REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB2112dH

Product name: Walkover Trio

Chemical active substances:

Thiencarbazone-methyl, 75 g/L
Mesotrione, 375 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: September 2024

zRMS Assessment : 31/03/2025

Version after commenting: 03/07/2025

List of references update: 10/07/2025

Version history

When	What
September 2024	Initial dossier submission by applicant for approval of new product.
March 2025	zRMS assessment
July 2025	After commenting round
July 2025	List of references update

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are:

- No data gap noticed.

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

- No data gap noticed.

Commodity/crop	Supported/ Not supported
~~Oilseed rape~~	~~supported~~
Maize	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 mesotrione, thiencarbazone-methyl and cyprosulfamide in plant protection product is provided as follows:

Comments of zRMS:	The analytical method code: DNA7206 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (thiencarbazone-methyl, mesotrione and cyprosulfamide). The method is successfully validated and accepted.

Reference:	KCP 5.1.1
Report	Validation of the methods of determination of active ingredients and specified impurities in a suspension concentrate formulation containing thiencarbazone-methyl, mesotrione and cyprosulfamide, in compliance with good laboratory practice, Fitmaurice T., 2023, DNA7206
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

Thiencarbazone-methyl and cyprosulfamide:

The mass of the formulation was accurately weighed into a volumetric flask and partially made to volume with Acetonitrile. The solution was sonicated for 5 minutes, allowed to cool back to room temperature (ambient) before being made to final volume with Acetonitrile. Each solution was injected once into the HPLC-UV under the following conditions:

HPLC-UV Conditions – Thiencarbazone-methyl and Cyprosulfamide:

Instrument:	Agilent 1100 Series HPLC-UV
Mode:	Isocratic Reverse Phase
Column:	Agilent Zorbax SB-Aq, 150mm x 4.6mm
Packing:	SB-Aq, 3.5µm
Eluent:	A: 25% Acetonitrile B: 75% Deionised Water adjusted to pH3 with Formic Acid
Flow Rate:	1.2mL/minute
Injection Volume:	10µL
Column Temperature:	30°C
Wavelength:	225nm
Data Collection:	LabSolutions
Retention Times:	Thiencarbazone-methyl approximately 18.7 to 18.9 minutes Cyprosulfamide approximately 29.7 to 30.1 minutes

Thiencarbazone-methyl reference standards were prepared in acetonitrile.
Cyprosulfamide reference standards were prepared in acetonitrile.

Mesotrione:

The mass of the formulation was accurately weighed into a volumetric flask and partially made to volume with Acetonitrile. The solution was sonicated for 5 minutes, allowed to cool back to room temperature (ambient) before being made to final volume with Acetonitrile. Each solution was injected once into the HPLC-UV under the following conditions:

HPLC-UV Conditions – Mesotrione:

Instrument:	Agilent 1100 Series HPLC-UV
Mode:	Isocratic Reverse Phase
Column:	Agilent Zorbax SB-Aq, 150mm x 4.6mm
Packing:	SB-Aq, 3.5µm
Eluent:	A: 25% Acetonitrile B: 75% Deionised Water adjusted to pH3 with Formic Acid
Flow Rate:	1.2mL/minute
Injection Volume:	5µL
Column Temperature:	30°C
Wavelength:	254nm
Data Collection:	LabSolutions
Retention Time:	Mesotrione approximately 13.9 to 14.0 minutes

Mesotrione reference standards were prepared in acetonitrile.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances mesotrione and thiencarbazone-methyl and the safener cyprosulfamide in plant protection product GLOB2112dH

<table>
<tr><th></th><th>Thiencarbazone-methyl</th><th>Mesotrione</th><th>Cyprosulfamide</th></tr>
<tr><td>Author(s), year</td><td colspan="3">Fitzmaurice T., 2023</td></tr>
<tr><td>Principle of method</td><td colspan="3">HPLC-UV</td></tr>
<tr><td>Linearity
(linear between mg/L / % range of the declared content)
(correlation coefficient, expressed as r)</td><td>0-1.00 mg/mL
R^2 = 0.9999
R = 1.0000</td><td>0-1.00 mg/mL
R^2 = 1.0000
R = 1.0000</td><td>0-1.00 mg/mL
R^2 = 1.0000
R = 1.0000</td></tr>
<tr><td>Precision – Repeatability
Mean
n = 6
(%RSD)</td><td>%RSD = 0.387
Hr = 0.196</td><td>%RSD = 0.297
Hr = 0.192</td><td>%RSD = 0.165
Hr = 0.0894</td></tr>
<tr><td>Accuracy
n = 6
(% Recovery)</td><td>Total recovery at 75 g/L:
Mean Recovery = 99.28%
%RSD = 0.846
Hr = 0.427

LOQ recovery at 5.00 g/L:
Mean Recovery = 100.6%
%RSD = 1.495
Hr = 0.503</td><td>Total recovery at 375 g/L:
Mean Recovery = 98.75%
%RSD = 0.922
Hr = 0.593

LOQ recovery at 5.00 g/L:
Mean Recovery = 108.2%
%RSD = 2.266
Hr = 0.771</td><td>Total recovery at 112 g/L:
Mean Recovery = 112.3%
%RSD = 0.996
Hr = 0.535

LOQ recovery at 5.00 g/L:
Mean Recovery = 99.78%
%RSD = 2.131
Hr = 0.716</td></tr>
<tr><td>Interference/ Specificity</td><td>The MS and UV spectra produced by the reference standard and the sample are the same. This shows that the method is specific for thiencarbazone-methyl.

There were no other significant peaks present at the same elution time as thiencarbazone-methyl. This shows that there are no interferences greater than 3%.
<table>
<tr><th>Analyte</th><th>Retention time</th></tr>
<tr><td>Solvent Blank</td><td>No Peak</td></tr>
<tr><td>Formulation Blank</td><td>No Peak</td></tr>
<tr><td>Thiencarbazone-methyl</td><td>18.7 Min</td></tr>
<tr><td>Mesotrione</td><td>15.3 Min</td></tr>
<tr><td>Cyprosulfamide</td><td>29.8 Min</td></tr>
<tr><td>Impurity 1 (R287431)</td><td>No Peak</td></tr>
<tr><td>Impurity 2 (R287432)</td><td>22.9 Min</td></tr>
<tr><td>Impurity 3 (R287432)</td><td>No Peak</td></tr>
</table></td><td>The MS and UV spectra produced by the reference standard and the sample are the same. This shows that the method is specific for mesotrione.

There were no other significant peaks present at the same elution time as mesotrione. This shows that there are no interferences greater than 3%.
<table>
<tr><th>Analyte</th><th>Retention time</th></tr>
<tr><td>Solvent Blank</td><td>No Peak</td></tr>
<tr><td>Formulation Blank</td><td>No Peak</td></tr>
<tr><td>Thiencarbazone-methyl</td><td>17.2 Min</td></tr>
<tr><td>Mesotrione</td><td>13.8 Min</td></tr>
<tr><td>Cyprosulfamide</td><td>27.1 Min</td></tr>
<tr><td>Impurity 1 (R287431)</td><td>40.9 Min</td></tr>
<tr><td>Impurity 2 (R287432)</td><td>20.2 Min</td></tr>
<tr><td>Impurity 3 (R287432)</td><td>No Peak</td></tr>
</table></td><td>The MS and UV spectra produced by the reference standard and the sample are the same. This shows that the method is specific for cyprosulfamide.

There were no other significant peaks present at the same elution time as cyprosulfamide. This shows that there are no interferences greater than 3%.
<table>
<tr><th>Analyte</th><th>Retention time</th></tr>
<tr><td>Solvent Blank</td><td>No Peak</td></tr>
<tr><td>Formulation Blank</td><td>No Peak</td></tr>
<tr><td>Thiencarbazone-methyl</td><td>18.7 Min</td></tr>
<tr><td>Mesotrione</td><td>15.3 Min</td></tr>
<tr><td>Cyprosulfamide</td><td>29.8 Min</td></tr>
<tr><td>Impurity 1 (R287431)</td><td>No Peak</td></tr>
<tr><td>Impurity 2 (R287432)</td><td>22.9 Min</td></tr>
<tr><td>Impurity 3 (R287432)</td><td>No Peak</td></tr>
</table></td></tr>
</table>

	Thiencarbazone-methyl	Mesotrione	Cyprosulfamide
Comment	-	-	-

Conclusion

The validation parameters for the Thiencarbazone-methyl, Mesotrione and Cyprosulfamide methodologies have been met for this study under SANCO/3030/99 rev.5 guidelines.

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

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

Comments of zRMS:	The analytical method code: DNA7206 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for analysis the content of the impurities in a suspension concentrate. The method is successfully validated and accepted.

Reference:	KCP 5.1.1
Report	Validation of the methods of determination of active ingredients and specified impurities in a suspension concentrate formulation containing thiencarbazone-methyl, mesotrione and cyprosulfamide, in compliance with good laboratory practice, Fitmaurice T., 2023, DNA7206
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Impurity 1 (R287431):

The mass of the formulation was accurately weighed into a volumetric flask and partially made to volume with Acetonitrile. The solution was sonicated for 5 minutes, allowed to cool back to room temperature (ambient) before being made to final volume with Acetonitrile. Each solution was injected once into the LC-QQQ under the following conditions:

LC-QQQ Conditions – Impurity 1 (R287431):

Instrument:	Agilent 6470 QQQ Mass Spectrometer
Mode:	Isocratic Reverse Phase (Standards) Gradient Reverse Phase (Samples)
Column:	Agilent Zorbax SB-Phenyl, 150mm x 4.6mm
Packing:	SB-Phenyl, 3.5µm
Eluent:	A: Acetonitrile B: Deionised Water adjusted to pH3 with Formic Acid
Flow Rate:	0.5mL/minute
Injection Volume:	10µL

Column Temperature:	25ºC		
Data Acquisition:	MassHunter		
Retention Time:	Approximately 3.6 to 3.7 minutes		
Ionisation:	Positive	Sheath Gas Temperature:	150ºC
Gas Temperature:	150ºC	Sheath Gas flow:	6L/minute
Gas Flow:	8L/minute	Capillary:	6000V
Nebulizer:	20psi	Nozzle Voltage:	2000V

Impurity 1 (R287431) MRM Precursor Ion: 345.02m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
345.02	235	100	222	28	5
345.02	206	100	222	56	5
345.02	152	100	222	72	5
345.02	151	100	222	75	5

The reference standards are assayed using an isocratic methodology with a 10 minute run-time. For the sample assays an additional gradient is employed to ensure that any residual co-formulants have been flushed from the column. This is to ensure that there is no potential carry-over between the sample assays and the following injections. The chromatographic conditions are identical for both methods at the retention time of Impurity 1 (R287431).

Gradient Conditions – Impurity 1 (R287431):

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	85	15
10.0	85	15
11.0	95	5
29.0	95	5
30.0	85	15
40.0	85	15

Between 10.0-11.0 minutes, the ratio of the eluent is changing from 85% to 95% for eluent A and from 15% to 5% for eluent B. Between 29.0-30.0 minutes, the ratio of the eluent is changing from 95% to 85% for eluent A and from 5% to 15% for eluent B.

Impurity 1 (R287431) reference standards were prepared in acetonitrile.

Impurity 2 (R287432):

The mass of the formulation was accurately weighed into a volumetric flask and partially made to volume with Acetonitrile. The solution was sonicated for 5 minutes, allowed to cool back to room temperature (ambient) before being made to final volume with Acetonitrile. Each solution was injected once into the HPLC-PDA under the following conditions:

HPLC-PDA Conditions – Impurity 2 (R287432):

Instrument:	Shimadzu HPLC-PDA
Mode:	Gradient Reverse Phase
Column:	Waters Sunfire C18, 150mm x 4.6mm
Packing:	C18, 3.5µm

Eluent:	A: Acetonitrile B: Deionised Water adjusted to pH3 with Formic Acid
Flow Rate:	1.0mL/minute
Injection Volume:	10µL
Column Temperature:	25ºC
Wavelength:	237nm
Data Collection:	LabSolutions
Retention Time:	Approximately 14.0 minutes

Gradient Conditions – Impurity 2 (R287432):

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	30	70
17.0	30	70
18.0	80	20
23.0	80	20
24.0	30	70
30.0	30	70

Between 17.0-18.0 minutes, the ratio of the eluent is changing from 30% to 80% for eluent A and from 70% to 20% for eluent B. Between 23.0-24.0 minutes, the ratio of the eluent is changing from 80% to 30% for eluent A and from 20% to 70% for eluent B.

Impurity 2 (R287432) reference standards were prepared in acetonitrile.

Impurity 3 (1,2-dichloroethane):

The mass of the formulation was accurately weighed into a Headspace Vial and made to volume with Deionised Water. Each solution was injected once into the GC-MSD with Headspace Sampler under the following conditions:

GC-MSD with Headspace Sampler Conditions – Impurity 3 (1,2-dichloroethane):

Instrument:	Shimadzu GC-MSD with HS-20 Headspace Sampler
Column:	VF-624MS (30m x 0.32mm x 1.8µm)
Temperatures:	
Column:	60ºC held for 5 minutes, then 12°C/minute to 280°C and held for 5 minutes
Injector:	33ºC
Scanning Range:	SIM 49m/z, 62m/z and 64m/z
Carrier Gas:	Helium
Data Collection:	GCMS Solutions
Retention Time:	Approximately 4.9 minutes
Headspace Conditions:	
Cycle Time:	28.33 minutes
Shake Strength:	4/5
Oven Temperature:	70°C
Loop Temperature:	150°C
Transfer Line:	160°C

Impurity 3 (1,2-dichloroethane) reference standards were prepared in methanol and diluted in deionised

water.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) GLOB2112dH

<table>
<tr><th></th><th>Impurity 1
(1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (R287431))
max. 0.00075 g/L</th><th>Impurity 2
(6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (R287432))
max. 0.75 g/L</th><th>Impurity 3
(1,2-dichloroethane)
max. 0.375 g/L</th></tr>
<tr><td>Author(s), year</td><td colspan="3">Fitzmaurice T., 2023</td></tr>
<tr><td>Principle of method</td><td>LC-QQQ</td><td>HPLC-PDA</td><td>GC-MSD</td></tr>
<tr><td>Linearity
(linear between mg/L)
(correlation coefficient, expressed as r)</td><td>0-0.50 mg/L
R^2 = 0.9983
R = 0.9991</td><td>0-4.00 mg/L
R^2 = 0.9999
R = 1.0000</td><td>0-5.00 mg/L
R^2 = 0.9953
R = 0.9976</td></tr>
<tr><td>Precision – Repeatability Mean
n = 6
(%RSD)</td><td>Not detectable above the LOQ level of 0.50 mg/kg in the formulation, equating to 1.6204 mg/kg in the active substance as manufactured.</td><td>Not detectable above the LOQ level of 0.10 g/kg in the formulation, equating to 0.3241g/kg in the active substance as manufactured</td><td>%RSD = 1.599
Hr = 0.270</td></tr>
<tr><td>Accuracy
n = 6
(% Recovery)</td><td>Total recovery at 0.05 mg/kg:
Mean Recovery = 113.6%
%RSD = 7.308
Hr = 0.885
LOQ recovery at 0.005 mg/kg:
Mean Recovery = 119.2%
%RSD = 5.573
Hr = 0.481</td><td>Total recovery at 1 g/kg:
Mean Recovery = 101.3%
%RSD = 0.486
Hr = 0.129
LOQ recovery at 0.10 g/kg:
Mean Recovery = 101.4%
%RSD = 10.057
Hr = 1.880*</td><td>Total recovery at 0.05 g/kg:
Mean Recovery = 91.95%
%RSD = 3.889
Hr = 0.645
LOQ recovery at 0.02 g/kg:
Mean Recovery = 116.1%
%RSD = 2.759
Hr = 0.413</td></tr>
<tr><td>Interference/ Specificity</td><td>The spectra produced by the reference standard and the sample are the same. This shows that the method is specific for Impurity 1 (R287431).
There were no other significant peaks present at the same elution time as Impurity 1 (R287431). This shows that there are no interferences greater than 3%.
<table>
<tr><th>Analyte</th><th>Retention time</th></tr>
<tr><td>Solvent Blank</td><td>No Peak</td></tr>
<tr><td>Formulation Blank</td><td>No Peak</td></tr>
<tr><td>Thiencarbazone-methyl</td><td>No Peak</td></tr>
<tr><td>Mesotrione</td><td>No Peak</td></tr>
<tr><td>Cyprosulfamide</td><td>No Peak</td></tr>
<tr><td>Impurity 1 (R287431)</td><td>3.7 Min</td></tr>
<tr><td>Impurity 2 (R287432)</td><td>No Peak</td></tr>
<tr><td>Impurity 3</td><td>No Peak</td></tr>
</table></td><td>The spectra produced by the reference standard and the sample are the same. This shows that the method is specific for Impurity 2 (R287432).
There were no other significant peaks present at the same elution time as Impurity 2 (R287432). This shows that there are no interferences greater than 3%.
<table>
<tr><th>Analyte</th><th>Retention time</th></tr>
<tr><td>Solvent Blank</td><td>No Peak</td></tr>
<tr><td>Formulation Blank</td><td>No Peak</td></tr>
<tr><td>Thiencarbazone-methyl</td><td>17.2 Min</td></tr>
<tr><td>Mesotrione</td><td>16.7 Min</td></tr>
<tr><td>Cyprosulfamide</td><td>18.6 Min</td></tr>
<tr><td>Impurity 1 (R287431)</td><td>No Peak</td></tr>
</table></td><td>The spectra produced by the reference standard and the sample are the same. This shows that the method is specific for Impurity 3 (1,2-dichloroethane).
There were no other significant peaks present at the same elution time as Impurity 3 (1,2-dichloroethane). This shows that there are no interferences greater than 3%.
<table>
<tr><th>Analyte</th><th>Retention time</th></tr>
<tr><td>Solvent Blank</td><td>No Peak</td></tr>
<tr><td>Formulation Blank</td><td>No Peak</td></tr>
<tr><td>Thiencarbazone-methyl</td><td>No Peak</td></tr>
<tr><td>Mesotrione</td><td>No Peak</td></tr>
<tr><td>Cyprosulfamide</td><td>No Peak</td></tr>
<tr><td>Impurity 1 (R287431)</td><td>No Peak</td></tr>
</table></td></tr>
</table>

	Impurity 1 (1-cyano-6-(methylsulfonyl)-7-nitro-9*H*-xanthen-9-one (R287431)) max. 0.00075 g/L	**Impurity 2 (6-(methylsulfonyl)-9-oxo-9*H*-xanthene-1-carbonitrile (R287432)) max. 0.75 g/L**	**Impurity 3 (1,2-dichloroethane) max. 0.375 g/L**
	(1,2-dichloroethane)	Impurity 2 (R287432): 13.9 Min Impurity 3 (1,2-dichloroethane): No Peak	Impurity 2 (R287432): No Peak Impurity 3 (1,2-dichloroethane): 4.9 Min
LOQ	0.50 mg/kg	0.10 g/kg	0.20 mg/kg
Comment	-	-	-

* The SANCO/3030/99 rev.5 guidelines state that a Horrat (Hr) >1 and ≤2 is acceptable in the case of a reasoned explanation.
The LOQ Recovery for Impurity 2 (R287432) gave a %RSD of 10.057 at a concentration of 0.101g/Kg. This concentration relates to a Horwitz value of 5.349 and provides an associated Horrat (Hr) value of 1.880. The Horrat (Hr) calculated value for the LOQ Recovery is therefore >1 and ≤2.
The LOQ is a measure of accuracy and provides a mean percentage recovery of 101.4% with each of the individual percentage recoveries also within the acceptable range of 75% to 125% for a concentration of 0.101g/Kg. The precision of the method at the LOQ level is influenced by the movement of the baseline noise, this is the reason that the Horrat value is >1 and ≤2. As the value is less than 2 with an acceptable recovery, it has been determined that this result does not impact the LOQ Recovery of the method, and is therefore acceptable.
The Recovery Precision performed at 1.00g/Kg is shown to have an acceptable Horrat (Hr) <1. Additionally, Impurity 2 (R287432) is not detected within the Formulation and as such it is determined that for the LOQ Recovery the Horrat (Hr) values being >1 and ≤2, does not impact upon the validity of the methodology and is therefore acceptable within the SANCO/3030/99 rev.5 guidelines.

Conclusion

The validation parameters for the Impurity 1 (R287431), Impurity 2 (R287432) and Impurity 3 (1,2-dichloroethane) methodologies have been met for this study under SANCO/3030/99 rev.5 guidelines.

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

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method is not available for the determination of mesotrione, thiencarbazone-methyl or cyprosulfamide in SC formulations.

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, and thiencarbazone-methyl 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 and MNBA				
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	Bolygo E., 1996 EU agreed EFSA, 2016
	Confirmatory (if required)	Not required		
	Primary	0.01 mg/kg	LC-MS/MS	Meyers T.J., Ryan J.., 1997 EU agreed EFSA, 2016
	Confirmatory (if required)	Not required		
	Primary	0.01 mg/kg	LC-MS/MS	Schneider, 2016
	Confirmatory (if required)	Not required		
	Primary	0.01 mg/kg	LC-MS/MS	Faessel V., 2018
	Confirmatory (if required)	Not required		
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**
Water (Ecotoxicology)	Primary and confirmatory	0.86 µg/L	HPLC-MS/MS	Kosak & Wydra, 2016
Water (Ecotoxicology)	Primary and confirmatory	0.4 µg/L	HPLC-MS/MS	Gonsior G., 2017
Water (Ecotoxicology)	Primary and confirmatory	246 µg/L	LC-MS/MS	Bauer J., 2024a
Water (Ecotoxicology)	Primary and confirmatory	0.259 µg/L	LC-MS/MS	Bauer J., 2024b
Water (Ecotoxicology)	Primary and confirmatory	0.1 µg/L	LC-MS/MS	Bauer J., 2024c
Aqueous contact application solution (Ecotoxicology)	Primary and confirmatory	4.03 µg/L	LC-MS/MS	Schabio S., 2024
Sucrose feeding solution (Ecotoxicology)	Primary and confirmatory	4.64 µg/L	LC-MS/MS	Schabio S., 2024
Aqueous contact application solution (Ecotoxicology)	Primary and confirmatory	4.03 µg/L	LC-MS/MS	Chwiesko D., 2024

Sucrose feeding solution (Ecotoxicology)	Primary and confirmatory	4.64 µg/L	LC-MS/MS	Chwiesko D., 2024
Sucrose feeding solution (Ecotoxicology)	Primary and confirmatory	1310 µg/kg	UHPLC-MS/MS	Venturi S., 2023
Aqueous solution (Ecotoxicology)	Primary and confirmatory	500 µg/L	UHPLC-MS/MS	Venturi S., 2024
Water (Ecotoxicology)	Primary and confirmatory	1 µg/L	LC-MS/MS	Dommes A.B., 2024a Dommes A.B., 2024b
Component of residue definition: sum of thiencarbazone-methyl (BYH 18636), BYH 18636-N-desmethyl and BYH 18636-MMT-glucoside expressed as thiencarbazone-methyl				
Plants (Residues)	Primary and confirmatory Method 00962	0.01 mg/kg	LC-MS/MS	Zimmer D., Philipowski C., 2006 EU agreed DAR, 2012
Plants (Residues)	Primary Method 00963	0.01 mg/kg	LC-MS/MS	Zimmer D., Philipowski C., 2006 EU agreed DAR, 2012
Component of residue definition: sum of thiencarbazone-methyl (BYH 18636) and BYH 18636-MMT expressed as thiencarbazone-methyl				
Animal products (Residues)	Primary and confirmatory Method 00990	0.01 mg/kg	LC-MS/MS	Brumhard B., 2006 EU agreed DAR, 2012
Component of residue definition: thiencarbazone-methyl (BYH 18636)				
Water (Ecotoxicology)	Primary and confirmatory	0.15 µg/L	LC-MS/MS	Minati R., 2024
Water (Ecotoxicology)	Primary and confirmatory	0.25 µg/L	LC-MS/MS	Bebon R., 2024
Water (Ecotoxicology)	Primary and confirmatory	48.7 µg/L	LC-MS/MS	Bauer J., 2024a
Water (Ecotoxicology)	Primary and confirmatory	0.051 µg/L	LC-MS/MS	Bauer J., 2024b
Water (Ecotoxicology)	Primary and confirmatory	0.02 µg/L	LC-MS/MS	Bauer J., 2024c
Sediment (Ecotoxicology)	Primary and confirmatory	0.51 µg/kg	LC-MS/MS	
Pore water (Ecotoxicology)	Primary and confirmatory	0.0175 µg/L	LC-MS/MS	
Aqueous contact application solution (Ecotoxicology)	Primary and confirmatory	3.98 µg/L	LC-MS/MS	Schabio S., 2024
Sucrose feeding solution (Ecotoxicology)	Primary and confirmatory	4.59 µg/L	LC-MS/MS	Schabio S., 2024
Aqueous contact application solution (Ecotoxicology)	Primary and confirmatory	3.98 µg/L	LC-MS/MS	Chwiesko D., 2024

Sucrose feeding solution (Ecotoxicology)	Primary and confirmatory	4.59 µg/L	LC-MS/MS	Chwiesko D., 2024
Sucrose feeding solution (Ecotoxicology)	Primary and confirmatory	260 µg/kg	UHPLC-MS/MS	Venturi S., 2023
Aqueous solution (Ecotoxicology)	Primary and confirmatory	100 µg/L	UHPLC-MS/MS	Venturi S., 2024
Water (Ecotoxicology)	Primary and confirmatory	1 µg/L	LC-MS/MS	Dommes A.B., 2024a Dommes A.B., 2024b

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)

The 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 mesotrione (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	Mesotrione	0.01 mg/kg*	Reg. (EU) 2017/626
Plant, high acid content		0.01 mg/kg*	Reg. (EU) 2017/626
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg*	Reg. (EU) 2017/626
Plant, high oil content		0.01 mg/kg*	Reg. (EU) 2017/626
Muscle	Mesotrione	0.01 mg/kg*	Reg. (EU) 2017/626
Milk		0.01 mg/kg*	Reg. (EU) 2017/626
Eggs		0.01 mg/kg*	Reg. (EU) 2017/626
Fat		0.01 mg/kg*	Reg. (EU) 2017/626
Liver, kidney		0.01 mg/kg*	Reg. (EU) 2017/626
Soil (Ecotoxicology)	Mesotrione	0.05 mg/kg	General limit for soil SANCO/825/00 rev. 8.1
Drinking water (Human toxicology)	Mesotrione	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			SANCO/825/00 rev. 8.1
Surface water (Ecotoxicology)	Mesotrione	0.0077 mg/L	EFSA, 2016 CEb50 (Lemna minor)
Air	Mesotrione	1.5 µg/m^3	EFSA, 2016 AOEL: 0.005 mg/kg bw/d
Tissue (meat or liver)	Mesotrione	0.01 mg/kg	general limits for tissues SANCO/825/00 rev. 8.1
Body fluids		0.05 mg/L	general limit for blood SANCO/825/00 rev. 8.1

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. For the detailed evaluation of no 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: mesotrione				
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	EFSA, 2016 Watson G., 2013
	ILV	0.01 mg/kg	LC-MS/MS	EFSA, 2016 Tessier V., 2012
High acid content	Primary	0.01 mg/kg	LC-MS/MS	EFSA, 2016 Watson G., 2013
High oil content	Primary	0.01 mg/kg	LC-MS/MS	EFSA, 2016 Watson G., 2013
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	EFSA, 2016 Watson G., 2013
	ILV	0.01 mg/kg	LC-MS/MS	EFSA, 2016 Tessier V., 2012

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:	EFSA, 2016
Not required, because:	-

The extraction method used in the analytical method for food and feed of plant origin is the same as the one used in the metabolism study and consists in acetonitrile/water (50:50 v/v). The extraction efficiency does not need to be proved again.

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in animal matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 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).

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	QuEChERS (LC-MS/MS)	EFSA, 2016 Watson G., 2013
	ILV	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Bernal J., 2013
Eggs	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Watson G., 2013
	ILV	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Bernal J., 2013
Muscle	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Watson G., 2013
Fat	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Watson G., 2013
Kidney, liver	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Watson G., 2013
	ILV (liver)	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 Bernal J., 2013

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 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	EFSA, 2016
Not required, because:	-

The extraction system employed is based predominantly on acetonitrile/water (50:50 v/v) which is similar

to that used for plant commodities. Animal metabolism data were not required and significant residues of mesotrione are not expected in animal commodities therefore this is acceptable.

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. For the detailed evaluation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-5: Validated methods for soil (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	0.002 mg/kg	LC-MS/MS	EFSA, 2016 Jutsum L. and Williams R., 2012; Jutsum L., 2013
Confirmatory	Not required (primary method is LC-MS/MS)		

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

Component of residue definition: metabolite AMBA			
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	EFSA, 2016 Jutsum L. and Williams R., 2012; Jutsum L., 2013
Confirmatory	Not required (primary method is LC-MS/MS)		

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

Component of residue definition: metabolite MNBA			
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	EFSA, 2016 Jutsum L. and Williams R., 2012; Jutsum L., 2013
Confirmatory	Not required (primary method is LC-MS/MS)		

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 mesotrione in surface and drinking water is given in the following tables. For the detailed valuation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-8: Validated methods for water (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**
Ground water	Primary	0.05 µg/L	LC-MS/MS	EFSA, 2016 Jutsum L. and Chamkesam N., 2013; Jutsum L., 2013
Drinking water	ILV	0.05 µg/L	LC-MS/MS	EFSA, 2016 Wiesner F. and Breyer N., 2013
Surface water	Primary	0.05 µg/L	LC-MS/MS	EFSA, 2016 Jutsum L. and Chamkesam N., 2013; Jutsum L., 2013

Table 5.3-9: Validated methods for water (if appropriate)

Component of residue definition: metabolite AMBA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Ground water	Primary	0.05 µg/L	LC-MS/MS	EFSA, 2016 Jutsum L. and Chamkesam N., 2013; Jutsum L., 2013
Drinking water	ILV	0.05 µg/L	LC-MS/MS	EFSA, 2016 Wiesner F. and Breyer N., 2013
Surface water	Primary	0.05 µg/L	LC-MS/MS	EFSA, 2016 Jutsum L. and Chamkesam N., 2013; Jutsum L., 2013

Table 5.3-10: Validated methods for water (if appropriate)

Component of residue definition: metabolite MNBA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Ground water	Primary	0.05 µg/L	LC-MS/MS	EFSA, 2016 Jutsum L. and Chamkesam N., 2013; Jutsum L., 2013
Drinking water	ILV	0.05 µg/L	LC-MS/MS	EFSA, 2016 Wiesner F. and Breyer N., 2013
Surface water	Primary	0.05 µg/L	LC-MS/MS	EFSA, 2016 Jutsum L. and Chamkesam N., 2013; Jutsum L., 2013

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 mesotrione in air is given in the following tables. For the detailed evaluation of no new/ additional studies please refer to Appendix 2.

Table 5.3-11: 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	0.45 µg/m^3	LC-MS/MS	EFSA, 2016 Jutsum L. 2013; Jutsum 2013

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)

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in body fluids and tissues is given in the following table. For the detailed evaluation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-12: Methods for body fluids and tissues (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 mg/kg	QuEchERS (LC-MS/MS)	EFSA, 2016 Watson G., 2013

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

5.3.2.8 Other studies/ information

/

5.3.3 Description of analytical methods for the determination of residues thiencarbazone-methyl (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 5.3-13: 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	Thiencarbazone-methyl	LOQ of 0.01 mg/kg	EFSA, 2013 Default MRL of 0.01 mg/kg
Plant, high acid content		LOQ of 0.01 mg/kg	
Plant, high protein/high starch content (dry commodities)		LOQ of 0.01 mg/kg	
Plant, high oil content		LOQ of 0.01 mg/kg	
Muscle	Sum of thiencarbazone-methyl and BYH 18636-MMT expressed as thiencarbazone-methyl	LOQ of 0.01 mg/kg	EFSA, 2013
Milk		LOQ of 0.01 mg/kg	
Eggs		LOQ of 0.01 mg/kg	
Fat		LOQ of 0.01 mg/kg	
Liver, kidney		LOQ of 0.01 mg/kg	
Soil (Ecotoxicology)	Thiencarbazone-methyl	1 µg/kg (LOQ)	EFSA, 2013 NOEC ≥ 1000 mg a.s./kg dw soil (earthworm repro)
Drinking water (Human toxicology)	Thiencarbazone-methyl	0.05 µg/L (LOQ)	EFSA, 2013 general limit for drinking water: 0.1 µg/L
Surface water (Ecotoxicology)	Thiencarbazone-methyl	0.05 µg/L (LOQ)	EFSA, 2013 Lowest EC50: 1.31 µg/L (growth rate) and 0.8 µg/L (biomass) Lowest NOEC: 0.21 µg/L
Air	Thiencarbazone-methyl	3.75 µg/m^3 (LOQ)	AOEL: 0.12 mg/kg bw/d EFSA, 2013
Body fluids	Sum of thiencarbazone-methyl and BYH 18636-MMT expressed as thiencarbazone-methyl*	0.05 mg/L (LOQ)	SANCO/825/00 rev. 8.1 Appendix 2

*not EU agreed

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 thiencarbazone-methyl in plant matrices is given in the following tables. For the detailed evaluation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-14: 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: thiencarbazone-methyl (BYH 18636)				
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 EM, includes a confirmatory procedure Method 00963	0.01 mg/kg	LC-MS/MS	Zimmer D, Philipowski C, 2006 EU agreed DAR, 2012
	ILV Method 00963	0.01 mg/kg	LC-MS/MS	Class T., 2006 EU agreed DAR, 2012
High acid content	Primary EM, includes a confirmatory procedure Method 00963	0.01 mg/kg	LC-MS/MS	Zimmer D, Philipowski C, 2006 EU agreed DAR, 2012
High oil content	Primary EM, includes a confirmatory procedure Method 00963	0.01 mg/kg	LC-MS/MS	Zimmer D, Philipowski C, 2006 EU agreed DAR, 2012
High protein/high starch content (dry)	Primary EM, includes a confirmatory procedure Method 00963	0.01 mg/kg	LC-MS/MS	Zimmer D, Philipowski C, 2006 EU agreed DAR, 2012
	ILV Method 00963	0.01 mg/kg	LC-MS/MS	Class T., 2006 EU agreed DAR, 2012

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 5.3-15: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Bongartz R., 2006 EU agreed DAR, 2012
Not required, because:	As residues are not expected to be ≥ LOQ based on the metabolism studies, extraction efficiency is not required according to SANTE 2017/10632.

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 thiencarbazone-methyl in animal matrices is given in the following tables. For the detailed evaluation of new studies it is referred to

Appendix 2.

Table 5.3-16: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: thiencarbazone-methyl and BYH 18636-MMT (expressed as thiencarbazone-methyl)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary EM, includes a confirmatory procedure Method 01022	0.01 mg/kg	LC-MS/MS	Zimmer D., Kuppels U., 2007 EU agreed DAR, 2012
	ILV Method 01022	0.01 mg/kg	LC-MS/MS	Class T., 2007 EU agreed DAR, 2012
Eggs	Primary, includes a confirmatory procedure	0.01 mg/kg	LC-MS/MS	Gustloff C., 2024
	ILV	0.01 mg/kg	LC-MS/MS	Senciuc M., 2024
Muscle	Primary EM, includes a confirmatory procedure Method 01022	0.01 mg/kg	LC-MS/MS	Zimmer D., Kuppels U., 2007 EU agreed DAR, 2012
	ILV Method 01022	0.01 mg/kg	LC-MS/MS	Class T., 2007 EU agreed DAR, 2012
Fat	Primary EM, includes a confirmatory procedure Method 01022	0.01 mg/kg	LC-MS/MS	Zimmer D., Kuppels U., 2007 EU agreed DAR, 2012
	ILV Method 01022	0.01 mg/kg	LC-MS/MS	Class T., 2007 EU agreed DAR, 2012
Kidney	Primary EM, includes a confirmatory procedure Method 01022	0.01 mg/kg	LC-MS/MS	Zimmer D., Kuppels U., 2007 EU agreed DAR, 2012
Liver	Primary EM, includes a confirmatory procedure Method 01022	0.01 mg/kg	LC-MS/MS	Zimmer D., Kuppels U., 2007 EU agreed DAR, 2012
	ILV Method 01022	0.01 mg/kg	LC-MS/MS	Class T., 2007 EU agreed DAR, 2012

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 5.3-17: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Schmeer K., 2007 EU agreed DAR, 2012
Not required, because:	As residues are not expected to be ≥ LOQ based on the metabolism studies, extraction efficiency is not required according to SANTE 2017/10632.

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 thiencarbazone-methyl in soil is given in the following tables. For the detailed evaluation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-18: Validated methods for soil (if appropriate)

Component of residue definition: thiencarbazone-methyl			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary Method 01028	1 µg/kg	HPLC-MS/MS	Brumhard B, Koch V. EU agreed DAR, 2012
Confirmatory	Not required since two MRM transitions were validated during the primary method.		

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 thiencarbazone-methyl in surface and drinking water is given in the following tables. For the detailed valuation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-19: Validated methods for water (if appropriate)

Component of residue definition: thiencarbazone-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary Confirmatory Method 01025	0.05 µg/L	HPLC-MS/MS	Krebber L., Leppelt L., 2007 EU agreed DAR, 2012
	ILV	Missing – to be matched at next renewal		
Surface water	Primary Confirmatory Method 01025	0.05 µg/L	HPLC-MS/MS	Krebber L., Leppelt L., 2007 EU agreed DAR, 2012

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 thiencarbazone-methyl in air is given in the following tables. For the detailed evaluation of no new/ additional studies please refer to Appendix 2.

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

Component of residue definition: thiencarbazone-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary Method RAGSM003	2.7 µg/m^3	HPLC-MS/MS	Ripperger R., 2007 EU agreed DAR, 2012
Confirmatory	Not required since two MRM transitions were validated during the primary method.		

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)

An overview on the acceptable methods and possible data gaps for analysis of thiencarbazone-methyl in body fluids and tissues is given in the following table. For the detailed evaluation of no new/ additional studies it is referred to Appendix 2.

Table 5.3-21: Methods for body fluids and tissues (if appropriate)

Component of residue definition: thiencarbazone-methyl and BYH18636-MMT expressed as thiencarbazone-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Tissues			
Primary Method 01022	0.01 mg/kg	HPLC-MS/MS	Zimmer D., Kuppels U., 2007 EU agreed DAR, 2012
Confirmatory	Not required since two MRM transitions were validated during the primary method.		
Body fluids			
~~Primary~~	~~Missing – to be matched at next renewal~~		

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

5.3.3.8 Other studies/ information

No other studies 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	Fitzmaurice T.	2023	Validation of the methods of determination of active ingredients and specified impurities in a suspension concentrate formulation containing thiencarbazone-methyl, mesotrione and cyprosulfamide, in compliance with good laboratory practice. DNA7206 David Norris Analytical Laboratories Ltd. GLP Unpublished	N	Globachem NV
KCP 5.1.2	Schneider E.	2016	Validation of the Analytical Method for the Determination of Mesotrione and its Metabolite Residues in Maize (Whole Plant and grain) B5117 Anadiag GLP Unpublished	N	Globachem NV Data protection started with Osorno 480 (178/2021)
KCP 5.1.2	Faessel V.	2018	Validation of the Analytical Method for the Analysis of Mesotrione in Oilseed rape whole plant B7315 ANADIAG GLP Unpublished	N	Globachem NV Study report never submitted before to PL

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 *(submitted as KCP 10.2.1)*	Kosak & Wydra	2016	Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test with a Subsequent Recovery Period 105732240 Ibacon GmbH GLP Unpublished	N	Syngenta *Globachem access*
KCP 5.1.2 *(submitted as KCP 10.2.1)*	Gonsior G.	2017	Mesotrione – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH S16-06273 GLP Unpublished	N	Syngenta *Globachem access*
KCP 5.1.1 *Submitted as KCA 8.2.7*	Minati R.	2024	Thiencarbazone-methyl: Toxicity to the Aquatic Plant Lemna gibba in a Pulsed Exposure Growth Inhibition Test 178651240 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.1 *Submitted as KCA 8.2.7*	Bebon R.	2024	Thiencarbazone-methyl: Toxicity to the aquatic plant Myriophyllum spicatum in a pulsed exposure growth inhibition test with a prior rooting phase 178651215 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted as KCP 10.2.1)*	Bauer J.	2024a	GLOB2112dH: Toxicity to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test 177011210 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted*	Bauer J.	2024b	GLOB2112dH: Toxicity to the Aquatic Plant *Lemna gibba* in a Static Growth Inhibition Test 177011240	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
as KCP 10.2.1)			Ibacon GmbH GLP Unpublished		
KCP 5.1.2 *(submitted as KCP 10.2.1)*	Bauer J.	2024c	GLOB2112dH: Toxicity to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test with a prior rooting phase 177011215 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted as KCP 10.3.1.1)*	Schabio S.	2024	GLOB2112dH: effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory 177011035 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted as KCP 10.3.1.1)*	Chwiesko D.	2024	GLOB2112dH: acute contact and oral toxicity to bumblebees (*Bombus terrestris* L.) in the laboratory 177011105 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted as KCP 10.3.1.2)*	Venturi S.	2023	Chronic oral effects of GLOB2112dH to adult worker honeybees (Apis mellifera L.) in a 10-day feeding laboratory test BT215/23 Biotechnology BT GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted as KCP 10.3.1.3)*	Venturi S.	2024	Effects of GLOB2112dH on honeybees (*Apis mellifera L.*) 22- day larval toxicity test with repeated exposure BT131/23 Biotechnology BT 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 *(submitted as KCP 10.6)*	Dommes A.B.	2024a	GLOB2112dH: Effects on terrestrial (non-target) plants : seedling emergence and seedling growth test 177011086 Ibacon GLP Unpublished	N	Globachem NV
KCP 5.1.2 *(submitted as KCP 10.6)*	Dommes A.B.	2024b	GLOB2112dH: Effects on terrestrial (non-target) plants : vegetative vigour test 177011087 Ibacon GLP Unpublished	N	Globachem NV
KCP 5.2	Gustloff C.	2024	Validation of analytical methods to determine residues of thiencarbazone-methyl and its metabolite in eggs Eurofins Agroscience Services Chem Gmbh S24-102708 GLP Unpublished	N	Globachem NV
KCP 5.2	Senciuc M.	2024	Independent Laboratory Validation of an Analytical Method for the Determination of Thiencarbazone-methyl and its metabolite BYH18636-MMT in Egg S24-102656 Eurofins Agroscience Services Eag Laboratories Gmbh GLP Unpublished	N	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
KCA 4.1.2	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	Syngenta *Out of data protection*
KCA 4.1.2	Meyers T.J., 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	Syngenta *Out of data protection*
KCA 4.1.2	Zimmer D., Philipowski C.	2006	Analytical method 00962 for the determination of residues of BYH18636 and its metabolites BYH18636-N-desmethyl and BYH18636-MMT-glucoside, and of AE 0001789 in/on plant matrices by HPLC-MS/MS Bayer CropScience AG 00962 GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.1.2	Zimmer D., Philipowski C.	2006	Analytical method 00963 for the determination of residues of BYH18636 and its metabolites BYH18636-N-desmethyl and BYH18636-MMT-glucoside in/on plant matrices by HPLC-MS/MS Bayer CropScience AG 00963 GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.1.2	Brumhard B.	2006	Analytical method 00990 for the determination of residues of BYH 18636 and its metabolites in animal matrices Bayer CropScience AG 00990 GLP	N	Bayer *Data out of protection*

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		
KCA 4.2	Watson G.	2013	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Crop Matrices by LC-MS/MS Report No. S12-03251 Syngenta File No ZA1296_10090 Eurofins Agroscience Services Ltd, Wilson, UK, GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Schlewitz P.	2016	Validation of the Analytical Method for the Determination of Mesotrione, MNBA and AMBA Residues in Orange whole fruit and Oilseed rape seeds B6202 Anadiag GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Schlewitz P.	2017	Validation of the Analytical Method for the Determination of Mesotrione, MNBA and AMBA Residues in Maize (whole plant) B6363 GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Tessier V.	2013	Mesotrione - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Mesotrione in Crop Matrices by LC-MS/MS Report No.: S12-04607 Syngenta File No ZA1296_10129 Eurofins Agroscience Services Chem SAS, Vergèze, France GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Gaffney V.	2017	Validation of an Analytical Method for the Determination of Residues of Mesotrione and Metabolites in Maize (Whole Plant and Grain), ILV VAL24/17 Laboratorio de Residuos SAPEC AGRO S.A.	N	Globachem NV *Matching data*

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP Unpublished		
KCA 4.2	Arias A.	2017	Validation of an Analytical Method for the Determination of Mesotrione, MNBA and AMBA in Oilseed rape seeds, ILV VAL10/17 Laboratorio de Residuos SAPEC AGRO S.A. GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Watson G.	2013	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS Report No: S12-03250 Syngenta File No ZA1296_10093 Eurofins Agroscience Services Ltd, Wilson, UK GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Lefresne S.	2017	Validation of the Analytical Method for the Determination of residues of Mesotrione (milk, egg, muscle, fat, liver and kidney) and body fluids (blood). B17S-G2-M-01 FREDON/ pays de la Loire/ GIRPA GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Bernal J.	2013	Bernal J., 2013 Mesotrione - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Mesotrione in Animal matrices by LC-MS/MS Report No: S12-04608 Syngenta File No ZA1296_10130 Eurofins Agroscience Services Chem SAS, Vergèze, France GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Arias A.	2017	Validation of an Analytical Method for the Determination of Mesotrione in Food of Animal Origin, ILV. VAL42/17	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**
			Laboratório SAPEC Agro, Setúbal, Portugal. GLP Unpublished		*Matching data*
KCA 4.2	Jutsum L., Williams R.	2013	Analytical Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil Report No: GRM007.10A Syngenta File No ZA1296_10092 Syngenta CEMAS, North Ascot, United Kingdom, GRM007.10A Not GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Jutsum L.	2013	Mesotrione – Validation of Draft Residue Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil Report No: CEMR-5657-REG Syngenta File No ZA1296_10088 CEMAS, North Ascot, United Kingdom GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Schneider E.	2016	Validation of the Analytical Method for the Determination of Mesotrione and its Metabolites Residues in Soil B5329 Anadiag Not GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Jutsum L., Chamkesam N.	2013	Analytical Method GRM007.09A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Water Report No: GRM007.09A Syngenta File No ZA1296_10092 CEMAS, North Ascot, United Kingdom Not GLP Unpublished	N	Syngenta *Matching data provided*

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.2	Jutsum L.	2013	Validation of Draft Residue Method GRM007.09A for the Determination of Mesotrione and its metabolites AMBA and MNBA in Water Report No: CEMR-5658-REG Syngenta File No ZA1296_10087 CEMAS, North Ascot, United Kingdom GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Schneider E.	2016	Validation of the Analytical Method for the Determination of Mesotrione and its Metabolite Residues In Surface and ground waters B5176 Anadiag GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Wiesner F., Breyer N.	2013	Mesotrione - Independent Laboratory Validation of Analytical Method GRM007.09A for the Determination of Residues of Mesotrione and its Metabolites in AMBA and MNBA Water Report No: S13-04185 Syngenta File No ZA1296_10174 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Ferreira Morais F.	2017	Validation of an Analytical Method for the Determination of Residues of Mesotrione and its Metabolites in Drinking Water, ILV. VAL12/17 Laboratorio de Residuos SAPEC AGRO, S.A. GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Jutsum L.	2013	Mesotrione - Residue Method GRM007.08B for the Determination of Mesotrione in Air Report GRM007.08B Syngenta File No ZA1296_10089 Syngenta CEMAS, North Ascot, United Kingdom	N	Syngenta *Matching data provided*

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Not GLP Unpublished		
KCA 4.2	Jutsum L.	2013	Mesotrione - Validation of Residue Method GRM007.08A for the Determination of Mesotrione in Air Report CEMR-5403-REG Syngenta File No ZA1296_10084 Syngenta CEMAS, North Ascot, United Kingdom GLP Unpublished	N	Syngenta *Matching data provided*
KCA 4.2	Schneider E.	2016	Validation of the Analytical Method for the Determination of Mesotrione Residues in Air B5330 Anadiag GLP Unpublished	N	Globachem NV *Matching data*
KCA 4.2	Krebber R., Leppelt L.	2007	Analytical method 01025 for the determination of thiencarbazone-methyl (BYH 18636) in drinking and surface water by HPLC-MS/MS Bayer CropScience AG 01025 GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.2	Class T.	2006	Independent laboratory validation of Bayer CropScience method No. 00963 for the determination of residues of BYH 18636 and its metabolites BYH 18636-N-desmethyl and BYH 18636-MMT-glucoside in/on plant materials by LC/MS/MS PTRL Europe P/B 1125 G GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.2	Bongartz R.	2006	[Dihydrotriazole-3-14C]BYH18636: Extraction efficiency of the residue analytical method for the determination of BYH18636 residues in plant matrices using aged radioactive residues Bayer CropScience AG MEF-05/504	N	Bayer *Data out of protection*

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP Unpublished		
KCA 4.2	Zimmer D., Kuppels U.	2007	Analytical method 01022 for the determination of residues of BYH18636 and BYH18636-MMT in animal matrices Bayer CropScience AG 01022 GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.2	Class T.	2007	Independent laboratory validation of Bayer CropScience method no. 01022 for the determination of residues of BYH 18636 and its metabolite BYH 18636-MMT in animal matrices by LC/MS/MS PTRL Europe P/B 1138 G GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.2	Schmeer K.	2007	[Dihydrotriazole-3-14C]BYH18636 and [thiophene-4-14C]BYH18636: Extraction efficiency of the residue analytical method for the determination of BYH18636 residues in animal matrices using aged radioactive residues Bayer CropScience AG MEF-06/292 GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.2	Brumhard B., Koch V.	2006	Analytical method 01028 for the determination of residues of BYH18636 in soil by HPLC-MS/MS Bayer CropScience AG 01028 GLP Unpublished	N	Bayer *Data out of protection*
KCA 4.2	Ripperger R.J.	2007	BYH 18636: Analytical method for the determination of BYH 18636 in air Bayer CropScience AG RAGSM003-1 GLP	N	Bayer *Data out of protection*

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		

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	Y/N	Owner

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
			Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished		

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)

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

A 2.1.1.1 Methods to determine mesotrione in plants

A 2.1.1.1.1 Analytical method for mesotrione in maize

Comments of zRMS:	Validation of the Analytical Method (Schneider E., 2016, R B5117) is acceptable and suitable for the determination of residues Mesotrione and its metabolite (MNBA) in maize (whole plant, grain).

Reference:	KCP 5.2
Report	Validation of the Analytical Method for the Determination of Mesotrione and its Metabolite Residues in Maize (Whole Plant and grain), Schneider E., 2016, R B5117
Guideline(s):	SANCO/825/00 rev.8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

10 g of blended sample were weighed into a 50 mL centrifuge tube and spiked if necessary. 10 mL hydrochloric acid 1M and 10 mL acetonitrile were added and shaken for 1 min. 4 g magnesium sulphate and 1 g NaCl were added and the whole sample was shaken for 1 min. The extract was centrifuged for 1 min. at 1800 rpm. After complete separation of the phases, the supernatent was filtered with PTFE 0.2 µm and 0.5 mL was transferred into a vial containing 20 µL of the triphenyl phosphate 2.5 µg/mL + 2.5% formic acid solution. The vial was homogenised prior to LC-MS/MS (negative ESI) analysis. Standard solutions of the active substance Mesotrione, the metabolite MNBA and the internal standard Triphenyl phosphate were prepared in acetonitrile. Transitions monitored were:
- for Mesotrione: m/z 338.1 → 291.1 for quantification and m/z 338.1 → 212.1 for confirmation.
- for MNBA: m/z 244.1 → 142.0 for quantification and m/z 244.1 → 170.0 for confirmation.

Analytical conditions:

Apparatus:	LC/MS/MS: XEVO
Column :	BEH HSS T3, particles 1.8 µm, 100*2.1 mm, 40°C, C18
Mobile Phase:	A= HPLC H_2O +0.1 % formic acid B= HPLC MeOH + 0.1% formic acid
Sample temperature:	15°C

Detector: IONISATION mode: ES
Polarity: Pos/Neg

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Number of fortified samples (n)
Whole plant	Mesotrione	0.01	103.9%	4.9%	5
		0.10	95.9%	6.1%	5
		All levels	99.9%	6.7%	10
	MNBA	0.01	95.1%	11.9%	5
		0.10	97.0%	5.9%	5
		All levels	96.0%	8.9%	10
Grain	Mesotrione	0.01	100.6%	6.1%	5
		0.10	102.9%	10.0%	5
		All levels	101.8%	7.9%	10
	MNBA	0.01	101.8%	14.6%	5
		0.10	104.3%	9.7%	5
		All levels	103.1%	11.7%	10

Table A 2: Characteristics for the analytical method used for validation of mesotrione residues in maize (whole plant and grain)

	Mesotrione	Metabolite MNBA
Specificity	For both analyte and both transition, chromatograms were provided for calibration standards (matrix matched), control and fortified samples at LOQ (grain and whole plant). No interfering signal was present accounting for more than 30% of the LOQ.	
Calibration (type, number of data points)	individual calibration data and calibration line equations are presented in appendix V of the study. 7 calibration concentrations were tested: 0.0, 3.0, 5.0, 10.1, 20.1, 50.4, 100.7, 120.8 ng/mL	individual calibration data and calibration line equations are presented in appendix V of the study. 7 calibration concentrations were tested: 0.0, 3.0, 5.1, 10.1, 20.2, 50.5, 101.0, 121.2 ng/mL
Calibration range	The linearity of the method was studied between 3 ng/mL and 120 ng/mL (0.003 mg/kg – 0.120 mg/kg) of mesotrione and its metabolite (MNBA) in matrix-matched calibration solutions. The linear correlation coefficients were typically > 0.990, showing a good linearity.	
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	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 Maize (whole plant and grain).	

Conclusion

The method is able to determine mesotrione and its metabolite (MNBA) in the presence of maize (whole plant and grain) at an LOQ of 0.01 mg/kg.
This was confirmed by analysing extracts using the qualifier transition.

A 2.1.1.1.2 Analytical method for mesotrione in oilseed rape

Comments of zRMS:	Validation of the Analytical Method (Faessel V., 2018, B7315) is acceptable and suitable for the determination of residues Mesotrione oilseed rape whole plant.

Reference:	KCP 5.2
Report	Validation of the analytical method for the analysis of mesotrione in oilseed rape whole plant, Faessel V., 2018, B7315
Guideline(s):	SANCO/825/00 rev.8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues are extracted with acetonitrile in acidic conditions in the presence of magnesium sulphate and sodium chloride. The extract obtained after centrifugation is then analysed by LC-MS/MS.

All standards were prepared in acetonitrile.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Number of fortified samples (n)
Oilseed rape whole plant	Mesotrione	0.01	78.3	3.1	5
		0.10	85.4	1.7	5
		All levels	81.8	5.1	10

Table A 4: Characteristics for the analytical method used for validation of mesotrione residues in oilseed rape (whole plant)

	Mesotrione
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 0.19604X-0.00014395 r^2 > 0.990 number of data points: 7

	Mesotrione
Calibration range	0.15 – 6 ng/mL (corresponding to 0.003 to 0.12 in mg/kg)
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.003 mg/kg / 0.01 mg/kg
Extract and standard stability	The stability of extracts during frozen storage was investigated. The results indicate a good stability up to 16 days. The stability of standard solutions during frozen storage was investigated. The results indicate a good stability up to 15 days.

Conclusion

The method is able to determine mesotrione in oilseed rape (whole plant) at an LOQ of 0.01 mg/kg.

A 2.1.1.2 Methods used in the ecotoxicology studies

A 2.1.1.2.1 Analytical method for mesotrione in Lemna study

Comments of zRMS:	The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: *„The analytical method 105732240A for the determination of mesotrione in test water supplemented with AAP medium was successfully validated according to SANCO/3029/99 rev. 4.* *The limit of quantification (LOQ) of the analytical method was 1 µg test item/L.* *The study has been accepted."* In accordance with the above and letter of Access to mesotrione Validation Data, Analytical Method is acceptable and suitable.

Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant Lemna gibba in a Semi-Static Growth Inhibition Test with a Subsequent Recovery Period, Kosak & Wydra, 2016, 105732240
Guideline(s):	Yes, OECD 221 (2016) and US EPA OPPTS 850.4400 (1996)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Five samples were fortified at the limit of quantification (LOQ; 0.86 µg/L mesotrione; analyzed with dilution factor 2), five at 5x LOQ (4.3 µg/L mesotrione; analyzed with dilution factor 2) and five at 75x LOQ (65 µg/L; analyzed with dilution factor 2 mesotrione). Fortified samples were analysed alongside untreated control samples.
Samples are shaken and diluted with acetonitrile by a factor of 2 prior to analysis by HPLC-MS/MS, monitoring for one transition (*m/z* = 357 → 228). The limit of quantification (LOQ) is 0.86 µg mesotrione /L corresponding to 0.43 µg mesotrione /L in final extract samples. Mesotrione is not

a chiral molecule and as such the method is non-enantiospecific.

Results and discussions

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

Matrix	Analyte	Fortification level (*n* = x)*	Mean recovery (%)	RSD (%)
20X AAP-growth medium	Mesotrione	0.86 µg/L** (5)	107	5
		4.3 µg/L (10)	105	6
		65 (5)	100	2

* analyzed with dilution factor 2
** Limit of quantification, defined by the lowest validated fortification level

Table A 6: Characteristics for the analytical method used for validation of mesotrione residues in 20X AAP-growth medium

	Mesotrione
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 2450 x + 109 r = 1.0000 number of data points: 7
Calibration range	0.249-39.776 µg/L (equivalent to 0.498-79.552 µg/L; considering a dilution factor of 2) The range extends from just above the LOQ level (115% of the LOQ) to at least 20% greater than the highest analyte fortification level. Although the range does not strictly meet the SANTE/2020/12830 rev.1 guideline range (30% of the LOQ to 20% above the highest level), the range is still acceptable, especially since the method was developed prior to the implementation of the guidance, and linearity has otherwise been sufficiently demonstrated.
Assessment of matrix effects is presented	No, matrix matched standards were used for calibration and quantification.
Limit of detection/quantification	0.30 µg /L corresponding to 0.15 µg/L in diluted final extract / 0.86 µg/L corresponding to 0.43 µg/L in diluted final extract
Stability of extract	Not applicable – method includes dilution steps only.
Stability of standard solution	Not tested. Validation performed prior to the implementation of SANTE/2020/12830 rev.1.

Conclusion

The LC-MS/MS analytical method has been demonstrated to be a sufficiently reliable and accurate procedure for the determination of mesotrione in 20X AAP-Growth medium with a limit of quantification (LOQ) of 0.86 µg/L in accordance with SANTE/2020/12830 rev. 1, using commercially available laboratory equipment and reagents.

A 2.1.1.2.2 Analytical method for mesotrione in Myriophyllum study

Comments of zRMS:	The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of

	product Calisto 100 SC. The following conclusion was made: „The analytical method S16-06273 for the determination of mesotrione in water supplemented with modified Andrews solution was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.4 µg test item/L. The study has been accepted.” In accordance with the above and letter of Access to mesotrione Validation Data, Analytical Method is acceptable and suitable.

Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	Mesotrione – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System, Gonsior G., 2017, S16-06273
Guideline(s):	Yes, OECD 239 (2014) and EC SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test medium samples were fortified with standard solutions of the test item ‘mesotrione technical’. Five samples were fortified at the limit of quantification (LOQ; 0.4 µg/L mesotrione) and five at a higher level (2000x LOQ; 800 µg/L mesotrione). Fortified samples were analysed alongside untreated control samples.
Aqueous samples are extracted with acetonitrile and shaken. If necessary, samples are further diluted with acetonitrile:water (1:1, v/v) prior to analysis by HPLC-MS/MS, monitoring for two transitions (*m/z* = 337.8 → 291 and *m/z* = 337.8 → 212). The limit of quantification (LOQ) of the method is 0.4 µg/L. Mesotrione is not a chiral molecule and as such the method is non-enantiospecific.

Results and discussions

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

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
Smart and Barko growth medium	Mesotrione	0.4 µg/L* (5)	93	3
		800 µg/L (5)	94	2

* Limit of quantification, defined by the lowest validated fortification level

Table A 8: Characteristics for the analytical method used for validation of mesotrione residues in Smart and Barko growth medium

	Mesotrione
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic $y = -9.36e04.x^2 + 2.32e06.x + 3.74e04$ r = 0.9984

	Mesotrione
	number of data points: 8
Calibration range	0.05-10 ng/L (equivalent to 0.1-2000 µg/L considering dilution factors for the different fortification levels).
Assessment of matrix effects is presented	No. Though - matrix effects are expected to be negligible as samples are diluted with acetonitrile and water (1;1, v/v) prior to analysis and calibration solutions are prepared in acetonitrile and purified water (1:1, v/v).
Limit of detection/quantification	0.120 µg /L / 0.4 µg/L
Stability of extract	Not applicable – method is direct injection.
Stability of standard solution	Not tested. However the study report states that the stability in solution of mesotrione is sufficient for the test purpose (at least 1 hour).

Conclusion

The LC-MS/MS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of mesotrione in overlaying water test medium with a limit of quantification (LOQ) of 0.4 µg/L in accordance with SANTE/2020/12830 rev.1, using commercially available laboratory equipment and reagents.

A 2.1.1.2.3 Analytical method for thiencarbazone-methyl in Lemna pulsed exposure study

Comments of zRMS:	Validation of the Analytical Method Minati R., 2024, R 178651240) is acceptable and suitable for the determination of residues Thiencarbazone-methyl in reconstituted water (20x AAP growth medium).

Reference:	KCP 5.2.1 (Submitted as KCA 8.2.7)
Report	Thiencarbazone-methyl: Toxicity to the aquatic plant *Lemna gibba* in a pulsed exposure growth inhibition test, Minati R., 2024, 178651240
Guideline(s):	Yes, OECD 221, 2006 and SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for thiencarbazone-methyl by LC-MS/MS.

All thiencarbazone-methyl standards were prepared in acetonitrile.

Results and discussions

Table A 9: Recovery results from method validation of thiencarbazone-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)
Reconstituted water (20x AAP growth medium)	Thiencarbazone-methyl	0.15 (5)	95	4
		70 (5)	102	2

Table A 10: Characteristics for the analytical method used for validation of thiencarbazone-methyl residues in reconstituted water (20x AAP growth medium)

	Thiencarbazone-methyl
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 559665 x + 2101 r = 0.9998 number of data points: 9
Calibration range	0.015 - 2.5 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.
Assessment of matrix effects is presented	Yes, > 20%, matrix matched solution used.
Limit of detection/quantification	0.015 µg/L / 0.15 µg/L
Extract and standard stability	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were analysed within 24 hours.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of thiencarbazone-methyl in reconstituted water (20x AAP growth medium).

A 2.1.1.2.4 Analytical method for thiencarbazone-methyl in Myriophyllum pulsed exposure study

Comments of zRMS:	Validation of the Analytical Method Bebon R., 2024, R 178651215 is acceptable and suitable for the determination of Thiencarbazone-methyl in Smart and Barko medium.

Reference:	KCP 5.2.1 (Submitted as KCA 8.2.7)
Report	Thiencarbazone-methyl: Toxicity to the aquatic plant *Myriophyllum spicatum* in a pulsed exposure growth inhibition test with a prior rooting phase, Bebon R., 2024, 178651215
Guideline(s):	Yes, OECD 239, 2014 and SANTE/2020/12830 Rev.2
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

Samples were analysed for thiencarbazone-methyl by LC-MS/MS.

All thiencarbazone-methyl standards were prepared in acetonitrile.

Results and discussions

Table A 11: Recovery results from method validation of thiencarbazone-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)
Smart and Barko medium	Thiencarbazone-methyl	0.25 (5)	109	1
		32 (5)	101	2

Table A 12: Characteristics for the analytical method used for validation of thiencarbazone-methyl residues in Smart and Barko medium

	Thiencarbazone-methyl
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 675934 x - 5582 r = 0.9997 number of data points: 9
Calibration range	0.025 - 2.5 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.
Assessment of matrix effects is presented	Yes, > 20%, matrix matched solution used.
Limit of detection/quantification	0.025 µg/L / 0.25 µg/L
Extract and standard stability	Storage stability of the test item in final extracts at 4 ± 4°C was proven for two days, covering the maximum storage of final extracts in this study. Standard stability was not investigated since freshly prepared standard solutions were used for all analyses.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of thiencarbazone-methyl in Smart and Barko medium.

A 2.1.1.2.5 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in algae study

Comments of zRMS:	Validation of the Analytical Method (Bauer J., 2024, R 177011210 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in reconstituted water (OECD medium).

Reference:	KCP 10.2.1
Report	GLOB2112dH: Toxicity to *Pseudokirchneriella subcapitata* in an algal growth inhibition test, Bauer J., 2024a, 177011210
Guideline(s):	Yes, OECD 201 (2011) and SANTE/2020//12830 Rev. 2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by LC-MS/MS.

All mesotrione standards were prepared in acetonitrile.
All thiencarbazone-methyl standards were prepared in acetonitrile.
All cyprosulfamide standards were prepared in acetonitrile.

Results and discussions

Table A 13: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
Reconstituted water (OECD medium)	Mesotrione	246 (5)	110	2
		36971 (5)	96	3
	Thiencarbazone-methyl	48.7 (5)	116	2
		7308 (5)	101	6
	Cyprosulfamide	74 (5)	112	4
		11085 (5)	98	3

Table A 14: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in reconstituted water (OECD medium)

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 55096 x - 3531 r = 0.9999 number of data points: 8	Linear y = 22242 x - 12 r = 0.9998 number of data points: 8	Linear y = 121794 x – 8090 r = 0.9999 number of data points: 8
Calibration range	0.75-25 µg/L	0.75-25 µg/L	0.75-25 µg/L

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	0.75 µg/L / 246 µg/L	0.75 µg/L / 48.7 µg/L	0.75 µg/L / 74 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in reconstituted water (OECD medium).

A 2.1.1.2.6 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in Lemna study

Comments of zRMS:	Validation of the Analytical Method Bauer J., 2024, 177011240 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in 20X AAP-Growth medium.

Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	GLOB2112dH: Toxicity to the aquatic plant *Lemna gibba* in a static growth inhibition test, Bauer J., 2024b, 177011240
Guideline(s):	Yes, OECD 221 (2006) and SANTE/2020/12830 Rev. 2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by LC-MS/MS.

All mesotrione standards were prepared in acetonitrile.
All thiencarbazone-methyl standards were prepared in acetonitrile.
All cyprosulfamide standards were prepared in acetonitrile.

Results and discussions

Table A 15: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
20X AAP-growth medium	Mesotrione	0.259 (4)	111	4
		38.8 (5)	109	2
	Thiencarbazone-methyl	0.051 (4)	101	2
		7.7 (5)	107	1
	Cyprosulfamide	0.078 (4)	114	4
		11.6 (5)	110	1

Table A 16: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in 20X AAP-growth medium

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 232656.2865 x + 266.4735 r = 0.9999 number of data points: 8	Linear y = 355173.1595 x + 531.0537 r = 1.0000 number of data points: 8	Linear y = 541405.3126 x – 1555.8107 r = 0.9998 number of data points: 8
Calibration range	0.06-6 µg/L	0.01-1 µg/L	0.02-2 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, > 20%, matrix matched solution used.	Yes, > 20%, matrix matched solution used.	Yes, > 20%, matrix matched solution used.
Limit of detection/quantification	0.06 µg/L / 0.259 µg/L	0.01 µg/L / 0.051 µg/L	0.02 µg/L / 0.078 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in 20X AAP-Growth medium.

A 2.1.1.2.7 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in Myriophyllum study

Comments of zRMS:	Validation of the Analytical Method Bauer J., 2024, 177011215 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in Smart and Barko medium, and for the determination of thiencarbazone-methyl in sediment and pore water.

Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	GLOB2112dH: Toxicity to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test with a prior rooting phase, Bauer J., 2024c, 177011215
Guideline(s):	Yes, OECD 239 (2014) and SANTE/2020/12830 Rev. 2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by LC-MS/MS.

All mesotrione standards were prepared in acetonitrile.
All thiencarbazone-methyl standards were prepared in acetonitrile.
All cyprosulfamide standards were prepared in acetonitrile.

Results and discussions

Table A 17: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
Smart and Barko medium (overlying water)	Mesotrione	0.1 µg/L (5)	110	7
		0.3 µg/L (5)	102	2
		38 µg/L (5)	95	0
	Thiencarbazone-methyl	0.02 µg/L (5)	115	5
		0.05 µg/L (5)	108	7
		7.6 µg/L (5)	96	2
	Cyprosulfamide	0.03 µg/L (5)	112	2
		0.08 µg/L (5)	103	4
		11.5 µg/L (5)	92	1
Sediment	Thiencarbazone-methyl	0.51 µg/kg (5)	92	17
		51 µg/kg (5)	94	4
Pore water	Thiencarbazone-methyl	0.0175 µg/L (5)	84	5
		4 µg/L (5)	105	2

Table A 18: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in Smart and Barko medium (overlying water)

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Calibration (type, number of data points)	Linear y=596612.1385x+4409.2926 r = 0.9997 number of data points: 8	Linear y=581455.2362x+247.1350 r = 0.9990 number of data points: 8	Linear y=1092611.1129x-983.8369 r = 0.9997 number of data points: 8
Calibration range	0.025–2.5 µg/L	0.005–0.5 µg/L	0.0075-0.75 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, > 20%, matrix matched solution used.	Yes, > 20%, matrix matched solution used.	Yes, > 20%, matrix matched solution used.
Limit of detection/quantification	0.025 µg/L / 0.1 µg/L	0.005 µg/L / µg/L	0.0075 µg/L / 0.03 µg/L
Extract and standard stability	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between the end of sample preparation and beginning of analysis.		

Table A 19: Characteristics for the analytical method used for validation of thiencarbazone-methyl residues in sediment and pore water

	Thiencarbazone-methyl	
	Sediment	Pore water
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic y=-6346.0430x²+174427.2567x+1477.1416 y=6804.1758x²+164187.5405x-387.7953 r ≥ 0.9999 number of data points: 8	Linear y = 340460.5194x + 1086.4302 r = 0.9999 number of data points: 6
Calibration range	0.02-1.5 µg/L	0.005-0.5 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	0.02 µg/L / 0.51 µg/kg	0.005 µg/L /0.0175 µg/L
Extract and standard stability	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between the end of sample preparation and beginning of analysis.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in Smart and Barko medium, and for the determination of thiencarbazone-methyl in sediment and pore water.

A 2.1.1.2.8 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in acute honey bee study

Comments of zRMS:	Validation of the Analytical Method Schabio S., 2024, 177011035 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in the contact application solution and the oral feeding solution used for the acute honeybee test.

Reference:	KCP 5.1.2 (submitted as KCP 10.3.1.1)
Report	GLOB2112dH: effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory, Schabio S., 2024, 177011035
Guideline(s):	Yes, OECD 213 and 214, SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by LC-MS/MS.

All mesotrione standards were prepared in acetonitrile/pure water (50/50 v/v).
All thiencarbazone-methyl standards were prepared in acetonitrile/pure water (50/50 v/v).
All cyprosulfamide standards were prepared in acetonitrile/pure water (50/50 v/v).

Results and discussions

Table A 20: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
Pure water + 0.1% v/v Triton X-100	Mesotrione	4.03 µg/L (5)	99	4
		16 µg/L (5)	102	9
	Thiencarbazone-methyl	3.98 µg/L (5)	98	4
		16 µg/L (5)	103	2
	Cyprosulfamide	6.06 µg/L (10)	94	4
		24 µg/L (5)	101	5
50% w/v sucrose solution	Mesotrione	4.64 µg/L (5)	110	4
		14.5 µg/L (5)	114	4
	Thiencarbazone-methyl	4.59 µg/L (5)	92	3
		14 µg/L (5)	85	4
	Cyprosulfamide	6.98 µg/L (10)	93	7
		22 µg/L (10)	88	8

Table A 21: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in contact application solution

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic $y = -963\ x^2 + 190329\ x - 22048$ r = 0.9995 number of data points: 8	Linear y = 88575 x + 12214 r = 0.9995 number of data points: 8	Linear y = 101912 x + 45125 r = 0.9994 number of data points: 8
Calibration range	1-30 µg/L	1-30 µg/L	1-30 µg/L
Residuals analysis	The regression residuals in the linear regression function are not randomly distributed. A polynomial trend was visible in the linear regression function and this trend was confirmed by the corresponding regression residual plot.	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	1 µg/L / 4.03 µg/L	1 µg/L / 3.98 µg/L	1 µg/L / 6.06 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Table A 22: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in oral feeding solution

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic $y = -617\ x^2 + 156880\ x + 79023$ r = 0.9997 number of data points: 8	Linear y = 89738 x + 2558 r = 0.9998 number of data points: 8	Linear y = 108953 x + 49734 r = 0.9990 number of data points: 8
Calibration range	1-30 µg/L	1-30 µg/L	1-30 µg/L
Residuals analysis	The regression residuals in the linear regression function are not randomly distributed. A polynomial trend was visible in the linear regression function and this trend was confirmed by the corresponding regression residual plot.	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Limit of detection/quantification	1 µg/L / 4.64 µg/L	1 µg/L / 4.59 µg/L	1 µg/L / 6.98 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in the contact application solution and the oral feeding solution used for the acute honeybee test.

A 2.1.1.2.9 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in acute bumble bee study

Comments of zRMS:	Validation of the Analytical Method Chwiesko D., 2024, 177011105 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in the contact application solution and the oral feeding solution used for the acute bumble bee test.

Reference:	KCP 5.2.1 (Submitted as KCP 10.3.1.1)
Report	GLOB2112dH: acute contact and oral toxicity to bumblebees (*Bombus terrestris* L.) in the laboratory, Chwiesko D., 2024, 177011105
Guideline(s):	Yes, OECD 246 and 247, SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by LC-MS/MS.

All mesotrione standards were prepared in acetonitrile/pure water (50/50 v/v).
All thiencarbazone-methyl standards were prepared in acetonitrile/pure water (50/50 v/v).
All cyprosulfamide standards were prepared in acetonitrile/pure water (50/50 v/v).

Results and discussions

Table A 23: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
Pure water + 0.1% v/v Triton X-100	Mesotrione	4.03 µg/L (5)	99	4
		16 µg/L (5)	102	9
	Thiencarbazone-	3.98 µg/L (5)	98	4

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
	methyl	16 µg/L (5)	103	2
	Cyprosulfamide	6.04 µg/L (10)	94	4
		24 µg/L (5)	101	2
50% w/v sucrose solution	Mesotrione	4.64 µg/L (5)	110	4
		14.5 µg/L (5)	114	4
	Thiencarbazone-methyl	4.59 µg/L (5)	92	3
		14 µg/L (5)	85	4
	Cyprosulfamide	6.96 µg/L (10)	93	7
		22 µg/L (10)	88	8

Table A 24: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in contact application solution

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic y = -963 x² + 190329 x – 22048 r = 0.9982 number of data points: 8	Linear y = 88575 x + ~~190329~~ 12214 r = ~~0.9997~~ 0.9995 number of data points: 8	Linear y = 101912 x + 45125 r = 0.9994 number of data points: 8
Calibration range	1-30 µg/L	1-30 µg/L	1-30 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	1 µg/L / 4.03 µg/L	1 µg/L / 3.98 µg/L	1 µg/L / 6.04 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Table A 25: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in oral feeding solution

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic y = - 617 x² + 156880 x + 79023 r = 0.9997 number of data points: 8	Linear y = 89738 x + 2558 r = 0.9998 number of data points: 8	Linear y = 108953 x + 49734 r = 0.9990 number of data points: 8
Calibration range	1-30 µg/L	1-30 µg/L	1-30 µg/L
Residuals analysis	The regression residuals in the linear regression function are not randomly distributed. A polynomial	The regression residuals are randomly distributed and no trends are visible.	

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
	trend was visible in the linear regression function and this trend was confirmed by the corresponding regression residual plot.		
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	1 µg/L / 4.64 µg/L	1 µg/L / 4.59 µg/L	1 µg/L / 6.96 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in the contact application solution and the oral feeding solution used for the acute bumble bee test.

A 2.1.1.2.10 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in chronic adult honey bee study

Comments of zRMS:	Validation of the Analytical Method Venturi S., 2023, BT215/23 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in the feeding solution used for the chronic oral honey bee test.

Reference:	KCP 5.2.1 (Submitted as KCP 10.3.1.2)
Report	Chronic oral effects of GLOB2112dH to adult worker honeybees (Apis mellifera L.) in a 10-day feeding laboratory test, Venturi S., 2023a, BT215/23
Guideline(s):	Yes, OECD TG No. 245 (2017) and SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by UHPLC-MS/MS.

All mesotrione standards were prepared in acetonitrile.
All thiencarbazone-methyl standards were prepared in acetonitrile.
All cyprosulfamide standards were prepared in acetonitrile.

Results and discussions

Table A 26: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
50% w/v sucrose solution	Mesotrione	1310 µg/kg	94.94	0.33
		1640 mg/kg	100.56	0.88
	Thiencarbazone-methyl	260 µg/kg	98.77	1.95
		320 mg/kg	101.09	0.66
	Cyprosulfamide	390 µg/kg	99.87	0.31
		500 mg/kg	102.01	0.83

Table A 27: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in 50% w/v sucrose solution

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic $y = -0.475820x^2 + 6026.234074x - 44515.755928$ r = 0.9997 number of data points: 5	Linear y = 1163.848037x - 1206.348422 r = 0.9996 number of data points: 5	Linear y = 2310.635302x - 3266.846143 r = 0.9998 number of data points: 5
Calibration range	40.1298-1738.9593 µg/L (corresponding to 0.4013-2173.6991 mg/kg)	7.9172-343.0770 µg/L (corresponding to 0.0792-428.8462 mg/kg)	12.0846-523.6650 µg/L (corresponding to 0.1208-654.5813 mg/kg)
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	30.9 µg/L / 1310 µg/kg	30.4 µg/L / 260 µg/kg	31 µg/L / 390 µg/kg
Extract and standard stability	The standard solutions and extracted solutions were analysed within 24 hours from preparation. Therefore, no test of stability was needed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in the feeding solution used for the chronic oral honey bee test.

A 2.1.1.2.11 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in chronic larvae honey bee study

Comments of zRMS:	Validation of the Analytical Method Venturi S., 2024, BT131/23 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in aqueous solution.

Reference:	KCP 5.2.1 (Submitted as KCP 10.3.1.3)
Report:	Effects of GLOB2112dH on honeybees (*Apis mellifera L.*) 22-day larval toxicity test with repeated exposure, Venturi S., 2024, BT131/23
Guideline(s):	Yes, OECD 239, 2021 and SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by UHPLC-MS/MS.

All mesotrione standards were prepared in acetonitrile/pure water.
All thiencarbazone-methyl standards were prepared in acetonitrile/pure water.
All cyprosulfamide standards were prepared in acetonitrile/pure water.

Results and discussions

Table A 28: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (*n* = x)	Mean recovery (%)	RSD (%)
Aqueous solution	Mesotrione	500 µg/L (5)	112.61	0.89
		25 g/L (5)	93.71	0.50
	Thiencarbazone-methyl	100 µg/L (5)	107.49	1.21
		5 g/L (5)	89.88	1.08
	Cyprosulfamide	152 µg/L (5)	111.42	0.90
		7 g/L (5)	92.93	0.94

Table A 29: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in aqueous solution

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 4534.023270x - 5482.780847 r = 0.99998690 number of data points: 5	Linear y = 875.762605x - 4678.038330 r = 0.99987349 number of data points: 5	Linear y = 2007.478320x - 3992.578797 r = 0.99997146 number of data points: 5
Calibration range	30.9290–1340.2584 µg/L (30.9290 µg/L–33.50646 g/L in matrix)	30.4209–1318.2369 µg/L (30.4209 µg/L–32.95592 g/L in matrix)	30.9563–1341.4385 µg/L (30.9563 µg/L–33.53596 g/L in matrix)
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	30.9290 µg/L / 500 µg/L	30.4209 µg/L / 0.10	30.9563 µg/L / 0.13

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
		mg/L	mg/L
Extract and standard stability	The standard solutions, as well as any extract, were analysed within 24 hours from preparation; therefore, no test of stability was needed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in aqueous solution.

A 2.1.1.2.12 Analytical method for mesotrione, thiencarbazone-methyl and cyprosulfamide in non-target plants study

Comments of zRMS:	Validations of the Analytical Method for studies Dommes A.B., 2024, 177011086 and Dommes A.B., 2024a, 177011086 is acceptable and suitable for the determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in water.

Reference:	KCP 5.2.1 (Submitted as KCP 10.6)
Report	GLOB2112dH: Effects on terrestrial (non-target) plants : seedling emergence and seedling growth test, Dommes A.B., 2024a, 177011086
Guideline(s):	Yes, OECD 208, 2006 and SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.2.1 (Submitted as KCP 10.6)
Report	GLOB2107H: Effects on terrestrial (non-target) plants : vegetative vigour test, Dommes A.B., 2024b, 177011087
Guideline(s):	Yes, OECD 227, 2006 and SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were analysed for mesotrione, thiencarbazone-methyl and cyprosulfamide by LC-MS/MS.

All mesotrione standards were prepared in acetonitrile/pure water (50/50 v/v).
All thiencarbazone-methyl standards were prepared in acetonitrile/pure water (50/50 v/v).
All cyprosulfamide standards were prepared in acetonitrile/pure water (50/50 v/v).

Results and discussions

Table A 30: Recovery results from method validation of mesotrione, thiencarbazone-methyl and cyprosulfamide using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)
Water	Mesotrione	6.45 (5)	88	2
		19 (4)	97	2
	Thiencarbazone-methyl	6.37 (5)	92	3
		19 (5)	99	4
	Cyprosulfamide	9.67 (5)	87	2
		29 (5)	99	4

Table A 31: Characteristics for the analytical method used for validation of mesotrione, thiencarbazone-methyl and cyprosulfamide residues in water

	Mesotrione	Thiencarbazone-methyl	Cyprosulfamide
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 527767 x + ~~517898~~ 261633 r = ~~0.9963~~ 0.9994 number of data points: 8	Linear y = 101309 x + 72081 34892 r = ~~0.9980~~ 0.9997 number of data points: 8	Linear y = 236543 x + ~~178368~~ 74611 r = ~~0.9970~~ 0.9994 number of data points: 8
Calibration range	1-35 µg/L	1-35 µg/L	1-35 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.		
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	1 µg/L / 6.45 µg/L	1 µg/L / 6.37 µg/L	1 µg/L / 9.67 µg/L
Extract and standard stability	The stock solution of the reference item was prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of mesotrione, thiencarbazone-methyl and cyprosulfamide in water.

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)

A 2.1.2.2.1 Analytical method for determination of thiencarbazone-methyl and BYH18636-MMT in eggs

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	Validation of the Analytical Method Gustloff C., 2024, S24-102708 is acceptable and suitable for the determination of thiencarbazone-methyl and its metabolite BYH18636-MMT in eggs.

Reference:	KCP 5.2
Report	Validation of analytical methods to determine residues of thiencarbazone-methyl and its metabolite in eggs, Gustloff C., 2024, S24-102708
Guideline(s):	Yes, SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of poultry's eggs were extracted two times with acetonitrile+water (4+1, v+v) using a GenoGrinder. After a concentration step the samples were resolved in water. Samples were analysed for thiencarbazone-methyl and its metabolite BYH18636-MMT by LC-MS/MS.

Results and discussions

Table A 32: Recovery results from method validation of thiencarbazone-methyl and BYH18636-MMT using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)
Eggs	Thiencarbazone-methyl	0.01 (5)	70	6.2
		0.1 (5)	70	4.7
	BYH18636-MMT	0.01 (5)	72	0.8
		0.1 (5)	72	1.5

Table A 33: Characteristics for the analytical method used for validation of thiencarbazone-methyl and BYH18636-MMT residues in eggs

	Thiencarbazone-methyl	BYH18636-MMT
Specificity	blank value < 30% LOQ	
Calibration (type, number of data points)	Linear r ≥ 0.99 number of data points: min. 5	

	Thiencarbazone-methyl	BYH18636-MMT
Calibration range	0.015 ng/mL to 1.5 ng/mL corresponding to 0.003 mg/kg to 0.30 mg/kg (expressed as thiencarbazone-methyl)	
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, ≥ 20%, matrix matched solution used.	
Limit of detection/quantification	0.003 mg/kg / 0.01 mg/kg	
Standard stability	Within ± 10% for at least 175 days for thiencarbazone-methyl and for at least 245 days for BYH 18636-MMT when prepared in acetone and stored at typically 1°C to 10°C in the dark. Within ± 10% for at least 12 days when prepared in acetonitrile and stored at typically 1°C to 10°C in the dark. Within ± 10% for at least 14 days when prepared in acetonitrile/water (1+9, v+v) and stored at typically 1°C to 10°C in the dark.	
Extract stability	Recoveries within 70% - 120% for at least 15 days when stored at typically 1°C to 10°C in the dark.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of thiencarbazone-methyl and its metabolite BYH18636-MMT in eggs.

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	Validation of the Analytical Method Senciuc M., 2024, S24-102656 is acceptable and suitable for the determination of thiencarbazone-methyl and its metabolite BYH18636-MMT in eggs.

Reference:	KCP 5.2
Report	Independent laboratory validation of an analytical method for the determination of thiencarbazone-methyl and its metabolite BYH18636-MMT in egg, Senciuc M., 2024, S24-102656
Guideline(s):	Yes, SANTE/2020/12830 rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of poultry's eggs were extracted two times with acetonitrile+water (4+1, v+v) using a Fast Prep 96. After a concentration step the samples were resolved in water. Samples were analysed for thiencarbazone-methyl and its metabolite BYH18636-MMT by LC-MS/MS.

Primary validation and independent laboratory validation were carried out at different locations and by different study personnel. No addition or modification to the original method other than optimisation of instrumental parameters was done with one exception: in the ILV study a pre-column was used.

For the determination of the confirmatory transition of BYH 18636-MMT by a second injection, additionally to the optimisation of instrumental parameters the following modifications were used:

- A SCIEX TripleQuad 6500 system was used
- Injection volume was 60 µL
- To protect the LC pump, acetonitrile with 0.1% water was used as mobile phase
- The following parameter were modified to improve sensitivity (DP, CXP and Dwell time)

No communication with the method developers or others familiar with the method was necessary to carry out the analysis.

Results and discussions

Table A 34: Recovery results from independent laboratory validation of thiencarbazone-methyl and BYH18636-MMT using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Eggs	Thiencarbazone-methyl	0.01 (5)	89	13
		0.1 (5)	96	9
	BYH 18636-MMT	0.01 (5)	90	13
		0.1 (5)	98	2

Table A 35: Characteristics for the analytical method used for independent laboratory validation of thiencarbazone-methyl and BYH18636-MMT residues in eggs

	Thiencarbazone-methyl	BYH18636-MMT
Specificity	blank value < 30% LOQ	
Calibration (type, number of data points)	Linear r ≥ 0.999 number of data points: min. 5	
Calibration range	0.015 ng/mL to 1.5 ng/mL corresponding to 0.003 mg/kg to 0.30 mg/kg (expressed as thiencarbazone-methyl)	
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, ≥ 20%, matrix matched solution used.	
Limit of determination/quantification	0.003 mg/kg / 0.01 mg/kg	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 2 and can be used for analytical determination of thiencarbazone-methyl and its metabolite BYH18636-MMT in eggs.

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 thiencarbazone-methyl

Please refer to A 2.1.