FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: **TERBUT 500 SC**

Product names: **TERBUT 500 SC/**

TAZOPRYM 500SC / CORNAO 500 SC

Chemical active substance:

Terbuthylazine, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: **Synthos Agro Sp. z o.o.**

Submission date: 04/2020

Finalisation date: 10/2021; 03/2022, 06.2022

Version history

When	What
October 2021	Assessment by the experts
March 2022	Final Registration Report
June 2022	Update according to Reg. 2021/824 , 21.05.2021 r.

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5.1 Conclusion and summary of assessment

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

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

- none

Commodity/crop	Supported/ Not supported
Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

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

Reference:	Małgorzata Wołoszynowska MSc.
Report	Terbut 500 SC Method development and validation for the determination of active substance and relevant impurities content in the formulation, Małgorzata Wołoszynowska MSc., 2018, Study code: BA-07-18
Guideline(s):	Yes, SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The content of active substance in the examined specimen was determined by high performance liquid chromatography HPLC with UV/Vis detector using reversed phase column. External standard method was used.

Examined material:
Examined material: Terbut 500 SC

Date of production: 01.2018
Batch number: 1/18
Manufacturer: Synthos Agro Sp. z o.o.
Code of examined item: 10/BA – 07/18

Reference material:
Terbuthylazine, IPO 710, batch no 3A/15, purity 99.7%

Equipment:
- Shimadzu liquid chromatograph equipped with UV/Vis detector, a thermostated column oven and autosampler
- ACE C18 column (5µm), 250 x 4.6 mm
- Analytical balance Mettler AT261 Delta Range®, accuracy 0.01 mg

Reagents
- Water for HPLC, Millipore
- Acetonitrile for HPLC, POCh

Chromatographic conditions
- Column temperature 30 ºC
- Mobile phase:
  A: acetonitrile
  B: H_2O

Time [min]	A [%]	B[%]
0.01 – 12.00	48	52
12.01 – 17.00	70	30
17.01 – 28.00	48	52

- Flow rate: 1.0 mL/min
- Wavelength λ = 220 nm
- Volume of sample solution injected: 5 µL

Retention time:
- Terbuthylazine: 13.2 min

Total analysis time is 28 minutes

Calculations
The analysed substance content [%] in examined specimen was calculated according to the equation:

$$f = (Awz) / (mwz* P)$$
$$X \% = (Apr* 100) / (Cpr * f)$$

Where:
f - average calculating factor
Apr – analyte peak area on specimen solution chromatogram
Awz – analyte peak area on standard solution chromatogram
P – purity of standard [%]
mwz – mass of the standard [mg]
Cpr – concentration of the examined specimen [mg].

Preparation of solutions

Standard solution
About 30 mg of terbuthylazine standard was weighed (with the accuracy of 0.01 mg) into 25 mL flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min.

Calibration curve
Into six 10 mL volumetric flasks the following amounts of standard were pipetted:

Solution No	1	2	3	4	5	6
Terbuthylazine	0.2 mL	0.6 mL	0.8 mL	1.0 mL	1.2 mL	1.6 mL

Acetonitrile was added to the mark and the solutions were stirred.

Specimen solution
About 50 mg of examined specimen was weighed (with the accuracy of 0.01 mg) into a 25 mL flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min. After cooling the solution was diluted 10-times. Six samples were prepared to assess the repeatability.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance Terbuthylazine in plant protection product Terbut 500 SC

	Terbuthylazine
Author(s), year	Małgorzata Wołoszynowska MSc
Principle of method	The content of active substance in the examined specimen was determined by high performance liquid chromatography HPLC with UV/Vis detector using reversed phase column. External standard method was used.
Linearity **Linear between** **0.0233 mg/mL and 0.1867 mg/mL, correspond to the following concentration range 24% to 192%** **Correlation coefficient = 0.9999** **Y= 44800109x +17765**	The linearity of the detector response was assessed using six standard solutions at the concentration range of terbuthylazine from 0.0233 mg/mL to 0.1867 mg/mL, which corresponds to the concentration range of 24% to 192% of terbuthylazine content in the preparation. All solutions were analysed twice. Correlation coefficient should be $r \geq 0.99$. The obtained result is acceptable.
Precision – Repeatability Mean **n = 6** **0.99 %RSD**	The method repeatability was assessed on the basis of six independent determinations of active substance content in Terbut 500 SC preparation. Acceptable relative standard deviation for main ingredient (~ 45%) is RSDr ≤ 1.51%. The obtained result 0.99% is acceptable.
Accuracy **n = 12** **100.05 % Recovery**	Accuracy of active substance determination in Terbut 500 SC was assessed by recovery value at two levels of concentration. Each of twelve 10 mL volumetric flasks were charged with approximately 20 mg placebo and weighed. About 0.2 mL of the terbuthylazine standard solution at concentration of 1.1669 mg/mL was added to the each of the first six flasks and acetonitrile was added up to the volume. To each of the remaining six flasks 1.6

	Terbuthylazine
	mL of terbuthylazine standard solution at the concentration of 0.7083 mg/mL was added and acetonitrile was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. The concentration of analyte in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the nominal and calculated contents were compared. For the main ingredient at concentration of > 10 % the average recovery value should be 100 ± 2 %. The obtained result of 100.05% is acceptable.
Interference/ Specificity	The chromatograms of placebo, solvent, standard solution and the examined specimen solution were performed and superimposed. There are no interferences between the analyte and other components of the specimen.
Comment	The validation parameters (linearity, LOQ, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

Conclusion

It was confirmed that chromatographic method of determination of the active compound Terbuthylazine is specific. No interference was observed. The validation parameters (linearity, LOQ, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.
Methodology validated in March 2018 according to SANCO /3030 /99 rev.4 ,test is accepted .
According to the results methodology fulfil the requirements according to SANCO /3030 /99 rev.5. too and it is accepted.

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

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

Reference:	Małgorzata Wołoszynowska MSc.
Report	Terbut 500 SC Method development and validation for the determination of active substance and relevant impurities content in the formulation, Małgorzata Wołoszynowska MSc., 2018, Study code: BA-07-18
Guideline(s):	Yes, SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The content of relevant impurities in the examined specimen was determined by high performance liquid chromatography HPLC with UV/Vis detector using reversed phase column. External standard method was used.

Examined material:

Examined material:	Terbut 500 SC
Date of production:	01.2018
Batch number:	1/18
Manufacturer:	Synthos Agro Sp. z o.o.
Code of examined item:	10/BA – 07/18

Reference material:

- Atrazine, IPO 005, batch no 6B/17, purity 98.8%
- Simazine, IPO 692, batch no 3D/14, purity 99.5%
- Propazine, IPO 575, batch no 3A/14, purity 99.8%

Equipment:

- Shimadzu liquid chromatograph equipped with UV/Vis detector, a thermostated column oven and autosampler
- ACE C18 column (5µm), 250 x 4.6 mm
- Analytical balance Mettler AT261 Delta Range®, accuracy 0.01 mg

Reagents

- Water for HPLC, Millipore
- Acetonitrile for HPLC, POCh

Chromatographic conditions

- Column temperature 40 ºC
- Mobile phase:

  A: acetonitrile
  B: H_2O

Time [min]	A [%]
0.01 – 20.00	30 - 80
20.01 – 35.00	80 – 30

- Flow rate: 1.0 mL/min
- Wavelength λ = 220 nm
- Volume of sample solution injected: 10 µL

Retention time:
simazine ~ 10.2 min.,
atrazine ~ 13.6 min.,
propazine ~ 16.4 min.
Total analysis time is 35 minutes

Calculations

The analysed substance content [%] in examined specimen was calculated according to the equation:

$$f = (Awz) / (mwz* P)$$
$$X \% = (Apr* 100) / (Cpr * f)$$

Where:

f - average calculating factor
Apr – analyte peak area on specimen solution chromatogram
Awz – analyte peak area on standard solution chromatogram
P – purity of standard [%]
mwz – mass of the standard [mg]
Cpr – concentration of the examined specimen [mg].

Preparation of solutions

Standard solution
About 10 mg of impurities standards was weighed (with the accuracy of 0.01 mg) into 25 mL flask with a screw cap and acetonitrile was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. After cooling solutions were diluted 10-times.

Calibration curve
Into six 25 mL volumetric flasks the following amounts of standard were pipetted:

Solution No	1	2	3	4	5	6
Atrazine	0.2 mL	0.25 mL	0.3 mL	0.35 mL	0.4 mL	0.5 mL
Propazine	0.2 mL	0.25 mL	0.3 mL	0.35 mL	0.4 mL	0.5 mL
Simazine	0.5 mL	0.55 mL	0.6 mL	0.8 mL	1.5 mL	2.0 mL

Acetonitrile was added to the mark and the solutions were stirred.

Specimen solution
About 100 mg of examined specimen was weighed (with the accuracy of 0.01 mg) into a 25 mL flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) Terbut 500 SC

	Atrazine	**Propazine**	**Simazine**
Author(s), year	Małgorzata Wołoszynowska MSc.	Małgorzata Wołoszynowska MSc.	Małgorzata Wołoszynowska MSc.
Principle of method	The content of relevant impurities in the examined specimen was determined by high performance liquid chromatography HPLC with UV/Vis detector using reversed phase column. External standard method was used.		
<u>Linearity - Atrazine</u> **Linear between** **0.000021 mg/mL to** **0.00062 mg/mL** **Correlation coefficient = 0.9983** **<u>Linearity – Propazine</u>** **Linear between** **0.000028 mg/mL to**	The linearity of the detector response was assessed using six standard solutions at the concentration range of atrazine from 0.000021 mg/mL to 0.00062 mg/mL All solutions were analysed twice. Correlation coefficient	The linearity of the detector response was assessed using six standard solutions at the concentration range of propazine from 0.000028 mg/mL to 0.00071 mg/mL All solutions were analysed twice. Correlation coefficient	The linearity of the detector response was assessed using six standard solutions at the concentration range of simazine from 0.00063 mg/mL to 0.0025 mg/mL. All solutions were analysed twice. Correlation coefficient

	Atrazine	Propazine	Simazine
0.00071 mg/mL **Correlation coefficient = 0.9989** **<u>Linearity – Simazine</u>** **Linear between 0.00063 mg/mL and 0.0025 mg/mL** **Correlation coefficient = 0.9982**	should be r ≥ 0.99. The obtained result is acceptable. Y= 114118235x - 419	should be r ≥ 0.99. The obtained result is acceptable. Y= 171529773x - 16679	should be r ≥ 0.99. The obtained result is acceptable. Y= 167824326 x-28459
<u>Atrazine</u> **Precision – Repeatability Mean n = 6 6.37 %RSD** **<u>Propazine</u>** **Precision – Repeatability Mean n = 6 7.08 %RSD** **<u>Simazine</u>** **Precision – Repeatability Mean n = 6 2.21 %RSD**	The method repeatability was assessed on the basis of six independent determinations of relevant impurities content in Terbut 500 SC preparation. Because peak of atrazine was not detected above either of the LOQ values in any of the solutions, six weights of placebo (approximately 100 mg) were fortified with atrazine at LOQ level (0.0001 mg/mL) were analyzed to determine reproducibility. Acceptable relative standard deviation for analyte (~ 0.0015%) is RSDr ≤ 10.07%. The obtained result 6.37% is acceptable.	The method repeatability was assessed on the basis of six independent determinations of relevant impurities content in Terbut 500 SC preparation. Because peak of atrazine was not detected above either of the LOQ values in any of the solutions, six weights of placebo (approximately 100 mg) were fortified with atrazine at LOQ level (0.0002 mg/mL) were analyzed to determine reproducibility. Acceptable relative standard deviation for analyte (~ 0.0018%) is RSDr ≤ 9.85%. The obtained result 7.08% is acceptable.	The method repeatability was assessed on the basis of six independent determinations of relevant impurities content in Terbut 500 SC preparation. Acceptable relative standard deviation for analyte (~ 0.038%) is RSDr ≤ 4.37%. The obtained result 2.21% is acceptable.
Accuracy **<u>Atrazine</u>** **n = 12 99.57 % Recovery** **<u>Propazine</u>** **n = 12 99.97 % Recovery** **<u>Simazine</u>** **n = 12 98.36 % Recovery**	Accuracy of impurities determination in Terbut 500 SC was assessed by recovery value at two levels of concentration. The concentration of analytes in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the nominal and calculated contents were compared.	Accuracy of impurities determination in Terbut 500 SC was assessed by recovery value at two levels of concentration. The concentration of analytes in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the nominal and calculated contents were compared.	Accuracy of impurities determination in Terbut 500 SC was assessed by recovery value at two levels of concentration. The concentration of analytes in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the nominal and calculated contents were compared.

	Atrazine	Propazine	Simazine
	For the impurities at concentration of > 0.1 % the average recovery value should be 100 ±25 %. The obtained result of 99.57% is acceptable.	For the impurities at concentration of > 0.1 % the average recovery value should be 100 ±25 %. The obtained result of 95.97% is acceptable.	For the impurities at concentration of > 0.1 % the average recovery value should be 100 ±25 %. The obtained result of 98.36% is acceptable.
Interference/ Specificity	The chromatograms of placebo, solvent, mixture of standards solutions and the examined specimen solution were performed and superimposed. There are no interferences between the analytes and other components of the specimen.	The chromatograms of placebo, solvent, mixture of standards solutions and the examined specimen solution were performed and superimposed. There are no interferences between the analytes and other components of the specimen.	The chromatograms of placebo, solvent, mixture of standards solutions and the examined specimen solution were performed and superimposed. There are no interferences between the analytes and other components of the specimen.
LOQ	The limit of quantification (LOQ) was defined as the lowest quantity of standard (approximately the height of the peak is a 10-fold amount of the baseline noise). LOQ for atrazine is 0.0000119 mg/mL. Limit of detection (LOD) is LOQ/2 i.e. about 0.0001%.	The limit of quantification (LOQ) was defined as the lowest quantity of standard (approximately the height of the peak is a 10-fold amount of the baseline noise). LOQ for propazine is 0.0000215 mg/mL. Limit of detection (LOD) is LOQ/2 i.e. about 0.0001%.	The limit of quantification (LOQ) was defined as the lowest quantity of standard (approximately the height of the peak is a 10-fold amount of the baseline noise). LOQ for simazine is 0.0000242 mg/mL. Limit of detection (LOD) is LOQ/2 i.e. about 0.0001%.
Comment	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.4.	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.4.	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.4.

Conclusion

It was confirmed that chromatographic methods of determination of the relevant impurities as Atrazine, Propazine, Simazine are specific. No interference was observed. The validation parameters (specificity, linearity, limit of quantification (LOQ), repeatability (precision)) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.4.

Methodology validated in March 2018 according to SANCO /3030 /99 rev.4 ,test is accepted . According to the results methodology fulfil the requirements according to SANCO /3030 /99 rev.5. too and it is accepted.

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

With respect to toxicological, eco-toxicological or environmental aspects Terbut 500 SC does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

For the determination of terbuthylazine the CIPAC method 234/TC/M/3 is applicable

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 for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.2-3: 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**
Plants, plant products – Maize whole plant and Maize grain (Residues)	Primary	DFG S19 0.02 mg/kg	GC-NPD	Dierterle R., 1993/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	DFG S7 0.02 mg/kg	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Plants, plant products – Maize whole plant and Maize grain (Residues)	Primary	DFG S7 0.02 mg/kg	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Plants, plant products – Maize	Primary	From Study SIP 1288 – This	GC-NPD	Freschi G., 2002c/ Additional Report to the DAR of

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**
whole plant and Maize grain (Residues)		method is as DFG S19 0.02 mg/kg		Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg Access from Syngenta to Oxon	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	DFG S7 0.02 mg/kg Access from Syngenta to Oxon	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Plants, plant products – Maize silage Maize grain (Residues)	Primary	Method from Workbook of Mutiresidue Methods for Pesticide Residue Analysis In Vegetable Products 0.02 mg/kg	GC-NPD	Freschi G., 2004c/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg Access from Syngenta to Oxon	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	DFG S7 0.02 mg/kg Access from Syngenta to Oxon	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Soil (Ecotoxicology)	Primary	0.1 mg/l	HPLC-DAD	Validation included in studies: (1) xxx, PhD, 2018 (2) xxxx, MSc, 2018a
	Confirmatory (if required)	Not provided		
Soil (Ecotoxicology)	Primary	0.5 mg/kg	HPLC-DAD	Validation included in the study: xxxx MSc, 2018b
	Confirmatory (if required)	Not provided		
Soil (Ecotoxicology)	Primary	0.5 mg/kg	HPLC-DAD	Validation included in studies: (1) xxxx, MSc, 2020 (2) xxxx MSc Eng., 2020
	Confirmatory (if required)	Not provided		
Water	Primary	0.001 mg/l	HPLC-DAD	Validation included in studies:

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**
(Ecotoxicology)				(1) Elżbieta Kulec-Płoszczyca, MSc, 2018 (2) Elżbieta Kulec-Płoszczyca, MSc, 2018 (3) Elżbieta Kulec-Płoszczyca, MSc, 2018 (4) xxxx
	Confirmatory (if required)	Not provided		
Water (Ecotoxicology)	Primary	0.001 mg/l	HPLC-DAD	Validation included in the study: Dennis Janota, Msc, 2019
	Confirmatory (if required)	Not provided		

Component of residue definition: MT1				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products – Maize whole plant (Residues)	Primary	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	The original method is highly specific therefore an additional confirmatory method is not necessary.		

Component of residue definition: MT14				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products – Maize whole plant (Residues)	Primary	REM 201.01 0.02 mg/kg	LC-MS-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	The original method is highly specific therefore an additional confirmatory method is not necessary.		

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of 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)	Terbuthylazine	0.1 mg/kg	Commission Regulation (EC) No 149/2008 of 29 January 2008
Soil (Ecotoxicology)	Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxyl-terbuthylazine (MT13)	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxy-terbuthylazine (MT13) plus desethyl-hydroxy-terbuthylazine (MT14) plus LM1, LM2, LM3, LM4, LM5 and LM6	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Terbuthylazine (MT0) plus desethyl-terbuthylazine (MT1) plus hydroxyl-terbuthylazine (MT13	µg/L	
Air	Terbuthylazine	Xxx µg/m^3	AOEL sys/AOEL inhal: xxx mg/kg bw/d
Tissue (meat or liver)	-	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 new/ additional studies it is referred to Appendix 2.

zRMS comment

EFSA Journal 2020;18(1):5980:

Methods of analysis for monitoring of residues (analytical technique, matrix groups, LOQs):

Dry and high-water content matrices:
GC-NPD, LOQ 0.02mg/kg (DFG S19) for terbuthylazine in cereal grain, ILV considered acceptable (EFSA, 2011). This method was reported validated for maize whole plant during the peer however an ILV and confirmatory methods are missing (United Kingdom, 2007; Spain, 2018)
High oil content matrices:
LC–MS/MS method for terbuthylazine, MT1 and MT14 with an individual LOQ of 0.02 mg/kg; ILV and confirmatory method are missing (United Kingdom, 2010a; Spain, 2018)

EURLs (EURLs, 2018) provided for routine analyses the following methods for terbuthylazine:
High water, acid content commodities and high oil commodities: •LC–MS/MS method (QuEChERS-method EN 15662:2008) with a LOQ 0.01 mg/kg, validated in tomato, orange, almonds and avocado; Dry matrices: •LC-QqQ-MS/MS method (QuEChERS-method EN 15662:2008) with a LOQ 0.01 mg/kg validated in wheat, oat, rice and rye; Special matrices: •LC–MS/MS method (QuEChERS-method EN 15662:2008) with a LOQ 0.01 mg/kg, validated in green tea.

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				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	DFG S7 0.02 mg/kg	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
High acid content	Primary	DFG S7 0.02 mg/kg	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February

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**
				2010
	ILV	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
High oil content	Primary	DFG S7 0.02 mg/kg	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
High protein/high starch content (dry)	Primary	DFG S19 0.02 mg/kg	GC-NPD	Dierterle R., 1993/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	REM 201.01 0.02 mg/kg	GC-MS	Ferguson L., 2009/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory (if required)	DFG S7 0.02 mg/kg	GC-NPD	Anon., 1987/Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010

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

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

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

Monitoring methods in food of animal origin **are not required** since no MRLs have been proposed.

zRMS comment

EFSA Journal 2020;18(1):5980:

Methods of analysis for monitoring of residues (analytical technique, matrix groups, LOQs):
LC–MS/MS multi-residue QuEChERS method (EN 15662:2009-02) for terbuthylazine in animal matrices; LOQ 0.01mg/kg; confirmatory method and ILV available for milk, meat, egg, liver, fat and kidney; LOD <0.00125 mg/kg (Spain, 2018)
Analytical method for enforcement of MT1 in milk, not available and required.
GC-NPD, LOQ 0.02mg/kg (DFG S19) for terbuthylazine in animal matrices; confirmatory method missing and ILV available (United Kingdom, 2007)
EURLs (EURLs, 2018) provided for routine analyses of food of animal origin for terbuthylazine an:
•LC–MS-Q-TOF QuEChERS with a screening detection limit (SDL) of 0.0025 mg/kg for terbuthylazine, validated in milk and milk products; meat (red and white), fish (high and low fat), various honeys
•LC–MS/MS QuEChERS EN-15662 method for monitoring terbuthylazine and MT1 with an individual LOQ of 0.01 mg/kg in milk (EURLs, 2018)

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. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-3: Validated methods for soil (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	REM 148.05 0,02 mg/kg	GC-NPD	Lutolf W., 1995a/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	REM 148.05 0,02 mg/kg	GC-MS	Lutolf W., 1995a/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Primary	REM 148.11 0,02 mg/kg	HPLC-MS/MS	Fugueiredo J., 2003 and Tribolet R., 2003/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
Primary	0,01 mg/kg	HPLC-MS/MS	Todd. M, 2002a/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

Component of residue definition: MT1			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	REM 148.11 0,02 mg/kg	HPLC-MS/MS	Fugueiredo J., 2003 and Tribolet R., 2003/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
Primary	0,01 mg/kg	HPLC-MS/MS	Todd. M, 2002a/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

Component of residue definition: MT13			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	REM 148.11 0,02 mg/kg	HPLC-MS/MS	Fugueiredo J., 2003 and Tribolet R., 2003/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
Primary	0,01 mg/kg	HPLC-MS/MS	Todd. M, 2002a/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

Component of residue definition: MT14			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	REM 148.11 0,02 mg/kg	HPLC-MS/MS	Fugueiredo J., 2003 and Tribolet R., 2003/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

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

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

Component of residue definition: Terbuthylazine, MT1, MT13				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	RAM 426/01 0.1 µg/L	HPLC-MS/MS	Robinson N J., 2004/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
	Confirmatory			
Surface water	Primary	RAM 426/01 0.1 µg/L	HPLC-MS/MS	Robinson N J., 2004/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
Drinking water	Primary	0.05 µg/L	HPLC-MS/MS	Todd M., 2002b/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
	Confirmatory			
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Todd M., 2002b/ Additional Report to the DAR of Terbuthylazine, B.5 Methods

Component of residue definition: Terbuthylazine, MT1, MT13				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				of analysis, February 2010
	Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

Component of residue definition: MT14				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	RAM 426/01 0.1 µg/L	HPLC-MS/MS	Robinson N J., 2004/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
	Confirmatory			
Surface water	Primary	RAM 426/01 0.1 µg/L	HPLC-MS/MS	Robinson N J., 2004/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

Component of residue definition: LM3, LM5, LM6				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	GRM015.02 0.05 µg/L	HPLC-MS/MS	Zietz, E., 2009 and 2009b/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
	ILV	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		
	Confirmatory			
Surface water	Primary	GRM015.02 0.05 µg/L	HPLC-MS/MS	Zietz, E., 2009 and 2009b/ Additional Report to the DAR of Terbuthylazine, B.5

Component of residue definition: LM3, LM5, LM6				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Methods of analysis, February 2010
	Confirmatory	HPLC-MS/MS is known to be highly specific, no confirmatory method is required.		

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

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

An overview on the acceptable methods and possible data gaps for analysis of Terbuthylazine in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

Table 5.3-10: 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	Tribolet R., 1992/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Confirmatory	1 µg/m^3	GC-MSD	Tribolet R., 1992/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010
Primary	0.5 µg/m^3	GC-NPD	Schultz M., and Ullrich-Mitzel A., 1995/ Additional Report to the DAR of Terbuthylazine, B.5 Methods of analysis, February 2010

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. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Analytical methods for body fluids and human issues are not required as Terbuthylazine is not classified as toxic or very toxic.

5.3.2.8 Other studies/ information

No other studies or information.

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	Małgorzata Wołoszynowska MSc.	2018	Terbut 500 SC Method development and validation for the determination of active substance and relevant impurities content in the formulation. Institute of Industrial Organic Chemistry Study code: BA-07-18 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Weronika Dec, PhD	2018	Validation included in the study. Terbut 500 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G/286/17 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Aneta Gierbuszewska, MSc	2018a	Validation included in the study. Terbut 500 SC Terrestrial Plant Test: Vegetative Vigour Test. Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G/287/17 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Aneta Gierbuszewska, MSc	2018b	Validation included in the study. Validation included in the report: Terbut 500 SC Earthworm Reproduction Test (*Eisenia andrei*). Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G/284/17 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Magdalena Wołany,	2020	Validation included in the study.	N	Synthos Agro

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
	MSc		Terbut 500 SC Collembolan (Folsomia candida) Reproduction Test. Research Network Łukasiewicz Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G/60/19 GLP Unpublished		Sp. z o.o.
KCP 5.1.2	Patrycja Holewik MSc Eng.	2020	Validation included in the study. Terbut 500 SC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil. Research Network Łukasiewicz Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G/61/19 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Elżbieta Kulec-Płoszczyca, MSc.	2018a	Validation included in the study. Terbut 500 SC Daphnia magna, acute immobilisation test. Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W/10/18 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Elżbieta Kulec-Płoszczyca, MSc.	2018b	Validation included in the study. Terbut 500 SC Pseudokirchneriella subcapitata SAG 61.81 Growth inhibition test. Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W/11/18 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Elżbieta Kulec-Płoszczyca, MSc.	2018c	Validation included in the study. Terbut 500 SC Lemna gibba CPCC 310, Growth inhibition test. Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W/12/18 GLP	N	Synthos Agro Sp. z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.1.2	xxxx	2018	Validation included in the study. Terbut 500 SC Rainbow Trout, Acute Toxicity Test, Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W/13/18 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Dennis Janota, Msc.	2019	Validation included in the study. Terbut 500 SC Navicula pelliculosa SAG 1050-3, Growth inhibition test. Research Network Łukasiewicz Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W/53/19 GLP Unpublished	N	Synthos Agro Sp. z o.o.

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	Dieterle, R.	1993	GS 13529, Applicability of Multiresidue Method DFG S 19 for Determination of GS 13529 in Maize (Grain and Whole Plant) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 121-92	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 Not Published Syngenta File N° GS13529/1080		
KCP 5.1.2	Ferguson L.	2009	Terbuthylazine – Independent Laboratory Validation of Analytical Method No. REM 201.01 for the Determination of Terbuthylazine (GS13529) and its Metabolites GS26379 and GS28620 in Whole Maize Plants and Rape Seed Syngenta - Jealott's Hill, Bracknell, United Kingdom; Oxon Italia, S.p.A, Pero, Italy Charles River Laboratories, Edinburgh, United Kingdom, 30377 GLP Not published Syngenta File No GS13529_10121	N	Oxon/ Syngenta
KCP 5.1.2	Anon.	1987	S7 multi-method for Triazine Herbicides, DFG Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis Volume 1. Pesticides Commission. DFG Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis Volume 1. Pesticides Commission. DFG Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis Volume 1. Pesticides Commission. Published Syngenta File N° N/0862	N	-
KCP 5.1.2	Freschi G.	2002c	Validation of The Method For Residues Analysis of Terbuthylazine In Maize Samples (Grain) Research Centre "E. Gagliardini", Salerano sul Lambro, Italy Oxon Italia S.P.A, Pero, Italy Report-no. SIP1288 GLP Not published	N	Oxon
KCP 5.1.2	Freschi G.	2004	Validation Of The Multiresidue Analytical Method For Quantification Of Terbuthylazine In Maize Specimens: Grain And Silage Research Centre "E. Gagliardini", Salerano sul Lambro, Italy Oxon Italia S.P.A, Pero, Italy Report-no. SIP1431	N	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
			GLP Not published		
KCP 5.2	Anon.	1987	S7 multi-method for Triazine Herbicides, DFG Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis Volume 1. Pesticides Commission. DFG Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis Volume 1. Pesticides Commission. DFG Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis Volume 1. Pesticides Commission. Published Syngenta File N° N/0862	N	-
KCP 5.2	Ferguson L.	2009	Terbuthylazine – Independent Laboratory Validation of Analytical Method No. REM 201.01 for the Determination of Terbuthylazine (GS13529) and its Metabolites GS26379 and GS28620 in Whole Maize Plants and Rape Seed Syngenta - Jealott's Hill, Bracknell, United Kingdom; Oxon Italia, S.p.A, Pero, Italy Charles River Laboratories, Edinburgh, United Kingdom, 30377 GLP Not published Syngenta File No GS13529_10121	N	Oxon/ Syngenta
KCP 5.2	Dieterle, R.	1993	GS 13529, Applicability of Multiresidue Method DFG S 19 for Determination of GS 13529 in Maize (Grain and Whole Plant) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 121-92 GLP Not Published Syngenta File N° GS13529/1080	N	Syngenta
KCP 5.2	Luetolf, W.	1995a	Determination of residues of parent compound by gas chromatography (GC), Soil Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No REM 148.05	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 Not Published Syngenta File N° GS13529/1276		
KCP 5.2	Figueiredo, J.	2003	Determination of GS13529 (Terbuthylazine) and its metabolites GS26379, GS28620, and GS23158 in Soil by LC-MS/MS. REM 148.11. Syngenta Crop Protection AG, Basel, Switzerland, Report No REM 148.11 Not GLP Not Published Syngenta File N° GS13529/1835	N	Syngenta
KCP 5.2	Todd M.	2002a	Validation Of Methodology For The Post-Registration Monitoring Of Residues Of Terbuthylazine And Its Two Major Metabolites Desethyl Terbuthylazine And 2-Hydroxy Terbuthylazine In Soil Huntingdon Life Sciences Limited, Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 228/024125 GLP Not Published	N	Oxon
KCP 5.2	Robinson, N.	2004	Residue Analytical Method for the Determination of Residues of Terbuthylazine (GS13529), GS23158, GS26379 and GS28620 in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Jealott's Hill, United Kingdom, Report No RAM 426/01 GLP Not Published Syngenta File N° GS13529/1916	N	Syngenta
KCP 5.2	Todd M.	2002b	Terbuthylazine: Validation Of Methodology For The Determination Of Residues Of Terbuthylazine And Its Two Major Metabolites Desethyl Terbuthylazine And 2-Hydroxy Terbuthylazine In Drinking And Surface Water Huntingdon Life Sciences Limited, Cambridgeshire, UK	N	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
			Oxon Italia S.P.A, Pero, Italy Report-no. OXN 229/024126 GLP Not Published		
KCP 5.2	Zietz E.	2009	Terbuthylazine - Validation of an Analytical Method (Draft GRM015.02A) for the Determination of Residues of the Terbuthylazine Metabolites CSCD648241 and GS16984 in Groundwater, Surface Water, and Drinking Water Syngenta - Jealott's Hill, Bracknell, United Kingdom; Oxon Italia, S.p.A, Pero, Italy SGS Institut Fresenius GmbH, D-65232 Taunusstein, Germany IF 08/01259634, T000964-09 GLP Not Published Syngenta File No GS13529_10092	N	Oxon/ Syngenta
KCP 5.2	Zietz E.	2009b	Terbuthylazine: Analysis of CSCD692760 (LM3) in groundwater samples from wells with documented uses of terbuthylazine on upstream fields in Germany Syngenta - Jealott's Hill, Bracknell, United Kingdom; Oxon Italia, S.p.A, Pero, Italy SGS Institut Fresenius GmbH, D-65232 Taunusstein, Germany IF-09/01393295, T0001794-09 Not GLP Not Published Syngenta File No GS13529_10097	N	Oxon/ Syngenta
KCP 5.2	Tribolet, R.	1992	Sampling of air and determination of residues of parent compound by gas chromatography Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No REM-148-03 GLP Not Published Syngenta File N° GS13529/1057	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 5.2	Schulz M., 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 Not Published	N	Oxon

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)

A 2.1.1.1.1 Analytical method 1 – Soil (Ecotoxicology)

A 2.1.1.1.1.1 Method validation

zRMS comment
Method is accepted.

Reference:	Validation included in the following reports: (1) Weronika Dec, PhD, 2018 (2) Aneta Gierbuszewska, MSc, 2018
Report	Validation included in the reports: (1) Terbut 500 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Weronika Dec, PhD, 2018, Study code: G/286/17 (2) Terbut 500 SC Terrestrial Plant Test: Vegetative Vigour Test, Aneta Gierbuszewska, MSc, 2018, Study code: G/287/17
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentration of Terbut 500 SC in water was determined using the validated high performance liquid chromatographic method with DAD detection. The validated analytical method was performed according to SANCO/3029/99 rev.4. The aim of analytical measurements of the study was to verify the concentration of the test item in all doses test solution.

Sample preparation for the chemical determinations
A 1 mL of water sample was taken and applied to the chromatographic column in a volume of 10.0 µL. The sample was diluted with deionized water.

Conditions of the chemical determinations

Reagents and solvents:
- deionized water,
- acetonitrile for HPLC,
- orthophosphoric acid, concentrated, pure p.a.,
- 0.05% solution of orthophosphoric acid (v/v),
- Terbut 500 SC, test item, 499.2 g/L, Synthos Argo,
- standard solution 1 mg/mL of Terbut 500 SC in acetonitrile,
- working solutions of Terbut 500 SC 100.0, 20.0, 10.0, 5.0, 1.0, 0.5, 0.1 and 0.05 µg/mL in deionized water.

Apparatus:
- laboratory glassware,
- analytical balance,
- Shimadzu Prominence-i chromatograph with DAD

The following liquid chromatography parameters were used:

Column:	Kinetex 2.6µm C18 100A, l = 100 mm, ϕ = 4,6 mm
mobile phase:	acetonitrile : 0.05% solution of orthophosphoric acid (70:30, v/v)
wave length:	223 nm
flow rate:	0.4 mL/min.
injected volume:	10 µL

Results and discussions

Confirmatory method not required due to specific method to the analytes. According to SANCO/3029/99 rev. 4

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Soil	Terbuthylazine	Control	-	-	-
		0.1	102.2	1.8	-
		10.0	98.2	4.0	-

Table A 2: Characteristics for the analytical method used for validation of terbuthylazine residues in soil (Ecotoxicology)

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The working solutions of captan at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded.

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
	The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999772for captan.
Calibration range	The range of linearity of the analytical graphs are from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%). The Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise. The Limit of Quantification (LOQ) for Terbut 500 SC analyzed in water is 0.1 mg/L and the Limit of Detection (LOD) is 0.03 mg/L.

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANCO/3029/99 rev.4 and fulfil its requirements.

A 2.1.1.1.2 Analytical method 2 – Soil (Ecotoxicology)

A 2.1.1.1.2.1 Method validation

zRMS comment
Method is accepted.

Reference: Validation included in the following report:

Aneta Gierbuszewska, MSc, 2018

Report Validation included in the report: Terbut 500 SC Earthworm Reproduction Test (*Eisenia andrei*), Aneta Gierbuszewska, MSc, 2018, Study code: G/284/17

Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentration of Terbut 500 SC in artificial soil was determined using the validated high performance liquid chromatographic method with DAD detection. The validated analytical method was performed according to SANCO/3029/99 rev.4. Samples collected at the beginning, during (after 28 days) and at the end of the experiment were analysed.

Sample preparation for the chemical determinations

First, 5 mL of mixture of acetonitrile: 0.05% solution of orthophosphoric acid (70:30, v/v), was added to 10 g of artificial soil sample, shaken for 2 minute, and sonicated for 10 minutes. The sample was centrifuged and filtered through filter paper. Then extraction was repeated with 5 mL of mixture of acetonitrile: 0.05% solution of orthophosphoric acid (70:30, v/v). Finally, 10 µL of the extract was introduced into a chromatographic column. The sample was diluted with of mixture of acetonitrile: 0.05% solution of orthophosphoric acid (70:30, v/v) (if necessary).

Conditions of the chemical determinations

Reagents and solvents:

- deionized water,
- acetonitrile for HPLC,
- orthophosphoric acid, concentrated, pure p.a.,
- 0.05% solution of orthophosphoric acid (v/v),
- mixture of acetonitrile: 0.05% solution of orthophosphoric acid (70:30, v/v),
- mixture of acetonitrile : deionized water (70 : 30, v/v),
- Terbut 500 SC, test item, 499.2 g/L, Synthos Argo,
- standard solution 1 mg/mL of Terbut 500 SC in acetonitrile,
- working solutions of Terbut 500 SC 100.0, 20.0, 10.0, 5.0, 1.0, 0.5, 0.1 and 0.05 µg/mL in mixture of acetonitrile : deionized water (70 : 30, v/v).

Apparatus:

- laboratory glassware,
- analytical balance,
- laboratory shaker,
- vacuum rotary evaporator,
- Shimadzu Prominence-i chromatograph with DAD

The following liquid chromatography parameters were used:

Column:	Kinetex 2.6µm C18 100A, l = 100 mm, ϕ = 4,6 mm
mobile phase:	acetonitrile : 0.05% solution of orthophosphoric acid (70:30, v/v)
wave length:	223 nm
flow rate:	0.4 mL/min.
injected volume:	10 µL

Results and discussions

Confirmatory method not required due to specific method to the analytes. According to SANCO/3029/99 rev. 4

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Soil	Terbuthylazine	Control	-	-	-
		0.5	100.4	1.9	-
		5.0	96.2	1.3	-

Table A 4: Characteristics for the analytical method used for validation of terbuthylazine residues in soil (Ecotoxicology)

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control artificial soil samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The working solutions of captan at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9998142 for captan.
Calibration range	The range of linearity of the analytical graphs are from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%). The Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise. The Limit of Quantification (LOQ) for Terbut 500 SC analyzed in artificial soil is 0.5 mg/kg and the Limit of Detection (LOD) is 0.15 mg/kg.

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analyti-

cal methods was performed according to SANCO/3029/99 rev.4 and fulfil its requirements.

A 2.1.1.1.3 Analytical method 3 – Soil (Ecotoxicology)

A 2.1.1.1.3.1 Method validation

zRMS comment
Method is accepted.

Reference:	Validation included in the following reports: (1) Magdalena Wołany, MSc, 2020 (2) Patrycja Holewik, MSc Eng., 2020
Report	Validation included in the following reports: (1) Terbut 500 SC Collembolan (*Folsomia candida*) Reproduction Test, Magdalena Wołany, MSc, 2020, Study code: G/60/19 (2) Terbut 500 SC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, Patrycja Holewik, MSc Eng., 2020, Study code: G/61/19
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentration of TERBUT 500 SC was been chemically determined using the validated high performance liquid chromatographic method with DAD detection. The validated analytical method was performed according to SANCO/3029/99 rev.4.

Sample preparation for the chemical determinations
5 mL of mixture acetonitrile and ortho-phosphoric acid 0.05% solution (70:30; v/v) were added to 10 g of an artificial soil sample, shaken for 2 minute, and sonicated for 10 minutes. The sample was centrifuged and filtered through filter paper. The extraction was repeated with 5 mL of mixture acetonitrile and orthophosphoric acid 0.05% solution (70:30; v/v). The eluate was diluted mixture acetonitrile and orthophosphoric acid 0.05% solution (70:30; v/v), if the necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Conditions of the chemical determinations
Reagents and solvents:
- deionized water,
- acetonitrile for HPLC,
- ortho-phosphoric acid 85% pure p.a.,
- ortho-phosphoric acid 0.05% solution,
- mixture acetonitrile and deionized water (70:30; v/v),
- mixture acetonitrile and ortho-phosphoric acid 0.05% solution (70:30; v/v),
- TERBUT 500 SC, standard,

- standard solution 1 mg/mL of TERBUT 500 SC in mixture acetonitrile and deionized water (70:30; v/v),
- working solutions of TERBUT 500 SC containing 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 20.0 and 100.0 µg/mL in mixture acetonitrile and deionized water (70:30; v/v).

Apparatus:

- laboratory glassware,
- analytical balance,
- technical balance
- ultrasonic cleaner,
- laboratory centrifuge,
- Shimadzu Prominence-i chromatograph with DAD.

The following liquid chromatography parameters were used:

Column:	Kinetex 2.6µm C18 100A, l = 100 mm, ϕ = 4,6 mm
mobile phase:	acetonitrile : 0.05% solution of orthophosphoric acid (70:30, v/v)
wave length:	223 nm
oven temperature:	35°C
flow rate:	0.4 mL/min.
injected volume:	10 µL

Results and discussions

Confirmatory method not required due to specific method to the analytes. According to SANCO/3029/99 rev. 4

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Soil	Terbuthylazine	Control	-	-	-
		0.5	94.0	3.6	-
		5.0	99.6	0.3	-

Table A 6: Characteristics for the analytical method used for validation of terbuthylazine residues in soil (Ecotoxicology)

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control artificial soil samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The working solutions of captan at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control artificial soil samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
	standard) are linear with coefficient (r^2) of 0.9999549 for captan.
Calibration range	The range of linearity of the analytical graphs are from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%). The Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise. The Limit of Quantification (LOQ) for TERBUT 500 SC analyzed in artificial soil is 0.5 mg/kg and the Limit of Detection (LOD) is 0.15 mg/kg.

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANCO/3029/99 rev.4 and fulfil its requirements.

A 2.1.1.1.4 Analytical method 4 – Water (Ecotoxicology)

A 2.1.1.1.4.1 Method validation

zRMS comment
Method is accepted.

Reference: Validation included in the following reports:

(1) Elżbieta Kulec-Płoszczyca, MSc, 2018
(2) Elżbieta Kulec-Płoszczyca, MSc, 2018
(3) Elżbieta Kulec-Płoszczyca, MSc, 2018
(4) xxxx., 2018

Report Validation included in the reports:

(1) Terbut 500 SC Daphnia magna, acute immobilisation test, Elżbieta Kulec-Płoszczyca, MSc, 2018, Study code: W/10/18
(2) Terbut 500 SC Pseudokirchneriella subcapitata SAG 61.81 Growth inhibition test, Elżbieta Kulec-Płoszczyca, MSc, 2018, Study code: W/11/18
(3) Terbut 500 SC Lemna gibba CPCC 310, Growth inhibition test, Elżbieta Kulec-Płoszczyca, MSc, 2018, Study code: W/12/18
(4) Terbut 500 SC Rainbow Trout, Acute Toxicity Test, xxxx., 2018, Study code: W/13/18

Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of the analytical part of the definitive test was to determine the concentrations of the test item using a validated liquid chromatographic method with DAD detection.

Sample preparation for the chemical determinations

Each sample in volume between 10 and 100 mL (i.e. control sample, test sample, sample fortified with standard) was applied to ENVI-18 column (3 mL, 500 mg), which was conditioned previously by washing twice with 5 mL of acetone, 5 mL of methanol and twice with 10 mL of deionised water, pH ≤ 2 (acidified HCl). Following the sample introduction the column was dried for 5 minutes by vacuum. The part of sample with affinity to the column was eluted with 10 mL of acetone, 10 mL of methanol. Eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was dissolved in mixture acetonitrile: deionized water (70:30, v/v) and 10 µL was applied to chromatographic column.

Conditions of the chemical determinations

Reagents and solvents:

- deionized water,
- deionized water, pH ≤ 2 (acidified HCl),
- acetone, pure p.a.,
- methanol, pure p.a.,
- acetonitrile for HPLC,
- hydrochloric acid, concentrated, pure p.a.,
- orthophosphoric acid, concentrated, pure p.a.,
- 0.05% solution of orthophosphoric acid (v/v),
- mixture of acetonitrile : deionized water (70 : 30, v/v),
- Terbut 500 SC was used as a standard, Synthos Argo,
- standard solution 1 mg/mL of Terbut 500 SC in acetonitrile,
- working solutions of Terbut 500 SC 100.0, 20.0, 10.0, 5.0, 1.0, 0.5, 0.1 and 0.05 µg/mL in mixture of acetonitrile : deionized water (70 : 30, v/v).

Apparatus:

- laboratory glassware,
- analytical balance,
- ENVI-18 column (3 mL, 500 mg),
- SPE-12G system,
- Shimadzu Prominence-i chromatograph with DAD

The following liquid chromatography parameters were used:

Column:	Kinetex 2.6 µm C18 100A, l = 100 mm, ϕ = 4,6 mm
mobile phase:	acetonitrile : 0.05% solution of orthophosphoric acid (70:30, v/v)
wave length:	223 nm
flow rate:	0.4 mL/min.
injected volume:	10 µL

Results and discussions

Confirmatory method not required due to specific method to the analytes. According to SANCO/3029/99 rev. 4

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	Terbuthylazine	Control	-	-	-
		0.001	91.4	6.2	-
		1.0	92.8	1.0	-

Table A 8: Characteristics for the analytical method used for validation of terbuthylazine residues in water Ecotoxicology)

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The working solutions of captan at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999142 for captan.
Calibration range	The range of linearity of the analytical graphs are from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%). Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise.

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated.
	The Limit of Quantification (LOQ) for Terbut 500 SC analyzed in water is 0.001 mg/L and the Limit of Detection (LOD) is 0.0003 mg/L.

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANCO/3029/99 rev.4 and fulfil its requirements.

A 2.1.1.1.5 Analytical method 5 – Water (Ecotoxicology)

A 2.1.1.1.5.1 Method validation

zRMS comment
Method is accepted.

Reference:	Validation included in the following report: Dennis Janota, Msc, 2019
Report	Terbut 500 SC Navicula pelliculosa SAG 1050-3, Growth inhibition test, Dennis Janota, Msc, 2019, Study code: W/53/19
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of the analytical part of the definitive test was to determine the concentrations of the test The aim of the analytical part of the definitive test was determine concentrations of the test item TERBUT 500 SC using validated high performance liquid chromatographic method with DAD detection. The validated analytical method was performed according to SANCO/3029/99 rev.4.

Sample preparation for the chemical determinations
Each sample of 10 - 100 mL volume was acidified by hydrochloric acid to pH ≤ 2 and applied to ENVI C18 (3 mL, 500 mg) column conditioned previously by sequential washing twice with 5 mL of acetone, twice with 5 mL of methanol and twice of 5 mL of deionised water pH≤2. Following the sample introduction the column was dried under vacuum for 5 minutes. The analytes were eluted with twice times of 5 mL acetone and twice times of 5 mL methanol. The eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was redissolved in mixture acetonitrile and deionized water (70:30; v/v).

An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Conditions of the chemical determinations

Reagents and solvents:

- deionized water,
- acetonitrile for HPLC,
- methanol pure p.a.,
- acetone pure p.a.,
- deionized water pH≤2 (acidified hydrochloric acid),
- hydrochloric acid 35-38% pure,
- ortho-phosphoric acid 85% pure p.a.,
- ortho-phosphoric acid 0.05% solution,
- mixture acetonitrile and deionized water (70:30; v/v),
- ENVI C18 column (3 mL, 500 mg),
- TERBUT 500 SC, standard,
- standard solution 1 mg/mL of TERBUT 500 SC in mixture acetonitrile and deionized water (70:30; v/v),
- working solutions of TERBUT 500 SC containing 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 20.0 and 100.0 µg/mL in mixture acetonitrile and deionized water (70:30; v/v).

Apparatus:

- laboratory glassware,
- analytical balance,
- SPE-12G system,
- laboratory vacuum rotary evaporator with water bath,
- Shimadzu Prominence-i chromatograph with DAD.

The following liquid chromatography parameters were used:

Column:	Kinetex 2.6 µm C18 100A, l = 100 mm, ϕ = 4,6 mm
mobile phase:	acetonitrile : 0.05% solution of orthophosphoric acid (60:30, v/v)
wave length:	223 nm
oven temperature:	35°C
flow rate:	0.4 mL/min.
injected volume:	10 µL

Results and discussions

Confirmatory method not required due to specific method to the analytes. According to SANCO/3029/99 rev. 4

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	Terbuthylazine	Control	-	-	-
		0.001	104.6	1.6	-
		1.0	104.0	0.8	-

Table A 10: Characteristics for the analytical method used for validation of terbuthylazine residues in water Ecotoxicology)

	Terbuthylazine
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The working solutions of captan at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999549 for captan.
Calibration range	The range of linearity of the analytical graphs are from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%). The Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise. The Limit of Quantification (LOQ) for TERBUT 500 SC analyzed in water is 0.001 mg/L and the Limit of Detection (LOD) is 0.0003 mg/L.

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANCO/3029/99 rev.4 and fulfil its requirements.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted