present in these chromatograms at the same elution time as prosulfocarb. This demonstrates that there were no analyte interferences.	In the specificity chromatograms 10 mg/L diflufenican eluted at 6.9 min with a response of 133073. Other significant peaks were accounted for by assaying samples of the three other active ingredients, a solvent blank and a sample of the formulation blank. There were no other peaks present in these chromatograms at the same elution time as diflufenican. This demonstrates that there were no analyte interferences.	In the specificity chromatograms 1.0 mg/L halauxifen-methyl eluted at 4.6 min with a response of 956427. Other significant peaks were accounted for by assaying samples of the three other active ingredients, a solvent blank and a sample of the formulation blank. There were no other peaks present in these chromatograms at the same elution time as halauxifen-methyl for any of the three other active ingredients or the solvent blank. The formulation blank provided an area of 711 for halauxifen-methyl. This equates to relative concentration of 0.003 g/L in the formulation which is 0.22% relative to the active ingredient concentration. This is below the 3% required in the SANCO guidelines. This demonstrates that there were no analyte interferences.	In the specificity chromatograms 0.1 mg/L cloquintocet-mexyl eluted at 6.4 min with a response of 127850. Other significant peaks were accounted for by assaying samples of the three other active ingredients, a solvent blank and a sample of the formulation blank. There were peaks present in these chromatograms at the same elution time as cloquintocet-mexyl for the three other active ingredients, solvent blank and the formulation blank. These are thought to be a small contamination within the HPLC system. The peaks all related to concentrations of less than 0.0004 g/L relative to the concentration in formulation, which is less than 0.03% relative to the active ingredient concentration. This is below the 3% required in the SANCO guidelines. This demonstrates that there were no analyte interferences.
Comment	-	-	-	-

Conclusion

The validation parameters for these methodologies have been met for this study under the SANCO/3030/99 rev. 5 guidelines.

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

GLOB1817H does not contain relevant impurities.

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

GLOB1817H does not contain relevant formulants.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods available for analysing one of these active substances/safener in the presence of the other active substances/safener.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: Prosulfocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	Primary	0.01 mg/kg	LC-MS/MS	XXXX F., 2010*
	Confirmatory (if required)	-	Not required	-
Soil (Environmental fate)	Primary	0.02 mg/kg	GC-MSD	Crook, 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Environmental fate)	Primary	0.1 µg/L	GC-MSD	Hargreaves, 1999/EU agreed
	Confirmatory (if required)	-	Not required	-
Air (Exposure)	Primary	0.00015 µg/m ³	GC-MSD	Kwaitkowski, 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Ecotoxicology)	Primary	97.82 µg/L	HPLC-MS/MS	XXXX D., 2021a
		3.354 µg/L	HPLC-MS/MS	XXXX D., 2021b
		13.92 µg/L	HPLC-MS/MS	XXXX D., 2021c
		1.467 µg/L	HPLC-MS/MS	XXXX D., 2021d
	Confirmatory (if required)	-	Not required	-
Soil (Ecotoxicology)	Primary	0.1 mg/kg	HPLC-UV	XXXX L., 2015
		0.02 mg/L	HPLC-UV	XXXX D., 2008
Other (Ecotoxicology)	Primary	5.73 mg/kg	HPLC-MS/MS	XXXX K., 2021
		20441 mg/L	HPLC-MS/MS	XXXX K., 2021
		5.55 mg/kg	HPLC-MS/MS	XXXX S., 2021
		4.64 mg/kg	HPLC-MS/MS	XXXX K., 2021
		2858 mg/L	HPLC-MS/MS	XXXX M., 2021
Metabolites of prosulfocarb: Prosulfocarb sulfoxide				
Water (Ecotoxicology)	Primary	6.21 µg/L	HPLC-MS/MS and UV/VIS	XXXX D., 2012a
		0.038 mg/L		XXXX D., 2012b
		0.1002 mg/L		XXXX D., 2012c
		0.1002 mg/L		XXXX D., 2012d
		0.01076 mg/L	HPLC-UV/VIS	XXXX D., 2012e
Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed

Plants, plant products,... (Residues)	Primary	0.01 mg/kg	GC-ECD	Class T., 2001/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Class T., 2001/EU agreed
Animal products, food of animal origin,... (Residues)	Primary	0.01 mg/kg (milk) 0.02 mg/kg (tissue)	GC-MS	Class T., 1999/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	Class T., 1999/EU agreed
Soil (Environmental fate)	Primary	0.002 mg/kg	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Environmental fate)	Primary	0.05 µg/L	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Air (Exposure)	Primary	0.4 µg/m ³	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Ecotoxicology)	Primary	2.064 µg/L	HPLC-MS/MS	XXXX D., 2021a
		0.07079 µg/L	HPLC-MS/MS	XXXX D., 2021b
		0.2939 µg/L	HPLC-MS/MS	XXXX D., 2021c
		0.03097 µg/L	HPLC-MS/MS	XXXX D., 2021d
	Confirmatory (if required)	-	Not required	-
Other (Ecotoxicology)	Primary	0.12 mg/kg	HPLC-MS/MS	XXXX K., 2021
		431.42 mg/L	HPLC-MS/MS	XXXX K., 2021
		0.12 mg/kg	HPLC-MS/MS	XXXX S., 2021
		0.10 mg/kg	HPLC-MS/MS	XXXX K., 2021
		605.3 mg/L	HPLC-MS/MS	XXXX M., 2021
Component of residue definition: Halauxifen-methyl (sum of halauxifen-methyl and X11393729 (halauxifen), expressed as halauxifen-methyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Ma M., Li Q., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Animal products, food of animal origin,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Ma M., Li Q., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Soil (Environmental fate)	Primary	0.05 µg/kg	LC-MS/MS	Blakeslee B.A., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-

Water (Environmental fate)	Primary	0.05 µg/L	LC-MS/MS	Rodrigues Junior A., Souza N, Li Q., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Air (Exposure)	Primary	0.82 µg/m ³	LC-MS/MS	Class T., 2011/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Ecotoxicology)	Primary	0.1923 µg/L	HPLC-MS/MS	XXXX D., 2021a
		0.006595 µg/L	HPLC-MS/MS	XXXX D., 2021b
		0.02738 µg/L	HPLC-MS/MS	XXXX D., 2021c
		0.002885 µg/L	HPLC-MS/MS	XXXX D., 2021d
	Confirmatory (if required)	-	Not required	-
Other (Ecotoxicology)	Primary	0.01 mg/kg	HPLC-MS/MS	XXXX K., 2021
		40.19 mg/L	HPLC-MS/MS	XXXX K., 2021
		0.01 mg/kg	HPLC-MS/MS	XXXX S., 2021
		0.01 mg/kg	HPLC-MS/MS	XXXX K., 2021
		5.640 mg/L	HPLC-MS/MS	XXXX M., 2021
Analyte: Cloquintocet-mexyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	McLean N., 2006/EU agreed
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Class T., 2005/EU agreed
Other (Ecotoxicology)	Primary	5.752 mg/L	HPLC-MS/MS	XXXX M., 2021

*Validation of the method used in the residue trials submitted in section B7.

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 substance 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 prosulfocarb (KCP 5.2)

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

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

Table 5.3-1: 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	Prosulfocarb	0.01 mg/kg	EFSA, 2007
Plant, high acid content		0.01 mg/kg	EFSA, 2007
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA, 2007
Plant, high oil content		0.01 mg/kg	EFSA, 2007
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	EFSA, 2007
Muscle	Not required		EFSA, 2007
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Prosulfocarb	0.02 mg/kg	EFSA, 2007
Drinking water (Human toxicology)	Prosulfocarb	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Prosulfocarb	0.1 µg/L	EFSA, 2007
Air	Prosulfocarb	0.00015 mg/m ³	AOEL: 0.007 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of prosulfocarb in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: Prosulfocarb				
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	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
High acid content	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
High oil content	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
Difficult (if required, depends on intended use)	Not required			

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
Required, available from:	The DFG S19 multi-residue method uses mixtures of acetone and water as an extraction solvent. Acetone/water mixtures and acetonitrile/water mixtures are considered to be of similar polarity. Prosulfocarb is equally soluble in both solvent mixtures. The solvent system is considered to be similar to that used in the carrot metabolism study (Derz, 2015). The extraction system is therefore validated and fit-for-purpose.
Not required, because:	-

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

Not required.

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

An overview on the acceptable methods and possible data gaps for analysis of prosulfocarb in soil is given in the following tables.

Table 5.3-4: Validated methods for soil (if appropriate)

Component of residue definition: Prosulfocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.02 mg/kg	GC-MSD	Crook, 2000/EU agreed
Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of prosulfocarb in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3-5: Validated methods for water (if appropriate)

Component of residue definition: Prosulfocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.1 µg/L	GC-MSD	Hargreaves, 1999/EU agreed
	ILV	missing		
	Confirmatory	-	Not required	-

Component of residue definition: Prosulfocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Surface water	Primary	0.1 µg/L	GC-MSD	Hargreaves, 1999/EU agreed
	Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

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 prosulfocarb in air is given in the following tables.

Table 5.3-6: Validated methods for air (if appropriate)

Component of residue definition: Prosulfocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.00015 µg/m ³	GC-MSD	Kwaitkowski, 2002/EU agreed
Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

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

Prosulfocarb and prosulfocarb metabolites are not defined as “relevant for monitoring” and are not categorised as toxic or very toxic in any recognised classification system. Consequently, analytical methods for post-approval control of residues in body fluids and tissues are not required.

5.3.2.8 Other studies/ information

No other studies were submitted.

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

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 5.3-7: 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	Diflufenican	0.01 mg/kg	EFSA, 2007
Plant, high acid content		0.01 mg/kg	EFSA, 2007
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA, 2007
Plant, high oil content		0.01 mg/kg	EFSA, 2007
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	EFSA, 2007
Muscle	Diflufenican	0.02 mg/kg	EFSA, 2007
Milk		0.01 mg/kg	EFSA, 2007
Eggs		0.02 mg/kg	EFSA, 2007
Fat		0.02 mg/kg	EFSA, 2007
Liver, kidney		0.02 mg/kg	EFSA, 2007
Soil (Ecotoxicology)	Diflufenican	0.002 mg/kg	EFSA, 2007
Drinking water (Human toxicology)	Diflufenican	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Diflufenican	0.05 µg/L	EFSA, 2007
Air	Diflufenican	0.4 µg/m ³	AOEL sys/AOEL inhal: 0.11 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of diflufenican in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-8: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Diflufenican				
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	Primary	0.01 mg/kg	GC-ECD	Bacher R., 2002/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Thom M., 2003/EU agreed

Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Bacher R., 2002/EU agreed
High acid content	Primary	0.01 mg/kg	GC-ECD	Bacher R., 2002/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Thom M., 2003/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Bacher R., 2002/EU agreed
High oil content	Primary	0.01 mg/kg	GC-ECD	Bacher R., 2002/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Thom M., 2003/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Bacher R., 2002/EU agreed
High protein/high starch content (dry)	Primary	0.01 mg/kg	GC-ECD	Class T., 2001/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Klumpp M., 2001/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Class T., 2001/EU agreed
Difficult (if required, depends on intended use)	Primary	Not required		
	ILV			
	Confirmatory (if required)			

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 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	As the same solvent, acetonitrile, is used in both methods, the method extraction can be considered comparable to the extraction in the metabolism study.
Not required, because:	-

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 diflufenican in animal matrices is given in the following tables.

Table 5.3-10: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	GC-MS	Class T., 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	Klumpp M., 2002/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-ECD	Class T., 1999/EU agreed
Eggs	Primary	0.02 mg/kg	GC-MS	Class T., 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	Klumpp M., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	Class T., 1999/EU agreed
Muscle	Primary	0.02 mg/kg	GC-MS	Class T., 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	Klumpp M., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	Class T., 1999/EU agreed
Fat	Primary	0.02 mg/kg	GC-MS	Class T., 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	Klumpp M., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	Class T., 1999/EU agreed
Kidney, liver	Primary	0.02 mg/kg	GC-MS	Class T., 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	Klumpp M., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	Class T., 1999/EU agreed

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

Table 5.3-11: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	As the same solvent, acetonitrile, is used in both methods, the method extraction can be considered comparable to the extraction in the metabolism study.
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of diflufenican in soil is given in the following tables.

Table 5.3-12: Validated methods for soil (if appropriate)

Component of residue definition: Diflufenican			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.002 mg/kg	LC-MS/MS	Bacher R., 2002/EU agreed
Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of diflufenican in surface and drinking water is given in the following tables.

Table 5.3-13: Validated methods for water (if appropriate)

Component of residue definition: diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Bacher R., 2002/EU agreed
	Primary	0.05 µg/L	LC-MS/MS	XXXX G., 2008
	ILV	0.05 µg/L	LC-MS/MS	XXXX H., 2016
	Confirmatory	-	Not required	-
Surface water	Primary	0.05 µg/L	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

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 diflufenican in air is given in the following tables.

Table 5.3-14: Validated methods for air (if appropriate)

Component of residue definition: diflufenican			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.4 µg/m ³	LC-MS/MS	Bacher R., 2002/EU agreed
Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for air it is referred to

Appendix 2.

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

Not required as diflufenican is not classified as toxic.

5.3.3.8 Other studies/ information

No other studies were submitted.

5.3.4 Description of analytical methods for the determination of residues halauxifen-methyl (KCP 5.2)

5.3.4.1 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 5.3-15: 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	Sum of halauxifen-methyl and X11393729 (halauxifen), expressed as halauxifen-methyl (restricted to cereals)	0.01 mg/kg	EFSA, 2014
Plant, high acid content		0.01 mg/kg	EFSA, 2014
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA, 2014
Plant, high oil content		0.01 mg/kg	EFSA, 2014
Plant, difficult matrices (hops, spices, tea)		Not required	
Muscle	Not required	0.01 mg/kg	EFSA, 2014
Milk		0.01 mg/kg	EFSA, 2014
Eggs		0.01 mg/kg	EFSA, 2014
Fat		0.01 mg/kg	EFSA, 2014
Liver, kidney		0.01 mg/kg	EFSA, 2014
Soil (Ecotoxicology)	Halauxifen-methyl	0.05 µg/kg	EFSA, 2014
Drinking water (Human toxicology)	Halauxifen-methyl	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Halauxifen-methyl and X11393729 (halauxifen)	0.05 µg/L	EFSA, 2014
Air	Halauxifen-methyl	0.82 µg/m ³	AOEL: 0.058 mg/kg bw/d
Tissue (meat or liver)	Halauxifen-methyl	Not required	Not classified as T / T+

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Body fluids		Not required	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of halauxifen-methyl in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-16: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Sum of halauxifen-methyl and X11393729 (halauxifen), expressed as halauxifen-methyl (restricted to cereals)				
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	Primary	0.01 mg/kg	LC-MS/MS	Daneva E., Taeufer A., 2011 and Ma M., 2012/EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Bacher R., 2011 and Robaugh D.A., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Daneva E., Taeufer A., 2011 and Ma M., 2012/EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Bacher R., 2011 and Robaugh D.A., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Daneva E., Taeufer A., 2011 and Ma M., 2012/EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Bacher R., 2011 and Robaugh D.A., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Daneva E., Taeufer A., 2011 and Ma M., 2012/EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Bacher R., 2011 and Robaugh D.A., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Difficult (if required, depends on intended use)	Not required			

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 5.3-17: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Residues are not expected to be found in cereal grain (representative and intended use). Therefore, extraction efficiency does not need to be addressed for this evaluation.
Not required, because:	-

5.3.4.3 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 halauxifen-methyl in animal matrices is given in the following tables.

It should be noted, however, that no residue definition has been set for food of animal origin.

Table 5.3-18: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Not required				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., 2011 and Ma M., Li Q., 2012/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Bacher R., 2011 and Langridge G., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Eggs	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., 2011 and Ma M., Li Q., 2012/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Bacher R., 2011 and Langridge G., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., 2011 and Ma M., Li Q., 2012/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Bacher R., 2011 and Langridge G., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-
Fat	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., 2011 and Ma M., Li Q., 2012/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Bacher R., 2011 and Langridge G., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-

Component of residue definition: Not required				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., 2011 and Ma M., Li Q., 2012/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Bacher R., 2011 and Langridge G., 2012/EU agreed
	Confirmatory (if required)	-	Not required	-

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

Table 5.3-19: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Residues are not expected to be found in products of animal origin for the proposed use.

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

An overview on the acceptable methods and possible data gaps for analysis of halauxifen-methyl in soil is given in the following tables.

Table 5.3-20: Validated methods for soil (if appropriate)

Component of residue definition: Halauxifen-methyl			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 µg/kg	LC-MS/MS	Blakeslee B.A., 2012/EU agreed
Confirmatory	Not required		

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of halauxifen-methyl in surface and drinking water is given in the following tables.

Table 5.3-21: Validated methods for water (if appropriate)

Component of residue definition: Halauxifen-methyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Rodrigues Junior A., Souza N., Li Q., 2012/EU agreed
	ILV	0.05 µg/L	LC-MS/MS	Gemrot F., 2012/EU agreed
	Confirmatory	Not required		
Component of residue definition: Halauxifen-methyl and X11393729 (halauxifen)				
Surface water	Primary	0.05 µg/L	LC-MS/MS	EU agreed
	Confirmatory	Not required		

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of halauxifen-methyl in air is given in the following tables.

Table 5.3-22: Validated methods for air (if appropriate)

Component of residue definition: Halauxifen-methyl			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.82 µg/m ³	LC-MS/MS	Class T., 2011/EU agreed
Confirmatory	Not required		

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.4.7 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 halauxifen-methyl in body fluids and tissues is given in the following table.

Table 5.3-23: Methods for body fluids and tissues (if appropriate)

Component of residue definition: not required			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Halauxifen-methyl is not classified as toxic or highly toxic; therefore monitoring methods for human tissues and body fluids are not required.		
Confirmatory			

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

5.3.4.8 Other studies/ information

No other studies are submitted.

5.3.5 Description of analytical methods for the determination of residues cloquintocet-mexyl (KCP 5.2)

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

No residue definitions for cloquintocet-mexyl has been set on EU level. Nevertheless, a method for determination of residues of cloquintocet-mexyl and its metabolite cloquintocet-acid in dry commodities is available.

Table 5.3-24: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Analyte	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Not required		
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)	Cloquintocet-mexyl Cloquintocet acid	0.01 mg/kg	UK, 2013 (DAR halauxifen-methyl)
Plant, high oil content	Not required		
Plant, difficult matrices (hops, spices, tea)			
Muscle	Not required		
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Not required		
Drinking water (Human toxicology)	Not required		
Surface water (Ecotoxicology)	Not required		
Air	Not required		
Tissue (meat or liver)	Not required		
Body fluids			

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

An overview on the acceptable methods and possible data gaps for analysis of cloquintocet-mexyl in plant matrices is given in the following tables.

Table 5.3-25: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Analyte: Cloquintocet-mexyl				
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	Not required			
High acid content	Not required			
High oil content	Not required			
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	McLean N., 2006/EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Class T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
Difficult (if required, depends on intended use)	Not required			

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 5.3-26: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	No residue definition set on EU level for cloquintocet-mexyl.

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

Not required since no residue definition has been set for cloquintocet-mexyl for food of animal origin.

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

Not required since no residue definition has been set for cloquintocet-mexyl for soil.

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

Not required since no residue definition has been set for cloquintocet-mexyl for water.

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

Not required since no residue definition has been set for cloquintocet-mexyl for air.

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

Not required.

5.3.5.8 Other studies/ information

No other studies are submitted.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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	XXXX D.	2020	Validation of the Methods of Determination of Prosulfocarb, Diflufenican, Halauxifen-methyl and Cloquintocet-Mexyl in an EC Formulation containing 667g/L Prosulfocarb, 14g/L Diflufenican, 1.33g/L Halauxifen-methyl and 1.33g/L Cloquintocet-Mexyl, in Compliance with Good Laboratory Practice DNA5656 David Norris Analytical Laboratories Ltd. GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP 10.2.1</i>	XXXX D.	2021a	Acute toxicity of GLOB1817H to <i>Daphnia magna</i> in a 48-hour semi-static test 2 48 ADL 0015 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP 10.2.1</i>	XXXX D.	2021b	Effects of GLOB1817H on <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test 20 48 AAL 0019 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2	XXXX D.	2021c	Effects of GLOB1817H on Lemna gibba in a growth inhibition test under semi-static test conditions	N	Globachem

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
<i>Submitted as KCP 10.2.1</i>			20 48 ALE 0017 Biochem Agrar GmbH GLP Unpublished		NV
<i>KCP 5.1.2 Submitted as KCP 10.2.1</i>	XXXX D.	2021d	Effect of GLOB1817H on <i>Myriophyllum spicatum</i> in a semi-static water-sediment system 20 48 AMS 0010 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
<i>KCP 5.1.2 Submitted as KCA 8.2.6.1</i>	XXXX, D.	2012a	Effects of Prosulfocarb sulfoxide on <i>Chlamydomonas reinhardtii</i> in an algal growth inhibition test 12 10 48 057 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
<i>KCP 5.1.2 Submitted as KCA 8.2.6.1</i>	XXXX, D.	2012b	Effects of Prosulfocarb sulfoxide on <i>Chlorella vulgaris</i> in an algal growth inhibition test 12 10 48 059 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
<i>KCP 5.1.2 Submitted as KCA 8.2.6.2</i>	XXXX, D.	2012c	Effects of Prosulfocarb sulfoxide on <i>Anabaena flos-aquae</i> in an algal growth inhibition test 12 10 48 058 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
<i>KCP 5.1.2 Submitted as KCA 8.2.6.2</i>	XXXX, D.	2012d	Effects of Prosulfocarb sulfoxide on <i>Navicula pelliculosa</i> in an algal growth inhibition test 12 10 48 053 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV

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 <i>Submitted as KCA</i> 8.2.6.2	XXXX, D.	2012e	Effects of Prosulfocarb sulfoxide on <i>Skeletonema costatum</i> in an algal growth inhibition test 12 10 48 060 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP</i> 10.3.1.1.1	XXXX, K.	2021	Acute toxicity of GLOB1817H to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions 20 48 BBA 0029 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP</i> 10.3.1.2	XXXX, S.	2021	Chronic toxicity of GLOB1817H to the honey bee <i>Apis mellifera</i> L. under laboratory conditions 20 48 BAC 0071 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP</i> 10.3.1.3	XXXX, K.	2021	GLOB1817H – Repeated exposure of the honey bee (<i>Apis mellifera</i> L.) larvae under laboratory conditions 20 48 BLC 0052 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP</i> 10.4.1.2	XXXX, L.	2015	Effects of prosulfocarb 800 g/L EC on earthworms under field conditions. Biochem Agrar Report Number 14 10 48 008 F GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCA</i> 8.1.3	XXXX, D.	2008	The bioaccumulation potential of prosulfocarb in earthworm (<i>Eisenia foetida foetida</i>). ENV8333/040822 Chemex Environmental International Ltd GLP	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.1.2 <i>Submitted as KCP 10.6</i>	XXXX, M.	2021	GLOB1817H: terrestrial plant test: vegetative vigour test STC/20/E1409 Stockbridge Technology Center Ltd GLP Unpublished	N	Globachem NV
KCP 5.1.2	XXXX F.	2010	Validation of the Analytical Method for the Determination of Prosulfocarb Residues in Potato Tubers, Sunflower Seeds and Winter Wheat Whole Plant + Amendment 1 to final report No R A9085 (2014) Anadiag R A9085 GLP Unpublished	N	Globachem NV
KCP 5.2	XXXX G.	2008	Development and validation of a method for the determination of diflufenican and two metabolites in surface water and drinking water. PGD-307 Central Science Laboratory GLP Unpublished	N	Sapac Group & Globachem NV & Punjab Chemicals and Crop Protection Ltd.
KCP 5.2	XXXX H.	2016	Validation of an analytical method for the determination of diflufenican in drinking water, ILV. VAL10/16 Laboratório de residuos Sapac Agro S.A. GLP Unpublished	N	Sapac Agro S.A. & Globachem NV

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

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
None					

The following tables are to be completed by MS

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for prosulfocarb

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

Reference is made to 5.2.1 for a summary of the methods used to determine the active substances in the formulated product.

The methods used to determine the active substances in the samples from the ecotoxicological studies are submitted and summarized in the section B9.

A 2.1.1.1 Analytical method for prosulfocarb in cereals

A 2.1.1.1.1 Method validation

Comments of zRMS:	The validation in potato tubers, sunflowers and winter wheat at 0,01 mg/kg has been accepted. Analysis of Prosulfocarb in LC/MS-MS, with monitoring of two transitions can be considered highly specific. The use of confirmation method is not necessary. On 2 validated levels a mean recovery is within the range 70-110 % with a RSD less than 20 %.
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Reference:	KCP 5.1.2
Report	Validation of the Analytical Method for the Determination of Prosulfocarb Residues in Potato Tubers, Sunflower Seeds and Winter Wheat Whole Plant + Amendment 1 to final report No. R A9085 (2014), XXXX F, 2010, R A9085.
Guideline(s):	Yes, SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and Methods

The method is based on the following reference: “Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method.”

Residues are extracted with acetonitrile/acetic acid 99.9 : 0.1 % in the presence of magnesium sulfate and sodium chloride. After centrifugation the extract is purified with magnesium sulfate and PSA. The internal standard (triphenylphosphate in acetonitrile) and formic acid are added to the extract before analysis by liquid chromatography using a MS/MS detector with the following conditions:

Apparatus	UPLC /MS /MS
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Column

Description	BEH C18	Supplier	WATERS	Particles	1.7 µm
Internal diam. x length	2.1*100 mm	Supplier reference	186002352	Temperature	40 °C
Development Column ANADIAG Number	130	Stationary Phase	C18	Comment	-

Mobile phase

A =	Methanol HPLC/ H2O HPLC 20:80 + 5 mM ammonium acetate	C =	-
B =	Methanol HPLC/ H2O HPLC 90:10 + 5 mM ammonium acetate	D =	-

Sample temperature	15 °C
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Elution

Elution	Time min	Flow ml/min	Composition (%)				Curve (type)
			A	B	C	D	
Pg1	0.00	0.40	100	0	-	-	1
Pg2	4.00	0.40	0	100	-	-	1
Pg3	6.40	0.40	0	100	-	-	1
Pg4	6.50	0.40	100	0	-	-	1
Pg5	8.00	0.40	100	0	-	-	1

Detector

IONISATION mode*	ES x	APCI
Polarity*	Pos x	Neg

*make a cross in the right choice

Active ingredient(s)	Cone voltage	Collision Voltage	Dwell time (ms)	TRANSITION 1	TRANSITION 2	RT (min.)
				Parent > Daughter	Parent > Daughter	
Prosulfocarb	20	15	50	252.1 > 91.2	-	≈ 4.8
	25	10		-	252.1 > 128.3	≈ 4.8
Triphenyl phosphate	40	27		327.0 > 215.0	-	≈ 4.5

Results and Discussion

The results of the validation of the 252.1 > 91.2 transition are given below and show that the method meets the requirements of the SANCO 825/00 rev. 8.1:

- Linearity:
 The linearity of the method was studied in matrix-matched calibration solutions between 3 ng/mL to 120 ng/mL of Prosulfocarb for potato tubers and sunflower seeds, and between 2 ng/mL to 60 ng/mL of Prosulfocarb for winter wheat whole plant. The linear correlation coefficients were typically > 0.990, showing a good linearity.
- Sensitivity:
 The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110 % with a RSD less than 20 % could be obtained.
 The LOQ was set at 0.01 mg/kg in potato tubers, sunflower seeds and winter wheat whole plant.
- Precision:
 Repeatability tests (5 recoveries at each fortification level) were performed at LOQ level and at 10 x LOQ for each matrix.

	Prosulfocarb
RSD for each fortification level	1.2 to 9.3 %
Overall RSD per sample material	2.0 to 10.8 %

All RSD determined were less than 20%, the method therefore fulfills the requirements of residue analytical methods.

- Recovery/accuracy:

The recovery results are presented hereunder. The accuracy of the method fulfills the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110 %.

Matrix	Active ingredient	Fortification level (mg/kg)	Mean recovery (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Potato tubers	Prosulfocarb	0.01	88.2	8.2	9.3	5
		0.10	101.3	7.6	7.5	5
		All levels	94.7	10.2	10.8	10
Sunflower seeds	Prosulfocarb	0.01	91.4	1.1	1.2	5
		0.10	88.7	1.1	1.3	5
		All levels	90.0	1.8	2.0	10
Winter wheat whole plant	Prosulfocarb	0.01	107.3	1.6	1.5	5
		0.10	105.3	3.9	3.7	5
		All levels	106.3	3.0	2.8	10

- Specificity:

Analysis of Prosulfocarb in LC/MS-MS, with monitoring of two transitions is considered as specific, thus the use of an alternative method was not necessary. However, to meet the requirements of the SANCO 825/00 rev. 8.1, the validation data of a second transition are provided below.

Table A 1: Validation data for a second transition at 0.01 ppm (252.1 > 128.3)

Crop/Matrix	Study	Analytical method
Winter wheat whole plant	Validation	Prosulfocarb in the untreated (n=2): <LOD Linearity: R ² = 0.996 Recovery whole plant (n=5): 99.7-108.1 (mean = 104.6%) Precision whole plant (n=5): 3.3%
Sunflower seeds	Validation	Prosulfocarb in the untreated (n=2): <LOD Linearity: R ² = 0.996 Recovery sunflower (n=5): 84.3-92.2% (mean = 89%) Precision sunflower: RSD (n=5): 3.3%
Potato tubers	Validation	Prosulfocarb in the untreated (n=2): <LOD Linearity: R ² = 0.996 Recovery potato (n=5): 71.4-91.3% (mean = 86.2%) Precision potato: RSD (n=5): 9.7%

Conclusion

The method meets the requirement of the SANCO 825/00 rev. 8.1 and can be used to reliably and accurately determine prosulfocarb in potato tubers, sunflowers and winter wheat to a limit of quantification of 0.01

mg/kg.

A 2.1.1.1.2 Analytical methods in water used in aquatic toxicity studies

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The method is considered acceptable. The method validation for prosulfocarb, diflufenican and halauxifen-methyl in water meets the requirements. All validation parameters are in required range.
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Reference:	KCP 5.2.1 (submitted as KCP 10.2.1)
Report	Acute toxicity of GLOB1817H to <i>Daphnia magna</i> in a 48 hour semi-static test, XXXX D., 2021a, 20 48 ADL 0015
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.1.2

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with deionised water and diluent in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 2: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Prosulfocarb	97.82 (5)	100.2	1.4
		1304 (5)	101.2	0.9
	Diflufenican	2.064 (5)	90.6	1.1
		27.53 (5)	93.2	0.8
	Halauxifen-methyl	0.1923 (5)	87.8	2.4
		2.565 (5)	90.4	0.9

Table A 3: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in reconstituted water

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Cubic $Y = -1.325X^3 + 343.5X^2 + 117602X + 181208$ $r^2 = 0.99990$ number of data points: 7	Linear $Y = 865847X + 9018$ $r^2 = 0.99968$ number of data points: 7	Quadratic $Y = -1986110X^2 + 5179720X - 6440$ $r^2 = 0.99973$ number of data points: 7
Calibration range	11.59 – 165.5 µg/L	0.2443 – 3.490 µg/L	0.02280 – 0.3258 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of determination/quantification	29.34 µg/L / 97.82 µg/L	0.6193 µg/L / 2.064 µg/L	0.05770 µg/L / 0.1923 µg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in reconstituted water.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb, diflufenican and halauxifen-methyl in aquatic matrix. RP-LC-MS/MS technique was employed. The validation parameters were in required range. The specificity of the method was assured by MS/MS detection and the absence of interfering peaks.
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Reference:	KCP 5.2.1 (submitted as KCP 10.2.1)
Report	Effects of GLOB1817H on <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test, XXXX D., 2021b, 20 48 AAL 0019
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with deionised water and/or diluent in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 4: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Prosulfocarb	3.354 (5)	103.2	1.2
		74.53 (5)	101.5	0.5
	Diflufenican	0.07079 (5)	100.9	9.3
		1.573 (5)	93.3	0.6
	Halauxifen-methyl	0.006595 (5)	88.2	1.9
		0.1466 (5)	88.5	1.2

Table A 5: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in OECD medium

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 176690X + 12164 r ² = 0.99993 number of data points: 8	Linear Y = 3664590X + 10807 r ² = 0.99982 number of data points: 8	Linear Y = 5986500X + 1397 r ² = 0.99996 number of data points: 8
Calibration range	0.7425 – 27.50 µg/L	0.01565 – 0.5798 µg/L	0.001461 – 0.05412 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of determination/quantification	1.006 µg/L / 3.354 µg/L	0.02124 µg/L / 0.07079 µg/L	0.001979 µg/L / 0.006595 µg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in OECD medium.

Comments of zRMS:	The method is considered acceptable. The purpose of the method was the determination of prosulfocarb, diflufenican and halauxifen-methyl in test solutions. RP-LC-MS/MS technique was applied. The validation parameters were in required range. The specificity of the method was assured by MS/MS detection and the absence of interfering peaks.
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Reference:	KCP 5.2.1 (submitted as KCP 10.2.1)
Report	Effects of GLOB1817H on <i>Lemna gibba</i> in a growth inhibition test under semi-static conditions, XXXX D., 2021c, 20 48 ALE 0017
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with diluent and/or deionised water in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 6: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
20x AAP medium	Prosulfocarb	13.92 (5)	108.9	1.4
		480.2 (5)	106.3	0.7
	Diflufenican	0.2939 (5)	95.9	1.6
		10.13 (5)	96.5	0.6
	Halauxifen-methyl	0.02738 (5)	95.5	2.1
		0.9442 (5)	94.4	2.4

Table A 7: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 20x AAP medium

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 154140X + 28666 r ² = 0.99988 number of data points: 7	Linear Y = 402287X + 10120 r ² = 0.99877 number of data points: 7	Linear Y = 4031440X + 3651 r ² = 0.99930 number of data points: 7
Calibration range	3.053 – 64.95 µg/L	0.06436 – 1.369 µg/L	0.006008 – 0.1278 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of determination/quantification	4.177 µg/L / 13.92 µg/L	0.08817 µg/L / 0.2939 µg/L	0.008215 µg/L / 0.02738 µg/L /

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in 20x AAP medium.

Comments of zRMS:	The method is considered acceptable.
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	The purpose of the method was the verification of the concentrations of prosulfocarb, diflufenican and halauxifen-methyl in the test solutions. RP-LC-MS/MS technique was applied. The validation parameters were in required range. The specificity of the method was assured by MS/MS detection and the absence of interfering peaks.
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Reference:	KCP 5.2.1 (submitted as KCP 10.2.1)
Report	Effects of GLOB1817H on <i>Myriophyllum spicatum</i> in a semi-static water-sediment system, XXXX D., 2021d, 20 48 AMS 0010
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with diluent and/or deionised water in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 8: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Smart and Barko medium	Prosulfocarb	1.467 (5)	95.8	2.5
		1334 (5)	99.6	1.0
	Diflufenican	0.03097 (5)	88.4	3.3
		28.15 (5)	89.4	0.7
	Halauxifen-methyl	0.002885 (5)	83.2	6.3
		2.623 (5)	87.5	1.2

Table A 9: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in Smart and Barko medium

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 189008X + 23020 r ² = 0.99971 number of data points: 8	Quadratic Y = -681034X ² + 6168920X - 11553 r ² = 0.99997	Linear Y = 5904100X + 1487 r ² = 0.99987 number of data points: 8

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
		number of data points: 8	
Calibration range	0.3243 – 16.21 µg/L	0.006837 – 0.3419 µg/L	0.0006382 – 0.03191 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of determination/quantification	0.4402 µg/L / 1.467 µg/L	0.009291 µg/L / 0.03097 µg/L	0.008656 µg/L / 0.002885 µg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in Smart and Barko medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. Only signals of the MS detector were used to calculate the results. The recoveries were within the required range. LOQ was set at 6,21 µg/L. UV detector data were reported as additional information.
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Reference:	KCP 5.2.1 (Submitted as KCA 8.2.6.1)
Report	Effects of Prosulfocarb sulfoxide on <i>Chlamydomonas reinhardtii</i> in an algal growth inhibition test, XXXX D., 2012a, 12 10 48 057 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 10: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb	MS	6.21 (5)	97	2.5

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
	sulfoxide		577.80 (5)	99	1.3
		UV	577.80 (5)	100	0.9
		UV detection could not be successfully validated, the sensitivity was too low. The results of the UV detector are reported as additional information.			

Table A 11: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 81.6976x - 83.746$ $r^2 = 0.9999$ MS/MS: Quadratic $y = 2.918629x^2 + 4249.561x + 11575.14$ $r^2 = 0.9993$ number of data points: 7
Calibration range	4.97 – 597.306 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	6.21 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 38,0 µg/L.
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Reference:	KCP 5.2.1 (Submitted as KCA 8.2.6.1)
Report	Effects of Prosulfocarb sulfoxide on <i>Chlorella vulgaris</i> in an algal growth inhibition test, XXXX D., 2012b, 12 10 48 059 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 12: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	38.0 (5)	104	3.5
			2003 (5)	98	5.4
		UV	38.0 (5)	89	5.3
			2003 (5)	100	1.0

Table A 13: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 44.3018x + 163.337$ $r^2 = 0.9999$ MS/MS: Quadratic $y = 0.2051259x^2 + 1214.308x + 14167.29$ $r^2 = 0.9994$ number of data points: 6
Calibration range	4 – 2407.5 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	38 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 100,15 µg/L.
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Reference:	KCP 5.2.1 (Submitted as KCA 8.2.6.2)
Report	Effects of Prosulfocarb sulfoxide on <i>Anabaena flos-aquae</i> in an algal growth inhibition test, XXXX D., 2012c, 12 10 48 058 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 14: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	3100.15 (5)	101.34	1.9
			1637 (5)	98	3.2
			51039 (5)	92	2.5
		UV	3100.15 (5)	93	5.7
			1637 (5)	101	1.5
			51039 (5)	103	0.3

Table A 15: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear y= 18.4253x+129.72 r ² = 0.9999 MS/MS: Quadratic y= 0.1192590x ² +706.9643x+32486.08 r ² = 0.9995 number of data points: 5
Calibration range	80.12 – 2003.04 µg/L

	Prosulfocarb sulfoxide
Assessment of matrix effects is presented	yes
Limit of quantification	100.15 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 100,2 µg/L.
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Reference:	KCP 5.2.1 (Submitted as KCA 8.2.6.2)
Report	Effects of Prosulfocarb sulfoxide on <i>Navicula pelliculosa</i> in an algal growth inhibition test, XXXX D., 2012d, 12 10 48 053 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 16: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	100.2 (5)	95.9	4.2
			1541 (5)	91	1.3
			50076 (5)	90	2.7
		UV	100.2 (5)	99	3.1
			15417 (5)	101	1.8
			500769 (5)	97	2

Table A 17: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 43.4484x - 290.748$ $r^2 = 0.9998$ MS/MS: Quadratic $y = 0.2507586x^2 + 1328.515x + 88046.21$ $r^2 = 0.9985$ number of data points: 5
Calibration range	78.1 – 2003.0 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	100.2 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 10,76 µg/L.
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Reference:	KCP 5.2.1 (Submitted as KCA 8.2.6.2)
Report	Effects of Prosulfocarb sulfoxide on <i>Skeletonema costatum</i> in an algal growth inhibition test, XXXX D., 2012e, 12 10 48 060 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-UV/VIS (250 nm).

Results and discussions

Table A 18: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	UV	10.76 (5)	99	4.5
			606.7 (5)	109	1.7

Table A 19: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear $y = 800.23x - 925.649$ $r^2 = 0.9999$ number of data points: 6
Calibration range	8.59 – 722.25 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	10.76 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

A 2.1.1.1.3 Analytical methods in soil used in ecotoxicological studies

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	The method has been accepted. The purpose of the method was to determine prosulfocarb in soil. The analytical method applied was based on the highly specific LC-MS/MS technique. Two transitions were applied. The method was validated in soil. The LOQ was set at 0.1 mg/kg dry soil. The mean recovery for soil was in the range of 70 to 110 % consistently with the requirements.
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Reference:	KCP 5.2.1 (Submitted as KCP 10.4.1.2)
Report	Effects of Prosulfoarb 800 g/L EC on earthworms under field conditions, XXXX L., Biochem Agrar, 14 10 48 008 F
Guideline(s):	Yes, ISO 11268-3 (1999), Kula <i>et al.</i> , 2006 - Technical recommendations to ISO 11268-3
Deviations:	No
GLP:	Yes

Acceptability: Yes
 Duplication /
 (if vertebrate study)

Materials and methods

The soil dry weight was determined by heating soil aliquots to a temperature of 105 °C and keeping them at that temperature until the soil weight was constant. The soil moisture analysis was carried out once for each soil specimen. 10 g of soil were weighed into an evaporating dish and thoroughly blended with 5 g of sea sand. A 22 mL extraction cell was closed at one end of the tube with a screw cap. Two round filters were placed into the bottom of the extraction cell and covered with a 0.5 cm layer of sea sand. The specimen was transferred into the extraction cell using a powder funnel. The evaporating dish and the powder funnel were rinsed with sea sand which was subsequently added to the cell. At this stage, the standard solution was applied to the blended soil/sea sand mixtures destined for fortification. The content of the cell was pressed using a piston to secure a firm consistency and filled up with sea sand to about 1 mm below the top of the cell tube. It was covered with a round filter and screwed hand-tight with another screw cap. The extraction was carried out using ASE (accelerated solvent extraction). The eluate of the ASE cell was transferred into a 50 mL volumetric flask, then filled up to the mark with “dilution solution” (CH₃OH/H₂O/HCOOH; 70/30/0.1; v/v/v) and mixed. An aliquot of approximately 1 mL of this final solution was filtered through an 0.2 µm PTFE filter into an injection vial and analysed using LC-MS/MS. Extracts above the calibration range were diluted with methanol/ultra pure water; 1/1; v/v. Standard solution in methanol were diluted with water in range of 0.251 to 15.06 ng/mL

Results and discussions

Table A 20: Recovery results from method validation of prosulfocarb using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Soil	Prosulfocarb	0.1 (5)	101	1.9
		1.0 (5)	103.9	1.3
		Overall (10)	102.4	2.1

Table A 21: Characteristics for the analytical method used for validation of prosulfocarb residues in soil

	Prosulfocarb
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear $y = -6516x + 446.3$ $r^2 = 0.9999$ number of data points: 8
Calibration range	0.251 – 15.06 ng/mL
Assessment of matrix effects is presented	yes
Limit of determination/quantification	≤ 30% of LOQ / 0.1 mg/kg

Conclusion

The method was sufficiently validated according to SANCO/3029/99, Rev.4 and can be used for analytical

determination of prosulfocarb in soil.

Comments of zRMS:	The method is acceptable. The purpose of HPLC method with UV detection was to determine prosulfocarb in the toxicity test exposure solutions. The method is considered suitable for bioaccumulation studies.
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Reference:	KCP 5.2.1 (Submitted as KCA 8.1.3)
Report	The bioaccumulation potential of prosulfocarb in earthworm (<i>Eisenia foetida</i>), XXXX D., 2008, ENV8333/040822
Guideline(s):	Yes, OECD Guideline for Testing of Chemicals 207: Earthworm acute toxicity tests (1984), OECD Guideline for Testing of Chemicals 222: Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>) (2004), OECD Guidelines for Testing of Chemicals 305, Bioconcentration: Flow-through Fish Test. (2006), OECD Guidelines for Testing of Chemicals, Bioaccumulation in sediment-dwelling Benthic Oligochaetes (Proposed December 2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Soil samples: extraction of the test sediment with dichloromethane/acetone in presence of sodium sulphate. Evaporation and redissolution in acetonitrile

Earthworm samples: the worms were ground and extracted with acetonitrile/acetone. Evaporation and redissolution in acetonitrile.

Standard solutions were diluted in acetonitrile.

All samples were analysed using HPLC-UV (220 nm).

Results and discussions

Table A 22: Recovery results from method validation of prosulfocarb using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = x)	Mean recovery (%)	RSD (%)
Soil	Prosulfocarb	2.5 (5)	99.9	1.18

Table A 23: Characteristics for the analytical method used for validation of prosulfocarb residues in soil

	Prosulfocarb
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear $y = -363269x + 5923.8$ $r^2 = 1.0$ number of data points: 9

	Prosulfocarb
Specificity	blank value < 30 % LOQ
Calibration range	0.028 – 25 mg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.01 mg/L / 0.02 mg/L

Conclusion

The method can be used for analytical determination of prosulfocarb in the earthworm bioaccumulation study.

A 2.1.1.1.4 Analytical methods used in other ecotoxicological studies

A 2.1.1.1.4.1 Method validation

Comments of zRMS:	The method is acceptable. The purpose of this LC-MS/MS method was to determine prosulfocarb, diflufenican and halauxifen methyl in the test and feeding solutions in bumblebee toxicity tests. The validation parameters were within the required range. The method is suitable for the intended purposes.
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Reference:	KCP 5.2.1 (submitted as KCP 10.3.1.1.1)
Report	Acute toxicity of GLOB1817H to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, XXXX K., 2021, 20 48 BBA 0029
Guideline(s):	Yes, OECD 246, OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Oral test

The samples were extracted prior the measurement. Therefore, 2.5 mL of acetonitrile were added to a sample aliquot of 0.400 ± 0.005 g. It was extracted by shaking on a FastPrep Instrument (3 cycles, 20 s at speed 5 m/s, 15 sec pause). Afterwards, the samples were centrifuged at 5000 rpm for 3 minutes and the extracts were diluted.

Contact test

The samples of were diluted in several steps prior to sample measurement.

All samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS)

All standards were prepared in methanol.

Results and discussions

Table A 24: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (n = x)	Mean recovery (%)	RSD (%)
50% (w/v) sucrose solution	Prosulfocarb	5.73 mg/kg (5)	89	2.5
		10238 mg/kg (5)	99	6.8
	Diflufenican	0.12 mg/kg (5)	95	2.3
		216.08 mg/kg (5)	110	7.4
	Halauxifen-methyl	0.01 mg/kg (5)	95	1.7
		20.13 mg/kg (5)	85	8.4
0.5% (v/v) TritonX in deionized water	Prosulfocarb	20441 mg/L (5)	99	1.2
		240479 mg/L (5)	100	1.8
	Diflufenican	431.42 mg/L (5)	86	2.2
		5076 mg/L (5)	92	1.7
	Halauxifen-methyl	40.19 mg/L (5)	84	1.3
		472.88 mg/L (5)	84	1.5

Table A 25: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 50% (w/v) sucrose solution

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic Y = -12.3X ² +40803X+726353 r ² > 0.9998 number of data points: 7	Linear Y = 13789X+819.2 r ² > 0.9998 number of data points: 7	Linear Y = 41110X+552.7 r ² > 0.998 number of data points: 7
Calibration range	106.97 – 891.43 µg/L	2.19 – 18.27 µg/L	0.21 – 1.75 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	5.73 mg/kg / 1.52 mg/kg	0.12 mg/kg / 0.03 mg/kg	0.01 mg/kg / 0.003 mg/kg

Table A 26: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 0.5% (v/v) TritonX in deionized water

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data)	Quadratic	Quadratic	Linear

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
points)	Y = -16.4X ² +53926X+938388 r ² > 0.9997 number of data points: 7	Y = -97.7X ² +19879X+6418 number of data points: 7	Y = 43728X+290.1 r ² > 0.9995 number of data points: 7
Calibration range	139.89 – 1165.71 µg/L	3.07 – 25.60 µg/L	0.27 – 2.29 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	20441 mg/L / 5595 mg/L	431.42 mg/L / 122.89 mg/L	40.19 mg/L / 10.97 mg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in 50% (w/v) sucrose solution and 0.5% (v/v) TritonX in deionized water.

Comments of zRMS:	The method is acceptable. The purpose of the method was to determine prosulfocarb, diflufenican and halauxifen-methyl in the test and feeding solutions in honeybee toxicity tests. The determination of active ingredients in sucrose solution was conducted using reversed-phase high-performance liquid chromatography combined with mass spectrometry (RP-HPLC-MS/MS). The specificity of the method was assured by multiple reaction monitoring (MRM) - detection with at least 2 transitions and the absence of interfering peaks. The validation parameters were within the required range. The method is suitable for the intended purposes.
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Reference:	KCP 5.2.1 (submitted as KCP 10.3.1.2)
Report	Chronic toxicity of GLOB1817H to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, XXXX S., 2021, 20 48 BAC 0071
Guideline(s):	Yes, OECD TG 245 (2017)
Deviations:	Yes, due to the higher abundance of the resulting ion, the mass transition m/z 345 → 250 was chosen for the quantification of halauxifen-methyl and m/z 345 → 285 was set for qualification. There is no impact on the analytical phase of the study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were extracted prior the measurement. Therefore, 2.5 mL of acetonitrile were added to a sample aliquot of 0.500 ± 0.005 g. It was extracted by shaking on a FastPrep Instrument (3 cycles, 20 s at speed 5 m/s, 15 sec pause). Afterwards, the samples were centrifuged at 5000 rpm for 3 minutes and the extracts were diluted.

All samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS)

All standards were prepared in methanol.

Results and discussions

Table A 27: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
50% (w/v) sucrose solution containing 0.1% (w/v) xanthan	Prosulfocarb	5.55 (5)	94	0.5
		3085 (5)	104	1.1
	Diflufenican	0.12 (5)	85	0.9
		65.11 (5)	94	1.3
	Halauxifen-methyl	0.01 (5)	88	1.4
		6.07 (5)	88	1.0

Table A 28: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic Y = -18.4X ² +52912X+392078 r ² > 0.99 number of data points: 6	Linear Y = 13307X+1354 r ² > 0.99 number of data points: 6	Linear Y = 37908X+1316 r ² > 0.99 number of data points: 6
Calibration range	111.19 – 654.07 µg/L	2.39 – 14.06 µg/L	0.22 – 1.29 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	5.55 mg/kg / 1.55 mg/kg	0.12 mg/kg / 0.03 mg/kg	0.01 mg/kg / 0.003 mg/kg

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan.

Comments of zRMS:	The method is acceptable. The purpose of this LC-MS/MS method was to determine prosulfocarb, diflufenican and halauxifen methyl in the test item stock solutions of a honeybee larvae toxicity tests. The specificity of the method was assured by multiple reaction monitoring (MRM) - detection with at least 2 transitions and the absence of interfering peaks. The validation parameters of the method were within the required range. The method is suitable for the intended purposes.
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Report	GLOB1817H – Repeated exposure to the honeybee (<i>Apis mellifera</i> L.) larvae under laboratory conditions, XXXX K., 2021, 20 48 BLC 0052
Guideline(s):	Yes, OECD 239 (2016)
Deviations:	Yes, because of a malfunction of the climatic chamber, the temperature and humidity were out of range on D8 for six hours. The temperature ranged in this time between 28.5 to 35.7°C (average 30.7°C) instead of 34.5 ± 0.5°C. The relative humidity ranged in this time between 18.9 to 97.1% (average 28.6% instead of 80 ± 5%). No impact is assumed as no effects on development of larvae in the untreated control were observed. Due to the higher abundance of the resulting ion, the mass transition m/z 345 → 250 was chosen for the quantification of halauxifen-methyl and m/z 345 → 285 was set for qualification. There is no impact on the analytical phase of the study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were extracted prior the measurement. Therefore, 2.5 mL of acetonitrile were added to a sample aliquot of 0.500 ± 0.005 g. It was extracted by shaking on a FastPrep Instrument (3 cycles, 20 s at speed 5 m/s, 15 s pause). Afterwards, the samples were centrifuged at 5000 rpm for 3 minutes and the extracts were diluted.

All samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS)

All standards were prepared in methanol.

Results and discussions

Table A 29: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Aqueous sugar solution	Prosulfocarb	4.64 (5)	88	2.0
		980.97 (5)	96	7.5
	Diflufenican	0.10 (5)	90	3.4
		20.70 (5)	99	6.7
	Halauxifen-methyl	0.01 (5)	87	0.8
		1.93 (5)	82	5.4

Table A 30: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in aqueous sugar solution

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 %	blank value < 30 %

	Prosulfocarb	Diflufenican	Halauxifen-methyl
		LOQ	LOQ
Calibration (type, number of data points)	Quadratic $Y = -4.54X^2 + 24703X + 92168$ $r^2 > 0.99$ number of data points: 8	Linear $Y = 6423X + 2313$ $r^2 > 0.99$ number of data points: 8	Linear $Y = 26311X + 590.5$ $r^2 > 0.99$ number of data points: 7
Calibration range	112.91 – 806.50 µg/L	2.47 – 17.67 µg/L	0.23 – 1.63 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	4.64 mg/kg / 1.33 mg/kg	0.10 mg/kg / 0.03 mg/kg	0.01 mg/kg / 0.003 mg/kg

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in aqueous sugar solution.

Comments of zRMS:	The method is acceptable. The purpose of this HPLC-UV method was to determine prosulfocarb, diflufenican, halauxifen methyl and cloquintocet mexyl in the spray solution (water). The validation parameters of the method were within the required range. The method is suitable for the intended purposes.
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Reference:	KCP 5.2.1 (submitted as KCP 10.6)
Report	GLOB1817H: terrestrial plant test: vegetative vigour test, XXXX M., 2021, STC/20/E1409
Guideline(s):	Yes, OECD 227 (2006)
Deviations:	pH of the soils being 8.2, rather than 7-8 as stated in the study plan. Relative humidity falling below 45%, rather than 70% - 25% as stated in the study plan. These deviations were not to the detriment of the plants as photographs of the untreated plants taken at harvest show. These deviations will not impact on the validity of the study, as demonstrated by the control performance and the fact that the validity criteria for the study were met.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The spray solution was diluted into the range of the calibration curve as follows:

The spray solution was sonicated for 20 minutes with intermittent shaking and stirred for 5 minutes prior to sampling. The stirring was continued as aliquots were removed for analysis.

Triplicate samples (10 mL) of the supplied spray solution were pipetted into separate 50 mL volumetric flasks and diluted to volume with acetonitrile.

Control samples were prepared by transferring aliquots (2 x 10 mL) of the supplied water to separate 50 mL volumetric flasks and diluted to volume with acetonitrile.

The final solutions were analysed by the HPLC method relative to a bracketing standard solution.

All standards were prepared in acetonitrile.

Results and discussions

Table A 31: Recovery results from method validation of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = x)	Mean recovery (%)	RSD (%)
Spray solution	Prosulfocarb	6193 (5)	99	0.3
		2858 (5)	100	0.5
	Diflufenican	130.7 (5)	87	0.3
		60.53 (5)	87	0.5
	Halauxifen-methyl	12.18 (5)	86	0.6
		5.640 (5)	86	1.2
	Cloquintocet-mexyl	12.42 (5)	83	1.2
		5.752 (5)	83	0.8

Table A 32: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl residues in spray solution

	Prosulfocarb	Diflufenican	Halauxifen-methyl	Cloquintocet-mexyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 5936x + 39680 r ² = 1.000 number of data points: 6	Linear y = 35200x – 1010 r ² = 1.000 number of data points: 6	Linear y = 133200x – 994.8 r ² = 1.000 number of data points: 6	Linear y = 116100x + 96.88 r ² = 1.000 number of data points: 6
Calibration range	160.2 – 1602 mg/L	3.562 – 35.62 mg/L	0.3536 – 3.536 mg/L	0.3446 – 3.446 mg/L
Assessment of matrix effects is presented	NR	NR	NR	NR
Limit of quantification/determination	2858 mg/L / 840 mg/L	60.53 mg/L / 18 mg/L	5.640 mg/L / 1.7 mg/L	5.752 mg/L / 1.7 mg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 rev.4 and can be used for analytical determination of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl in spray solution.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for diflufenican

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

A 2.2.1.1.1 Analytical methods in water used in aquatic toxicity studies

Please refer to A 2.1.1.1.2.

A 2.2.1.1.2 Analytical methods used in other ecotoxicological studies

Please refer to A 2.1.1.1.4.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

A 2.2.2.4.1 Analytical method for diflufenican in drinking water

A 2.2.2.4.1.1 Method validation

Comments of zRMS:	The method has been accepted. The method is intended to determine diflufenican and metabolites AEB107137 (2-[3-(trifluoromethyl)phenoxy]nicotinic acid)) and AE0592370 (N-(2,4-difluorophenyl)-2-hydroxy-N-(3-trifluoromethylphenyl) nicotinamide)) in surface and drinking water by solid phase extraction (SPE) followed by LC-MS/MS. For each individual analyte, one ion transition was used for quantification and a further ion transition was also monitored for confirmation. The method was validated at 2 fortification levels for all analytes – at the LOQ of 0.05 µg/L and at 0.5 µg/L. The mean recovery at each fortification level was within the range 70 — 110% and corresponding RSD values were < 20%. No residues or interferences were detected in control surface water samples. The method is considered highly specific.
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Reference:	KCP 5.2
Report	Development and validation of a method for the determination of diflufenican and two metabolites in surface water and drinking water, XXXX G., 2008, PGD-307.
Guideline(s):	Yes, SANCO/825/00 rev. 7
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Method Description

Concentrations of diflufenican and its metabolites AEB107137 and AE0592370 determined by liquid chromatography (LC) coupled with tandem mass spectrometric detection (LC-MS/MS).

Extraction

- Dispense 200ml of water sample and fortify recovery samples with diflufenican and metabolites according to the table below.

Fortification level (µg/L)	Volume (mL)	Concentration of diflufenican and metabolites in fortification solution (µg/ml)
0.05	0.2	0.05
0.5	0.2	0.5

- For SPE extraction, use vacuum where required. Condition an Oasis HLB 6cc, 500mg, LP cartridge with 5ml methanol followed by 5ml of water.

- Load the sample onto the cartridge and discard the eluate. Wash the cartridge with 4ml of 5% methanol in water and discard the eluate. For surface water samples, elute the cartridge with 10ml of methanol, collecting the eluate in an appropriate vessel. For drinking water samples, elute the cartridge with 12ml of methanol, collecting the eluate in an appropriate vessel.

- Evaporate the eluate to dryness at $\leq 35^{\circ}\text{C}$ in a TurboVap LV concentration workstation. Re-dissolve the residue (with the aid of ultra-sonication and a vortex mixer) in 1ml of methanol.

- To prepare the matrix-matched standards, evaporate portions of blank matrix, e.g. 200µL, to dryness at $\leq 35^{\circ}\text{C}$ in a TurboVap LV concentration workstation. Re-dissolve the residue (with the aid of ultra-sonication and vortex mixer) in the same volume, e.g. 200µL, of calibration standard.

Water characteristics

Test	Surface Water
Used for method validation of	diflufenican
Sampling point	River Derwent at Stamford bridge, East Riding of Yorkshire, United Kingdom
pH	7.8
Total hardness (as CaCO ₃)	246 mg/L
DOC (diluted organic carbon)	5.0 mg/L
Suspended solids	15 mg/L

Chromatographic conditions

HPLC

LC System	Agilent 1100 LC binary pump			
LC Column	Waters, Atlantis dC18, 3 µm, 2.1 x 150 mm			
LC Injection Volume	10 µL.			
LC Method	Solvent A1: 10 mM ammonium acetate (surface water) Solvent A2: 5 mM ammonium acetate + 0.01% acetic acid (drinking water, LOQ) Solvent A3: 5 mM ammonium acetate + 0.1% formic acid (drinking water, 10xLOQ) Solvent B: Methanol Mobile Phase Composition: Time (min) Flow rate (mL/min) % A % B 0.00 0.2 90 10 8 0.2 5 95			

	23	0.2	5	95
	23.5	0.2	90	10
	32	0.2	90	10
Retention Time	Around 15.4 minutes			

MS/MS

MS System	AB Sciex API 2000 mass spectrometer with TurboIonspray ESI source.
MS/MS Conditions for diflufenican:	MS/MS transition for quantification: 395.1 m/z > 266.2 m/z MS/MS transition for confirmation: 395.1 m/z > 245.8 m/z
MS/MS Conditions for AEB107137:	MS/MS transition for quantification: 284.1 m/z > 266.0 m/z MS/MS transition for confirmation: 284.1 m/z > 246.0 m/z
MS/MS Conditions for AE0592370:	MS/MS transition for quantification: 395.1 m/z > 122.0 m/z MS/MS transition for confirmation: 395.1 m/z > 140.0 m/z

Reagents and apparatus

Reagents	Apparatus
Water	Balance
Methanol	Microsyringe
Hydrochloric acid, 1M	Oasis HLB 6cc, 500mg, LP cartridges
5% methanol in water	SPE vacuum manifold and pump
Ammonium Acetate	TurboVap LV concentration workstation
Acetic Acid	Ultrasonice bath
Formic Acid	Vortex mixer
	Common laboratory glassware

Results and discussions

Table A 33: Recovery results from method validation of diflufenican using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)
Surface water	Diflufenican	0.05	84.2	11.5
		0.5	71.4	14.4
Surface water	AEB1071137	0.05	103.6	2.5
		0.5	102.3	6.3
Surface water	AE0592370	0.05	102.3	5.9
		0.5	98.2	3.4
Drinking water	Diflufenican	0.05	75.8	8.6
		0.5	71.9	4.6
Drinking water	AEB1071137	0.05	98.1	5.1
		0.5	98.5	8.2
Drinking water	AE0592370	0.05	100.5	6.8
		0.5	94.9	6.1

Table A 34: Characteristics for the analytical method used for validation of diflufenican residues in surface water and drinking water

	Diflufenican	AEB1071137	AE0592370
Specificity	LC-MS/MS is considered a specific technique as two different mass transitions were monitored; therefore additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided which indicate no significant interference between the relevant peaks; diflufenican, AEB107137 and AE0592370 and any of the water commodity matrices (>30% of the LOQ). Representative mass spectra for diflufenican have been provided that confirm the mass transitions are appropriate. The method is considered to have the required specificity.		
Calibration (type, number of data points)	Calibration was generated using standards prepared in blank matrix extracts (matrix matched standards). Linear calibration functions were calculated and plotted by regression analysis. Regression coefficients (r) were always > 0.99.		
Calibration range	The linear range is considered appropriate to the test sample concentrations used in this method. This includes 30% of the LOQ to 20% above the highest fortification level. The calibration range used for the low level validation samples was 0.002 to 0.1 µg/mL. The calibration range used for the high level validation samples was 0.002 to 0.2 µg/mL		
Assessment of matrix effects is presented	There were no significant matrix effects (<30%) observed for any matrices		
Limit of determination/quantification	0.05 µg/L		

Conclusion

The method for diflufenican and its metabolites AE B107137 and AE 0592370 in surface water and drinking water was successfully validated and met the requirements of SANCO/825/00 rev. 7.

A 2.2.2.4.1.2 Independent laboratory validation

Comments of zRMS:	The ILV for diflufenican has been accepted. The objective of the current study was an ILV of the analytical method validated in the previous study (A 2.2.2.4.1.1). Two MRM transitions were monitored for diflufenican. The recovery tests were done at two levels, LOQ and 10 times LOQ, with five replicates each, for each matrix. The validation parameters were within the required range.
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Reference:	KCP 5.2
Report	Validation of an analytical method for the determination of diflufenican in drinking water, ILV, XXXX H., 2016, VAL10/16.
Guideline(s):	Yes, SANCO/825/00 rev 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The primary method to determine diflufenican in matrices of water origin was independently validated by Laboratório de Resíduos, SAPEC AGRO, Portugal, a different test facility to the primary method. The method conditions and sample preparations are identical to those given in the primary method: PGD-307. Quantitation was performed using liquid chromatography electrospray ionization with tandem mass spectrometric detection (LC-MS/MS); the method monitored two ion transitions (quantitation transition m/z 395.3 \rightarrow 266.2 and confirmation transition m/z 395.3 \rightarrow 246.3 for diflufenican.

Results and discussions

Table A 35: Recovery results from independent laboratory validation of diflufenican using the analytical method

Matrix	Analyte	Fortification level ($\mu\text{g/L}$) (n = x)	Mean recovery (%)	RSD (%)
Drinking water	Diflufenican	0.050	78.8	6.7
		0.50	78.0	2.4

Table A 36: Characteristics for the analytical method used for independent laboratory validation of diflufenican residues in drinking water

	Diflufenican
Specificity	Chromatograms have been provided which indicate no significant interference between the relevant peak diflufenican and the matrix drinking water (>30% of the LOQ).
Calibration (type, number of data points)	The linearity of the detector response was checked by injecting several matrix matched standard solutions. The correlation coefficients obtained were higher than 0.99.
Calibration range	The validated calibration range for both levels was from 0.010 $\mu\text{g/L}$ to 1.0 $\mu\text{g/L}$ (0.002 $\mu\text{g/mL}$ to 0.20 $\mu\text{g/mL}$), for the drinking water matrix. The correlation coefficients obtained were higher than 0.99.
Assessment of matrix effects is presented	There were no significant matrix effects (<30%) observed for any matrices.
Limit of determination/quantification	0.05 $\mu\text{g/L}$

Conclusion

The method for diflufenican in drinking water is successfully validated according to SANCO/825/00 rev 8.1. The method is acceptable as ILV for the primary method.

A 2.2.2.4.1.3 Confirmatory method

No confirmatory method is required since LC-MS/MS is considered a specific technique as two different mass transitions were monitored.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.3 Analytical methods for halauxifen-methyl

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.3.1.1.1 Analytical methods in water used in aquatic toxicity studies

Please refer to A 2.1.1.1.2.

A 2.3.1.1.2 Analytical methods used in other ecotoxicological studies

Please refer to A 2.1.1.1.4.

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted