

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: M-100SC-OR2-C

Product name(s): Juzan Extra 100 SC

Chemical active substance:

Mesotrione, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: CIECH Sarzyna S.A.

Submission date: 05/2022

Finalisation date: 01/2023; 05/2023

Version history

When	What
May 2022	First submission for the product authorisation.
January 2023	zRMS assessment
May2023	The final version of RR after commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

CIECH Sarzyna S.A. has a Letter of Access to alternative data package for active substance Mesotrione. Data Matching List covers all the protected studies from the main notifier.

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

Noticed data gaps are:

- none

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

Noticed data gaps are:

- none

Commodity/crop	Supported/ Not supported
Maize (ZEAMX)	Supported
Sugar maize (ZEAMS)	Supported
Popcorn (ZEAME)	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 for analysis of mesotrione in plant protection product is provided as follows:

Comments of zRMS:	The proposed analytical method is suitable for the determination of active substance mesotrione in plant protection product Juzan Extra 100 SC. The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. Proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.
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Reference: KCP 5.1.1

Report M-100SC-OR2-C. Method validation for determination of the active substance and three relevant impurities content in the formulation, Bajdor K., Wołoszynowska M., 2020, 1/20/041/K

Guideline(s): Yes (SANCO/3030/99 rev. 5)
Deviations: No
GLP: Yes
Acceptability: Yes ~~No/Supplementary~~

Materials and methods

Method validation for determination of the Mesotrione content was performed using high performance liquid chromatography (HPLC) with DAD detector and external standard method.

Test Item	Juzan Extra 100SC (M-100SC-OR2-C)
Active substance	Mesotrione 100 g/L
Chromatographic conditions:	
Apparatus	Shimadzu liquid chromatograph equipped with DAD detector
Column	Reprosil Gold 100 C18-XDB, 250 x 4.6mm x 5µm
Oven temperature	30 °C
Mobile phase flow	v = 1.0 mL/min
Wavelength	λ = 220 nm
Injection volume	5 µL
Mobile phase composition	0.1% aqueous solution of H ₃ PO ₄ (40%+60%; v/v)
Retention time(s)	12.5 ± 0.5 min.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances mesotrione in plant protection product Juzan Extra 100 SC

	Mesotrione
Author(s), year	Bajdor K. Wołoszynowska M., 2020
Principle of method	Method validation for determination of the Mesotrione content was performed using high performance liquid chromatography (HPLC) with DAD detector and external standard method.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	<p>The linearity of the detector response was assessed using five standard solutions at the concentration range of Mesotrione 60.93 mg/l to 142.17 mg/l (61% - 142% mesotrione content).</p> <p>Linear equation: y = 18 174x – 35 763</p> <p>Correlation coefficient: 0.9996 (acceptance criterion R² ≥ 0.99) The obtained results are acceptable.</p>
Precision – Repeatability Mean n = 6 (%RSD)	<p>RSD = 1.11% acceptance criterion: < 1.90%</p> <p>Hr = 0.58 acceptance criterion: ≤ 1</p>

	Mesotrione				
Recovery n = 12 (6 for every level)	Total recovery:				
	Level	Avarage Recovery on levels [%]	Mean Recovery [%]	SD	RSD [%]
	Level 1	100.25	100.15	3.20	3.19
	Level 2	100.06		1.46	1.46
Accuracy n = 12 (% Recovery)	100.15% acceptance criterion: 100 ± 3				
Interference/ Specificity	No interference. The chromatograms of solvent, standard solution, placebo solution and the examined specimen solution were performed and superimposed.				
Comment	Suitable for verification of analyte in the nominal concentration range				

Conclusion

The method has good precision, accuracy and linearity and fulfils requirements of SANCO/3030/99 rev. 5 which guarantee correctness of mesotrione determination in the preparation Juzan Extra 100 SC.

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

Mesotrione contains three relevant impurities: 1,2-dichloroethane, 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (IMP 1) (R287431) and 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (IMP 2) (R287432).

An overview on the acceptable methods for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	The proposed analytical methods are suitable for the determination of relevant impurities (1,2-dichloroethane, R287431, R287432) in plant protection product Juzan Extra 100 SC. The proposed analytical methods have been fully validated in terms of specificity, linearity, repeatability, and accuracy. LOQs are acceptable. The proposed methods fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical methods has been accepted.
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Reference: KCP 5.1.1

Report M-100SC-OR2-C. Method validation for determination of the active substance and three relevant impurities content in the formulation, Bajdor K., Wołoszynowska M., 2020, 1/20/041/K

Guideline(s): Yes (SANCO/3030/99 rev. 5)

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Materials and methods - 1,2-dichloroethane

Method validation for determination of the 1,2-dichloroethane content was performed by headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard method.

Chromatographic conditions:

Apparatus	VARIAN CP-38000 Gas Chromatograph with FID
Column	Rxi-1301Sil MS capillary column, 30 m x 0.25 mm x 1.0 µm (RESTEK)
Oven temperature	45°C (1 min), 10°C/min- 110°C, 20°C/min- 250°C
Mobile phase flow	v = 2.0 mL/min
Auxiliary gases flow	<ul style="list-style-type: none">• Nitrogen: 25 ml/min• Hydrogen: 30ml/min• Air: 300 ml/min

Retention time(s) 4.00 ± 0.05 min.

Materials and methods – IMP 1 (R287431), IMP 2 (R287432)

Method validation for determination of the 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (R287431) (IMP 1) and 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (R287432) (IMP 2) content was performed using UHPLC chromatography with MS/MS detection using external standard method.

Chromatographic conditions:

Apparatus	Sciex QTRAP 4500 mass spectrometer with UHPLC
Column	Synergi Furion-RP 80A, 50 x 2 mm, 4 µm, Phenomenex
Column temperature	30°C
Mobile phase flow	v = 0.4 mL/min
Volume of sample injected	5 µl
Mobile phase	5 mmol aqueous solution of ammonium acetate + acetonitrile with 0.1% of formic acid
Retention time(s)	IMP 1 (R287431): 3.6 ± 0.1 min IMP 2 (R287432): 3.4 ± 0.1 min

A triple quadrupole MS equipped with a positive electrospray ionization source was used in the MRM mode.

The MS conditions of MRM for IMP 1 (R287431):

- Precursor ion m/z : 362.0
- Product ions m/z : 234.9 and 206.1

The MS conditions of MRM for IMP 2 (R287432):

- Precursor ion m/z : 317.0
- Product ions m/z : 221.0 and 209.1

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) Juzan Extra 100 SC

	1,2-dichloroethane max. 1 mg/kg	IMP 1 (R287431) max. 2 mg/kg	IMP 2 (R287432) max. 2 g/kg
Author(s), year	Bajdor K., Wołoszynowska M., 2020		
Principle of method	Method validation for determination of the 1,2-dichloroethane content was performed by headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard method. Confirmation: GC-MS technique with electron impact ionization (EI).	Method validation for determination of IMP 1 (R287431) and IMP 2 (R287432) content was performed using ultra high performance liquid chromatography (UHPLC) with MS/MS detection and external standard method.	
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was assessed using six solutions in the range of 1,2-dichloroethane content from 0.0005 mg to 0.010 mg (corresponding to 5% - 105% w/w of maximum content of 1,2-dichloroethane in 100 mg of preparation). Linear equation: $y = 880\,621.8x - 33.55$ Correlation coefficient: 0.9994 (acceptance criterion $R^2 \geq 0.99$) The obtained results are acceptable.	The linearity of the detector response was assessed using six solutions at the concentration range of IMP 1 from 0.4959 ng/ml to 9.0922 ng/ml (corresponding to 7.08% - 129.89% of maximum content of IMP 1 in preparation). Linear equation: $y = 2\,909.9x - 60.096$ Correlation coefficient: 0.9969 (acceptance criterion $R^2 \geq 0.99$) The obtained results are acceptable.	The linearity of the detector response was assessed using six solutions at the concentration range of IMP 2 from 0.9368 ng/ml to 32786.5 ng/ml (corresponding to 0.01% - 468.38% of maximum content of IMP 2 in preparation). Linear equation: $y = 2\,292.6x + 629\,853$ Correlation coefficient: 0.9985 (acceptance criterion $R^2 \geq 0.99$) The obtained results are acceptable.
Precision – Repeatability Mean (%RSD)	n = 6 RSD = 2.52% acceptance criterion: < 7.37% Hr = 0.32 - 0.34 acceptance criterion: ≤ 1	n = 5 RSD = 4.85% acceptance criterion: < 17.83% Hr = 0.27 acceptance criterion: ≤ 1	n = 5 RSD = 5.49 % acceptance criterion: < 8.11 % Hr = 0.68 acceptance criterion: ≤ 1
Recovery	Assessed at two levels of concentration (n=12). Mean total recovery [%]=100.6	Assessed at two levels of concentration (n=12). Mean marginal	Assessed at two levels of concentration (n=12). Mean marginal recovery [%]=109.53

	1,2-dichloroethane max. 1 mg/kg	IMP 1 (R287431) max. 2 mg/kg	IMP 2 (R287432) max. 2 g/kg
	RSD[%]=1.68	recovery [%]=109.8 RSD[%]=3.56	RSD[%]=3.30
Accuracy n = 6 12 (% Recovery)	100.6% acceptance criterion: 100 ± 30	109.8% acceptance criterion: 100 ± 30	109.53% acceptance criterion: 100 ± 30
Interference/ Specificity	To prove specificity the following chromatograms were performed and superimposed: mixture DMSO and deionized water, placebo of M-100SC-OR2-C, specimen of M-100SC-OR2-C. No interference	The chromatograms of solvent, standard solution, placebo solution and the examined specimen solution were performed and superimposed. No interference	
LOQ	Limit of quantification is 0.0005 mg what corresponds to 0.0005% of maximum 1,2-dichloroethane content in M-100SC-OR2-C preparation.	Limit of quantification is 0.4959 ng/ml what corresponds to 0.000014 g/kg of IMP 1 in M-100SC-OR2-C preparation i.e. 0.000139 g/kg of Mesotrione	Limit of quantification is 0.93676 ng/ml what corresponds to 0.000026 g/kg of IMP 2 in M-100SC-OR2-C preparation i.e. 0.000262 g/kg of Mesotrione
Comment	-	-	-

Conclusion

The method has good precision, accuracy and linearity and fulfils requirements of SANCO/3030/99 rev. 5 which guarantee correctness of relevant impurities determination in the preparation Juzan Extra 100 SC.

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

The other formulants and also components of other formulants of Juzan Extra 100 SC are not of toxicological and/or ecotoxicological or environmental concern and therefore it is not necessary to submit the analytical methods for determination of other formulants or components of other formulants of above product.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no existing CIPAC method for mesotrione.

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 mesotrione for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new

studies it is referred to Appendix 2.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: mesotrione*				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Maize (Residues)	Primary	0.01 mg/kg	HPLC	EU DAR, 1999, Alferness, P.L, 1996a DP 59666 Meyers, T.J, et al 1997 DP 59670
Maize (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Peda T., Niewelt S (analytical phase), 2021, 21SGS76
Deionized water (Ecotoxicology)	Primary	0.007636 mg/L	HPLC UV-DAD	Biesiada M., 2020, 0016/0090/FA
Sucrose solution (Ecotoxicology)	Primary	0.031768 mg/kg	HPLC UV-DAD	Biesiada M., 2020, 0016/0092/FA
Water (Ecotoxicology)	Primary	0.33 µg/kg	LC-MS/MS	Dec W., 2021, EMI/4/10/2021
			LC-MS/MS	Dec W., 2021, EMI/4/9/2021
AAP (Ecotoxicology)	Primary	0.7 µg/L	LC-MS/MS	Szlauer S., 2022, EMI/4/3/2021
AAP (Ecotoxicology)	Primary	0.2 µg/L	LC-MS/MS	Szlauer S., 2022, EMI/4/6/2021
Elendt M7 (Ecotoxicology)	Primary	0.7 µg/L	LC-MS/MS	Szlauer S., 2022, EMI/4/5/2021
AAP medium (Ecotoxicology)	Primary	0.7 µg/L	LC-MS/MS	Szlauer S., 2022, EMI/4/2/2021

*There are significant residues of AMBA and it's conjugates in forage and fodder, and MNBA is significant in pre- emergence treated forage, for future uses for which these parts of the plant would be fed to animals, for example cereal straw, consideration should be given to including these metabolites in the risk assessment.

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 of mesotrione (KCP 5.2)

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

The residue definition proposed in the original assessment was mesotrione+MNBA (expressed as mesotrione) for monitoring and risk assessment. However, it has been concluded that MNBA does not contrib-

ute significantly to the residue s found in human food commodities. Therefore this metabolite has been removed from the residue definition for monitoring and risk assessment.

EFSA review of the existing maximum residue levels (MRLs) for mesotrione according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2015;13(1):3976) also concluded that the residue definition for monitoring and risk assessment should be mesotrione (parent only) cereal grains and pulses and oilseeds.

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
Maize (grain, forage and fodder)	Mesotrione	0.01 mg/kg	Regulation (EU) No 2017/626
Muscle	Not required for the representative use (provisional)	0.01 mg/kg	Regulation (EU) No 2017/626
Milk		0.01 mg/kg	Regulation (EU) No 2017/626
Eggs		0.01 mg/kg	Regulation (EU) No 2017/626
Fat		0.01 mg/kg	Regulation (EU) No 2017/626
Liver, kidney		0.01 mg/kg	Regulation (EU) No 2017/626
Soil (Ecotoxicology)	Mesotrione (MNBA, AMBA)	LOQ = 0.002 mg/kg	AOEL: 0.005 mg/kg bw/d EFSA Journal 2016;14(3):4419
Drinking water (Human toxicology)	Mesotrione (MNBA, AMBA)	LOQ = 0.05 µg/L	general limit for drinking water EFSA Journal 2016;14(3):4419
Surface water (Ecotoxicology)	Mesotrione (MNBA, AMBA)	LOQ = 0.05 µg/L	EFSA Journal 2016;14(3):4419
Air	Mesotrione	LOQ = 0.45 µg/m ³	AOEL: 0.005 mg/kg bw/d/ LOQ of 0.45 µg/m ³ EFSA Journal 2016;14(3):4419
Tissue (meat or liver)	Mesotrione	LOQ = 0.01 mg/kg	Not classified as T / T+
Body fluids		LOQ = 0.01 mg/kg	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 mesotrione in plant matrices is given in the following tables.

zRMS:

Mesotrione residues can be monitored in food and feed of plant origin by the QuEChERS method (LC-MS/MS) with LOQs of 0.01 mg/kg in each commodity group (EFSA Journal 2016;14(3):4419).

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: mesotrione				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Maize (grain, forage and fodder)	Primary	0.01 mg/kg	HPLC-FL	EU DAR, 1999, Alferness, 1996, TMR0643B
	ILV	0.01 mg/kg	HPLC-FL	EU DAR, 1999, Bolygo, 1996, RJ2149B
	Primary	0.01 mg/kg	GC-MSD	EU DAR, 1999, Meyers et al, 1997, TMR0689B

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	In compliance with SANCO/825/00 rev. 8.1 the extraction procedure has to be addressed for all matrix groups for which residues \geq LOQ are expected. For mesotrione non of residue value exceed LOQ.

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

Residue monitoring method for food of animal origin is not required as no MRLs were set, however mesotrione can be determined in food and feed of animal origin by the QuEChERS method (LC-MS/MS) with LOQs of 0.01 mg/kg in all animal matrices.

Applicant presents below analytical methods for animal matrices available and evaluated in EU DAR 1999.

zRMS:

Adequate methods exist to monitor Mesotrione residues in animal matrices.

Mesotrione can be determined in food and feed of animal origin by the QuEChERS LC-MS/MS method (LOQ = 0.01 mg/kg).

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: mesotrione				
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	capillary GC (HP Ultra 1), mass selective detector	EU DAR 1999, Meyers et al., 1998, DP 59670
Eggs	Primary	0.01 mg/kg	capillary GC (HP	EU DAR 1999, Meyers et al.,

Component of residue definition: mesotrione				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
			Ultra 1), mass selective detector	1998, DP 59670
Liver (beef)	Primary	0.01 mg/kg	capillary GC (HP Ultra 1), mass selective detector	EU DAR 1999, Meyers et al., 1998, DP 59670
Ground beef	ILV	0.01 mg/kg	capillary GC (HP Ultra 1), mass selective detector	EU DAR 1999, Meyers et al., 1998, DP 59670

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	In compliance with SANCO/825/00 rev. 8.1 the extraction procedure has to be addressed for all matrix groups for which residues \geq LOQ are expected. For mesotrione non of residue value exceed LOQ.

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 mesotrione in soil is given in the following tables.

Please note that an Applicant – Ciech Sarzyna S.A. – has a LoA to Data Matching List which covers all the new, protected studies from the main notifier.

zRMS:

Adequate method exists to monitor Mesotrione residues as well as the metabolites AMBA and MNBA in soil.

Residues of Mesotrione in soil (Jutsum L. (2013) (EFSA Journal 2016;14(3):4419):

LC-MS/MS

LOQ = 0.002 mg/kg

AMBA in soil:

LC-MS/MS

LOQ = 0.002 mg/kg

MNBA in soil:

LC-MS/MS

LOQ = 0.002 mg/kg

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

Component of residue definition: mesotrione (MNBA, AMBA)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.005 mg/kg	reversed phase HPLC-FSD	EU DAR, Vo. 3, 1999 Alferness, 1996 TMR0661B (ZA1296/0443)

All analytical methods are active substance data and were evaluated during the EU review of mesotrione. No additional studies have been performed.
These data have been provided and are considered to adequate.

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 mesotrione in surface and drinking water is given in the following tables.
Please note that an Applicant – Ciech Sarzyna S.A. – has a LoA to Data Matching List which covers all the new, protected studies from the main notifier.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: mesotrione (MNBA)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Water	Primary	0.05 µg/L	GC-MSD	EU DAR, Vol.3, 1999, Meyers, 1997 TMR0707B (ZA1296/0445)

All analytical methods are active substance data and were evaluated during the EU review of mesotrione. No additional studies have been performed.
These data have been provided and are considered to adequate.

zRMS:

Adequate method exists to monitor Mesotrione residues as well as the metabolites AMBA and MNBA in drinking and surface water (Jutsum L. (2013a) (EFSA Journal 2016;14(3):4419)).

LC-MS/MS

LOQ = 0.05 µg/L

AMBA in water:

LC-MS/MS

LOQ = 0.05 µg/L

MNBA in water:

LC-MS/MS

LOQ = 0.05 µg/L

An ILV for drinking water is required according to Regulation (EU) No 284/2013.

Acceptable ILV method is available (EU agreed EFSA Journal 2016; 14 (3) : 4419; Wiesner F, Breyer N, 2013 (RAR 2015). LOQ = 0.05 µg/L for Mesotrione, AMBA and MNBA.

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 mesotrione in air is given in the following tables.

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: mesotrione			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 µg/m ³	HPLC-UV	EU DAR Vol.3 , 1999 Leung, 1997 RR 97-031B (ZA1296/0456)

All analytical methods are active substance data and were evaluated during the EU review of mesotrione. No additional studies have been performed.

These data have been provided and are considered to adequate.

zRMS:

Adequate method exists to monitor Mesotrione residues in air.

LC-MS/MS

LOQ = 0.45 µg/m³

EU agreed EFSA Journal 2016; 14 (3): 4419

Jutsum L, 2013b, Jutsum L, 2013c (RAR 2015)

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

Analytical methods for the determination of residues in body fluids and tissues are not required as mesotrione is not classified as toxic or highly toxic.

zRMS: An analytical method for mesotrione in body fluids is required according to Regulation (EC) No 283/2013. The QuEChERS method (LC-MS/MS) can be used for monitoring mesotrione residues in blood with a LOQ of 0.01 mg/kg.

5.3.2.8 Other studies/ information

No other studies have been provided.

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	Bajdor K.	2020	M-100SC-OR2-C, Method validation for determination of the active substance and three relevant impurities content in the formulation Łukasiewicz Research Network – Institute of Industrial Organic Chemistry 1/20/041/K GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/01 (KCP 10.1.2/01)	Peda T. Niewelt S. (analytical phase)	2021	Magnitude of the residues of Mesotrione in maize (Raw Agricultural Commodity) after one application of M-100SC-OR2-C – four decline curve studies in Poland, Germany, Hungary and Northern France – 2021 SGS Polska Sp. z o. o. 21SGS76 GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/02	Biesiada M.	2020	Validation of analytical method for the determination of active substance – mesotrione in deionized water SORBOLAB Research Laboratory LLC 0016/0090/FA GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/03	Biesiada M.	2020	Validation of analytical method for the determination of active substance – mesotrione in 50% sucrose solution SORBOLAB Research Laboratory LLC	N	CIECH Sarzyna S.A.

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
			0016/0092/FA GLP Unpublished		
KCP 5.1.2/04 (KCP 10.6.2/01)	Dec W.	2021	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test according to OECD Guideline No. 208 (2006) Ecomelius Institute Sp. z o. o. EMI/4/10/2021 GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/05 (KCP 10.6.2/02)	Dec W.	2021	Terrestrial Plant Test: Vegetative Vigour Test according to OECD Guideline No. 227 (2006) Ecomelius Institute Sp. z o. o. EMI/4/9/2021 GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/06 (KCP 10.2.1/03)	Szlauer S.	2022	Freshwater Alga and Cyanobacteria, Growth Inhibition Test according to OECD Guidline No. 201 (2011) Ecomelius Institute Sp. z o. o. EMI/4/3/2021 GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/07 (KCP 10.2.1/04)	Szlauer S.	2022	Lemna sp. Growth Inhibition Test according to OECD Guidline No. 221 (2006) Ecomelius Institute Sp. z o. o. EMI/4/6/2021 GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/08 (KCP 10.2.1/01)	Szlauer S.	2022	Daphnia sp., Acute Immobilisation Test according to OECD Guidline No. 202 (2004) Ecomelius Institute Sp. z o. o. EMI/4/5/2021 GLP Unpublished	N	CIECH Sarzyna S.A.

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 5.1.2/09 (KCP 10.2.1/02)	Szlauer S.	2022	Freshwater Alga and Cyanobacteria, Growth Inhibition Test according to OECD Guideline No. 201 (2011) Ecomelius Institute Sp. z o. o. EMI/4/2/2021 GLP Unpublished	N	CIECH Sarzyna S.A.

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
KCA 4.1.2/01	Alferness PL	1999	ZA1296: Liquid Chromatographic Determination with Fluorescence Detection of ZA1296 & 4- (Methylsulfonyl)-2-Nitrobenzoic Acid in Crops after Conversion to 2-Amino-4- (Methylsulfonyl)- Benzoic Acid - A Modification of TMR0643B Zeneca Agrochemicals, Jealott's Hill, United Kingdom, TMR0882B Not GLP, not published Syngenta File No ZA1296/0121	N	SYN
II, 4.2	Meyers, T.J.and Ryan, J.	1997	ZA 1296: Determination of ZA 1296 and its Metabolite MNBA in Corn by Gas Chromatography with Mass-Selective Detection (WRC-96-163) Zeneca Report No. TMR0689B	N	SYN
III, 5.2.1.1/02	Bolygo, E.	1996	ZA 1296: Independent Laboratory Confirmation of an Analytical Method for Liquid Chromatographic Determination with Fluorescence Detection of ZA 1296 and 4- (methylsulfonyl)-2-nitrobenzoic acid in Crops after Conversion to 2-amino-4-(methylsulfonyl)- benzoic acid Zeneca Report No. RJ2149B	N	SYN
II, 4.2.1.1/ 03	Meyers, T.J. et al.	1998	ZA 1296: Determination of ZA 1296 in milk, eggs and animal products by gas chromatography with mass-selective detection - Addendum (WRC-98-006) Zeneca Report No. TMR0739B ADD	N	SYN

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
III, 5.2.1.1/01 01	Alferness, P.L.	1996	ZA 1296: Liquid Chromatographic Determination with Fluorescence Detection of ZA 1296, 4-methylsulfonyl-2-nitrobenzoic acid and 2-amino-4-methylsulfonyl-benzoic acid in Soil (WRC-96-107) Zeneca Report No. TMR0661B	N	SYN
II, 4.2.3.1/ 01	Meyers, T.J.	1997	ZA 1296: Determination of ZA 1296 or a total of ZA 1296 and MNBA in water by gas chromatography with mass selective detection (WRC-97-025) Zeneca Report No. TMR0707B	N	SYN
II, 4.2.4.1/ 01	Leung, S.C	1997	ZA 1296: Analytical Method for the Determination of ZA 1296 in Air (WRC-97-069) Zeneca Report No. RR 97-031B	N	SYN

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for mesotrione

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

A 2.1.1.1 Determination of residues in maize – decline studies.

A 2.1.1.1.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/01
Report	Magnitude of the residues of Mesotrione in maize (Raw Agricultural Commodity) after one application of M-100SC-OR2-C – four decline curve studies in Poland, Germany, Hungary and Northern France – 2021, Peda T. , Niewelt S., 2021, 21SGS76
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP 10.1.2/01. A summary for analytical part is provided below.

Materials and methods

Specimen extraction and determination of residues of mesotrione were performed according to the multi-QuEChERS method.

Quantification was performed by use of LC-MS/MS detection.

LC-MS/MS conditions

Apparatus	Shimadzu 8050
Column	HPLC column: Arion Polar C18, 3 micro, 120x2.1 mm
Pumps	Binary Gradient Total flow: 0.25mL/min Mobile phase A: 5 mmol ammonium formate + 0.1% formic acid in water Mobile phase B: 5 mmol ammonium formate + 0.1% formic acid in methanol
Oven Temp.	50°C
Interface	ESI
Acquisition mode	MRM
Retention time	6.26 min

Results and discussions

Table A 1: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mL/kg) (<i>n</i> = 5)	Mean recovery (%)	RSD (%)	Comments
<i>m/z</i> 338.00 > 290.85					
Water	Mesotrione	0.01	99.2	1.90	-
		0.1	100.4	2.18	-
<i>m/z</i> 338.0>211.95					
Water	Mesotrione	0.01	97.6	4.41	
		0.1	100.8	2.06	

Table A 2: Characteristics for the analytical method used for validation of mesotrione residues in water medium

	Mesotrione
Specificity	LC-MS/MS method was used during the study.Two mass transitions were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for matrix blank samples. No interferences at above 30% of the LOQ were detected at the retention time of the active substance in matrix blank sample.
Calibration	n= 6 The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at six concentration levels ranging from 1 ppb to 500 ppb of mesotrione. <i>m/z</i> 338.00 > 290.85 R ² = 0.9999491; R= 0.9999746; y=0.616994x-0.000567045 <i>m/z</i> 338.00>211.95 R ² =0.99995683; R= 0.9997841; y=0.221031x-0.000113843
Calibration range	1 ppb to 500 ppb of mesotrione
Assessment of matrix effects is presented	In order to compensate for matrix effects, there were used matrix-matched standards (matrix-matched calibration) and IL-IS for quantification.
Stability	As required in SANTE/2020/12830, Rev.1., if the extracts contain an IL-IS for quantification, testing on final extract stability is not required since the IL-IS will compensate for losses during extract storage. However extract stability was proven by the corresponding procedural recovery samples, which were stored under the same conditions together with the extracts of the specimens for residue analysis.
Limit of determination/quantification	LOQ: 0.010 mg/kg

	LOD: 0.002 mg/kg
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Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev.1.

A 2.1.1.2 Determination of residues in water used in support of ecotoxicological studies 1 - Chronic toxicity test for honey bee larvae.

A 2.1.1.2.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/02
Report	Validation of analytical method for the determination of active substance mesotrione in deionized water, Biesiada M., 2020, 0016/0090/FA
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes

The validation report is linked to the Chronic toxicity test for honey bee larvae, point KCP 10.3.1.2/02.

Materials and methods

The content of the active substance - mesotrione in deionized water was conducted by the method of high-performance liquid chromatography with UV-DAD detection on the basis of a signal from the active substance. Identification of the active substance in the test item was made by comparing the UV spectra an retention times of the standard substances (mesotrione) and a test item sample.

Column Kinetex	5 μ C18 100A 150 x 4.60 mm
Detection	UV-DAD 272 nm
Injection volume	40 μ L
Column oven temperature	40°C
Mobile phase flow	2.0 mL/min
Mobile phase	0.1% (v/v) orthophosphoric acid (A):acetonitrile (B)
Mobile phase composition	50% (A):50% (B)
Time of analysis	2.4 minutes

Results and discussions

Table A 3: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Deionized water	Mesotrione	0.17984	100.18	0.31	-
		2.51776	100.28	0.10	-

Table A 4: Characteristics for the analytical method used for validation of mesotrione residues in deionized water

	Mesotrione
Specificity	<ul style="list-style-type: none"> - under the analysis conditions, retention times of the active substance in the solution of the test item and the solution of standard are comparable (with differences less than 2%); - interfering peaks derived from other substances with area larger than 3% active substance peak area in the test item solution do not occur at retention time of active substance; - comparison of UV spectra of active substance standard and the test item allows to identify the active substance in a test item sample
Calibration	<p>To determine the linearity of the method a 5 solutions of the test item at concentrations of 0.05503 mg/L, 0.61146 mg/L, 1.43872 mg/L, 2.15808 mg/L and 3.05728 mg/L were prepared.</p> <p>The correlation coefficient was $r=0.999$. The acceptance criterion $r \geq 0.99$ was fulfilled.</p> <p>Function was linear in a full range. A calibration curve is described by the following equation: $f(x) = 51041.8x - 266.405$</p>
Calibration range	Calibration curve: 0.05503 – 3.05728 mg/L
Assessment of matrix effects is presented	no
Limit of determination/quantification	<p>LOD = 0.002520 mg/L</p> <p>LOQ = 0.007636 mg/L</p>

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4.

A 2.1.1.2.1.1 Confirmatory method

No confirmatory method is required

A 2.1.1.3 Determination of residues in 50% sucrose solution used in support of ecotoxicological studies 2 - Honey bee chronic oral toxicity.

A 2.1.1.3.1 Method validation

Comments of zRMS: The method is acceptable

Reference: KCP 5.1.2/03
Report Validation of analytical method for the determination of active substance mesotrione in 50% sucrose solution, Biesiada M., 2020, 0016/0092/FA
Guideline(s): Yes (SANCO/3029/99 rev. 4)
Deviations: No
GLP: Yes
Acceptability: Yes

The validation report is linked to the Honey bee chronic oral toxicity study, KCP 10.3.1.2/01.

Materials and methods

The content of the active substance mesotrione after extraction from 50% sucrose solution was conducted by the method of high-performance liquid chromatography with UV-DAD detection on the basis of a signal from the active substance. Identification of the active substance in the test item was made by comparing the UV spectra and retention times of the standard substance (mesotrione) and a test item sample.

Column Kinetex 5 μ C18 100A 150 x 4.60 mm
Detection UV-DAD 271 nm
Injection volume 25 μ L
Column oven temperature 40°C
Mobile phase flow 1.8 mL/min
Mobile phase 0.1% (v/v) orthophosphoric acid (A):acetonitrile (B)
Mobile phase composition 60% (A) : 40% (B)
Time of analysis 3.9 minutes

Results and discussions

Table A 5: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Sucrose solution	Mesotrione	1.45834	100.60	2.15	-
		8.32788	100.17	3.48	-

Table A 6: Characteristics for the analytical method used for validation of mesotrione residues in deionized water

	Mesotrione
Specificity	<ul style="list-style-type: none"> - under the analysis conditions, retention times of the active substance in the solution of the test item and the solution of standard are comparable (with differences less than 2%); - interfering peaks derived from other substances with area larger than 3% active substance peak area in the test item solution do not occur at

	retention time of active substance; - comparison of UV spectra of active substance standard and the test item allows to identify the active substance in a test item sample
Calibration	Function was linear in a full range. A calibration curve is described by the following equation: $f(x) = 117099x + 4489.64$
Calibration range	To determine the linearity of the method 5 solutions of active substance standard at concentrations of 0.10032 mg/kg; 1.71218 mg/kg; 4.00160 mg/kg; 7.30062 mg/kg and 10.02849 mg/kg were prepared. The correlation coefficient was $r=0.999$. The acceptance criterion $r \geq 0.99$ was fulfilled.
Assessment of matrix effects is presented	no
Limit of determination/quantification	LOD = 0.010483 mg/kg LOQ = 0.031768 mg/kg

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4.

A 2.1.1.3.1.1 Confirmatory method

No confirmatory method is required

A 2.1.1.4 Determination of residues in water used in support of ecotoxicological studies 3 - Terrestrial Plant Test 1.

A 2.1.1.4.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/04 (KCP 10.6.2/01)
Report	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, W. Dec, EMI/4/10/2021
Guideline(s):	SANTE/2020/12830 Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP 10.6.2/01. A summary for analytical part is provided below.

Materials and methods

The concentration of Mesotrione in water was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

LC-MS conditions

Apparatus	Agilent Infinity 1290, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Column	HiP Sampler G4226A, Binary Pump G4220A Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm, Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar, Column Comp. G1316C
Injection	10 µL
Oven temperature	40.00 °C
Mobile phase flow	30%: water + amonium formate (0,1%), 70%: methanol + formic acid (0,05%)
Auxiliary gases flow	0.200 mL/min
Mass spectrometer conditions	
Gas Temp	300 °C
Gas Flow	12 L/min
Nebulizer	10 psi
SheathGasHeater	200 °C
SheathGasFlow	12 L/min

Results and discussions

Table A 7: Recovery results from method validation of Mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.33	111.8	4.3	-
		3.3	114.8	2.3	-

Table A 8: Characteristics for the analytical method used for validation of mesotrione residues in deionized water

	Mesotrione
Specificity	<p>Specificity was determined on the basis of chromatograms obtained from matrix blank sample (prepared according to Sample preparation) and the fortified sample (at LOQ level).</p> <p>The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response is equal 8.7% of mean LOQ response which is acceptable.</p> <p>Two ion transitions were recorded: Transition (Target): 340.1→ 227.9 Transition (Qualifier): 340.1→ 104 Specificity was verified using the ion transition ratio of 23.6% ±30%.</p>
Calibration	<p>Linearity was determined by preparing a series of standard solutions of mesotrione at the concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, 10 µg/L.</p> <p>n=6 y=3453.9C + 68.1 R²= 0.9922</p>

Calibration range	0.1-10 µg/L
Assessment of matrix effects is presented	Standard solutions used for the calibration curve were prepared using water as a solvent. This procedure is for the determination of mesotrione in water, so a matrix effect will not occur.
Standard stability	Two standard solution of mesotrione were prepared: one of the standard has been stored in freezer for 185 days, second standard was prepared freshly. Difference is less than 10% so standard solution of mesotrione stored in freezer (<-18 °C) for at least 6 months is stable.
Limit of determination/quantification	LOD = 0.1 µg/kg LOQ = 0.33 µg/kg

Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830 Rev.1.

A 2.1.1.5 Determination of residues in water used in support of ecotoxicological studies 4 - Terrestrial Plant Test 2.

A 2.1.1.5.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/05 (KCP 10.6.2/02)
Report	Terrestrial Plant Test: Vegetative Vigour Test, W. Dec, 2021, EMI/4/9/2021
Guideline(s):	SANTE/2020/12830 Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP 10.6.2/02. A summary for analytical part is provided below.

Materials and methods

The concentration of Mesotrione in water was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

LC-MS conditions

Apparatus	Agilent Infinity 1290, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI) HiP Sampler G4226A, Binary Pump G4220A
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm, Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar, Column Comp. G1316C
Injection	10 µL

Oven temperature	40.00 °C
Mobile phase flow	30%: water + amonium formate (0,1%), 70%: methanol + formic acid (0,05%)
Auxiliary gases flow	0.200 mL/min
Mass spectrometer conditions	
Gas Temp	300°C
Gas Flow	12 L/min
Nebulizer	10 psi
SheathGasHeater	200°C
SheathGasFlow	12 L/min

Results and discussions

Table A 9: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.33	111.8	4.3	-
		3.3	114.8	2.3	-

Table A 10: Characteristics for the analytical method used for validation of mesotrione residues in deionized water

	Mesotrione
Specificity	<p>Specificity was determined on the basis of chromatograms obtained from matrix blank sample (prepared according to Sample preparation) and the fortified sample (at LOQ level).</p> <p>The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response is equal 8.7% of mean LOQ response which is acceptable.</p> <p>Two ion transitions were recorded: Transition (Target): 340.1 → 227.9 Transition (Qualifier): 340.1 → 104</p> <p>Specificity was verified using the ion transition ratio of 23.6% ±30%.</p>
Calibration	<p>Linearity was determined by preparing a series of standard solutions of mesotrione at the concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, 10 µg/L.</p> <p>n=6 y=3453.9C + 68.1 R2= 0.9922</p>
Calibration range	0.1-10 µg/L
Assessment of matrix effects is presented	Standard solutions used for the calibration curve were prepared using water as a solvent. This procedure is for the determination of mesotrione in water, so a matrix effect will not occur.
Limit of determination/quantification	LOD = 0.1 µg/kg LOQ = 0.33 µg/kg

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4.

A 2.1.1.6 Determination of residues in water used in support of ecotoxicological studies 5 - *Raphidocelis subcapitata* (*Pseudokirchneriella subcapitata*).

A 2.1.1.6.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/06 (KCP 10.2.1/03)
Report	Freshwater Alga and Cyanobacteria, Growth Inhibition Test, S. Szlauer, 2022, EMI/4/3/2021
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP 10.2.1/03. A summary for analytical part is provided below.

Materials and methods

The concentration of Mesotrione in AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

LC-MS conditions

Apparatus	Agilent Infinity 1290, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar, Column Comp. G1316C
Injection	10 µL
Oven temperature	50.00 °C
Mobile phase flow	75%: water + ammonium formate (0,1%), 25%: methanol + formic acid (0,05%)

Mass spectrometer conditions

Gas Temp	300°C
Gas Flow	12 L/min
Nebulizer	20 psi
SheathGasHeater	300 °C
SheathGasFlow	12 L/min

Results and discussions

Table A 11: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
AAP	Mesotrione	0.7	102.8	9.1	-
		7	95	2.7	-

Table A 12: Characteristics for the analytical method used for validation of mesotrione residues in AAP medium

	Mesotrione																										
Specificity	<p>Specificity was determined on the basis of chromatograms obtained from matrix blank sample (prepared according to Sample preparation) and the fortified sample (at LOQ level).</p> <p>The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response (734, n = 2) is equal to 15% of mean LOQ response which is acceptable.</p> <p>Two ion transitions were recorded: Transition (Target): 340.1→ 227.9 Transition (Qualifier): 340.1→ 104</p> <p>Specificity was verified using the ion transition ratio of 24.5% ± 30% (relative). Specificity of the method was confirmed.</p>																										
Calibration	<p>Linearity was determined by preparing a series of standard solutions of Mesotrione at the concentrations of 0.2, 0.7, 1, 3, 6, 10, 15 µg/L.</p> <p>Signal = 6794 C + 6.2 R²: 0.9998</p>																										
Calibration range	0.2 – 15 µg/L																										
Assessment of matrix effects is presented	<p>Assessment of matrix effects were performed by comparing the analyte response of the test item M100SC-OR2-C prepared in ultrapure water (UP) to one prepared in AAP medium.</p> <table><tr><th>Solvent</th><th>Measurement repetition</th><th>Response (area)</th><th>Mean Response</th><th>Standard deviation</th><th>Relative standard deviation [%]</th></tr><tr><td rowspan="3">UP</td><td>1</td><td>67545</td><td rowspan="3">68554</td><td rowspan="3">912</td><td rowspan="3">1.3</td></tr><tr><td>2</td><td>69321</td></tr><tr><td>3</td><td>68795</td></tr><tr><td rowspan="3">AAP</td><td>1</td><td>65298</td><td rowspan="3">66983</td><td rowspan="3">1547</td><td rowspan="3">2.3</td></tr><tr><td>2</td><td>67311</td></tr><tr><td>3</td><td>68339</td></tr></table> <p>Difference did not exceed 20% so matrix effects are not considered significant.</p>	Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]	UP	1	67545	68554	912	1.3	2	69321	3	68795	AAP	1	65298	66983	1547	2.3	2	67311	3	68339
Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]																						
UP	1	67545	68554	912	1.3																						
	2	69321																									
	3	68795																									
AAP	1	65298	66983	1547	2.3																						
	2	67311																									
	3	68339																									
Stability	<p>Sample has been stored in ambient temperature for 2 days.</p> <p>Recovery of analyte meets the acceptance criteria 70-120% so analyte is stable for at least for 2 days.</p>																										

	Mean recovery [%] = 90.5
Limit of determination/quantification	LOQ: 0.7 µg/L LOD: 0.2 µg/L

Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev.1.

A 2.1.1.7 Determination of residues in water used in support of ecotoxicological studies 6 - *Lemna sp.* Growth Inhibition Test.

A 2.1.1.7.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/07 (KCP 10.2.1/04)
Report	<i>Lemna sp.</i> Growth Inhibition Test, S. Szlauer, 2022, EMI/4/6/2021
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP 10.2.1/04. A summary for analytical part is provided below

Materials and methods

The concentration of Mesotrione in 20×AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

LC-MS conditions

Apparatus	Agilent Infinity 1290, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar, Column Comp. G1316C
Injection	10 µL
Oven temperature	50.00 °C
Mobile phase flow	75%: water + ammonium formate (0,1%), 25%: methanol + formic acid (0,05%)

Mass spectrometer conditions

Gas Temp	300 °C
Gas Flow	12 L/min
Nebulizer	20 psi
SheathGasHeater	300 °C
SheathGasFlow	12 L/min

Results and discussions

Table A 13: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
AAP	Mesotrione	0.2	84.7	7.0	-
		2	103	2.0	-

Table A 14: Characteristics for the analytical method used for validation of mesotrione residues in AAP medium

	Mesotrione																										
Specificity	<p>Specificity was determined on the basis of chromatograms obtained from matrix blank sample (prepared according to Sample preparation) and the fortified sample (at LOQ level).</p> <p>The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response (94, n = 2) is equal to 11% of mean LOQ response which is acceptable.</p> <p>Two ion transitions were recorded: Transition (Target): 340.1→ 227.9 Transition (Qualifier): 340.1→ 104</p> <p>Specificity was verified using the ion transition ratio of 24.0% ± 30% (relative). Specificity of the method was confirmed.</p>																										
Calibration	<p>Linearity was determined by preparing a series of standard solutions of Mesotrione at the concentrations of 0.06, 0.2, 0.6, 1, 2, 3 µg/L.</p> <p>Signal = 4241 C + 176 R²: 0.9997</p>																										
Calibration range	0.06– 3 µg/L																										
Assessment of matrix effects is presented	<p>Assessment of matrix effects were performed by comparing the analyte response of the test item M100SC-OR2-C prepared in ultrapure water (UP) to one prepared in AAP medium.</p> <table><tr><th>Solvent</th><th>Measurement repetition</th><th>Response (area)</th><th>Mean Response</th><th>Standard deviation</th><th>Relative standard deviation [%]</th></tr><tr><td rowspan="3">UP</td><td>1</td><td>14049</td><td rowspan="3">14528</td><td rowspan="3">515</td><td rowspan="3">3.5</td></tr><tr><td>2</td><td>14462</td></tr><tr><td>3</td><td>15072</td></tr><tr><td rowspan="3">AAP</td><td>1</td><td>9622</td><td rowspan="3">9373</td><td rowspan="3">238</td><td rowspan="3">2.5</td></tr><tr><td>2</td><td>9349</td></tr><tr><td>3</td><td>9147</td></tr></table> <p>Difference did not exceed 20% so matrix effects are not considered significant.</p>	Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]	UP	1	14049	14528	515	3.5	2	14462	3	15072	AAP	1	9622	9373	238	2.5	2	9349	3	9147
Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]																						
UP	1	14049	14528	515	3.5																						
	2	14462																									
	3	15072																									
AAP	1	9622	9373	238	2.5																						
	2	9349																									
	3	9147																									
Stability	Sample has been stored in ambient temperature for 1 day.																										

	Recovery of analyte meets the acceptance criteria 70-120% so analyte is stable for at least for 1 day. Mean recovery [%] = 107.2
Limit of determination/quantification	LOQ: 0.2 µg/L LOD: 0.06 µg/L

Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev.1.

A 2.1.1.8 Determination of residues in water used in support of ecotoxicological studies 7 - *Daphnia sp.*, Acute Immobilisation Test

A 2.1.1.8.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/08 (KCP 10.2.1/01)
Report	Daphnia sp., Acute Immobilisation Test, 2022, S. Szlauer, EMI/4/5/2021
Guideline(s):	SANTE/2020/12830 Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP 10.2.1/01. A summary for analytical part is provided below.

Materials and methods

The concentration of Mesotrione in Elendt M7 medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

Chromatographic conditions

Apparatus	Agilent Infinity 1290, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Column	HiP Sampler G4226A, Binary Pump G4220A, Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar, Column Comp. G1316C
Injection	10 µL
Elution	Isocratic
Oven temperature	50.00 °C
Mobile phase flow	75%: water + ammonium formate (0,1%), 25%: methanol + formic acid (0,05%)

Mass spectrometer conditions

Gas Temp	300 °C
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Gas Flow 12 L/min
 Nebulizer 20 psi
 SheathGasHeater 300 °C
 SheathGasFlow 12 L/min

Results and discussions

Table A 15: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Elendta M7	Mesotrione	0.7	117.6	6.9	-
		7.0	99.0	2.9	-

Table A 16: Characteristics for the analytical method used for validation of mesotrione residues in AAP medium

	Mesotrione																								
Specificity	<p>Specificity was determined on the basis of chromatograms obtained from matrix blank sample and the fortified sample (at LOQ level).</p> <p>The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response (734, n = 2) is equal to 15% of mean LOQ response which is acceptable.</p> <p>Two ion transitions were recorded: Transition (Target): 340.1→ 227.9 Transition (Qualifier): 340.1→ 104</p> <p>Specificity was verified using the ion transition ratio of 24.5% ± 30% (relative). Specificity of the method was confirmed.</p>																								
Calibration	<p>Linearity was determined by preparing a series of standard solutions of Mesotrione at the concentrations of 0.2, 0.7, 1, 3, 6, 10, 15 µg/L.</p> <p>n=7 Signal = 6794 C + 6.2 R²: 0.9998</p>																								
Calibration range	0.2 – 15 µg/L																								
Assessment of matrix effects is presented	<p>Assessment of matrix effects were performed by comparing the analyte response of the test item M100SC-OR2-C prepared in ultrapure water (UP) to one prepared in Elendta M7 medium.</p> <table><tr><th>Solvent</th><th>Measurement repetition</th><th>Response (area)</th><th>Mean Response</th><th>Standard deviation</th><th>Relative standard deviation [%]</th></tr><tr><td rowspan="3">UP</td><td>1</td><td>67545</td><td rowspan="3">68554</td><td rowspan="3">912</td><td rowspan="3">1.3</td></tr><tr><td>2</td><td>69321</td></tr><tr><td>3</td><td>68795</td></tr><tr><td rowspan="2">Elendta M7</td><td>1</td><td>72618</td><td rowspan="2">71477</td><td rowspan="2">1336</td><td rowspan="2">1.9</td></tr><tr><td>2</td><td>71804</td></tr></table>	Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]	UP	1	67545	68554	912	1.3	2	69321	3	68795	Elendta M7	1	72618	71477	1336	1.9	2	71804
Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]																				
UP	1	67545	68554	912	1.3																				
	2	69321																							
	3	68795																							
Elendta M7	1	72618	71477	1336	1.9																				
	2	71804																							

	<table><tr><td></td><td>3</td><td>70007</td><td></td><td></td><td></td></tr></table> <p>Difference did not exceed 20% so matrix effects are not considered significant.</p>		3	70007			
	3	70007					
Stability	Sample has been stored in ambient temperature for 2 days. Recovery of analyte meets the acceptance criteria 70-120% so analyte is stable for at least for 2 days. Mean recovery [%] = 98.7						
Limit of determination/quantification	LOQ: 0.7 µg/L LOD: 0.2 µg/L						

Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev.1.

A 2.1.1.9 Determination of residues in water used in support of ecotoxicological studies 8 – *Navicula pelliculosa*.

A 2.1.1.9.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/09 (KCP 10.2.1/02)
Report	Freshwater Alga and Cyanobacteria, 2022, S. Szlauer, EMI/4/2/2021
Guideline(s):	SANTE/2020/12830 Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The test report is included in the dRR Part 9, point KCP KCP 10.2.1/02. A summary is provided below.

Materials and methods

The concentration of Mesotrione in AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

Chromatographic conditions

Apparatus	Agilent Infinity 1290, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Column	HiP Sampler G4226A, Binary Pump G4220A, Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar, Column Comp. G1316C
Injection	10 µL
Elution	Isocratic
Oven temperature	50.00 °C

Mobile phase flow 75%: water + amonium formate (0,1%),
25%: methanol + formic acid (0,05%)

Mass spectrometer conditions

Gas Temp 300 °C
Gas Flow 12 L/min
Nebulizer 20 psi
SheathGasHeater 300 °C
SheathGasFlow 12 L/min

Results and discussions

Table A 17: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
AAP medium	Mesotrione	0.7	102.8	9.1	-
		7.0	95.0	2.7	-

Table A 18: Characteristics for the analytical method used for validation of mesotrione residues in AAP medium

	Mesotrione												
Specificity	<p>Specificity was determined on the basis of chromatograms obtained from matrix blank sample (prepared according to Sample preparation) and the fortified sample (at LOQ level).</p> <p>The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response (734, n = 2) is equal to 15% of mean LOQ response.</p> <p>Two ion transitions were recorded: Transition (Target): 340.1→ 227.9 Transition (Qualifier): 340.1→ 104</p> <p>Specificity was verified using the ion transition ratio of 24.5% ± 30% (relative). Specificity of the method was confirmed.</p>												
Calibration	<p>Linearity was determined by preparing a series of standard solutions of Mesotrione at the concentrations of 0.2, 0.7, 1, 3, 6, 10, 15 µg/L.</p> <p>n=7 Signal = 6794 C + 6.2 R²: 0.9998</p>												
Calibration range	0.2 – 15 µg/L												
Assessment of matrix effects is presented	<p>Assessment of matrix effects were performed by comparing the analyte response of the test item M100SC-OR2-C prepared in ultrapure water (UP) to one prepared in AAP medium</p> <table><tr><td>Solvent</td><td>Measurement repetition</td><td>Response (area)</td><td>Mean Response</td><td>Standard deviation</td><td>Relative standard deviation [%]</td></tr><tr><td></td><td></td><td></td><td></td><td></td><td></td></tr></table>	Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]						
Solvent	Measurement repetition	Response (area)	Mean Response	Standard deviation	Relative standard deviation [%]								

	UP	1	67545	68554	912	1.3
		2	69321			
		3	68795			
	AAP medium	1	65298	66983	1547	2.3
		2	67311			
		3	68339			
Difference did not exceed 20% so matrix effects are not considered significant.						
Stability	Sample has been stored in ambient temperature for 2 days. Recovery of analyte meets the acceptance criteria 70-120% so analyte is stable for at least for 2 days. Mean recovery [%] = 90.5					
Limit of determination/quantification	LOQ: 0.7 µg/L LOD: 0.2 µg/L					

Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev.1.

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