FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CHR/H/TERIZ 650 WG

Product name(s): Undito 650 WG, Jotamun 650 WG, Metodus 650 WG

Chemical active substance(s):

Terbuthylazine, 400 g/kg

Mesotrione, 150 g/kg

Isoxaflutole, 100 g/kg

Central zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT – renewal of authorisation (Poland)

Applicant: Innvigo Sp. z o.o.

Submission date: October 2019

Update: November 2021

Finalisation date: November 2021; June 2023

Version history

When	What
October 2019	New data for isoxaflutole based on the renewal of active substance. New data marked in yellow
November 2021	References and description of equivalent studies to the protected data on isoxaflutole were added.
November 2021	Evaluation of new data (isoxaflutole)
June 2023	Final Registration Report

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5 Analytical methods

Applicant supplemented the section of analytical methods with references and description of equivalent studies to protected data.
Data matching table and studies have been evaluated by Poland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/TERIZ 650 WG (Undito 650 WG / Jotamun 650 WG / Metodus 650 WG) INN-VIGO is allowed to refer to EU approved reports.

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.

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

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 terbuthylazine, isoxaflutole and mesotrione in the CHR/H/TERIZ 650 WG plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of terbuthylazine, isoxaflutole and mesotrione in plant protection product is provided as follows:

Reference:	KCP 5.1.1
Report	CHR/H/TERIZ 650 WG Isoxaflutole/Mesotrione/Terbuthylazine 100/150/400 Development and validation of the method for determination of active substances content in the formulation, E.J. Gwóźdź, 2015,BA-25/15, Authority registration No: 17/2015/DPL
Guideline(s):	SANCO /3030 /99 rev.4.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

Determination of isoxaflutole, mesotrione and terbuthylazine content was performed using HPLC-UV method developed and validated in Analytical Department of IPO. The content of active substances in the formulation is the following:

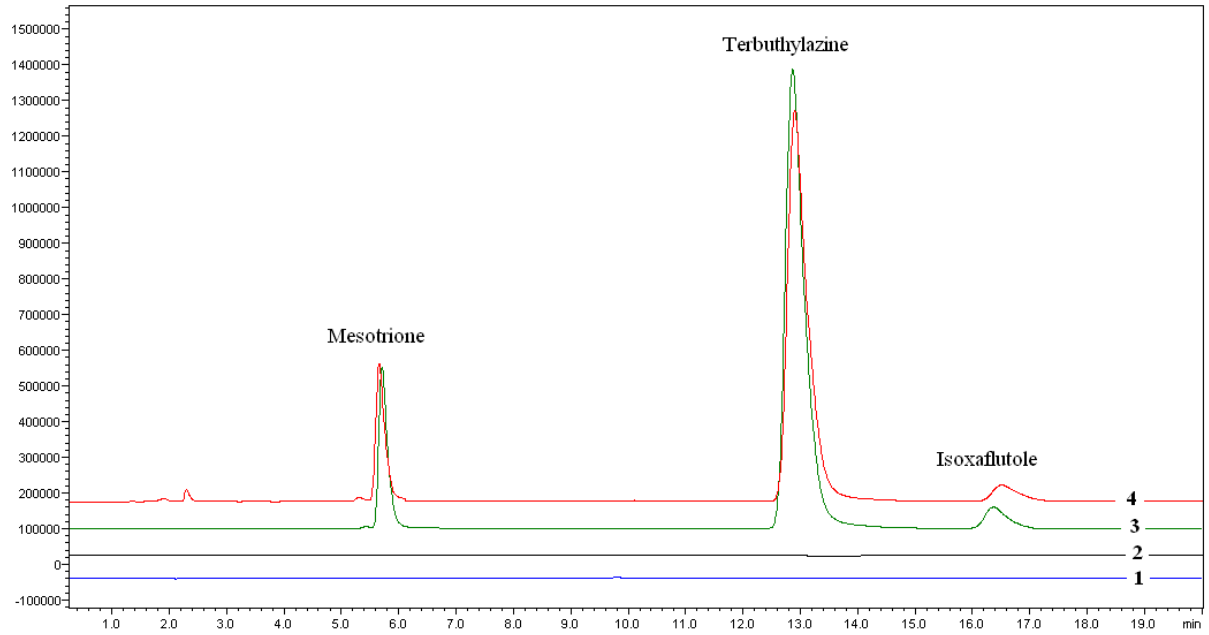
Isoxaflutole: **9.83 ± 0.26 %**
Mesotrione: **14.15 ± 0.27 %**
Terbuthylazine: **39.43 ± 0.73 %**

It was confirmed that the method is specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances Isoxaflutole, mesotrione and terbuthylazine in plant protection product CHR/H/TERIZ 650 WG

	Isoxaflutole	**Mesotrione**	**Terbuthylazine**
Author(s), year	E.J. Gwóźdź, 2015	E.J. Gwóźdź, 2015	E.J. Gwóźdź, 2015
Principle of method	HPLC-UV	HPLC-UV	HPLC-UV
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of analytical method was assessed using five standard solutions of izoxaflutole of concentration in the range from 0.1011 mg/mL to 0.3033 mg/mL (from 51 % to 152 % of nominal content in the sample). Each standard solution was injected two times into the column. Equation from calibration curve: y=7970599x + 53752 R^2=0.9999	The linearity of analytical method was assessed using five standard solutions of mesotrione of concentration in the range from 0.1497 mg/mL to 0.4490 mg/mL (from 50 % to 150 % of nominal content in the sample). Each standard solution was injected two times into the column. Equation from calibration curve: y=16368969x + 154037 R^2=0.9996	The linearity of analytical method was assessed using five standard solutions of terbuthylazine of concentration in the range from 0.4098 mg/mL to 1.2293 mg/mL (from 51 % to 154 % of nominal content in the sample). Each standard solution was injected two times into the column. Equation from calibration curve: y=32883474x + 3317568 R^2=0.9985
Precision – Repeatability Mean n = 6 (%RSD)	The repeatability of the method was assessed on the basis of six determinations of izoxaflutole content in the examined material. The repeatability was expressed as relative standard deviation. Acceptable relative standard deviation for active substance (9.8 %) should be RSDr ≤ 2.80 %. 1.9% In the present study RSDr = 0.67 x RSD = 1.67 %. The obtained result is acceptable. The confidence interval (x ± 0.26 %) was assessed on the base of the results.	The repeatability of the method was assessed on the basis of six determinations of mesotrione content in the examined material. The repeatability was expressed as relative standard deviation. Acceptable relative standard deviation for active substance (14 %) should be RSDr ≤ 2.70 %. In the present study RSDr = 0.67 x RSD = 1.21 %. The obtained result is acceptable. The confidence interval (x ± 0.27 %) was assessed on the base of the results.	The repeatability of the method was assessed on the basis of six determinations of terbuthylazine content in the examined material. The repeatability was expressed as relative standard deviation. Acceptable relative standard deviation for active substance (40 %) is RSDr ≤ 1.50 %. In the present study RSDr = 0.67 x RSD = 1.19 %. The obtained result is acceptable. The confidence interval - x ± 0.73 % was assessed on the base of the results.
Accuracy	The accuracy of izoxaflutole	The accuracy of mesotrione determination	The accuracy of terbuthylazine

	Isoxaflutole	Mesotrione	Terbuthylazine
n = 12 (2 X 6) (% Recovery)	determination in CHR/H/TERIZ was estimated by the recovery measurement. About 7 mg (level I) and 10 mg (level II) of the sample were weighed into twelve 5 mL volumetric flasks. To six of these flasks 0.5 mL of 0.2527 mg/mL standard solution of izoxaflutole was added and to the other six flasks – 1.0 mL. The volume was filled to the mark with acetonitrile and the content was mixed and iltered. All solutions were analyzed and detector responses were recorded. The average recovery value for the isoxaflutole is 99.13% should be 100 ± 2 %. The obtained result is acceptable.	in CHR/H/TERIZ was estimated by the recovery measurement. About 7 mg (level I) and 10 mg (level II) of the sample were weighed into twelve 5 mL volumetric flasks. To six of these flasks 0.5 mL of 0.5537 mg/mL standard solution of mesotrione was added and to the other 6 flasks - 1 mL. The volume was filled to the mark with acetonitrile and the content was mixed and filtered. All solutions were analyzed and detector responses were recorded. The average recovery value for the mesotrione is 98.81% should be 100 ± 2 %. The obtained result is acceptable.	determination in CHR/H/TERIZ was estimated by the recovery measurement. About 7 mg (level I) and 10 mg (level II)) of the sample were weighed into twelve 5 mL volumetric flasks. To six of these flasks 0.5 mL of 1.0244 mg/mL standard solution of terbuthylazine was added and to the other 6 flasks – 1.0 mL. The volume was filled to the mark with acetonitrile and the content was mixed and filtered. All solutions were analyzed and detector responses were recorded. The average recovery value for the Terbuthylazine is 98.64% should be 100 ± 2 %. The obtained result is acceptable.
Interference/ Specificity	Chromatograms of the acetonitrile, solution of placebo standards mixture solution and examined sample solution were performed and superimposed. There were no peaks from the placebo interfering with determined compounds. 		
Comment	1- placebo solution, 2- acetonitrile, 3 - standards mixture solution, 4 – sample solution		

Conclusion

Determination of isoxaflutole, mesotrione and terbuthylazine content was performed using HPLC-UV method developed and validated in Analytical Department of IPO. The content of active substances in the formulation is the following:
Isoxaflutole: 9.83 ± 0.26 %
Mesotrione: 14.15 ± 0.27 %
Terbuthylazine: 39.43 ± 0.73 %
It was confirmed that the method is specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

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 terbuthylazine, isoxaflutole and mesotrione in plant protection product is provided as follows:

Reference:	KCP 5.2.1/01
Report	Validation of the method of determination of terbuthylazine, isoxaflutole, mesotrione and a specified Mesotrione impurity in a WG Formulation, in compliance with GLP. Study no: DNA 3319; DNAL
Guideline(s):	SANCO /3030 /99 rev.4.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Analytical methodology of mesotrione, tarbuthylazine and isoxaflutole details:

Analysis of Mesotrione:

The assay of Mesotrione was performed using approximately 0.2 g of Formulation. The mass of the sample (DNA3232/1) was accurately recorded, transferred to a 100 mL volumetric flask and made to partial volume with Methanol. The samples were then sonicated for 5 mins and made up to volume with Methanol. These solutions were then used for assay by injecting each solution once into the HPLC-DAD under the following conditions:

Mesotrione HPLC-DAD Conditions

Instrument:	Agilent 1200/1260 series HPLC-DAD
Mode:	Gradient
Column:	Phenomenex Synergi Hydro, 250mm x 4.6mm x 4µm.
Eluent:	A: Acetonitrile: B: Deionised Water adjusted to pH3.2 with Formic Acid pH

Retention Time (mins)	Percentage of Eluent A	Percentage of Eluent B
0	30	70
3	30	70
25	80	20
32	80	20
35	30	70
40	30	70

Between 3-25 minutes, the ratio of the eluent is changing from 30% to 80% for eluent A and from 70% to 20% for eluent B. Between 32-35 minutes, the ratio of the eluent is changing from 80% to 30% for eluent A and from 20% to 70% for eluent B.

Wavelength:	270nm
Injection Volume:	10µl
Flow Rate:	1.0ml/min
Data Collection:	Chemstation
Retention Times:	Approximately 11.5-11.8 minutes for HPLC 5 Approximately 12.2 minutes for HPLC 3

Analysis of the Terbuthylazine and Isoxaflutole:

The assay of Terbuthylazine and Isoxaflutole was performed using approximately 0.2g of the sample (DNA3232/1). The mass of the Formulation was accurately recorded, transferred to a 100 mL volumetric flask and made to partial volume with Methanol. The samples were then sonicated for 5 mins and made up to volume with Methanol. These solutions were then used for assay by injecting each solution once into the HPLC-DAD under the following conditions:

Terbuthylazine and Isoxaflutole HPLC-DAD Conditions

Instrument:	Agilent 1200 series HPLC-DAD
Mode:	Isocratic
Column:	Grace, (250mm x 4.6mm x 5µm)
Packing:	C18 5µm
Eluent:	50% Acetonitrile: 50% Deionised Water with Formic Acid pH 3.0
Wavelength:	270nm
Injection Volume:	10µl
Flow Rate:	1.0ml/min
Data Collection:	Chemstation
Retention Times:	Terbuthylazine approximately 14.0 mins Isoxaflutole approximately 16.1 mins

Validation parameters

Mesotrione:

The validation parameters for the Mesotrione methodology have been met for this study under the Sanco/3030/99 rev. 4 guidelines. A summary of these results are shown in Table 1.

Table 1: Validation Summary Table – Mesotrione

Validation Parameter	Results Obtained	Acceptance Criteria under Sanco 3030/99 rev4
Linearity	R^2 = 0.9999	R^2 = >0.99
Sample Precision	%RSD = 1.28	%RSD less than 1.78 at 150g/Kg
Recovery at 150g/Kg	Mean Recovery = 99.49%	Between 98%-102%
LOQ Recovery at 2.5g/Kg	Mean Recovery = 103.5%	Between 95%-105%
Selectivity (Spectral Analysis)	The UV and MS Spectra for Mesotrione confirm the species identification	To confirm the species identity in the associated spectra traces
Specificity	Mesotrione eluted at 12.2 minutes, and there were no other significant peaks present at the same elution time as Mesotrione	To show no interference

Terbythylazine

The validation parameters for the Terbuthylazine methodology have been met for this study under the Sanco/3030/99 rev. 4 guidelines. A summary of these results are shown in Table 2.

Table 2: Validation Summary Table – Terbuthylazine

Validation Parameter	Results Obtained	Acceptance Criteria under Sanco 3030/99 rev4
Linearity	R^2 = 1.000	R^2 = >0.99
Sample Precision	%RSD = 0.905	%RSD less than 1.54 at 400g/Kg
Recovery at 400g/Kg	Mean Recovery = 100.4%	Between 98%-102%
LOQ Recovery at 1g/Kg	Mean Recovery = 98.84%	Between 97%-103%
Selectivity (Spectral Analysis)	The UV and MS Spectra for Terbuthylazine confirm the species identification	To confirm the species identity in the associated spectra traces
Specificity	Terbuthylazine eluted at 13.9 minutes, and there were no other peaks present at the same elution time as Terbuthylazine	To show no interference.

Izoxaflutole

The validation parameters for the Isoxaflutole methodology have been met for this study under the Sanco/3030/99 rev. 4 guidelines. A summary of these results are shown in Table 3.

Table 3: Validation Summary Table – Isoxaflutole

Validation Parameter	Results Obtained	Acceptance Criteria under Sanco 3030/99 rev4
Linearity	R^2 = 1.000	R^2 = >0.99
Sample Precision	%RSD = 0.971	%RSD less than 1.90 at 100g/Kg
Recovery at 100g/Kg	Mean Recovery = 99.36%	Between 97%-103%
LOQ Recovery at 0.5g/Kg	Mean Recovery = 98.00%	Between 95%-105%
Selectivity (Spectral Analysis)	The UV and MS Spectra for Isoxaflutole confirm the species identification	To confirm the species identity in the associated spectra traces
Specificity	Isoxaflutole eluted at 16.1 minutes, and there were no other peaks present at the same elution time as Isoxaflutole	To show no interference.

RMS Comments:

It was confirmed that the method is specific.
The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

The validation parameters for the Mesotrione Impurity (l-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one) methodology have been met for this study under the Sanco/3030/99 rev. 4 guidelines. A summary of these results are shown in table below.

Materials and method

The assay of the Mesotrione Impurity l-cyano-6-(methylsulfonyl)-7-nitro-9Hxanthen- 9-one was performed using approximately 0.5g of each batch of Formulation. The mass of the sample (DNA3232/l) was accurately recorded, transferred to a 25ml volumetric flask and made to final volume with Acetonitrile. These solutions were then used for assay by injecting each solution once into the LCMS Q-ToF under the following conditions:

LCMS 0-ToF LC Conditions - Mesotrione Impurity:
Instrument: Agilent 1200 Series HPLC-DAD
Mode: Isocratic
Column: YMC J-Sphere ODS-H80, 150mm x 4.6mm
Packing: ODS-H80, S-4µm 8run
Eluent: 45% Acetonitrile : 55% Water adjusted to pH3 with Formic Acid
Injection Volume: 10µl
Flow Rate: l.0 ml/min

LCMS Q-ToF MS Conditions - Mesotrione Impurity:

Instrument:	Agilent 6500 Series Q-ToF Mass Spectrometer
Mode:	ESI Jet Spray Source
Ionisation:	Positive
MS Scan Range:	50-1000 m/z
Extracted Ions:	$C_{15}H_8N_2O_6S$ which equates to 345.0176, 366.9995, 382.9735, and 383.9764m/z
Acquisition Rate:	1 Spectra/Second
Acquisition Time:	1000 ms/Spectra
Retention Time:	Approximately 8.6 minutes
Gas Temperature:	360 °C
Drying Gas Flow:	8 L/min
Nebulizer:	60 psig
Sheath Gas:	250 °C
Sheath Gas Flow:	7 L/min
VCap:	3000 V
Nozzle Voltage:	2000 V
Fragmentor:	150 V
Skimmer:	65 V
OCT 1 RF Vpp:	750 V
Collision Energy:	0 V

~~Validation - Results and discussions~~

The validation parameters for the Mesotrione Impurity (1-cyano-6-(methylsulfonyl)-7-nitro-9*H*- xanthen-9-one) methodology have been met for this study under the Sanco/3030/99 rev. 4 guidelines. A summary of these results are shown in Table 4.

Table 4: Validation Summary Table – Mesotrione Impurity

Validation Parameter	**Results Obtained**	**Acceptance Criteria under Sanco 3030/99 rev4**
Linearity	$R^2 = 0.9999$	$R^2 = >0.99$
Sample Precision	No Mestrione Impurity Detected	N/A
Recovery Precision	%RSD = 3.79	%RSD less than 8.41 at 0.0005%
Recovery at 0.0005%	Mean Recovery = 96.16%	Between 75%-125%
LOQ Recovery at 0.0001%	Mean Recovery = 113.4%	Between 75%-125%
Selectivity (Spectral Analysis)	The UV and MS Spectra for Mesotrione Impurity confirms the species identification	To confirm the species identity in the associated spectra traces
Specificity	Mesotrione Impurity eluted at 5.7 minutes, and there were no other peaks present at the same elution time as Mesotrione Impurity	To show no interference.

Conclusion

It was confirmed that the method is specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

Reference:	KCP 5.2.1/02
Report	CHR/H/TERIZ 650 WG Development and validation of the method for determination of the relevant impurities (simazine, atrazine and propazine) content in the formulation. Study no: BA-06/17; E.Gwóźdź, IPO, 2017
Guideline(s):	SANCO /3030 /99 rev.4.
Deviations:	NO
GLP:	YES
Acceptability:	YES

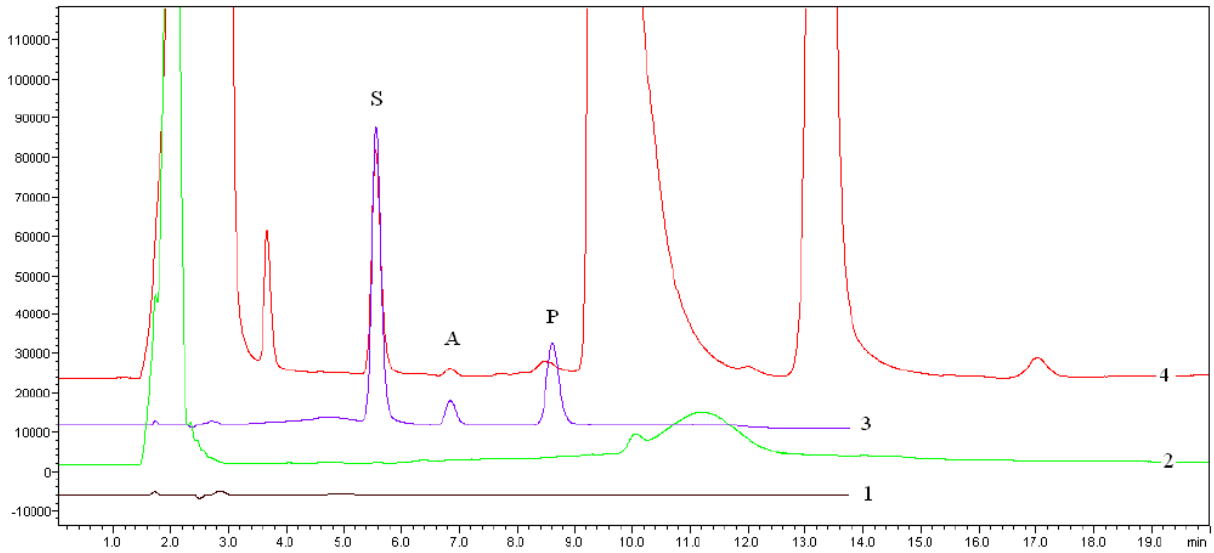
Material and methods

Determination of the content of relevant impurities was performed using HPLC-UV method developed in IPO Analytical Department, using external standards.
Chromatographic method was validated. It was confirmed that the method is specific. No interference was observed. The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities (simazine, atrazine and propazine) content in the formulation plant protection product CHR/H/TERIZ 650 WG

<table>
<tr><th></th><th>Terbuthylazine – relevant impurities simazine, atrazine and propazine</th></tr>
<tr><td>Author(s), year</td><td>E.Gwóźdź, 2017</td></tr>
<tr><td>Principle of method</td><td>HPLC-UV</td></tr>
<tr><td>Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)</td><td>
<table>
<tr><th>Statistical data</th><th>Impurity I- simazine</th><th>Impurity II- atrazine</th><th>Impurity III - propazine</th></tr>
<tr><td>Correlation coefficient</td><td>0.9999</td><td>0.9998</td><td>1.0000</td></tr>
<tr><td>Slope</td><td>172128944</td><td>157551092</td><td>138171837</td></tr>
<tr><td>Intercept</td><td>-20481</td><td>-806</td><td>-1214</td></tr>
<tr><td>Criterion achieved ($R^2 > 0.99$)</td><td>yes</td><td>yes</td><td>yes</td></tr>
</table>
</td></tr>
<tr><td>Precision – Repeatability Mean n = 6 (%RSD)</td><td>Limit of quantification (LOQ) and limit of detection (LOD)
The limit of quantification (LOQ) was defined as the lowest concentration of impurities standards used for calibration curves:
-simazine 0.0002 mg/mL (0.01 % by mass),
-atrazine 0.000005 mg/mL (0.0002%)
-propazine 0.00004 mg/ml (0.002 %).
The limit of detection is LOQ/2.</td></tr>
</table>

<table>
<tr><td></td><td colspan="3">Terbuthylazine – relevant impurities simazine, atrazine and propazine</td></tr>
<tr><td>Accuracy
n = 12 (2 X 6)
(% Recovery)</td><td colspan="3">
<table>
<tr><th>Statistical Data</th><th>Impurity I- simazine</th><th>Impurity II- atrazine</th><th>Impurity III - propazine</th></tr>
<tr><td>Recovery [%]</td><td>97.7 - 102.3</td><td>97.2 - 102.8</td><td>106.4 – 114.7</td></tr>
<tr><td>Acceptable recovery [%]</td><td>80 ÷ 120</td><td>75 ÷ 125</td><td>80 ÷ 120</td></tr>
<tr><td>Criterion achieved</td><td>yes</td><td>yes</td><td>yes</td></tr>
</table>
</td></tr>
<tr><td>Interference/ Specificity</td><td colspan="3">Chromatograms of the acetonitrile, solution of placebo standards mixture solution and examined sample solution were performed and superimposed. There were no peaks from the placebo interfering with determined compounds.
 

1 - Acetonitrile, 2- solution of placebo , 3- solution of standards mixture, 4- sample solution</td></tr>
<tr><td>Comment</td><td>-</td><td>-</td><td>-</td></tr>
</table>

Conclusion

Determination of relevant impuritiers of terbuthylazine in the formulation CHR/H/TERIZ 650 WG content was performed using HPLC-UV method developed and validated in Analytical Department of IPO. The content of active substances in the formulation is the following:
- Simazine - 1.52 ± 0.01 g/kg (SANCO specification no more than 12 g/kg of the formulation)
- Atrazine - 0.06 ± 0.001 g/kg (SANCO specification no more than 0.40 g/kg of the formulation)
- Propazine – 0.19 ± 0.01 g/kg (SANCO specification no more than 4.0 g/kg of the formulation)It was confirmed that the method is specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

Reference:	KCP 5.2.1/03
Report	Validation of the method of determination of two specified impurities within a WG Formulation containing Terbuthylazine, Isoxaflutole and Mesotrione in compliance with GLP. Study no: DNA 4178; DNAL
Guideline(s):	SANCO /3030 /99 rev.4.
Deviations:	NO
GLP:	YES
Acceptability:	YES

IMPURITY 1 - R287432: (6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile)

Summary of the validation:

Validation Parameter	Results Obtained	Acceptance Criteria under SANCO/3030/99 rev.4
Linearity	$R^2 = 0.9968$	$R^2 = >0.99$
Sample Precision	No detectable Impurity 1 above the LOQ Level of 0.005g/Kg, equating to 0.033g/Kg in the active substance as manufactured	n/a
Recovery Precision	%RSD = 1.397	%RSD less than 5.97 at 0.049g/Kg (0.0049%)
Recovery at 0.05g/Kg (0.005%)	Mean Recovery = 97.23%	Between 75%-125%
LOQ Recovery at 0.005g/Kg (0.0005%)	Mean Recovery = 103.7%	Between 75%-125%
Selectivity (Spectral Analysis)	The MS Spectra for Impurity 1 confirms the species identification	To confirm the species identity in the associated spectra traces
Specificity	Impurity 1 eluted at 3.9 minutes, and there were no other peaks present at the same elution time as Impurity 1	To show no interference

Analytical Method:

The Sample Precision of Impurity 1 (R287432: (6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile)) was performed using approximately 0.05g of sample (DNA3232/1). The mass of the sample was accurately recorded, transferred to a 50ml volumetric flask, made to partial volume with Acetonitrile and sonicated for 5 minutes. After cooling to room temperature (ambient) the solution was made to final volume with Acetonitrile. Six separate solutions were prepared in this way and then used for assay by injecting each solution once into the LC-QQQ under the following conditions:

LC-QQQ Conditions:

Instrument:	Agilent 6470 QQQ Mass Spectrometer
Mode:	Isocratic Reverse Phase
Column:	Waters Sunfire C18, 150mm x 4.6mm
Packing:	C18, 3.5µm
Eluent:	70% Acetonitrile with 0.1% Acetic Acid : 30% Water with 0.1% Acetic Acid
Flow Rate:	0.5ml/min
Injection Volume:	10µl
Column Temperature:	25°C
MS Scan Range:	n/a
Retention Time:	Approximately 3.9 minutes
Data Acquisition:	MassHunter

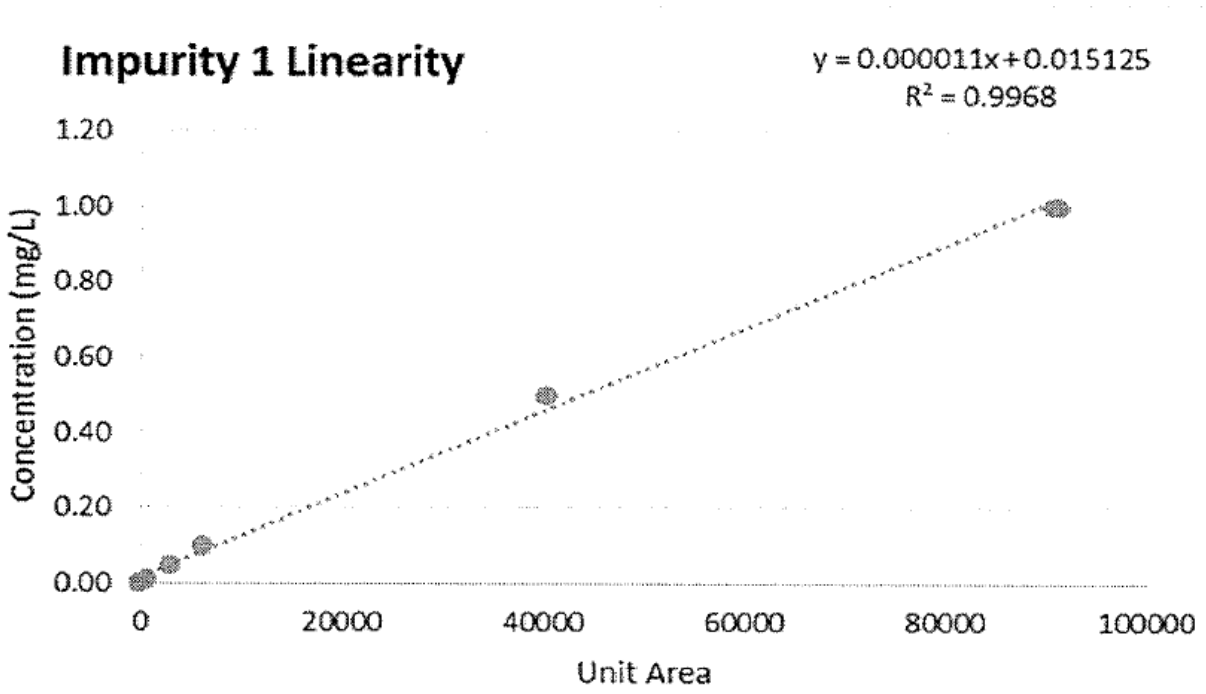
Ionisation:	Positive	Sheath Gas:	300°C
Gas Temperature:	150°C	Sheath Gas flow:	6L/min
Gas Flow:	8 L/min	Capillary:	6000V
Nebulizer:	30psi	Nozzle Voltage:	2000V

MRM Precursor Ion: 300m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
300	221	200	237	32	5
300	209	200	237	32	5
300	193	200	237	44	5

Linearity:

The linearity was determined from fourteen injections of seven concentrations of standard ranging from a blank to 1.00mg/L. The samples were prepared for analysis at a sample concentration of 1.0mg/ml. From the Sample Precision it is known that the sample (DNA3232/1) contains no detectable Impurity 1 above the LOQ level of 0.005g/Kg, therefore Recovery Precision was performed at 0.05g/Kg which equates to a concentration of 0.05mg/L and falls within the limits of the linearity range. Table 3 shows the individual peak area response for each duplicate standard at the specified concentration. The mean area of each duplicate injection for each standard concentration has been plotted on a graph displayed below. The plot possesses a correlation coefficient of 0.9968, based on individual values.



Precision:

To show the Sample Precision six samples of approximately 0.05g of sample DNA3232/1 were prepared in 50ml of Acetonitrile and injected into the LC-QQQ. The results obtained are shown in Table 4. Impurity 1 was not detected above the LOQ Level of 0.005g/Kg, equating to 0.033g/Kg in the active substance as manufactured.

EFSA Specification = Maximum 2g/kg in the active substance as manufactured

Table 4: Sample Precision of Impurity 1

Description	Raw Data	Retention Time (minutes)	Unit Area	Response Factor	Concentration (mg/L)	Weight (g)	Percentage (w/w) in Formulation	g/Kg in Formulation	g/Kg Relative to Mesotrione Content
0.05mg/L Impurity 1	M0709S13	3.88	2943	0.0000170					
Sample Precision A	M0709V1	3.91	71		0.001207	0.0535	< 0.0005	< 0.005	< 0.033
Sample Precision B	M0709V2	3.89	54		0.000918	0.0497	< 0.0005	< 0.005	< 0.033
Sample Precision C	M0709V3	3.90	59		0.001003	0.0509	< 0.0005	< 0.005	< 0.033
0.05mg/L Impurity 1	M0709S14	3.88	2937	0.0000167					
Sample Precision D	M0709V4	3.89	71		0.001185	0.0527	< 0.0005	< 0.005	< 0.033
Sample Precision E	M0709V5	3.89	66		0.001101	0.0503	< 0.0005	< 0.005	< 0.033
Sample Precision F	M0709V6	3.89	80		0.001335	0.0515	< 0.0005	< 0.005	< 0.033
0.05mg/L Impurity 1	M0709S15	3.88	3057						
						Mean	< 0.0005	< 0.005	< 0.033
						SD	n/a	n/a	n/a
						%RSD	n/a	n/a	n/a

Where:
Response Factor = 0.05/mean bracketing standard area
Concentration (mg/L) = Response Factor x area
Percentage in Formulation = Concentration x (100/1) x (0.05/weight) x (Standard Purity/100)/1000
g/Kg in Formulation = Percentage in Formulation x 10
g/Kg Relative to Mesotrione Content = g/Kg in Formulation / Declared Mesotrione Content x 1000
Declared Mesotrione Content = 150g/Kg
Standard Purity = 88.1%
SD = standard deviation
%RSD = percentage relative standard deviation = (SD/mean) x 100

Recovery:

From the Sample Precision it is known that the sample DNA3231/1 contains no detectable Impurity 1 above the LOQ Level of 0.005g/Kg. Therefore a Recovery Precision was performed at 0.05g/Kg. This equates to 0.05mg/L as the samples were made at 1.0mg/ml concentration.

Therefore, the Recovery Precision samples were prepared for analysis by spiking samples of DNA3231/1 at 0.05mg/L using the certified reference standard material. This was achieved by weighing approximately 0.05g of DNA3231/1 into a 50ml volumetric flask, spiked with 2.5ml of 1.00mg/L Impurity 1 reference standard solution and made to volume with Acetonitrile. Six separate solutions were prepared in this way and then injected into the LC-QQQ. The results are shown in Table 5 and indicate a percentage recovery range of 94.99% to 98.96%, with a mean of 97.23%, a standard deviation of 1.358 and a percentage relative standard deviation of 1.397.

Specificity:

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the Specificity chromatograms Impurity 1 eluted at 3.9 minutes and other significant peaks were accounted for by assaying a solvent blank, the Formulation Blank (DNA3232/2) and reference standards for Mesotrione, Terbuthylazine, Isoxaflutole, Impurity 2 and the Impurity R287431. There were no significant peaks present in these chromatograms at the same elution time as Impurity 1.

There is a small amount of Impurity 1 present in the reference standard for Mesotrione. This has no impact upon the analysis of Impurity 1 within the sample (DNA3232/1).

IMPURITY 2 – 1,2-dichloroethane

Validation Summary:

Validation Parameter	Results Obtained	Acceptance Criteria under SANCO/3030/99 rev.4
Linearity	$R^2 = 0.9977$	$R^2 = >0.99$
Sample Precision	No detectable Impurity 2 above the LOQ Level of 0.005g/Kg, equating to 0.033g/Kg in the active substance as manufactured	n/a
Recovery Precision	%RSD = 1.092	%RSD less than 5.16 at 0.129g/Kg (0.0129%)
Recovery at 0.15g/Kg (0.015%)	Mean Recovery = 86.18%	Between 75%-125%
LOQ Recovery at 0.005g/Kg (0.0005%)	Mean Recovery = 94.55%	Between 75%-125%
Selectivity (Spectral Analysis)	The MS Spectra for Impurity 2 confirms the species identification	To confirm the species identity in the associated spectra traces
Specificity	Impurity 2 eluted at 6.9 minutes, and there were no other peaks present at the same elution time as Impurity 2	To show no interference

Analytical methods:

The Sample Precision of Impurity 2 (1,2-dichloroethane) was performed using approximately 0.10g of sample (DNA3232/1). The mass of the sample was accurately recorded, transferred to a 20ml Headspace Vial to which 5ml of Dimethylsulfoxide (DMSO) was added. Six separate solutions were prepared in this way and then used for assay by injecting each solution once into the GC-MSD with Headspace Sampler under the following conditions:

GC-MSD conditions:

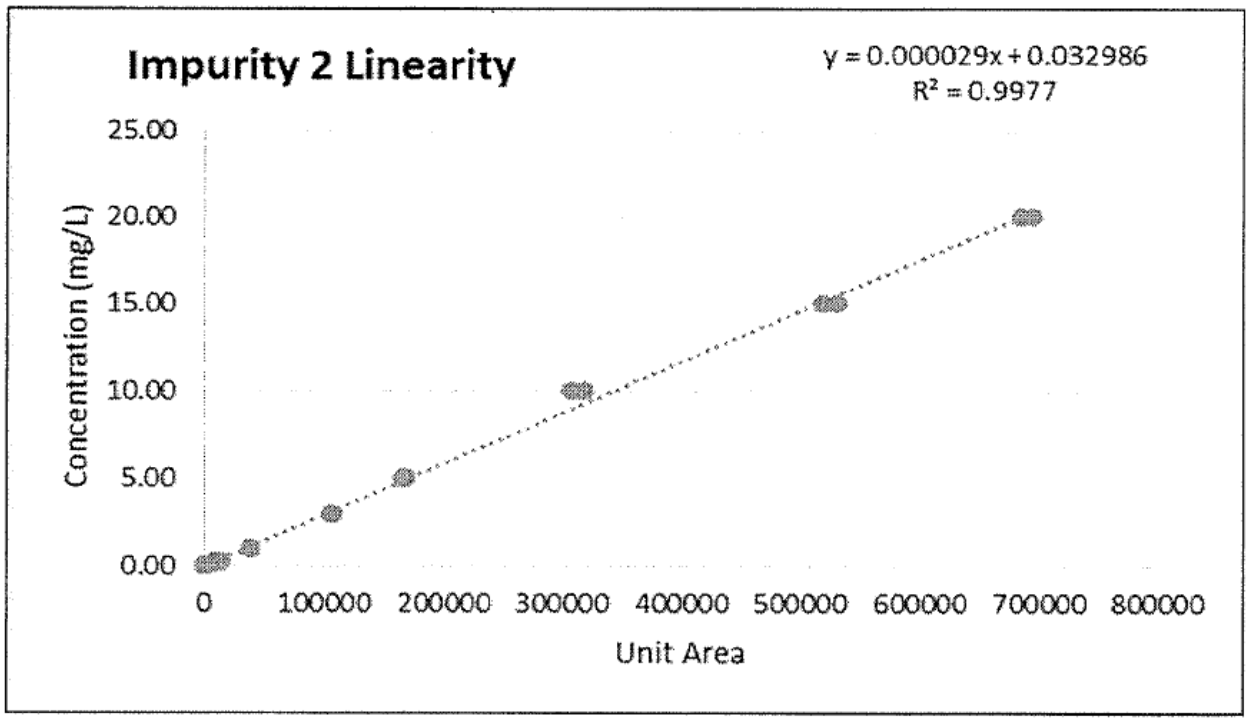
Instrument:	Shimadzu GC-MSD with HS-20 Headspace Sampler
Column:	Rtx-1, (30m x 0.32mm x 5.0µm)
Temperatures:	
Column:	30°C for 2 minutes, then 10°C/min to 220°C, held for 4 minutes
Injector:	26.5°C
Carrier gas:	Helium
Detector:	SIM: 49m/z, 62m/z and 64m/z SCAN: 30-250m/z (for MS Spectra)
Data Collection:	GCMS Solutions
Retention Time:	Approximately 6.9 minutes

Headspace Conditions:	
Cycle Time:	25.0 minutes
Shake Strength:	4/5

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

Linearity:

The linearity was determined from eighteen injections of nine concentrations of standard ranging from a blank to 20.0mg/L. The samples were prepared for analysis at a sample concentration of 20mg/ml. From the Sample Precision it is known that the sample (DNA3232/1) contains no detectable Impurity 2 above the LOQ Level of 0.005g/Kg. Recovery Precision was therefore performed at 0.15g/Kg which equates to a concentration of 3.00mg/L and falls within the limits of the linearity range. Table 7 shows the individual peak area response for each duplicate standard at the specified concentration. The mean area of each duplicate injection for each standard concentration has been plotted on a graph displayed overleaf. The plot possesses a correlation coefficient of 0.9977, based on individual values.



Sample Precision:

To show the sample precision six samples of approximately 0.10g of sample DNA3232/1 were prepared in 5ml of Dimethylsulfoxide (DMSO) and injected into the GC-MSD with Headspace Sampler. The results obtained are shown in Table 8 Impurity 2 was not detected above the LOQ Level of 0.005g/Kg, equating to 0.033g/Kg in the active substance as manufactured.

EFSA Specification = Maximum 1g/kg in the active substance as manufactured

Table 8: Sample Precision of Impurity 2

Description	Raw Data	Retention Time (minutes)	Unit Area	Response Factor	Concentration (mg/L)	Weight (g)	Percentage (w/w) in Formulation	g/Kg in Formulation	g/Kg Relative to Mesotrione Content
3.0mg/L Impurity 2	D0510S18	6.93	105971	0.0000286					
Sample Precision A	D0510P1	6.60	3376		< 0.1	0.1015	< 0.0005	< 0.005	< 0.033
Sample Precision B	D0510P2	6.93	3125		< 0.1	0.1052	< 0.0005	< 0.005	< 0.033
Sample Precision C	D0510P3	6.93	3002		< 0.1	0.0989	< 0.0005	< 0.005	< 0.033
3.0mg/L Impurity 2	D0510S19	6.93	103830	0.0000285					
Sample Precision D	D0510P4	6.93	2899		< 0.1	0.0990	< 0.0005	< 0.005	< 0.033
Sample Precision E	D0510P5	6.93	2518		< 0.1	0.1003	< 0.0005	< 0.005	< 0.033
Sample Precision F	D0510P6	6.93	2446		< 0.1	0.1027	< 0.0005	< 0.005	< 0.033
3.0mg/L Impurity 2	D0510S20	6.92	106451						
						Mean	< 0.0005	< 0.005	< 0.033
						SD	n/a	n/a	n/a
						%RSD	n/a	n/a	n/a

Where:
Response Factor = 3.0/mean bracketing standard area
Concentration (mg/L) = Response Factor x area
Percentage in Formulation = Concentration x (100/20) x (0.10/weight) x (Standard Purity/100)/1000
g/Kg in Formulation = Percentage in Formulation x 10
g/Kg Relative to Mesotrione Content = g/Kg in Formulation / Declared Mesotrione Content x 1000
Declared Mesotrione Content = 150g/Kg
Standard Purity = 99.97%
SD = standard deviation
%RSD = percentage relative standard deviation = (SD/mean) x 100

Recovery precision:

From the Sample Precision it is known that the sample DNA3231/1 contains no detectable Impurity 2 above the LOQ Level of 0.005g/Kg. Therefore a Recovery Precision was performed at 0.15g/Kg. This equates to 3.00mg/L as the samples were made at 20mg/ml concentration.

Impurity 2 is present in the sample (DNA3232/1) below the LOQ level, therefore the Recovery Precision was performed by spiking Impurity 2 onto the Formulation Blank (DNA3232/2).

Therefore, the Recovery Precision samples were prepared for analysis at 3.00mg/L using the certified reference standard material. This was achieved by weighing approximately 0.10g of Formulation Blank DNA3232/2 into a 20ml Headspace vial, spiked with 150µl of 100mg/L Impurity 2 reference standard solution and made to 5ml volume with Dimethylsulfoxide (DMSO). Six separate solutions were prepared in this way and then injected into the GC-MSD with Headspace Sampler. The results are shown in Table 9 and indicate a percentage recovery range of 85.16% to 87.80% with a mean of 86.18%, a standard deviation of 0.941 and a percentage relative standard deviation of 1.092.

Specificity:

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte. In the Specificity chromatograms Impurity 2 eluted at 6.9 minutes and other significant peaks were accounted for by assaying a solvent blank, the Formulation Blank (DNA3232/2) and reference standards for Mesotrione, Terbuthylazine, Isoxaflutole, Impurity 1 and the Impurity R287431. There were no significant peaks present in these chromatograms at the same elution time as Impurity 2. This demonstrates that there were no analyte interferences.

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

Please refer to PART C – Confidential data.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Analytical methods for determination of Terbuthylazine, mesotrione, isoxaflutole impurities and relevance of CIPAC methods in CHR/H/TERIZ were not evaluated as part of the EU review of any of three active substances. Therefore, all relevant data are provided and are considered adequate.

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 terbuthylazine, mesotrione, isoxaflutole 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 5.2-2a: Validated methods for the generation of pre-authorization data

Component of residue definition: Terbuthylazine				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food/feed of plant origin (Residues)	Primary	0.02 mg/kg	GC-NPD	Diertelre, 1993 II A 4.2.1, IIIA 5.2 DAR Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
	Confirmatory (if required)	0.02 mg/kg	HPLC MS/MS	Ferguson, 2009 II A 4.2.1, IIIA 5.2 DAR Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
Animal products, food of animal origin (Residues)	Primary	According to the EFSA Journal 2011; 9(1):1969, no methods required as MRLs for animal tissues have not been set.		
	Confirmatory (if required)			
Soil (Environmental fate)	Primary	0.02 mg/kg	GC-MS	Lutolf W.,, 1995 II A 4.2.2 to 4.2.4, IIIA 5.2 DAR Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
	Confirmatory (if required)	0.01 mg/kg	HPLC-MS/MS	Figueiredo J, 2003 II A 4.2.2 to 4.2.4, IIIA 5.2 DAR Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
Water (surface, ground and	Primary	0.1 mg/kg	RP HPLC-MS/MS	Robinson.,2004 II A 4.2.2 to 4.2.4, IIIA 5.2 DAR

Component of residue definition: Terbuthylazine				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
drinking water) (Environmental fate)				Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
	Confirmatory (if required)	Not required		
Air (Environmental fate)	Primary	1µg/m^3	GC-NPD	Tribolet.,1992 II A 4.2.2 to 4.2.4, IIIA 5.2 DAR Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
	Confirmatory (if required)	1µg/m^3	GC-MS	Tribolet.,1992 II A 4.2.2 to 4.2.4, IIIA 5.2 DAR Terbuthylazine – Additional report, B.5: Methods of analysis January 2010. EU agreed EFSA Journal 2011; 9(1):1969
Soil, water,... (Efficacy)	Primary	Not required		
	Confirmatory (if required)			
Feed, body fluids,... (Toxicology)	Primary	No data submitted or required as terbuthylazine is not classified as toxic or very toxic		
	Confirmatory (if required)			
Body fluids, air, (Exposure)	Primary	No data submitted or required as terbuthylazine is not classified as toxic or very toxic		
	Confirmatory (if required)			
Soil, water. (Ecotoxicology)	Primary	All data was evaluted during Annex I inclusion , and new studies are necessery. All methods are described separatly in DAR Vol3 B9 Ecotoxicology 2007. Please refer to the DAR Vol 3 B9 2007. No general analytical methods were developed for risk assessment apart those reported as specific in studies in support of ecotoxicological studies.		
	Confirmatory (if required)			

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

Component of residue definition: Isoxaflutole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food/feed of plant origin	Primary	0.01 mg/kg	HPLC-MS/MS	Schoening, R.; Wolters, A.;2006II KCA 4.1.2- ISoxaflutole RAR

Component of residue definition: Isoxaflutole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(Residues)				Vol 3 CA-B5 Modification M001 of analytical method 00985 for the determination of residues of isoxaflutole and its metabolites AEB197278-AE0540092 (RPA202248) and AE0317309-AEB197555 (RPA203328) in/on corn plant material by HPLC-MS/MS EFSA Journal 2016;14(3):4416 to which is equivalent Jörg Semrau, 2018, Study code: S17-04903 Study code: S17-04904 Study code: S17-04905 Study code: S17-04906 Study code: S17-04983
	Confirmatory (if required)	Not required		
Animal products, food of animal origin (Residues)	Primary	0.01 mg/kg	LC – MS/MS	Winter O., Amann S.; 2013 KCA 4.2 Isoxaflutole RAR Vol 3 CA-B5 Validation of the BCS-method-01300/M008 (based on QuEChERS) for the determination of residues of Isoxaflutoe and its Metabolite RPA 202248 in animal tissue Not required, no residue definition is available. However, the study equivalent to Winter O., Amann S., 2013 is available (Knop M., 2019, S19-04083)
	Confirmatory (if required)	Not equired		
Soil (Environmental fate)	Primary	LOQ = 0.0002 mg/kg (isoxaflutole) LOQ = 0.0002 mg/kg (RPA 202248)	LC MS/MS	Netzband, 2008 KCA 4.3/32 Isoxaflutole RAR Vol 3 CA-B5 Analytical method for the determination of residues of isoxaflutole (IFT) and its metabolite RPA 202248 (DKN) in soil using LC/MS/MS EU approved EFSA Journal 2016;14(3):4416 (the study was not conducted in compliance with GLP and

Component of residue definition: Isoxaflutole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				therefore the data protection was not claimed)
	Confirmatory (if required)	Not required		
Water (surface, ground and drinking water) (Environmental fate)	Primary	LOQ = 0.00005 mg/L (isoxaflutole) LOQ = 0.00005 mg/L (RPA 202248)	LC MS/MS	Krebber, R.; Leppelt, L.; 2012 KCA 4.2 Isoxaflutole RAR Vol 3 CA-B5 Analytical method 01333 for the determination of isoxaflutole and its metabolite AE 0540092 in drinking and surface water by HPLC-MS/MS EU approved EFSA Journal 2016;14(3):4416 (the study was not conducted in compliance with GLP and therefore the data protection was not claimed)
	Confirmatory (if required)	Not required		
Air (Environmental fate)	Primary	0.002 µg/m^3	LC-UV	Corgier, M. M.; Turier, G. P. 1995 KCA 4.2 Isoxaflutole RAR Vol 3 CA-B5 Analytical method for the determination of isoxaflutole (RPA201772) in air EU approved EFSA Journal 2016;14(3):4416
	Confirmatory (if required)	Not required		
Soil, water (Efficacy)	Primary	Not required		
	Confirmatory (if required)			
Feed, body fluids, (Toxicology)	Primary	No general analytical methods were developed for risk assessment apart those reported as specific in studies on route and rate. For details please refer to section CA-B.6 Isoxaflutole RAR (november) 2015		
	Confirmatory (if required)			
Body fluids, air, (Exposure)	Primary	Not available		
	Confirmatory (if required)			
Soil, water. (Ecotoxicology)	Primary	Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies. No general analytical methods were developed for risk assessment apart those reported as specific in studies on route and rate. For details please refer to section CA-B9 Isoxaflutole RAR (november) 2015.		
	Confirmatory (if required)			

Table 5.2-4c: Validated methods for the generation of pre-authorization data

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food/feed of plant origin (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Crook S., 2002 KCA 5.1.2- Mesotrione RAR Vol 3 CA-B5 Mesotrione: Residue Analytical Method for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Samples EFSA Journal 2016;14(3):4419
	Confirmatory (if required)	Not required		
Animal products, food of animal origin (Residues)	Primary	0.01 mg/kg	LC – MS/MS	Watson G., 2013b KCA 5.2.2- Mesotrione RAR Vol 3 CA-B5 Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS EFSA Journal 2016;14(3):4419
	Confirmatory (if required)	Not equired		
Soil (Environmental fate)	Primary	Mesotrione: LOQ 0.002 mg/kg MNBA: LOQ 0.002 mg/kg AMBA: LOQ 0.002 mg/kg	LC MS/MS	Jutsum L, Williams R, 2013 KCA 5.2. Mesotrione RAR Vol 3 CA-B5 Mesotrione - Analytical Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil EU approved EFSA Journal 2016;14(3):4419
	Confirmatory (if required)	Not required		
Water (surface, ground and drinking water) (Environmental fate)	Primary	Mesotrione: LOQ 0.05 µg/L MNBA: LOQ 0.05 µg/L AMBA: LOQ 0.05 µg/L	LC-MS/MS (surface and ground water, ILV available for drinking water)	Jutsum L., Chamkesam N, 2013 KCA 5.2. Mesotrione RAR Vol 3 CA-B5 Mesotrione – Analytical Method GRM007.09A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Water EU approved EFSA Journal 2016;14(3):4419
	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**
Air (Environmental fate)	Primary	0.45 µg/m^3	LC-MS/MS	Jutsum L., 2013b KCA 5.2. Mesotrione RAR Vol 3 CA-B5 Mesotrione - Residue Method GRM007.08B for the Determination of Mesotrione in Air EFSA Journal 2016;14(3):4419
	Confirmatory (if required)	Not required		
Soil, water (Efficacy)	Primary	Not required		
	Confirmatory (if required)			
Feed, body fluids, (Toxicology)	Primary	LOQ 0.01 mg/kg in blood	LC-MS/MS	Watson G., 2013b KCA 5.2. Mesotrione RAR Vol 3 CA-B5 Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS EFSA Journal 2016;14(3):4419
	Confirmatory (if required)	Not required		
Body fluids, air, (Exposure)	Primary	LOQ 0.01 mg/kg in blood	LC-MS/MS	Watson G., 2013b KCA 5.2. Mesotrione RAR Vol 3 CA-B5 Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS EFSA Journal 2016;14(3):4419
	Confirmatory (if required)			
Soil, water. (Ecotoxicology)	Primary	Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies. No general analytical methods were developed for risk assessment apart those reported as specific in studies on route and rate. For details please refer to section CA-B9 mesotrione RAR 2015.		
	Confirmatory (if required)			

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

Data provided on Annex I inclusion is sufficient for post-authorizations methods. No new methods are necessary since all data is described and presented in Table 5.2-3 in point KCP 5.1.2.

However since there are presented new residues studies for CHR/H/TERIZ 650 WG in maize, new method for residues is presented below.

zRMS comment: Method is accepted

Reference:	KCP 5.3/01-05
Report	Determination of residues of terbuthylazine, mesotrione and isoxaflutole after one application of TERIZ 650 WG in maize at 1 site in Northern Europe 2017. Jörg Semrau, Dr. Sönke Lakaschus, Sabrina Fritzsch, 2018, Study code: S17-04983, S17-04903, S17-04904, S17-04905, S17-04906
Guideline(s):	EU Guidance Document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data requirements Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice and Compliance Monitoring (as revised in 1997) ENV/MC/CHEM(98)17 SANCO/3029/99 rev.4.
Deviations:	NO
GLP:	Yes

The analytical method QuEChERS **Błąd! Nie można odnaleźć źródła odwołania.** was validated for the determination of terbuthylazine (MT0) and its metabolites desethyl-terbuthylazine (MT1) and desethyl-hydroxy-terbuthylazine (MT14), of mesotrione, as well as isoxaflutole (RPA 201772) and its metabolites RPA 202248 and RPA 203328 in maize (whole plant and grain) according to SANCO/3029/99, rev. 4 within this analytical phase by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the recoveries. Five (5) fortifications of untreated control samples at the level of LOQ (0.01 mg/kg) and five (5) fortifications at the level of tenfold LOQ (0.1 mg/kg) were performed.

The validation data was generated in sole analytical set(s), *i. e.* separately from the analytical sets for residue sample analysis.

No residues above 30 % of the LOQ were detected in the control (untreated) test portions used for recovery determinations.

All mean recovery values at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ) comply with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4. with evaluation of two (2) mass transitions.

The coefficients of determination (R^2) of linear regression of the calibration plots were ≥ 0.98 and thus demonstrated linearity of the detection system over the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a sample.

Matrix effects on LC-MS/MS detection were investigated: Matrix effects were < ± 20 % and deemed to be insignificant for mesotrione (maize (whole plant and grain)), terbuthylazine (MT0) (maize (whole

plant), desethyl-terbuthylazine (MT1) (maize (whole plant)), RPA 202248 (maize (whole plant and grain)) and RPA 203328 (maize (whole plant and grain)).

Matrix effects were ≥ ± 20 % and deemed to be significant for terbuthylazine (MT0) (maize (grain), desethyl-terbuthylazine (MT1) (maize (grain), terbuthylazine (MT14) (maize (whole plant and grain)) and isoxaflutole (RPA 201772) (maize (whole plant and grain)).

Extract Stability

Mesotrione, terbuthylazine (MT0) and desethyl-terbuthylazine (MT1) were found to be stable in final extracts of maize (grain) for 10 days when stored at 1 °C to 10 °C in the dark.

Mesotrione, terbuthylazine (MT0), desethyl-terbuthylazine (MT1) and desethyl-hydroxy-terbuthylazine (MT14) were found to be stable in final extracts of maize (whole plant) for 12 days when stored at 1 °C to 10 °C in the dark.

Desethyl-hydroxy-terbuthylazine (MT14) in maize (grain) and isoxaflutole (RPA 201772), RPA 202248 and RPA 203328 were found to be stable in final extracts of maize (whole plant and grain) for 13 days when stored at 1 °C to 10 °C in the dark.

Desethyl-hydroxy-terbuthylazine (MT14) was found to be stable in final extracts of maize maize (grain) for 13 days when stored at 1 °C to 10 °C in the dark.

Method summary

Test method:	Mesotrione, terbuthylazine (MT0), desethyl-terbuthylazine (MT1)
Method Reference(s)	The method was developed based on Multi-residue method QuEChERS **Błąd! Nie można odnaleźć źródła odwołania.** and modified where necessary.
Extraction	Addition of water and extraction with acetonitrile
Liquid/Liquid Partition	Addition of magnesium sulphate, sodium chloride followed by centrifugation
Extract clean-up	Further dilution with acetonitrile/water (20/80, v/v)
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS) LC-MS/MS method 1 for mesotrione, terbuthylazine (MT0), desethyl-terbuthylazine (MT1)
Limit(s) of Quantification (LOQ)	0.01 mg/kg

Test method:	Desethyl-hydroxy-terbuthylazine (MT14), isoxaflutole (RPA 201772), RPA 202248 and RPA 203328)
Method Reference(s)	The method was developed based on Multi-residue method QuEChERS **Błąd! Nie można odnaleźć źródła odwołania.** and modified where necessary.
Extraction	Addition of acidified water and extraction with acetonitrile
Liquid/Liquid Partition	Addition of magnesium sulphate, sodium chloride followed by centrifugation
Extract clean-up	Further dilution with acidified acetonitrile/water (20/80, v/v) at pH 3
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS) LC-MS/MS method 1 for desethyl-hydroxy-terbuthylazine (MT14)

	LC-MS/MS method 2 for isoxaflutole (RPA 201772), RPA 202248 and RPA 203328)
Limit(s) of Quantification (LOQ)	0.01 mg/kg

Method Performance

Selectivity

The analytes /were determined in the final specimen extracts by use of LC-MS/MS detection.

For each analyte, one (1) mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of specimens.

Untreated samples for accompanying control sample work up, for determination of (procedural) recoveries and, if needed, for preparation of matrix-matched standards originated from the current study.

At least one (1) control sample per each matrix type and analytical set was analysed to investigate the residue level of the analytes and to check for any background interferences at the expected retention times of the analytes.

The blank values at the expected retention times of the analytes of the control sample materials that were used for determinations of the (procedural) recoveries did not exceed 30 % of the LOQ.

Correction for blank values was performed for desethyl-hydroxy-terbuthylazine (MT14) even if they were below 30 % of the LOQ. Since blank peaks were not observed for terbuthylazine (MT0), desethyl-terbuthylazine (MT1), mesotrione, isoxaflutole (RPA 201772), and RPA 203328, blank correction was not necessary.

Furthermore, at least one (1) reagent blank sample, which is a sample work up without matrix present, was conducted with each analytical set during validation and field sample analyses. Reagent blank values did not exceed 30 % of the LOQ.

Matrix Effects

The effect of matrix on the LC-MS/MS response was assessed by comparing peak areas of matrix-matched standards of 90 % matrix amount with solvent standards at identical concentrations.

Matrix effects on LC-MS/MS detection were investigated: Matrix effects were < ± 20 % and deemed to be insignificant for mesotrione (maize (whole plant and grain)), terbuthylazine (MT0) (maize (whole plant), desethyl-terbuthylazine (MT1) (maize (whole plant)), RPA 202248 (maize (whole plant and grain)) and RPA 203328 (maize (whole plant and grain)).

Matrix effects were ≥ ± 20 % and deemed to be significant for terbuthylazine (MT0) (maize (grain), desethyl-terbuthylazine (MT1) (maize (grain), terbuthylazine (MT14) (maize (whole plant and grain)) and isoxaflutole (RPA 201772) (maize (whole plant and grain)).

However, matrix-matched standards were used for quantification throughout the analytical phase, in order to compensate any possible matrix effects. Solvent standards were used for the analysis of diluted samples.

Linearity

The linearity of the detector response was demonstrated by single determination of matrix-matched or solvent calibration standards at a minimum of five (5) concentration levels ranging from 0.21 ng/mL to 10 ng/mL. This covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) specimen extract.

The calibration curves obtained for both mass transitions / all analytes and all matrices were linear with coefficients of determination (R^2) ≥ 0.98. Linear regression was performed with 1/x-weighting.

Quantification

Quantification was performed using a calibration curve that fulfilled the above given criteria. The injection of standard solutions was spread evenly over the whole analytical sequence. The linear regression equation was used for calculation of the analyte concentrations.
If necessary, specimen extracts and extracts from high level recovery samples were diluted with solvent to be within the calibration range. Diluted sample extracts (at least by a factor of 10) were quantified using solvent calibration standards instead of matrix-matched calibration standards.

Method Validation

Apart from the determination of procedural recoveries during analysis of specimens, a sole validation set in accordance to SANCO/3029/99 rev. 4. was conducted for maize (whole plant and grain) prior to the analysis of the specimens.

Five (5) recovery determinations at 0.01 mg/kg (LOQ) and five (5) recovery determinations at 0.1 mg/kg (10x LOQ) were performed.

All analytes were fortified jointly and quantified separately.

At least one (1) reagent blank and two (2) control samples were analysed.

Only for the purpose of validation, two (2) mass transitions were evaluated and representative ion chromatograms along with the product ion mass spectrum are in **Błąd! Nie można odnaleźć źródła odwołania.** and **Błąd! Nie można odnaleźć źródła odwołania.**. One of the two mass transitions is proposed as quantification transition but both selected mass transitions proved to be interchangeably applicable for quantification and confirmation.

The following recoveries were obtained:

Matrix	Fortification level	Recovery					Mean Recovery	RSD	n	Overall Mean Recovery	Overall RSD
	(mg/kg)	(%)					(%)	(%)		(%)	(%)
Mesotrione, MRM 338-291 (Quantification)											
whole plant	0.01	80	75	77	78	82	78	3.4	5	78	3.3
	0.1	77	75	77	81	75	77	3.2	5		
Mesotrione, MRM 338-212 (Confirmation)											
whole plant	0.01	80	81	73	83	82	80	5.0	5	79	4.7
	0.1	79	77	76	82	73	77	4.3	5		
Mesotrione, MRM 338-291 (Quantification)											
grain	0.01	74	72	74	74	74	74	1.2	5	73	1.6
	0.1	72	71	72	72	74	72	1.5	5		
Mesotrione, MRM 338-212 (Confirmation)											
grain	0.01	72	71	70	71	73	71	1.6	5	71	1.6
	0.1	70	72	70	71	73	71	1.8	5		

Matrix	Fortification level	Recovery					Mean Recovery	RSD	n	Overall Mean Recovery	Overall RSD
	(mg/kg)	(%)					(%)	(%)		(%)	(%)
Terbuthylazine (MT0), MRM 202-146 (Quantification)											
whole plant	0.01	90	87	91	93	97	92	4.1	5	92	2.9
	0.1	94	92	94	92	92	93	1.2	5		
Terbuthylazine (MT0), MRM 202-79 (Confirmation)											
whole plant	0.01	89	85	87	88	96	89	4.7	5	92	4.8
	0.1	94	94	98	94	95	95	1.8	5		
Terbuthylazine (MT0), MRM 202-146 (Quantification)											
grain	0.01	88	87	86	92	89	88	2.6	5	90	2.5
	0.1	92	89	93	89	90	91	2.0	5		
Terbuthylazine (MT0), MRM 202-79 (Confirmation)											
grain	0.01	83	92	91	88	83	87	4.9	5	88	3.6
	0.1	90	89	90	87	86	88	2.1	5		
Desethyl-terbuthylazine (MT1), MRM 230-174 (Quantification)											
whole plant	0.01	96	99	94	96	98	97	2.0	5	94	3.2
	0.1	93	90	92	93	91	92	1.4	5		
Desethyl-terbuthylazine (MT1), MRM 230-96 (Confirmation)											
whole plant	0.01	95	97	93	94	95	95	1.6	5	95	1.8
	0.1	95	93	97	98	94	95	2.2	5		
Desethyl-terbuthylazine (MT1), MRM 230-174 (Quantification)											
grain	0.01	91	89	93	86	91	90	2.9	5	88	3.7
	0.1	85	85	84	84	87	85	1.4	5		
Desethyl-terbuthylazine (MT1), MRM 230-96 (Confirmation)											
grain	0.01	86	84	88	80	88	85	3.9	5	85	3.1
	0.1	84	85	82	83	87	84	2.3	5		
Desethyl-hydroxy-terbuthylazine (MT14), MRM 184-128 (Quantification)											
whole plant*	0.01	72	69	68	69	70	70	2.2	5	72	4.3
	0.1	72	75	74	75	77	75	2.4	5		
Desethyl-hydroxy-terbuthylazine (MT14), MRM 184-69 (Confirmation)											
whole plant*	0.01	74	69	72	73	72	72	2.6	5	71	2.7
	0.1	69	71	70	68	71	70	1.9	5		
Desethyl-hydroxy-terbuthylazine (MT14), MRM 184-128 (Quantification)											
grain*	0.01	77	77	78	75	84	78	4.4	5	78	5.0
	0.1	77	83	78	77	70	77	6.0	5		
Desethyl-hydroxy-terbuthylazine (MT14), MRM 184-69 (Confirmation)											
grain*	0.01	66	72	70	65	83	71	10	5	74	9.0
	0.1	79	83	78	73	69	76	7.1	5		

Matrix	Fortification level	Recovery					Mean Recovery	RSD	n	Overall Mean Recovery	Overall RSD
	(mg/kg)	(%)					(%)	(%)		(%)	(%)
Isoxaflutole (RPA 201772), MRM 360-251 (Quantification)											
whole plant	0.01	87	84	88	88	91	88	2.9	5	87	2.6
	0.1	87	87	86	84	90	87	2.5	5		
Isoxaflutole (RPA 201772), MRM 358-79 (Confirmation)											
whole plant	0.01	89	86	90	89	84	88	2.9	5	88	2.8
	0.1	91	89	85	85	89	88	3.1	5		
Isoxaflutole (RPA 201772), MRM 360-251 (Quantification)											
grain	0.01	78	88	83	84	85	84	4.4	5	81	9.0
	0.1	64	77	75	86	86	78	12	5		
Isoxaflutole (RPA 201772), MRM 358-79 (Confirmation)											
grain	0.01	91	87	86	82	85	86	3.8	5	87	4.6
	0.1	96	90	86	87	84	89	5.3	5		
RPA 202248, MRM 358-64 (Quantification)											
whole plant	0.01	84	84	88	84	84	85	2.1	5	83	3.1
	0.1	81	82	80	80	86	82	3.0	5		
RPA 202248, MRM 358-79 (Confirmation)											
whole plant	0.01	88	88	90	88	89	89	1.0	5	85	4.5
	0.1	82	81	82	80	83	82	1.4	5		
RPA 202248, MRM 358-64 (Quantification)											
grain	0.01	85	86	84	82	82	84	2.1	5	83	2.9
	0.1	82	79	80	80	85	81	2.9	5		
RPA 202248, MRM 358-79 (Confirmation)											
grain	0.01	90	87	88	85	85	87	2.4	5	83	4.9
	0.1	81	79	80	79	80	80	1.0	5		
RPA 203328, MRM 267-159 (Quantification)											
whole plant	0.01	84	81	85	81	88	84	3.5	5	86	4.0
	0.1	89	89	87	88	91	89	1.7	5		
RPA 203328, MRM 267-223 (Confirmation)											
whole plant	0.01	84	82	87	80	72	81	7.0	5	84	6.2
	0.1	88	87	86	87	90	88	1.7	5		
RPA 203328, MRM 267-159 (Quantification)											
grain	0.01	85	85	86	84	85	85	0.83	5	80	9.0
	0.1	77	67	69	75	83	74	8.7	5		
RPA 203328, MRM 267-223 (Confirmation)											
grain	0.01	83	84	83	84	83	83	0.66	5	79	8.6
	0.1	76	66	69	74	83	74	8.9	5		

*Recoveries are corrected for the mean peak area of the control sample extract(s)
No observable peak was detected in any control sample extract, except for desethyl-hydroxy terbuthylazine (MT14)
Recoveries are without any blank correction

The mean recovery at each fortification level was in the range of 70 - 110 % with a relative standard deviation of ≤ 20 % for both mass transitions of all analytes in all tested matrices and thus comply with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4.

Extract Stability

Following the first analysis, the final extracts fortified at the 10x LOQ level together with one control sample extract were stored at 1 °C to 10 °C for up to 13 days in the dark. After this period, the final extracts were re-analysed against freshly prepared calibration standards. One mass transition per analyte was evaluated. The results obtained are summarised in the table below. Detailed data are presented in **Błąd! Nie można odnaleźć źródła odwołania.**.

Matrix	Fortification level	Mean Recovery 1st Injection	Rel. Std. Dev. 1st Injection	Mean Recovery 2nd Injection	Rel. Std. Dev. 2nd Injection	Days of storage (1st to 2nd injection)	Difference
	(mg/kg)	(%)	(%)	(%)	(%)		(%)
Mesotrione, MRM 338-291 (quantification)							
whole plant	0.10	77	3.2	77	2.8	12	0.26
grain	0.10	72	1.5	72	2.7	10	0.28
Terbuthylazine (MT0), MRM 202-146 (quantification)							
whole plant	0.10	93	1.2	104	2.6	12	12
grain	0.10	91	2.0	105	3.4	10	15
Desethyl-terbuthylazine (MT1), MRM 230-174 (quantification)							
whole plant	0.10	92	1.4	92	4.9	12	-0.22
grain	0.10	85	1.4	85	5.1	10	0.00
Desethyl-hydroxy-terbuthylazine (MT14), MRM 184-128 (quantification)							
whole plant	0.10	75	2.4	81	7.5	12	9.1
grain	0.10	77	6.0	71	6.9	13	-7.5
Isoxaflutole (RPA 201772), MRM 360-251 (quantification)							
whole plant	0.10	87	2.5	71	4.0	13	-18
grain	0.10	78	12	99	2.1	13	27[1]
RPA 202248, MRM 358-64 (quantification)							
whole plant	0.10	82	3.0	88	1.5	13	7.1
grain	0.10	81	2.9	89	1.2	13	9.9
RPA 203328, MRM 267-159 (quantification)							
whole plant	0.10	89	1.7	94	2.1	13	5.4
grain	0.10	74	8.7	94	3.5	13	26[1]

The mean recovery value(s) of the re-analysed extracts were in the range of 70 – 120 % and within ± 20 % of the original result. Therefore, extracts are considered to be stable when stored at 1 °C to 10 °C for 10 days (mesotrione, terbuthylazine (MT0) and desethyl-terbuthylazine (MT1) in maize (grain)), 12 days (mesotrione, terbuthylazine (MT0), desethyl-terbuthylazine (MT1) and desethyl-hydroxy-terbuthylazine (MT14) in maize (whole plant)) or 13 days (desethyl-hydroxy-terbuthylazine (MT14) in

[1] Since the mean recovery of the stability test is higher than that of the first analysis, the extract can be considered stable and the difference of > 20 % can be neglected.

maize (grain) and isoxaflutole (RPA 201772), RPA 202248 and RPA 203328 in maize (whole plant and grain) in the dark.

Conclusion
The method was successfully validated for determination of terbuthylazine (MT0) and its metabolites desethyl-terbuthylazine (MT1) and desethyl-hydroxy-terbuthylazine (MT14), of mesotrione, as well as isoxaflutole (RPA 201772) and its metabolites RPA 202248 and RPA 203328 in maize (whole plant and grain) with an LOQ of 0.01 mg/kg and up to 0.1 mg/kg according to the guidance document(s) SANCO/3029/99 rev. 4.
With regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the specimens of the study.

5.3.1 Analysis of the plant protection product (KCP 5.2)

For all three active substances terbuthylazine, isoxaflutole and mesotrione all presented methods are sufficient and no new methods are necessary. Please refer to KCP 5.2

5.3.2 Description of analytical methods for the determination of residues of Terbuthylazine (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 protein/high starch content (dry commodities) –Maize	Terbuthylazine	LOQ 0.02 mg/kg	EFSA Journal 2011; 9(1):1969
Muscle	Not necessary for the representative uses.	EFSA Journal 2011; 9(1):1969	EFSA Journal 2011; 9(1):1969
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxyl-terbuthylazine (MT13)	LOQ 0.01 mg/kg	EFSA Journal 2011; 9(1):1969
Drinking water (Human toxicology)	Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxy-terbuthylazine (MT13) plus desethyl-hydroxy-terbuthylazine (MT14) plus LM1, LM2, LM3, LM4,	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
	LM5 and LM6		
Surface water (Ecotoxicology)	Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxyl-terbuthylazine (MT13)	12 µg a.s/L	EFSA Journal 2011; 9(1):1969
Air	Terbuthylazine	1 µg/m^3	AOEL sys/AOEL inhal: 0.0032 mg/kg bw/d
Tissue (meat or liver)	Terbuthylazine	Not required	notclassified as T / T+
Body fluids		Not required	notclassified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of Terbuthylazine in plant matrices is given in the following tables. For the detailed evaluation of 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: Terbuthylazine (MT0)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry) Maize	Primary	0.02 mg/kg	GC-NPD	Syngenta : Diertelrle, 1993. EU agreed Oxon: Freschi 2002c, EU agreed
	ILV	0.02mg/kg	LC MS/MS	Syngenta: Ferguson 2009 EU agreed OXON: not available
	Confirmatory (if required)		Not required	

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	DAR 2010 (additional report) Vol3 B5
Not required, because:	No new studies neccesary since all studies described in DAR are sufficient.

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

According to the EFSA Journal 2011; 9(1):1969 for all representative uses in maize, there is no requirement for presenting methods for methods for food and feed of animal origin. There is no residue definition for monitoring purposes.

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 Terbuthylazine in soil is given in the following tables. No new methods are necessary.

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

Component of residue definition: Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxyl-terbuthylazine (MT13)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ=0.01 mg/kg	LC MS/MS	REM 148.11.: Figueriredo J, 2003; Trobolet R., 2003 EU Approved
Confirmatory	Not required		

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

An overview on the acceptable methods and possible data gaps for analysis of terbuthylazine in surface and drinking water is given in the following tables. No new method is necessary.

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

Component of residue definition: Terbuthylazine (MT0) plus desethyl-terbuthylazine. (MT1) plus hydroxy-terbuthylazine (MT13) plus desethyl-hydroxy-terbuthylazine (MT14) plus LM1, LM2, LM3, LM4, LM5 and LM6				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	LOQ= 0.1 µg/L for parent, MT1, MT13, MT14 LOQ=0.05 µg/L for LM5, LM6, LM3	Reverse phase HPLC-MS/MS	DAR(additional report) 2010, IIA 4.2.2 to 4.2.4, IIIA 5.2 Syngenta: RAM 426/01 Robinson N.J, 2004 Glanzel, A. 2005 Syngenta HPLC MS/MS method Zietz, E. 2009 OXON:Todd,N.,2002
	ILV		Not available	
	Confirmatory		Not required	

Component of residue definition: Terbuthylazine (MT0) plus desethyl-terbuthylazine. (MT1) plus hydroxy-terbuthylazine (MT13) plus desethyl-hydroxy-terbuthylazine (MT14) plus LM1, LM2, LM3, LM4, LM5 and LM6				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface water	Primary	LOQ= 0.1 µg/L for parent, MT1, MT13, MT14 LOQ=0.05 µg/L for LM5, LM6, LM3		Syngenta: REM 148.05:Lutolf W., 1995a REM 148.11.:Figueriredo J, 2003; Trobolet R., 2003 Oxon: Todd M, 2002a Gillis N.A, 1997 Todd M., 1999
	Confirmatory		Not required	

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

An overview on the acceptable methods and possible data gaps for analysis of terbuthylazine in air is given in the following tables. No new method necessary.

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

Component of residue definition: terbuthylazine			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1 µg/m^3	GC-NPD	Syngenta: Tribolet R, 1992 Oxon: Schulz M, and Ullrich-Mitzel A., 1995
Confirmatory		Not required	

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

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

An overview on the acceptable methods and possible data gaps for analysis of terbuthylazine in body fluids and tissues is given in the following table. No new methods are necessary.

No methods are necessary, since no MRLs for animal tissues have not been set. No data submitted or required as terbuthylazine is not classified as toxic or very toxic.

5.3.2.8 Other studies/ information

No other studies are provided.

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

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

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

Table 5.3.7: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high protein/high starch content (dry commodities) –Maize	RPA 203328	LOQ 0.01 mg/kg	EFSA Journal 2016;14(3):4416
Muscle	Not necessary for the representative uses.	-	EFSA Journal 2016;14(3):4416
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole	LOQ = 0.0002 mg/kg (isoxaflutole) LOQ = 0.0002 mg/kg (RPA 202248)	EFSA Journal 2016;14(3):4416
Drinking water (Human toxicology)	Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole	Limit: 0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole	1 µg a.s/L from most sensetive species Americamysis bahia	EFSA Journal 2011; 9(1):1969
Air	Isoxaflutole	LOQ = 0.002 mg/m^3	AOEL sys/AOEL inhal: 0.012 mg/kg bw/d
Tissue (meat or liver)	Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole	Not required	notclassified as T / T+
Body fluids		Not required	notclassified as T / T+

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

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

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

Component of residue definition: RPA 203328				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry) Maize	Primary	0.01 mg/kg	HPLC-MS/MS	Winter O., Amann S.;2013 to which is equivalent Knop M., 2019, S19-04082
	ILV	0.01mg/kg	LC MS/MS	Mewis A.; 2013 to which is equivalent Imart C., 2019, S19-04084
	Confirmatory (if required)		Not required	

Table 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	RAR Volume 3 – Annex CA - B.5 : 01300/M008 extraction efficiency EN 15662 (multi-residue QuEChERS) HPLC-MS/MS. Additionally, according to RAR the extraction efficiencies has been checked during the old metabolism study ;Veerasekaran, P.; Crudace, A.;1993;M-274733-01 and Veerasekaran, P.; Crudace, A.;1993;M-274674-01:
Not required, because:	No new studies neccesary since all studies described in DAR are sufficient.

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

According to the EFSA Journal 2016;14(3):4416 for all representative uses in maize, there is no requirement for presenting methods for methods for food and feed of animal origin. There is no residue definition for monitoring purposes.

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 isoxaflutole in soil is given in the following tables. No new methods are necessary.

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

Component of residue definition: Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ = 0.0002 mg/kg (isoxaflutole) LOQ = 0.0002 mg/kg (RPA 202248)	LC MS/MS	Netzband,2008 (the study was not conducted in compliance with GLP and therefore the data protection was not claimed)
Confirmatory	Not required		

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

An overview on the acceptable methods and possible data gaps for analysis of isoxaflutole in surface and drinking water is given in the following tables. No new studies are necessary.

Table 5.3-11: Validated methods for water (if appropriate)

Component of residue definition: Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	LOQ = 0.00005 mg/L (isoxaflutole) LOQ = 0.00005 mg/L (RPA 202248)	LC-MS/MS	Krebber,2012 (the study was not conducted in compliance with GLP and therefore the data protection was not claimed)
	ILV	LOQ = 0.00005 mg/L (isoxaflutole) LOQ = 0.00005 mg/L (RPA 202248)	LC-MS/MS	Stanislowski, 2013 (the study was not conducted in compliance with GLP and therefore the data protection was not claimed)
	Confirmatory		Not required	
Surface water	Primary	LOQ = 0.00005 mg/L (isoxaflutole) LOQ = 0.00005 mg/L (RPA 202248)	LC-MS/MS	Krebber,2012 (the study was not conducted in compliance with GLP and therefore the data protection was not claimed)
	Confirmatory		Not required	

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

An overview on the acceptable methods and possible data gaps for analysis of isoxaflutole in air is given in the following tables. No new methods are necessary.

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

Component of residue definition: isoxaflutole			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.002 mg/m^3	LC-UV	Corgier, 1995
Confirmatory		Not required	

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

No methods were supplied for the determination of isoxaflutole or metabolites in these matrices. A case has been made for not providing these as the active substance is not classified as toxic or very toxic which is acceptable.

5.3.4 Description of analytical methods for the determination of residues of mesotrione (KCP 5.2)

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

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

Table 5.3-13: 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 protein/high starch content (dry commodities) –Maize	Mesotrione	LOQ= 0.01 mg/kg	EFSA Journal 2016;14(3):4419
Muscle	Not required (provisional)	LOQ= 0.01 mg/kg	EFSA Journal 2016;14(3):4419
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Mesotrione and metabolite A (open)	Mesotrione: LOQ 0.002 mg/kg MNBA: LOQ 0.002 mg/kg AMBA: LOQ 0.002 mg/kg	EFSA Journal 2016;14(3):4419
Drinking water (Human toxicology)	Mesotrione and metabolite A (open)	Limit: 0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Mesotrione and metabolite A (open)	7.7 µg a.s/L from most sensetive species Lemna gibba	EFSA Journal 2016;14(3):4419

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Air	Mesotrione	LOQ = 0.45 µg/m^3	AOEL sys/AOEL inhal: 0.005 mg/kg bw/d
Tissue (meat or liver)	Mesotrione	Not required	notclassified as T / T+
Body fluids		Not required	notclassified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in plant matrices is given in the following tables. For the detailed evaluation of 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: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry) Maize	Primary	0.01 mg/kg	LC-MS/MS	Watson G, 2013a
	ILV	0.01mg/kg	LC MS/MS	Tessier V 2013
	Confirmatory (if required)		Not required	

Table 5.3-15: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Data to address the extraction efficiency of the QuEChERS multi-residue method in accordance with the requirements of SANCO 825/00 rev. 8.1 have not been provided specifically for the use of the method for determination of mesotrione in the aforementionedcrops. The extraction system employed is based predominantly on acetonitrile/water (50:50 v/v).
Not required, because:	

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

According to the EFSA Journal 2016;14(3):4419 for all representative uses in maize, there is no requirement for presenting methods for methods for food and feed of animal origin. There is no residue definition for monitoring purposes.

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

Component of residue definition: Not required (provisional)				
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	LC MS/MS	Watson G., 2013b
	ILV	0.01 mg/kg		Bernal J., 2013
	Confirmatory (if required)		Not required	
Eggs	Primary	0.01 mg/kg	LC MS/MS	Watson G., 2013b
	ILV	0.01 mg/kg		Bernal J., 2013
	Confirmatory (if required)		Not required	
Muscle	Primary	0.01 mg/kg	LC MS/MS	Watson G., 2013b
	ILV		Not available	
	Confirmatory (if required)		Not required	
Fat	Primary	0.01 mg/kg	LC MS/MS	Watson G., 2013b
	ILV		Not available	
	Confirmatory (if required)		Not required	
Kidney, liver	Primary	0.01 mg/kg	LC MS/MS	Watson G., 2013b
	ILV	0.01 mg/kg		Bernal J., 2013
	Confirmatory (if required)		Not required	

Table 5.3-17: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Data to address the extraction efficiency of the QuEChERS multi-residue method in accordance with the requirements of SANCO 825/00 rev. 8.1 have not been provided specifically for the use of the method for determination of mesotrione in animal products. 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.

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

An overview on the acceptable methods and possible data gaps for analysis of Terbuthylazine in soil is given in the following tables. No new methods are necessary.

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

Component of residue definition: Mesotrione and metabolite A (open)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	Mesotrione: LOQ 0.002 mg/kg MNBA: LOQ 0.002 mg/kg AMBA: LOQ 0.002 mg/kg)	LC MS/MS	Jutsum L, Williams R.W., 2012, Jutsum L, 2013
Confirmatory	Not required		

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in surface and drinking water is given in the following tables. No new studies are necessary.

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

Component of residue definition: Mesotrione and metabolite A (open)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	Mesotrione: LOQ 0.05 µg/L MNBA: LOQ 0.05 µg/L AMBA: LOQ 0.05 µg/L	LC-MS/MS	Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a
	ILV	Mesotrione: LOQ 0.05 µg/L MNBA: LOQ 0.05 µg/L AMBA: LOQ 0.05 µg/L	LC-MS/MS	Wiesner F, Breyer N, 2013
	Confirmatory		Not required	
Surface water	Primary	Mesotrione: LOQ 0.05 µg/L MNBA: LOQ 0.05 µg/L AMBA: LOQ 0.05 µg/L	LC-MS/MS	Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a
	Confirmatory		Not required	

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in air is given in the following tables. No new methods are necessary.

Table 5.3-20: 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	LOQ 0.45 µg/m3	LC-MS/MS	Jutsum L, 2013b
Confirmatory		Not required	

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

No methods were supplied for the determination of mesotrione or metabolites in these matrices. A case has been made for not providing these as the active substance is notclassified as toxic or very toxic which is acceptable.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1	E.J. Gwóźdź	2015	CHR/H/TERIZ 650 WG Isoxaflutole/Mesotrione/Terbuthylazine 100/150/400 g/kg Development and validation of the method for determination of active substances content in the formulation Study code: BA-25/15 Analytical Department of Institute of Industrial Organic Chemistry (IPO), Warsaw, Poland GLP Unpublished	N	Chemirol
KCP 5.2.1/01	Pomeroy D.	2015	Validation of the method of determination of terbuthylazine, isoxaflutole, mesotrione and a specified mesotrione impurity in a WG formulation in compliance with Good laoratory Practice. Study code: DNA3319 David Norris analytical Laboratories Ltd. Units 13-15, Swan Business Park, Dartford, UK GLP Unpublished	N	Chemirol
KCP 5.2.1/02	E.. Gwóźdź	2017	CHR/H/TERIZ 650 WG Development and validation of the method for determination of the relevant impurities (simazine, atrazine and propazine) content in the formulation Study code: BA-06/17 Analytical Department of Institute of Industrial Organic Chemistry (IPO), Warsaw, Poland GLP Unpublished	N	Chemirol
KCP 5.2.1/03	Pomeroy D.	2017	Validation of the method of determination of two specified impurities within a WG formulation containing Terbuthylazine, Isoxaflutole and Mesotrione, in compliance with Good Laboratory Practice Study code: DNA4178 David Norris analytical Laboratories Ltd.	N	Chemirol

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
			Units 13-15, Swan Business Park, Dartford, UK GLP Unpublished		
KCP 5.3/01	Jörg Semrau	2018	Determination of residues of terbuthylazine, mesotrione and isoxaflutole after one application of TERIZ 650 WG in maize at 1 site in Northern Europe 2017 Eurofins, Germany Study no.: S17-04983 (field phase) GLP unpublished	N	Chemirol
KCP 5.3/02	Jörg Semrau	2018	Determination of residues of terbuthylazine, mesotrione and isoxaflutole after one application of TERIZ 650 WG in maize at 1 site in Northern Europe 2017 Eurofins, Germany Study no.: S17-04903 (field phase) GLP unpublished	N	Chemirol
KCP 5.3/03	Jörg Semrau	2018	Determination of residues of terbuthylazine, mesotrione and isoxaflutole after one application of TERIZ 650 WG in maize at 1 site in Northern Europe 2017 Eurofins, Germany Study no.: S17-04904 (field phase) GLP unpublished	N	Chemirol
KCP 5.3/04	Jörg Semrau	2018	Determination of residues of terbuthylazine, mesotrione and isoxaflutole after one application of TERIZ 650 WG in maize at 1 site in Northern Europe 2017	N	Chemirol

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
			Eurofins, Germany Study no.: S17-04905 (field phase) GLP unpublished		
KCP 5.3/05	Jörg Semrau	2018	Determination of residues of terbuthylazine, mesotrione and isoxaflutole after one application of TERIZ 650 WG in maize at 1 site in Northern Europe 2017 Eurofins, Germany Study no.: S17-04906 (field phase) GLP unpublished	N	Chemirol
KCP 5.1.1/01	Dr Knop, M.	2019	Development and Validation of an Analytical Method for the Determination of Isoxaflutole and RPA202248 in Different Animal Matrices Study Code S19-04083 Eurofins Agroscience Services EcoChem GmbH GLP/GEP: Yes Unpublished	N	CHEMIROL
KCP 5.2/04	Dr Knop, M.	2019	Development and Validation of an Analytical Method for the Determination of Isoxaflutole and RPA202248 in Different Plant Matrices Study Code S19-04082 Eurofins Agroscience Services EcoChem GmbH GLP/GEP: Yes Unpublished	N	CHEMIROL
KCP 5.2/05	Imart, C.	2019	Independent Laboratory Validation of Analytical Method for the Determination of Isoxaflutole and RPA 202248 in Foodstuffs of Plant Origin Study Code	N	CHEMIROL

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
			S19-04084 Eurofins Agroscience Services Chem SAS 75 B Avenue de Pascalet 30310 Vergèze France GLP/GEP: Yes Unpublished		

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
KCP 5.1.2/01	Dieterle, R.	1993	GS13529, Applicability of Multiresidue Method DFG S 19 for determination of GS 13529 in maize(grain and whole plant) Company Report No: 121-92 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta
KCP 5.1.2/02	Ferguson, L.	2009	Terbuthylazine – Independent Laboratory validation of analytical method no. REM 201.01 for the determination of terbuthylazine (GS 13529) and its Metabolites GS26379 and GS28620 in whole Maize Plants and Rape seed. Company Report No: GS13529_10121 Syngenta-Jealott Hill Bracknell UK, Oxon Italia S.p.A.,Pero, Italy Charles River Laboratries, Edinburgh, UK, 30377 GLP Unpublished	N	Syngenta/ Oxon

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 5.1.2/03	Luetolf, W.	1995a	Determination of residues of parent compund by gas chromatography (GC), Soil Company Report No: REM 148.05 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta
KCP 5.1.2/04	Figueiredo J	2003	Determination of GS13529 (Terbuthylazine) and its metabolites GS26379, GS28620 and GS23158 in soil by LC-MS/MS.REM 148.11 Report No: REM 148.11 Syngenta Crop Protection,AG, Basel GLP no Unpublished	N	Syngenta
KCP 5.1.2/05	Robinson,N.	2004	Residue analytical method for the determiation of residues of terbuthylazine (GS 13529), GS23158, GS26379 and GS28620 in Water Report No: REM 426/01 Syngenta Crop Protection,AG, Basel,Switzerland Syngenta, Jealotts Hill, UK GLP Unpublished	N	Syngenta
KCP 5.1.2/06	Tribolet, R.	1992	Sampling of air and determination of residues of parent compund by gas chromatography Company Report No: REM 148-03 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta
KCP 5.1.2/07	Tribolet, R.	1996	Validation by analysis of fortified specimens and determination of recoveries. Validation of method REM 148.03 in air Company Report No: 140/95 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta

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 5.1.2/08	Schoening, R.; Wolters, A.	2006	Modification M001 of analytical method 00985 for the determination of residues of isoxaflutole and its metabolites AEB197278-AE0540092 (RPA202248) and AE0317309-AEB197555 (RPA203328) in/on corn plant material by HPLC-MS/MS Bayer CropScience, Report No.: 00985/M001 GLP Unpublished	N	Bayer Crop Science
KCP 5.1.2/09	Winter, O.; Amann, S.	2013	Validation of the BCS-method- 1300/M009 (based on QuEChERS) for the determination of residues of isoxaflutoe and itsmetabolite RPA 202248 in animal tissues Eurofins Agroscience Services Chem GmbH (EAS Chem), Hamburg, Germany Bayer CropScience, Report No.: S12-00056, GLP Unpublished	N	Bayer Crop Science
KCP 5.1.2/10	Netzband, D. J.	2008	Analytical method for the determination of residues of isoxaflutole (IFT) and its metabolite RPA 202248 (DKN) in soil using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: IS-003-S08-01, GLP Unpublished	N	Bayer Crop Science
KCP 5.1.2/11	Krebber, R.; Leppelt, L.	2012	Analytical method 01333 for the determination of isoxaflutole and its metabolite AE 0540092 in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report No.: MR-11/110,	N	Bayer Crop Science

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		
KCP 5.1.2/12	Corgier, M. M.; Turier, G. P.	1995	Analytical method for the determination of isoxaflutole (RPA201772) in air Rhone-Poulenc Agro, Lyon, France Bayer CropScience, Report No.: R014776, GLP Unpublished	N	Bayer Crop Science
KCP 5.1.2/13	Crook S.	2002	Mesotrione: Residue Analytical Method for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Samples Syngenta Crop Protection AG, Basel, Switzerland Syngenta – Jealott's Hill International, Bracknell, Berkshire, United Kingdom, RAM 366/01, 2704-01 Syngenta File No ZA1296/0752 GLP no Unpublished	N	Syngenta
KCP 5.1.2/14	Watson G.	2013	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, S12-03250 GLP Unpublished	N	Syngenta
KCP 5.1.2/15	Jutsum L., Chamkesam N.	2013	Mesotrione – Analytical Method GRM007.09A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Water Syngenta CEMAS, North Ascot, United Kingdom, GRM007.09A Not GLP Unpublished	N	Syngenta
KCP	Jutsum L.	2013	Mesotrione - Residue Method GRM007.08B for the Determination of Mesotrione in Air	N	Syngenta

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**
5.1.2/16			Syngenta CEMAS, North Ascot, United Kingdom, GRM007.08B Not GLP Unpublished		
KCP 5.2/01	Dieterle, R.	1993	GS13529, Applicability of Multiresidue Method DFG S 19 for determination of GS 13529 in maize(grain and whole plant) Company Report No: 121-92 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta
KCP 5.2/02	Ferguson, L.	2009	Terbuthylazine – Independent Laboratory validation of analytical method no. REM 201.01 for the determination of terbuthylazine (GS 13529) and its Metabolites GS26379 and GS28620 in whole Maize Plants and Rape seed. Company Report No: GS13529_10121 Syngenta-Jealott Hill Bracknell UK, Oxon Italia S.p.A.,Pero, Italy Charles River Laboratries, Edinburgh, UK, 30377 GLP Unpublished	N	Syngenta/ Oxon
KCP 5.2/03	Luetolf, W.	1995a	Determination of residues of parent compund by gas chromatography (GC), Soil Company Report No: REM 148.05 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta
KCP 5.2/04	Figueiredo J	2003	Determination of GS13529 (Terbuthylazine) and its metabolites GS26379, GS28620 and GS23158 in soil by LC-MS/MS.REM 148.11 Report No: REM 148.11 Syngenta Crop Protection,AG, Basel GLP no	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
KCP 5.2/05	Todd M.	1999	Validation and determination of residues in soil samples generated from field disssipation trials held in northern Europe. Huntingdon Life Science limited, UK Oxon ItaliaS.P.A, Pero, Italy Report No OXN 228/993260 GLP Unpublished	N	Oxon
KCP 5.2/06	Todd M.	2002	Terbuthylazine: Validation od methodology for the determination of residues of terbuthylazine and its two major metabolites desethylterbuthylazineand 2-hydroxyterbuthylazine in soil Oxon ItaliaS.P.A, Pero, Italy Report No OXN 229/024125 GLP Unpublished	N	OXON
KCP 5.2/07	Todd M.	2002	Terbuthylazine: Validation od methodology for the determination of residues of terbuthylazine and its two major metabolites desethylterbuthylazineand 2-hydroxyterbuthylazine in drinking and surface water Oxon ItaliaS.P.A, Pero, Italy Report No OXN 229/024126 GLP Unpublished	N	OXON
KCP 5.2/08	Robinson,N.	2004	Residue analytical method for the determiation of residues of terbuthylazine (GS 13529), GS23158, GS26379 and GS28620 in Water Report No: REM 426/01 Syngenta Crop Protection,AG, Basel,Switzerland Syngenta, Jealotts Hill, UK GLP Unpublished	N	Syngenta
KCP	Tribolet, R.	1992	Sampling of air and determination of residues of parent compund by gas chromatography	N	Syngenta

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
5.2/09			Company Report No: REM 148-03 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished		
KCP 5.2/10	Tribolet, R.	1996	Validation by analysis of fortified specimens and determination of recoveries. Validation of method REM 148.03 in air Company Report No: 140/95 Novartis Crop Protection AG Basel, Switzerland/Ciba-Geigy Ltd.,Basel Switzrland GLP Unpublished	N	Syngenta
KCP 5.2/11	Schulz M, and Ullrich-Mitzel A	1995	Analytical method for the determination of terbuthylazine in air RCC AG Itingen, Switzerland Oxon Italia S.P.A, Pero Italy Report no: 385615 GLP Unpublished	N	Oxon
KCP 5.2/12	Schoening, R.; Wolters, A.	2006	Modification M001 of analytical method 00985 for the determination of residues of isoxaflutole and its metabolites AEB197278-AE0540092 (RPA202248) and AE0317309-AEB197555 (RPA203328) in/on corn plant material by HPLC-MS/MS Bayer CropScience, Report No.: 00985/M001 GLP Unpublished	N	Bayer Crop Science
KCP 5.2/13	Winter, O.; Amann, S.	2013	Validation of the BCS-method- 1300/M009 (based on QuEChERS) for the determination of residues of isoxaflutoe and itsmetabolite RPA 202248 in animal tissues Eurofins Agroscience Services Chem GmbH (EAS Chem), Hamburg, Germany	N	Bayer Crop Science

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
			Bayer CropScience, Report No.: S12-00056, GLP Unpublished		
KCP 5.2/14	Netzband, D. J.	2008	Analytical method for the determination of residues of isoxaflutole (IFT) and its metabolite RPA 202248 (DKN) in soil using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: IS-003-S08-01, GLP Unpublished	N	Bayer Crop Science
KCP 5.2/15	Krebber, R.; Leppelt, L.	2012	Analytical method 01333 for the determination of isoxaflutole and its metabolite AE 0540092 in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report No.: MR-11/110, GLP Unpublished	N	Bayer Crop Science
KCP 5.2/16	Corgier, M. M.; Turier, G. P.	1995	Analytical method for the determination of isoxaflutole (RPA201772) in air Rhone-Poulenc Agro, Lyon, France Bayer CropScience, Report No.: R014776, GLP Unpublished	N	Bayer Crop Science
KCP 5.2/17	Crook S.	2002	Mesotrione: Residue Analytical Method for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Samples Syngenta Crop Protection AG, Basel, Switzerland Syngenta – Jealott's Hill International, Bracknell, Berkshire, United Kingdom, RAM 366/01, 2704-01 Syngenta File No ZA1296/0752	N	Syngenta

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 no Unpublished		
KCP 5.2/18	Watson G.	2013	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, S12-03250 GLP Unpublished	N	Syngenta
KCP 5.2/19	Jutsum L., Chamkesam N.	2013	Mesotrione – Analytical Method GRM007.09A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Water Syngenta CEMAS, North Ascot, United Kingdom, GRM007.09A Not GLP Unpublished	N	Syngenta
KCP 5.2/20	Jutsum L.	2013	Mesotrione - Residue Method GRM007.08B for the Determination of Mesotrione in Air Syngenta CEMAS, North Ascot, United Kingdom, GRM007.08B Not GLP Unpublished	N	Syngenta

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for terbuthylazine

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

No new or additional studies have been submitted

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

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

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

zRMS comment: Method is accepted

Reference:	KCP 5.2/04
Report	Development and Validation of an Analytical Method for the determination of Isoxaflutole and RPA202248 in Different Matrices, M. Knop, 2019, S19-04082
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The validated method consisted in an extraction of the analyte from the matrices with acetonitrile after addition of water. A salt mixture containing magnesium sulfate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase diluted with water. Quantification is performed by use of LC-MS/MS detection. Two mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30% of LOQ was detected in any of the reagent blanks or the control sample extracts of any matrix, so that a high level of selectivity was demonstrated.

Results and discussions

The validation of the analytical method was carried out under GLP compliance according to the SANCO/825/00 rev.8.1 guideline. The evaluated validation parameters are reported in the following paragraphs.
Linearity was checked by a 7-points calibration curve (single injection). All the obtained calibration curves had R^2 values in accordance with that prefixed (R2 > 0.995). Accuracy and precision were verified by means of recovery tests carried out at the following 2 spiking levels for each tested matrix (cucumber, oranges, rape seed, wheat grain):
- 0.01 mg/kg corresponding to the target LOQ
- 0.1 mg/kg corresponding to 10 x LOQ

The mean recovery per level, found for both primary and confirmatory transition for all the matrixes, were in compliance with the guideline requirements (mean recovery per level in the range 70-120% and RSD% per level ≤ 20%).
Matrix effects on the detection of Isoxaflutole in extracts of cucumber, rape seed, oranges and wheat grain were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification.
Matrix effects on the detection of RPA202248 in extracts of oranges were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification.
Matrix effects on the detection of RPA202248 in extracts of cucumber ,rape seed and wheat grain were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.

Table A 1: Recovery results from method validation of isoxaflutole and RPA202248 using the analytical method in different matrices

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comment
Cucumber	Isoxaflutole	0.01	112	3	Primary transition 358/79
		0.1	100	1	
		0.01	107	4	Confirmatory transition 358/278
		0.1	100	0	
Oranges		0.01	103	4	Primary transition 358/79
		0.1	103	4	
		0.01	98	2	Confirmatory transition 358/278
		0.1	101	3	
Rape seed		0.01	100	4	Primary transition 358/79
		0.1	108	4	
		0.01	105	7	Confirmatory transition 358/278
		0.1	108	5	
Wheat grain		0.01	98	5	Primary transition 358/79
		0.1	113	4	
		0.01	102	7	Confirmatory transition 358/278
		0.1	114	4	
Cucumber	RPA202248	0.01	93	2	Primary transition 358/79
		0.1	95	2	
		0.01	92	3	Confirmatory

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comment
		0.1	96	2	transition 358/278
Oranges		0.01	111	2	Primary transition 358/79
		0.1	104	1	
		0.01	107	2	Confirmatory transition 358/278
		0.1	102	1	
Rape seed		0.01	97	4	Primary transition 358/79
		0.1	104	6	
		0.01	85	11	Confirmatory transition 358/278
		0.1	101	5	
Wheat grain		0.01	85	5	Primary transition 358/79
		0.1	101	3	
		0.01	82	5	Confirmatory transition 358/278
		0.1	100	3	

Table A 2: Characteristics for the analytical method used for validation of isoxaflutole and RPA202248 residues

	Isoxaflutole and RPA202248
Specificity	A reagent blank and two (2) control samples per matrix/analyte were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analytes. For both mass transitions, the samples showed no significant interference above 30 % of LOQ at the retention time of the analytes in any investigated matrix, therefore showing that the method is highly specific.
Calibration (type, number of data points)	The detector response was assessed by single determination of matrix-matched calibration standards at a minimum of seven (7) concentration levels ranging from 0.30 ng/mL to 100 ng/mL. This range corresponds to a fortification level of at least 0.0012 mg/kg to 0.20 mg/kg and thus covers the range from no more than 12 % of the limit of quantification (LOQ) and at least + 20 % of the highest analyte concentration detected in a (diluted) sample extract. For all analyses of isoxaflutole and for the analysis of RPA202248 in oranges and rape seed, a linear calibration was used. The calibration curves obtained for both ion mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.995. For the analysis of RPA202248 in wheat grain and cucumber, a quadratic calibration was used. The calibration curves obtained for both ion mass transitions showed correlation coefficients (R) of ≥ 0.995.
Calibration range	0.30 ng/mL to 100 ng/mL which corresponds to 0.0012 mg/kg to 0.20 mg/kg
Limit of determination/quantification	The limit of quantification was defined by the lowest fortification level successfully tested and was 0.01 mg/kg

Conclusion

The analytical method is considered fully suitable for the analysis of isoxaflutole and RPA202248 in high

water content, high acid content, high oil content and dry/high starch content matrices.

A 2.1.2.1.1.2 Independent laboratory validation

zRMS comment: Method is accepted

Reference:	KCP 5.2/05
Report	Independent Laboratory Validation of Analytical Method for the Determination of Isoxaflutole and RPA202248 in Foodstuffs of Plant Origin, Camille Imart, 2019, S19-04084
Guideline(s):	Yes (EEC guideline SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The independent validation study followed the analytical steps of the primary method. The analysis was conducted using LC-MS/MS. The confirmatory test has been run by following two different transitions and processing the data of both transitions, obtaining acceptable data for linearity, repeatability and recovery for each one for each matrix.

Results and discussions

Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.
Matrix effects on the detection of isoxaflutole and RPA202248 were found to be insignificant (< 20 %) in extracts of cucumber but were found to be significant (≥ 20%) in extracts of rape seeds Therefore, matrix-matched standards were used for quantification.
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five (5) concentration levels ranging from 0.3 ng/mL to 100 ng/mL and covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract.
The calibration curves obtained for both mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.990.
Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). The analytes were fortified and quantified separately. The following recoveries were obtained:

Table A 3: Recovery results from independent laboratory validation of isoxaflutole and RPA202248 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Cucumber	Isoxaflutole	0.01	95	5	m/z 358 → 79
		0.10	92	2	
		0.01	96	4	m/z 358 → 278
		0.10	93	1	
Rape seeds		0.01	77	10	m/z 358 → 79
		0.10	83	5	
		0.01	85	17	m/z 358 → 278
		0.10	81	8	
Cucumber	RPA202248	0.01	98	1	m/z 358 → 79
		0.10	99	1	
		0.01	97	2	m/z 358 → 278
		0.10	99	2	
Rape seeds		0.01	96	3	m/z 358 → 79
		0.10	96	1	
		0.01	97	3	m/z 358 → 278
		0.10	95	0	

Table A 4: Characteristics for the analytical method used for independent laboratory validation of isoxaflutole and RPA202248 residues

	Isoxaflutole and RPA202248
Specificity	LC-MS/MS determination was conducted by monitoring two (2) mass transitions (*m/z* 358→79 and *m/z* 358→278). Due to enhanced sensitivity mass transition 358→79 *m/z* is proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation. A reagent blank and two (2) control samples per matrix/analyte were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte(s). For both mass transitions, the samples showed no significant interference above 30 % of LOQ at the retention time of the analyte(s) in both investigated matrices, therefore showing that the method is highly specific. Blank correction was not performed.
Calibration (type, number of data points)	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five (5) concentration levels ranging from 0.30 ng/mL or 0.50* ng/mL (*for isoxaflutole in rape seeds) to 100 ng/mL. This range corresponds to a fortification level of 0.0006 mg/kg to 0.20 mg/kg in cucumber and to a fortification level of 0.0012 mg/kg or 0.001* mg/kg (* for isoxaflutole) to 0.40 mg/kg in rape seeds and thus covers the range from no more than 30 % of the limit of quantification (LOQ) and at least + 20 % of the highest analyte concentration detected in a (diluted) sample extract. The calibration curves obtained for both ion mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.990. Linear regression was

	Isoxaflutole and RPA202248
	performed with 1/x-weighting.
Calibration range	0.30 ng/mL or 0.50* ng/mL (*for isoxaflutole in rape seeds) to 100 ng/mL Which corresponds to 0.0006 mg/kg to 0.20 mg/kg in cucumber and 0.0012 mg/kg or 0.001* mg/kg (* for isoxaflutole) to 0.40 mg/kg in rape seeds
Assessment of matrix effects is presented	Yes. Matrix effects were < ± 20 % and deemed to be insignificant for both analytes in cucumber but were ≥ ± 20 % and deemed to be significant for both analytes in rape seeds. Therefore, matrix-matched standards were used for quantification throughout the study.
Limit of determination/quantification	an LOQ of 0.01 mg/kg was confirmed for isoxaflutole and its metabolite RPA202248 in cucumber and rape seeds. The LOD was set at 30 % of the LOQ which is 0.003 mg/kg.

Conclusion

The results of the independent laboratory validation confirm the results of the validation study and demonstrate that analytical method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries.

A 2.1.2.1.1.3 Confirmatory method

No new or additional studies have been submitted

A 2.1.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.1.2.1.2 Analytical method 2

No new or additional studies have been submitted

A 2.1.2.1.3 Extraction efficiency

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 1

A 2.1.2.2.1.1 Method validation

zRMS comment: Method is accepted

Reference:	KCP 5.1.1/01
Report	Development and Validation of an Analytical Method for the Determination of Isoxaflutole and RPA202248 in Different Animal Matrices, Matthias Knop, S19-04083, 2019
Guideline(s):	Yes (SANCO/825/00 rev.8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of milk, egg, fat, meat, liver and kidney were extracted with acetonitrile after addition of water. A salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation an aliquot of the acetonitrile phase was diluted with water. Quantification was performed by use of LC-MS/MS detection.

For the detection of isoxaflutole, meat was extracted with ethyl acetate. After centrifugation the solvent was evaporated and re-dissolved in water and acetonitrile. Quantification is performed by use of LC-MS/MS detection.

Results and discussions

Accuracy was determined by fortification of control samples with known amounts of the test items and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). The analytes were fortified and quantified separately. The following recoveries were obtained:

Table A 5: Recovery results from method validation of isoxaflutole and RPA202248 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Mass Transition
Milk	Isoxaflutole	0.01 mg/kg	101	2	mz 358 → 79
		0.1 mg/kg	103	4	
		0.01 mg/kg	101	3	mz 358 → 278
		0.1 mg/kg	103	4	
	RPA202248	0.01 mg/kg	83	1	mz 358 → 79
		0.1 mg/kg	94	2	
		0.01 mg/kg	82	2	mz 358 → 278
		0.1 mg/kg	95	1	
Egg	Isoxaflutole	0.01 mg/kg	102	2	mz 358 → 79
		0.1 mg/kg	101	2	
		0.01 mg/kg	103	2	mz 358 → 278

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Mass Transition
		0.1 mg/kg	101	2	
	RPA202248	0.01 mg/kg	87	2	mz 358 → 79
		0.1 mg/kg	93	2	
		0.01 mg/kg	86	2	mz 358 → 278
		0.1 mg/kg	92	2	
Fat	Isoxaflutole	0.01 mg/kg	105	2	mz 358 → 79
		0.1 mg/kg	115	3	
		0.01 mg/kg	105	2	mz 358 → 278
		0.1 mg/kg	115	3	
	RPA202248	0.01 mg/kg	75	5	mz 358 → 79
		0.1 mg/kg	75	5	
		0.01 mg/kg	74	5	mz 358 → 278
		0.1 mg/kg	74	6	
Meat	Isoxaflutole	0.01 mg/kg	79	3	mz 358 → 79
		0.1 mg/kg	79	3	
		0.01 mg/kg	80	4	mz 358 → 278
		0.1 mg/kg	77	3	
	RPA202248	0.01 mg/kg	102	6	mz 358 → 79
		0.1 mg/kg	101	4	
		0.01 mg/kg	103	7	mz 358 → 278
		0.1 mg/kg	100	2	
Liver	Isoxaflutole	0.01 mg/kg	93	3	mz 358 → 79
		0.1 mg/kg	89	7	
		0.01 mg/kg	94	3	mz 358 → 278
		0.1 mg/kg	90	7	
	RPA202248	0.01 mg/kg	81	1	mz 358 → 79
		0.1 mg/kg	91	3	
		0.01 mg/kg	82	3	mz 358 → 278
		0.1 mg/kg	91	2	
Kidney	Isoxaflutole	0.01 mg/kg	89	3	mz 358 → 79
		0.1 mg/kg	97	3	
		0.01 mg/kg	102	5	mz 358 → 278
		0.1 mg/kg	99	2	
	RPA202248	0.01 mg/kg	86	9	mz 358 → 79
		0.1 mg/kg	99	3	
		0.01 mg/kg	89	9	mz 358 → 278

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Mass Transition
		0.1 mg/kg	99	4	

Table A 6: Characteristics for the analytical method used for validation of active substance and metabolite residues in matrix

	Isoxaflutole and RPA202248
Specificity	LC-MS/MS determination was conducted by monitoring two (2) mass transitions (*m/z* 358→79 and *m/z* 358→278). Due to enhanced sensitivity mass transition 358→79 *m/z* was used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation. A reagent blank and two (2) control samples per matrix/analyte were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analytes. For both mass transitions, the samples showed no significant interference above 30 % of LOQ at the retention time of the analytes in any investigated matrix, therefore showing that the method is highly specific.
Calibration (type, number of data points)	All calibrations solutions cover the range from no more than 30 % of the limit of quantification (LOQ) and at least + 20 % of the highest analyte concentration detected in a (diluted) sample extract. For all analyses of isoxaflutole and for the analysis of RPA202248 meat, a linear calibration was used. The calibration curves obtained for both ion mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.995. Linear regression was performed with 1/x-weighting. For the analysis of RPA202248 in milk, egg, fat, liver and kidney, a quadratic calibration was used in order to obtain sufficiently large calibration intervals to cover the concentration of the samples. The calibration curves obtained for both ion mass transitions showed correlation coefficients (R) of ≥ 0.995. Regression was performed with 1/x-weighting.
Calibration range	<table><tr><th>Matrix</th><th>Calibration range</th><th>Corresponding fortification level</th></tr><tr><td colspan="3">Isoxaflutole</td></tr><tr><td>Milk</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td>egg</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td>fat</td><td>0.25 - 20 ng/mL</td><td>0.0025 - 0.2 mg/kg</td></tr><tr><td>meat</td><td>0.3 - 100 ng/mL</td><td>0.003 - 1.0 mg/kg</td></tr><tr><td>liver</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td>kidney</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td colspan="3">RPA202248</td></tr><tr><td>Milk</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td>egg</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td>fat</td><td>0.25 - 60 ng/mL</td><td>0.001 - 0.24 mg/kg</td></tr><tr><td>meat</td><td>0.25 - 20 ng/mL</td><td>0.001 - 0.08 mg/kg</td></tr><tr><td>liver</td><td>0.25 - 100 ng/mL</td><td>0.001 - 0.4 mg/kg</td></tr><tr><td>kidney</td><td>0.15 - 10 ng/mL</td><td>0.003 - 0.2 mg/kg</td></tr></table>
Assessment of matrix effects is presented	Yes Matrix effects were < ± 20 % and deemed to be insignificant isoxaflutole and RPA202248 in all tested matrices. However, matrix-matched standards were used for quantification throughout the study.
Limit of determination/quantification	limit of quantification 0.01 mg/kg limit of detection 0.003 mg/kg

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in food-

stuff of animal origin.

A 2.1.2.2.1.2 Independent laboratory validation

No new or additional studies have been submitted

A 2.1.2.2.1.3 Confirmatory method

No new or additional studies have been submitted

A 2.1.2.2.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.1.2.2.2 Analytical method 2

No new or additional studies have been submitted

A 2.1.2.2.2.1 Extraction efficiency

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2 Analytical methods for Isoxaflutole

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

No new or additional studies have been submitted

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

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

No new or additional studies have been submitted

A 2.2.2.1.1 Analytical method 1

A 2.2.2.1.1.1 Method validation

No new or additional studies have been submitted

A 2.2.2.1.1.2 Independent laboratory validation

No new or additional studies have been submitted

A 2.2.2.1.1.3 Confirmatory method

No new or additional studies have been submitted

A 2.2.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.2.2.1.2 Analytical method 2

No new or additional studies have been submitted

A 2.2.2.1.3 Extraction efficiency

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

No new or additional studies have been submitted

A 2.2.2.2.1 Analytical method 1

A 2.2.2.2.1.1 Method validation

No new or additional studies have been submitted

A 2.2.2.2.1.2 Independent laboratory validation

No new or additional studies have been submitted

A 2.2.2.2.1.3 Confirmatory method

No new or additional studies have been submitted

A 2.2.2.2.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.2.2.2.2 Analytical method 2

No new or additional studies have been submitted

A 2.2.2.2.2.1 Extraction efficiency

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.3 Analytical methods for mesotrione

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

No new or additional studies have been submitted

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

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

No new or additional studies have been submitted

A 2.3.2.1.1 Analytical method 1

A 2.3.2.1.1.1 Method validation

No new or additional studies have been submitted

A 2.3.2.1.1.2 Independent laboratory validation

No new or additional studies have been submitted

A 2.3.2.1.1.3 Confirmatory method

No new or additional studies have been submitted

A 2.3.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.3.2.1.2 Analytical method 2

No new or additional studies have been submitted

A 2.3.2.1.3 Extraction efficiency

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

No new or additional studies have been submitted

A 2.3.2.2.1 Analytical method 1

A 2.3.2.2.1.1 Method validation

No new or additional studies have been submitted

A 2.3.2.2.1.2 Independent laboratory validation

No new or additional studies have been submitted

A 2.3.2.2.1.3 Confirmatory method

No new or additional studies have been submitted

A 2.3.2.2.1.4 Extraction efficiency

No new or additional studies have been submitted

A 2.3.2.2.2 Analytical method 2

No new or additional studies have been submitted

A 2.3.2.2.2.1 Extraction efficiency

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted