

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: TERBUT 500 SC

Product name(s): La Zina 500 SC; Tekno 500 SC

Chemical active substance:

Terbuthylazine, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: PUH Chemirol Sp. z o.o.

Submission date: November 2019

MS Finalisation date: December 2021, June 2022

Version history

When	What
December 2021	Finalisation of the assessment by zRMS-PL.
June 2022	Final Version after Commenting period

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

PPP (product name/code):	TERBUT 500 SC	Formulation type:	SC ^(a, b)
Active substance 1:	terbuthylazine	Conc. of as 1:	500 g/l ^(c)
Active substance 2:	-	Conc. of as 2:	^(c)
Active substance 3:	-	Conc. of as 3:	^(c)
Safener:	-	Conc. of safener:	^(c)
Synergist:	-	Conc. of synergist:	^(c)
Applicant:	PUH Chemirol Sp. z o.o.	Professional use:	<input checked="" type="checkbox"/>
Zone(s):	central ^(d)	Non professional use:	<input type="checkbox"/>
Verified by MS:	yes/ no		

Field of use: herbicide

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use- No. ^(e)	Member state(s)	Crop and/ or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I	Pests or Group of pests controlled (additionally: developmen- tal stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/synergist per ha ^(f)
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		

Zonal uses (field or outdoor uses, certain types of protected crops)													
1	PL,CZ	Maize (ZEAMX)	F	Dicotyledonous weeds	Spray, medium sprayer	Spring BBCH 12-16	a)1 b)1	n/a	a) 1.0 l/ha b) 1.0 l/ha	a) 0.5 kg a.s./ha b) 0.5 kg a.s./ha	200-400	n/a	
1	PL,CZ	Maize (ZEAMX)	F	Dicotyledonous weeds	Spray, medium sprayer	Spring BBCH 00-05	a)1 b)1	n/a	a) 1.0 l/ha b) 1.0 l/ha	a) 0.5 kg a.s./ha b) 0.5 kg a.s./ha	200-400	n/a	
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)													
3													
4													
Minor uses according to Article 51 (zonal uses)													
5													
6													
Minor uses according to Article 51 (interzonal uses)													
7													
8													

Remarks table heading:

(a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
 (b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
 (c) g/kg or g/l

(d) Select relevant
 (e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
 (f) No authorization possible for uses where the line is highlighted in grey. Use should be crossed out when the notifier no longer supports this use.

Remarks columns:

1 Numeration necessary to allow references
 2 Use official codes/nomenclatures of EU Member States
 3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)
 4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
 5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named.
 6 Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated.

7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
 8 The maximum number of application possible under practical conditions of use must be provided.
 9 Minimum interval (in days) between applications of the same product
 10 For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products.
 11 The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
 12 If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
 13 PHI - minimum pre-harvest interval
 14 Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

zRMS comment:

The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the ZRMS are presented in grey commenting boxes. Minor changes are introduced directly as text in blue. Not agreed or not relevant information is struck through and shaded for transparency.

The COMMISSION IMPLEMENTING REGULATION (EU) 2021/824 for a.s. **terbuthylazine**, should be taken into account by MSs in the evaluation of the zonal registration of the product.

COMMISSION IMPLEMENTING REGULATION (EU) 2021/824 of 21 May 2021 amending Implementing Regulations (EU) No 540/2011 and (EU) No 820/2011 as regards the conditions of approval of the active substance terbuthylazine.

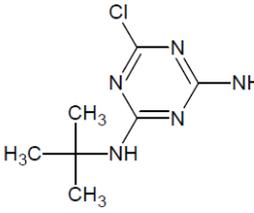
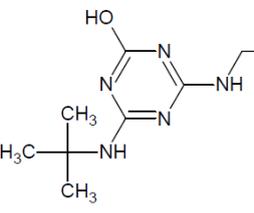
For the implementation of the uniform principles, as referred to in Article 29(6) of Regulation (EC) No 1107/2009, the conclusions of the review report on terbuthylazine, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 17 June 2011 and updated by the Standing Committee on Plants, Animals, Food and Feed on 24 March 2021 shall be taken into account.

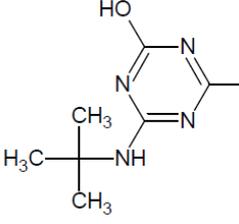
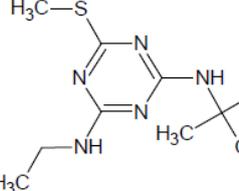
In this overall assessment Member States shall pay particular attention to:

- the consumer risk assessment from exposure to metabolites of terbuthylazine,
- the protection of groundwater, when the active substance is applied in regions with vulnerable soil and/or climatic conditions,
- the risk to mammals and earthworms.

Conditions of use shall include risk mitigation measures and the obligation to carry out monitoring programmes to verify potential groundwater contamination in vulnerable zones, where appropriate

9.1.1.1 Table 9.1-3 Metabolites of Terbuthylazine

9.1.1.2 Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Risk assessment required?
MT1 desethyl-terbuthylazine (GS 26379)	201.7		Soil (lab): max 25.1% AR Maximum occurrence observed in sediment/water studies: 7.3 %	Yes
MT13 Hydroxy-terbuthylazine Or 2-hydroxy terbuthylazine GS 23158	211.3		Soil (Lab): max 34.5% AR Maximum occurrence observed in sediment/water studies: 20.0 %	Yes

9.1.1.2 Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Risk assessment required?
MT14 desethyl-hydroxyterbutylazine or desethyl-2-hydroxy terbutylazine GS 28620	183.2		Soil (Lab): mx 1.7% AR Maximum occurrence observed in sediment/water studies: N/A (soil metabolite only)	Yes
MT26	241.4		Maximum occurrence observed in sediment/water studies: 7.4%	Yes

9.1.1.3 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

TERBUT 500 SC pose no unacceptable risk to birds ~~mammals~~ with according to the label.
 TERBUT 500 SC pose no unacceptable risk for mammals only for pre-emergence application.
 Further refinement is required for post emergence application to mammals.

9.1.1.4 Effects on aquatic organisms (KCP 10.2)

Based on the predicted rates of TERBUT 500 SC in aquatic species, the TER values describing the risk for aquatic species following exposure to TERBUT 500 SC according to the GAP of the formulation TERBUT 500 SC achieve the acceptability criteria PEC/RAC<1 with applying:

- 5 m vegetated buffer zone

9.1.1.5 Effects on bees (KCP 10.3.1)

All hazard quotients (HQ) are considerably less than 50, indicating that TERBUT 500 SC applied at the maximum use rate in maize poses low risk to bees. According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees) the Applicant should provide the chronic test on adult bees and chronic test for larvae for formulated product.

Thus, concerned Member States must decide on the consideration of data requirements and the risk assessment at national level.

9.1.1.6 Effects on arthropods other than bees (KCP 10.3.2)

All hazard quotients (HQ) are considerably less than 2, indicating that TERBUT 500 SC applied at the maximum use rate in cereals winter poses no risk to non-target arthropods. No risk mitigation needed.

9.1.1.7 Effects on non-target soil meso- and macrofauna (KCP

10.4), Effects on soil microbial activity (KCP 10.5)

The long term risk to earthworms and other non-target soil organisms (meso- and macrofauna) was assessed as low for TERBUT 500 SC in a first-tier risk assessment. But a potential high risk was indicated on the long-term time scale for earthworms, but based on Risk refinement for terbuthylazine it can be concluded that application of formulation TERBUT 500 SC is unlikely to pose a long term risk to earthworms and other non-target soil organisms (meso- and macrofauna).

The Predicted Environmental Concentrations of the formulation TERBUT 500 SC and its active substance terbuthylazine in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed after 28 days or more of exposure, indicating that the proposed use of TERBUT 500 SC poses an acceptable risk to soil microorganisms.

9.1.1.8 Effects on non-target terrestrial plants (KCP 10.6)

Based on the predicted rates of TERBUT 500 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to TERBUT 500 SC according to the GAP of the formulation TERBUT 500 SC achieve the acceptability criteria $TER \geq 5$ with applying:

- 5 m buffer zone or
- 1 m and use of 75% drift reducing nozzles

9.1.1.9 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

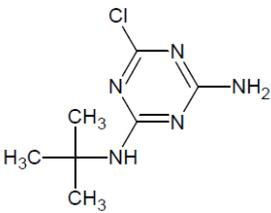
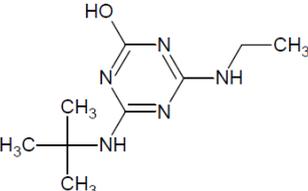
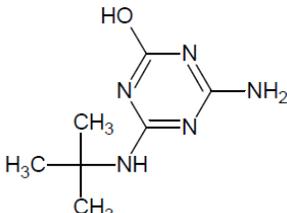
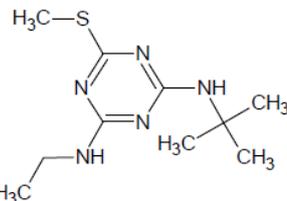
Table 9.1-2: Critical use pattern of TERBUT 500 SC grouped according to crop, application rate, number of applications, timing, etc.

Grouping according to crop, application rate, number of applications, timing criterion			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
1	Maize BBCH 00 500 g as/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of TERBUT 500 SC is indicated in the table.

Table 9.1-3 Metabolites of Terbutylazine

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Risk assessment required?
MT1 desethyl-terbutylazine (GS 26379)	201.7		Soil (lab): max 25.1% AR Maximum occurrence observed in sediment/ water studies: 7.3 %	Yes
MT13 Hydroxy-terbutylazine Or 2-hydroxy terbutylazine GS 23158	211.3		Soil (Lab): max 34.5 % AR Maximum occurrence observed in sediment/ water studies: 20.0 %	Yes
MT14 desethyl-hydroxyterbutylazine or desethyl-2-hydroxy terbutylazine GS 28620	183.2		Soil (Lab): mx 1.7% AR Maximum occurrence observed in sediment/ water studies: N/A (soil metabolite only)	Yes
MT26	241.4		Maximum occurrence observed in sediment/ water studies: 7.4 %	Yes

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with terbutylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of TERBUT 500 SC were not evaluated as part of the EU assessment of terbutylazine.

However, the provision of further data on the TERBUT 500 SC is not considered essential, because studies from Annex I inclusion can be used in Annex I inclusion.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Terbutylazine	Oral 1 d Acute	LD50 = 1236 mg a.s./kg bw	EFSA Journal 2011; 9(1):1969
Mallard duck	Terbutylazine	Dietary	LC50 > 395	EFSA Journal 2011;

Species	Substance	Exposure System	Results	Reference
		8 d Short-term	mg a.s./kg bw/d	9(1):1969
Japanese quail	Terbutylazine	Dietary Reproductive toxicity	NOEL = 13.85 mg a.s./kg bw/d	EFSA Journal 2011; 9(1):1969

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for birds from all other intended uses in groups maize (see 9.1.2).

9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of TERBUT 500 SC in maize

Intended use						
Active substance/product		terbutylazine				
Application rate (g/ha)		1 × 500				
Acute toxicity (mg/kg bw)		1236				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Growth stage						
Screening step	Small omnivorous bird	158.8	1.0	79.40	15.6	
Reprod. toxicity (mg/kg bw/d)		13.85				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}	
Growth stage						
Screening step	Small omnivorous bird	64.8	0.53	17.17	0.8	
Bare soil BBCH < 10	Small granivorous bird “finch” Small seeds 100% weed seeds	11.4	-	-	4.6	
Bare soil BBCH < 10	Small insectivorous bird “wag-tail” ground invertebrates without interception 100% soil dwelling invertebrates	5.9	-	-	8.9	
Bare soil BBCH < 10	Small omnivorous bird “lark” Combination (ground invertebrates without interception) 50% seeds, 50% ground arthropods	8.2	-	-	6.4	

Maize BBCH 10 - 19	Small insectivorous bird “wag-tail” ground invertebrates without interception 50% ground arthropods, 50% foliar arthropods	11.3	-	-	4.6
Maize BBCH 10 - 29	Medium granivorous bird "gamebird" Small seeds 100% seed	3.0	-	-	17.4
Maize BBCH 10 - 29	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves	22.7	-	-	2.3
Maize BBCH 10 - 29	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	-	-	4.8
Maize Leaf development BBCH 10 to 19	Small insectivorous/worm feeding species “thrush” ground invertebrates without interception 100% soil dwelling invertebrates	5.7	-	-	9.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Based on TIER I assessment TERBUT 500 SC pose unacceptable long term risk for
 - pigeon at BBCH 10-29
 - lark at BBCH 10-29
 - finch at BBCH <10
 - wagatail at BBCH 10-19

Risk refinement is necessary. Therefore, please see to point 9.2.2.2

9.2.2.2 Higher-tier risk assessment

Refined assessment of long-term risk for pigeon (BBCH 10-29), lark (BBCH 10-29), finch (BBCH <10) and wagatail (BBCH 10-19) is based generally on:

- New value of RUD
- Diet composition
- Deposition factor

Refined RUD and DT50:

According to DAR Terbutylazine Vol.3 Annex B.9 the field residue studies provided by notifier – eleven studies from Balkar, 2006 and two studies from Lucini, 2008 - show realistic value of residue unite dose, dissipation time in maize, weeds and invertebrates. According to this studies, DT50 was reduced to 2.8 days and ftwa reduce to 0.19 for maize. Also, new value of RUD are provided: 34.5 mg/kg for maize, 24.5 mg/kg for weeds and 1.93 mg/kg for invertebrates. Therefore, new value of RUD, DT50 and ftWA are using in risk refinement are given below:

Exposure source	Mean RUD (mg/kg)	DT ₅₀ (days)	f _{twa}
Maize	34.5	2.8	0.19
Weeds	24.5	10	0.53
Invertebrates	1.93	10	0.53

Refined Deposition factor:

According to Appendix A of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) it is assumed that the voles are feeding on 100% foliage and no account is given to interception. However, leaf cover in treated crop is estimated to intercept approximately 25% for maize according to Appendix E of the EFSA Guidance Document, quoting FOCUS 2001¹ and 2005² and FOCUS GW³. Therefore, the risk assessment has been refined by applying a deposition factor of 0.75.

Risk refinement for finch:

According to APPENDIX A of EFSA Journal 2009; 7(12):1438 the FIR/bw's value equal 0.28. Therefore, this value and new value of RUD for weeds was used in risk refinement for finch.

Table 9.2-3: Higher-tier assessment of the long term risk for finch due to the terbuthylazine use of TERBUT 500 SC in maize

Intended use	Maize							
Active substance/product	Terbuthylazine							
Application rate (g/ha)	1 X 500							
Reprod. toxicity (mg/kg bw/d)	13.85 mg/kg bw/d							
TER criterion	5							
Focal species	Food category, % in diet	FIR/bw_a	RUD_a	MAF_m × TWA	PT	F_{twa}	DDD_m (mg/kg bw/d)	TER_t
Common shrew	weeds seeds, 100%	0.28	24.5	0.53	1	0.53	1.82	7.61

PD: proportion of food item in the diet; RUD: residues per unit dose; MAF: multiple application factor; F_{twa}: time weighted average factor; PT: proportion of diet obtained in the treated area; DDD: daily dietary dose; TER: toxicity exposure ratio

^a EFSA default values

Risk refinement for pigeon:

The woodpigeon *Columba palumbus* is a widespread and common or abundant species in agricultural and forested landscapes, and partly also in urban areas.

¹ FOCUS (Forum for the Co-ordination of pesticide fate models and their Use), 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.

² FOCUS (Forum for the Co-ordination of pesticide fate models and their Use), 2005. A Comparison of Crop Interception values in FOCUS Ground Water and Surface Water Scenarios1.

³ FOCUS (2014) "Assessing Potential for Movements of Active Substances and their Metabolites to Ground Waters in the EU " - Report of the FOCUS Ground Water Work Group, Version 3 of 10 October 2014. EC Document Reference Sanco/13144/2010 version 3, 613pp

Woodpigeons feed on a wide range of plant material, with seeds or green leaves dominating, depending on season. Seeds from newly sown cereal, pea or rape fields and all types of grain from stubble fields are apparently preferred when available. The summer diet is highly variable and may include up to 5% invertebrates (Christensen et al. 1996⁴). Ljunggren (1968)⁵ studied adult crop contents in a rural population of woodpigeons in SW Sweden. The results are presented as percentage of food items by number are given below:

Time of year	Food type	% of food items
Jan – Apr	Plant leaves	52
	Cereal grain	46
	Weed seeds	2
	Rape seeds	1
	Peas	1
May – Aug	Rape seeds	28
	Cereal grain	26
	Peas	16
	Weed seeds	15
	Plant leaves	13
Sep – Nov	Cereal grain	68
	Peas	12
	Plant leaves	9
	Rape seeds	7
	Weed seeds	3

According to Appendix G of EFSA/2009/1438 ratios of daily intake rate to body weight 480g based on given diet composition was calculated:

Food category	PD (% diety)	FE (kJ/ dry g)	MC (%)`	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Rape seeds	28	21.7	9.9	0.76	4.16	-	10.03	0.02
Cereal grain	26	18.4	14.7	0.76	3.10	-	9.31	0.02
Peas	16	21.7	9.9	0.76	2.38	-	5.73	0.01
Weed seeds	15	21.7	9.9	0.76	2.23	-	5.37	0.01
Plant leaves	13	17.6	76.4	0.53	0.29	-	4.66	0.01
Total	-	-	-	-	12.15	435.25	-	-

The estimated daily exposure, i.e. the uptake of a compound via a single food item:

Table 9.2-4: Higher-tier assessment of long-term exposure for woodpigeon due to the use TERBUT 500 SC

Intended use		maize							
Active substance		terbuthylazine							
Application rate (g/ha)		500							
Reprod. toxicity (mg/kg bw/d)		13.85							
TER criterion		5							
Crop	Food category, % in diet	FIR/bw	RUD	DF	MAF _m × TWA	Dose (kg	DDD _m (mg/kg bw/d)	DDD _m (mg/kg bw/d)	TER _{LT}

⁴ Christensen, K.D., Falk, K. & Petersen, B.S. 1996: Feeding Biology of Danish Farmland Birds. Working Report No. 12 1996, Danish Environmental Protection Agency.

⁵ Ljunggren, L. 1968. Seasonal studies of Wood Pigeon populations. I. Body weight, feeding habits, liver and thyroid activity. Viltrevy 5: 435-504.

						s.a./ha)		sum	
maize BBCH 12-16	Rape seeds, 28%	0.02	28.7	0.75	0.53	0.5	0.12	0.41	34.2
	Cereal grain, 26%	0.02	15.0	0.75	0.53		0.06		
	Peas, 16%	0.01	28.7	0.75	0.53		0.07		
	Weed seeds, 15%	0.01	28.7	0.75	0.53		0.06		
	Plant leaves, 13%	0.01	54.2	0.75	0.53		0.10		

Risk refinement for wagatail:

According to APPENDIX A of EFSA Journal 2009; 7(12):1438 the wagatail’s diet combine from 50% ground arthropods and 50% foliar arthropods. Assume the wagatail’s diet include from 100% arthropod. Therefore, the FIR/bw’s value of 0.79 and new value of RUD for arthropod was used in risk refinement for wagatail.

Table 9.2-5: Higher-tier assessment of the long term risk for wagatail due to the terbuthylazine use of TERBUT 500 SC in maize

Intended use	Maize							
Active substance/product	Terbuthylazine							
Application rate (g/ha)	1 X 500							
Reprod. toxicity (mg/kg bw/d)	13.85 mg/kg bw/d							
TER criterion	5							
Focal species	Food category, % in diet	FIR/bw_a	RUD_a	MAF_m × TWA	PT	Ftwa	DDD_m (mg/kg bw/d)	TER_t
Yellow wagatail	Arthropods	0.79	1.93	0.53	1	0.53	0.40	35

PD: proportion of food item in the diet; RUD: residues per unit dose; MAF: multiple application factor; Ftwa: time weighted average factor; PT: proportion of diet obtained in the treated area; DDD: daily dietary dose; TER: toxicity exposure ratio

Risk refinement for lark:

According to Appendix G of EFSA/2009/1438 ratios of daily intake rate to body weight of 28.5 for skylark based on given diet composition in APPENDIX A of EFSA Journal 2009; 7(12):1438 :

Food category	PD (% dietary)	FE (kJ/dry g)	MC (%)`	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Ground arthropods	50	19.4	84.3	76	1.16	-	7.84	0.27
Weed seeds	25	21.7	9.9	80	3.92	-	3.92	0.15
Crop leaves	25	17.6	76.4	76	0.79	-	3.92	0.14
Total	-	-	-	-	5.87	103.6	-	-

The estimated daily exposure, i.e. the uptake of a compound via a single food item:

Table 9.2-6: Higher-tier assessment of long term exposure for skylark due to the use of TERBUT 500 SC

Intended use	maize
Active substance	terbuthylazine
Application rate (g/ha)	500

Reprod. toxicity (mg/kg bw/d)		13.85							
TER criterion		5							
Crop Growth stage	Food category, % in diet	FIR/bw	RUD	DF	MAF_m × TWA	Dose (kg s.a./ha)	DDD_m (mg/kg bw/d)	DDD_m (mg/kg bw/d) sum	TER_{LT}
maize BBCH 12-16	Ground arthropods, 50%	0.27	1.93	0.75	0.53	0.5	0.10	1.17	11.8
	Weed seeds, 25%	0.15	24.5	0.75	0.53		0.73		
	Crop leaves, 25%	0.14	34.5	0.75	0.19		0.34		

zRMS comments:

The risk assessment provided by the Applicant was evaluated and verified by zRMS.

Based on the Tier 1 risk assessment for birds not all of the long-term TER values are greater than the trigger value of 5 indicating potential long-term risk to birds from terbuthylazine for the proposed use.

The scenarios which indicate a potential risk and require further consideration are given below:

Pre - emergence application < 10 BBCH:

- at 0.50 kg a.s./ha :Small granivorous bird “finch” Small seeds 100% weed seeds

Post -emergence application 10-14 BBCH:

- At 0.50 kg a.s./ha: Small omnivorous bird (“lark”) in maize (BBCH 10-29)
- At 0.50 kg a.s./ha: Medium herbivorous/granivorous bird (“pigeon”) in maize (BBCH 10-29)
- At 0.50 kg a.s./ha: Small insectivorous bird (“wagtail”) in maize (BBCH 10-19)

Refined Long-term Risk Assessment

The scenarios which did not pass the tier 1 risk assessment and require refinement are detailed in Table below:

Scenarios requiring refinement of long-term risk (TER_{LT}) to birds.

Active substance	Formulation	Application rate (g a.s./ha)	Scenario	
			Crop / growth stage	Generic focal species / diet
Terbuthylazine		500	Maize < 10 BBCH	Small granivorous bird “finch” Small seeds 100% weed seeds
Terbuthylazine		500	Maize BBCH 10-29	Medium herbivorous/granivorous bird “pigeon” Non-grass herbs 100% leaves
Terbuthylazine		500	Maize BBCH 10-29	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods

Terbuthylazine		500	Maize BBCH 10-19	Small insectivorous bird “wagtail” Ground invertebrates without interception 50% ground arthropods, 50% foliar arthropods
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Refined long-term risk assessment for birds the a.s.-terbulathozine

1. Identification of relevant focal species and PT value for birds

Based on the results from generic field study evaluated in the DAR (Wolf, 2005 for the a.s.-terbulathozine) conducted in Austria from pre-drilling to BBCH growth stage of 14 for maize (2 April – 20 May 2004) the focal species was identified in maize by RMS.

As the study started some weeks before the drilling and was completed when maize reached BBCH 14 growth stage this assessment time period spans the time window in which terbuthylazine is recommended for use for Terbut 500 SC.

In this study, the main species observed in the drilled maize fields was skylark (39% of all species).

The skylark is a widespread species species in the CEU in arable crops; it consumes predominantly plant material and insects. Due to its small size and a relatively high FIR/bw the exposure to the skylark is likely to represent a worst case for the proposed use.

The second species most abundant in drilled maize fields was common pheasant (16% of all species).

However, as pheasants are omnivorous the risk to this species will be covered by evaluation performed for skylark, which is also omnivorous, but represents worst case in terms of bodyweight.

The next species was pied wagtail (15% of all species). Another species was common kestrel (8% of all species), which is the predator, so the risk is covered in ZRMS opinion by evaluation of the risk of secondary poisoning. Carrion crow represented 7% of all observed species. This species is also omnivorous, so evaluation performed for skylark covers risk for carrion crow, similarly as in case of pheasants.

Remaining species (barn swallow, collared dove and lapwing) represented less than 5% of all observed species, but the risk to these species will be covered by evaluation performed for skylark and wood pigeon. No granivorous birds were observed in study by Wolf (2005).

As no granivorous birds were observed in study by Wolf (2005) confirming that this species is not relevant for drilled maize fields.

According to the Terbuthylazine Addendum to Additional Report B.9 (June 2010) the skylark is deemed to be a key focal species for both insectivorous and herbivorous birds feeding on early post-emergence maize, as the evidence in relation to the levels of exposure on plant and insects indicates potentially much higher residue levels in plant materials. Therefore the use of skylark as a focal species is considered acceptable by the ZRMS for the risk assessment.

Besides skylark, the EFSA Conclusion for terbuthylazine (EFSA Journal 2011; 9(1):1969) proposed to

consider the medium herbivorous birds in the risk assessment because it cannot be excluded that the risk to medium herbivorous birds is not covered by the risk assessment to skylark being a small omnivorous bird. For the current evaluation refinement of the long risk assessment to birds for post emergence application three species were considered: skylark, wood pigeon and wagtail.

2. PT values:

For all three species the default PT value of 100% is used in the risk assessment.

3. Proportion of food type in the diet (PD)

Dietary information on skylarks are available from the generic field study of Wolf (2005), provided in Austria relevant for the intended uses in Central in pre- and early post-emergence maize fields based on faecal analysis (see Table below).

Diet composition of skylarks for pre- and early post-emergence maize according to Wolf (2005) for skylark.

Feed item	Fraction in the diet [%] ^{a)}
Animals (mainly ground-dwelling invertebrates)	81
Seeds (mainly weed seeds)	16.4
Foliage (mainly weeds)	2.6

^{a)} based on volume reported from faecal analysis

The ZRMS accepts the diet given by Wolf (2005) for skylark diet feeding in maize, when Terbut 500 SC is recommended for use (consist of seeds, foliage and invertebrates).

According to the data on skylark's diet from the terbuthylazine DAR, Vol. 3 Annex B.9 (2007) the proportions of the diet components vary, therefore both a mixed diet and a single diet item (e.g. 100% foliage) will be considered in the subsequent risk assessment.

Wood pigeon

The ZRMS calculated the exposure based on diet for wood pigeon -100% leaves.

As presented above, for Tier 1 assessments, the medium herbivorous wood pigeon is conservatively assumed to only feed from green plant material which bears the highest residue levels.

Wagtail

The default tier 1 assessment diet will be used in the risk assessment i.e. 50% ground dwelling invertebrates without interception and 50% foliar arthropods.

4. Refinements of residues in maize plants and insects

In the Terbuthylazine Final Addendum to the Additional Report (September, 2010), a revised RUD for maize plants was calculated for terbuthylazine and the metabolite MT1 (desethyl-terbuthylazine). As MT1 was not measured in the residue studies, the formation of MT1 in whole plants (i.e. 2.0%) in the plant metabolism study was used. The estimated residue of MT1 was added to the mean residue of terbuthylazine to calculate a RUD for parent and MT1. The arithmetic mean initial residue from the reported residue trials including the maximum formation of MT1 was 31.9 mg/kg (standardised to 1 kg a.s./ha).

Also in the Terbutylazine Final Addendum to the Additional Report (September, 2010), the Ftwa value used for maize is 0.19 derived from a residue trial where the DT₅₀ value of 2.8 days was determined (Lucini, 2008).

The mean residue value on insects was also reported in the Terbutylazine Final Addendum to the additional Report (September, 2010), where a RUD of 1.93 mg a.s./kg was determined in a field trial by Bakker (2006).

Refined reproductive risk assessments for birds exposed to terbuthylazine.

Long term TER values after refinement for skylark for pre and post emergence application.

Feed item	Fraction in diet (PD _i)	FIR _{i, total} fresh [g fresh weight/d]	Application Rate [kg a.s./ha]	RUD of feed item [mg a.s./kg]	Deposition factor (DF)	MAF	f _{TWA}	PT	Body weight [g]	DDD/ETE [mg/kg bw/day]
Maize – Pre- and post-emergence use										
Small omnivorous scenario (BBCH 10-29) – skylark										
Maize shoots (grasses and cereal shoots)	0.45	21.02*	0.50	31.9	1	1	0.19	1.0	37.2*	0.76
Weed foliage (non grass herbs)	0.05			24.5			0.53			0.18
Weed seeds	0.20			24.5			0.53			0.72
Insects (arthropods)	0.30			1.93			0.53			0.083
NOAEL [mg a.s./kg bw/d]		13.85 (Tier 1)								
TER		7.95								

*values from the DAR for TBT

Refined TER values for selected focal species for wood pigeon

For the risk assessment all the previously mentioned refinements have been considered for each of the focal species.

Wood pigeon:

Long term TER values after refinement for the wood pigeon.

Crop	Maize
Scenario	BBCH 10-29
Application rate (kg a.s./ha)	0.50
FIR/bw	0.79
Diet	leaves
PD	1
Mean RUD (mg a.s./kg)	31.9*
Ftwa	0.19
PT	1
DDD (mg a.s./kg)	2.39

NOEC	13.85
TER	5.8
TER trigger	5

*According to EFSA 2011

For the wood pigeon the TER value was above trigger value of 5 indicating an acceptable risk for wood pigeon.

Wagtail:

Long term TER values after refinement for the wagtail.

Crop	Maize	
Scenario	BBCH 10-29	
Application rate (kg a.s./ha)	0.5	
FIR/bw	0.79	
Diet	Ground dwelling insects without interception	Foliar dwelling Insects
PD	0.5	0.5
Mean RUD (mg a.s./kg)	1.93	21.0*
Ftwa	0.53	0.53
DDD (mg a.s./kg)		
PT	1.0	
DDDsum (mg a.s./kg)	2.39	
NOEC	13.85	
TER	5.8	
TER trigger	5	

DDD_{sum} is calculated by summing the individual DDD for the components and multiplying by the PT.

* based on worst case RUD values (EFSA appendix F)

Overall conclusion for long-term risk to birds:

A series of refinements for the long-term risk assessment of terbuthylazine were considered: relevant focal species, dissipation data, FIR and residues. The all TER_{LT} values were above trigger of 5 indicating acceptable risk for birds.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

Leaf scenario

Since TERBUT 500 SC is not a product for spray applications / not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 151, terbuthylazine belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for birds from all other intended uses in groups maize (see 9.1.2).

Effective application rate (g/ha) =	500		
Acute toxicity (mg/kg bw) =	1236	quotient =	0.40
Reprod. toxicity (mg/kg bw/d) =	13.85	quotient =	36

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of terbuthylazine amounts to 3.4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for birds from all other intended uses in groups maize (see 9.1.2).

Table 9.2-7: Assessment of the risk for earthworm-eating birds due to exposure to terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

Parameter	terbuthylazine	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.5726	
log P_{ow} / P_{ow}	3.4/ 2512	
K _{oc}	151	
foc	0.02	Default
BCF _{worm}	10.26	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw}) = (0.84 + 0.12 × P_{ow}) / foc × K _{oc}

Parameter	terbutylazine	comments
PEC _{worm}	5.87	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	6.17	DDD = PEC _{worm} × 1.05
NOEL (mg/kg bw/d)	13.85	
TER _{It}	2.24	

TER values shown in bold fall below the relevant trigger.

Risk refinement for risk for earthworm-eating mammals due to exposure to Terbutylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

To address this risk, the joint Notifiers have submitted an earthworm bioaccumulation study (Batscher 2007) to measure more realistic body burdens within earthworms from the proposed uses. Therefore, a new refined risk assessment has been performed using this measured BAF (0.86) in place of the BCF calculated in the TIER I assessment.

Table 9.2-8: Risk refinement for risk for earthworm-eating mammals due to exposure to Terbutylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

Parameter	Terbutylazine	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.5726	EFSA Journal 2011; 9(1):1969
log P _{ow} / P _{ow}	3.4/2512	Confirmatory Data Terbutylazine November 2015
Koc	151	Confirmatory Data Terbutylazine November 2015
foc	Organic carbon content of soil (0.02 taken as a default value)	Default
BAF	0.86	DAR Terbutylazine Volume 3 B9 2010
PEC _{worm}	0.4924	PEC _{worm} = PEC _{soil} × BAF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.5170	DDD = PEC _{worm} × 1.05
NOEL (mg/kg bw/d)	13.85	EFSA Journal 2011; 9(1):1969
TER _{It}	26.79	Above trigger value 5

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of terbutylazine in water.

Table 9.2-9: Assessment of the risk for fish-eating birds due to exposure terbutylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize

Parameter	terbutylazine	comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0001404	FOCUS STEP 4 D4 Pond
BCF _{fish}	34	EFSA Journal 2011; 9(1):1969
BMF	not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.004774	PEC _{fish} = PEC _{water} × BCF _{fish}

Parameter	terbuthylazine	comments
Daily dietary dose (mg/kg bw/d)	0.0007590	DDD = $PEC_{\text{fish}} \times 0.159$
NOEL (mg/kg bw/d)	13.85	EFSA Journal 2011; 9(1):1969
TER _{tr}	18 248	

TER values shown in bold fall below the relevant trigger.

zRMS comments:

The risk assessment risk for fish-eating birds and earthworms-eating birds due to exposure terbuthylazine via bioaccumulation in fish (secondary poisoning) and in earthworms for the intended use in maize is considered as acceptable.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

TERBUT 500 SC pose no unacceptable to mammals with according to the label.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report (new studies).

However, the provision of further data on the formulation TERBUT 500 SC is not considered essential, because the selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Terbuthylazine	Acute	LD ₅₀ = 1000 mg/kg bw	EFSA Journal 2011; 9(1):1969
Rat	Terbuthylazine	Long-term	NOAEL = 3.3 mg/kg bw/d	EFSA Journal 2011; 9(1):1969

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for mammals from all other intended uses in groups maize (see 9.1.2).

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of TERBUT 500 SC in maize

Intended use					
Active substance/product	terbuthylazine				
Application rate (g/ha)	1 × 500				
Acute toxicity (mg/kg bw)	1000				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Screening step	Small herbivorous mammal	136.4	1.0	68.20	14.7
Reprod. toxicity (mg/kg bw/d)	3.3				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Screening step	Small herbivorous mammal	72.3	0.53	19.16	0.17
Bare soil BBCH < 10	Small omnivorous mammal “mouse” Combination (ground invertebrates without interception) 50% weed seeds, 50% ground arthropods	5.7	-	-	2.2
Maize BBCH 10 - 19	Small insectivorous mammal “shrew” ground dwelling invertebrates without interception 100% ground arthropods	4.2	-	-	3.0
Maize BBCH 10 -29	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass	72.3	-	-	0.2
Maize BBCH 10 -29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	-	-	1.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Based on TIER I assessment TERBUT 500 SC pose unacceptable long term risk for

- mouse at BBCH <10
- shrew at BBCH 10-19
- vole at BBCH 10-29
- mouse at BBCH 10-29

Risk refinement is necessary. Therefore, please see to point 9.3.2.2

9.3.2.2 Higher-tier risk assessment

Refined assessment of long-term risk for mouse (BBCH<10), shrew (BBCH 10-19), vole (BBCH 10-29) and mouse (10-29) is based generally on :

- new value of RUD
- focal species
- diet composition
- deposition factor.

Refined RUD and DT50:

According to DAR Terbutylazine Vol.3 Annex B.9 the field residue studies provided by notifier – eleven studies from Balkar, 2006 and two studies from Lucini, 2008 - show realistic value of residue unit dose, dissipation time in maize, weeds and invertebrates. According to this studies, DT50 was reduced to 2.8 days and f_{twa} reduce to 0.19 for maize. Also, new value of RUD are provided: 34.5 mg/kg for maize, 24.5 mg/kg for weeds and 1.93 mg/kg for invertebrates. Therefore, new value of RUD, DT50 and f_{twa} are using in risk refinement are given below:

Exposure source	Mean RUD (mg/kg)	DT ₅₀ (days)	f_{twa}
Maize	34.5	2.8	0.19
Weeds	24.5	10	0.53
Invertebrates	1.93	10	0.53

Focal species:

According to Additional Report to the DAR a generic field data (Wolf, 2005), based on monitoring/trapping of mammals and radio-tracking of wood mice in maize growing farmland area, are available. To identify the mammal species that use plain fields, maize and sugar beet fields as part of their natural home range, live trapping of small mammals with mark-recapture was conducted. Based on this data it was determined that vole and shrew were not species occurring regularly in maize during pre-emergence and early post emergence and that Wood mouse is the relevant focal species for these exposure scenarios. Therefore, wood mouse is presented as the focal species in the refined risk assessment for terbutylazine.

Also, according to the DAR, the hare was deemed appropriate as the focal species for the proposed uses of terbutylazine, because the hare occurred within maize field during mammal monitoring study.

Refined Deposition Factor:

According to Appendix A of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) it is assumed that the voles are feeding on 100% foliage and no account is given to interception. However, leaf cover in treated crop is estimated to intercept approximately 25% for maize according to

Appendix E of the EFSA Guidance Document, quoting FOCUS 2001⁶ and 2005⁷ and FOCUS GW⁸. Therefore, the risk assessment has been refined by applying a deposition factor of 0.75.

Refined risk for mouse:

Radio-tracking studies of wood mice caught in arable land have been conducted in the UK and are reported by Prosser (2010) and Additional Report to the DAR. The PT for wood mice for the proposed uses of terbuthylazine on pre-emergence/early post-emergence maize (i.e. bare soil; BBCH < 10). The results of this study indicate the 90th percentile PT of wood mice in newly drilled cereals fields (i.e. bare soil) to be 0.51 (data for consumers only). According to Appendix G of EFSA/2009/1438 ratios of daily intake rate to body weight 21.7 g for mouse based on given diet composition in APPENDIX A of EFSA Journal 2009; 7(12):1438 are below for BBCH 00-05:

Food category	PD (% dietary)	FE (kJ/dry g)	MC (%)`	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Arthropods	50	19.4	84.3	87	1.30	-	4.75	0.22
Weed seed	50	21.7	9.9	84	8.25	-	4.75	0.22
Total	-	-	-	-	9.55	58.83	-	-

Table 9.3-3 Higher-tier assessment of the long term risk for mouse due to the terbuthylazine use of CHR/H/TERIZ in maize

Intended use	Maize							
Active substance/product	Terbuthylazine							
Application rate (g/ha)	1 X 500							
Reprod. toxicity (mg/kg bw/d)	3.3 mg/kg bw/d							
TER criterion	5							
Focal species	Food category, % in diet	FIR/bw_a	RUD_a	MAF_m × TWA	PT^b	Ftwa	DDD_m (mg/kg bw/d)	TER_t
<i>Wood mice</i>	Weed seeds, 50 %	0.22	24.5	0.53	0.51	0.53	0.3642	
	Ground arthropods, 50%	0.22	1.93				0.02869	
	whole diet						0.3929	8.40

PD: proportion of food item in the diet; RUD: residues per unit dose; MAF: multiple application factor; Ftwa: time weighted average factor; PT: proportion of diet obtained in the treated area; DDD: daily dietary dose; TER: toxicity exposure ratio

^a EFSA default values

^b Based on radio tracking work by Prosser (2010)

According to Appendix G of EFSA/2009/1438 ratios of daily intake rate to body weight 21.7 g for mouse based on given diet composition in APPENDIX A of EFSA Journal 2009; 7(12):1438 are below for BBCH 12-16:

⁶ FOCUS (Forum for the Co-ordination of pesticide fate models and their Use), 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.

⁷ FOCUS (Forum for the Co-ordination of pesticide fate models and their Use), 2005. A Comparison of Crop Interception values in FOCUS Ground Water and Surface Water Scenarios1.

⁸ FOCUS (2014) "Assessing Potential for Movements of Active Substances and their Metabolites to Ground Waters in the EU " - Report of the FOCUS Ground Water Work Group, Version 3 of 10 October 2014. EC Document Reference Sanco/13144/2010 version 3, 613pp

Food category	PD (% diety)	FE (kJ/ dry g)	MC (%)`	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Arthropods	25	19.4	84.3	87	0.65	-	2.38	0.11
Weed seeds	50	21.7	9.9	84	8.25	-	4.75	0.22
Weeds	25	17.6	76.4	47	0.48	-	2.36	0.11
Total	-	-	-	-	9.38	58.83	-	-

¹ For risk assessment puposes, the insect larvae are assumed to be picked from the ground.

² Mono- or dicotyledonous, depending on the crop. Here considered as weeds.

Table 9.3-4 Higher-tier assessment of the long term risk for mouse due to the therbutylazine use of TERBUT 500 SC in maize

Intended use	Maize							
Active substance/product	Terbutylazine							
Application rate (g/ha)	1 X 500							
Reprod. toxicity (mg/kg bw/d)	3.3 mg/kg bw/d							
TER criterion	5							
Focal species	Food category, % in diet	FIR/bw _a	RUD _a	MAF _m × TWA	PT ^b	Ftwa	DDD _m (mg/kg bw/d)	TER _{it}
<i>Wood mice</i>	Weed seeds, 50 %	0.22	24.5	0.53	0.51	0.53	0.3642	
	Ground arthro- pods, 25%	0.11	1.93				0.007173	
	Weeds, 25%	0.11	24.5				0.09106	
	whole diet					0.4624	7.14	

PD: proportion of food item in the diet; RUD: residues per unit dose; MAF: multiple application factor; Ftwa: time weighted average factor; PT: proportion of diet obtained in the treated area; DDD: daily dietary dose; TER: toxicity exposure ratio

^a EFSA default values

Refined risk to shrew:

According to APPENDIX A of EFSA Journal 2009; 7(12):1438 the FIR/bw's value equal 0.55. Therefore, this value and refined vale of RUD for arthropod was used in risk refined for shrew.

Table 9.3-5 Higher-tier assessment of the long term risk for shrew due to the terbutylazine use of TERBUT 500 SC in maize

Intended use	Maize							
Active substance/product	Terbutylazine							
Application rate (g/ha)	1 X 500							
Reprod. toxicity (mg/kg bw/d)	3.3 mg/kg bw/d							
TER criterion	5							
Focal species	Food category, % in diet	FIR/bw _a	RUD _a	MAF _m × TWA	PT	Ftwa	DDD _m (mg/kg bw/d)	TER _{it}
Common shrew	Ground arthro- pods, 100%	0.55	1.93	0.53	1	0.53	0.2813	11.73

PD: proportion of food item in the diet; RUD: residues per unit dose; MAF: multiple application factor; Ftwa: time weighted average factor; PT: proportion of diet obtained in the treated area; DDD: daily dietary dose; TER: toxicity exposure ratio

^a EFSA default values

Refined risk to vole – focal species refinement.

According to Additional Report to the DAR a generic field data (Wolf, 2005), based on monitoring/trapping of mammals and radio-tracking of wood mice in maize growing farmland area, are available. To identify the mammal species that use plain fields, maize and sugar beet fields as part of their natural home range, live trapping of small mammals with mark-recapture was conducted. Based on this data it was determined that vole and shrew were not species occurring regularly in maize during pre-emergence and early post emergence and that Wood mouse is the relevant focal species for these exposure scenarios. Therefore, wood mouse is presented as the focal species in the refined risk assessment for terbuthylazine. Risk assessment for mouse is provided above.

Also, according to the DAR, the hare was deemed appropriate as the focal species for the proposed uses of terbuthylazine, because the hare occurred within maize field during mammal monitoring study. The hare's diet consists of 90% maize and 10% weeds. Therefore, daily intake and FIR/bw calculation was based on these values and are presented below

Calculation of daily intake and FIR/bw ratio for a hare of body mass 3800 g; scenario post-emergence application of formulation in maize at stage of BBCH 12 – 16

Food category	PD (% diety)	FE (kJ/dry g)	MC (%)`	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Maize	90	17.6	76.4	47	1.76	-	1109.04	0.29
Weeds	10	17.8	88.1	76	0.16	-	123.23	0.03
Total	-	-	-	-	1.92	2363.44	-	-

According to Appendix G of EFSA/2009/1438 ratios of daily intake rate to body weight 21.7 g for mouse based on given diet composition below

Table 9.3-6: Higher-tier assessment of long-term exposure for hare due to the use of TERBUT 500 SC

Intended use		maize								
Active substance		terbuthylazine								
Application rate (g/ha)		1 x 500								
Acute toxicity (mg/kg bw/d)		1000								
TER criterion		10								
Crop	Food category, % in diet	FIR/bw	RUD	DF	Dose (kg s.a./ha)	DDD _m (mg/kg bw/d)	DDD _m (mg/kg bw/d) sum	TER _{LT}		
maize BBCH 12-16	Maize, 90%	0.29	103.2	0.25	0.5	3.73	4.59	218		
	Weeds, 10%	0.03	70.3	0.75		0.86				
Intended use		maize								
Active substance		terbuthylazine								
Application rate (g/ha)		1 x 500								
Reprod. toxicity (mg/kg bw/d)		3.3								
TER criterion		5								
Crop	Food category, % in diet	FIR/bw	RUD	DF	MAF _m × TWA	Dose (kg s.a./ha)	DDD _m (mg/kg bw/d)	DDD _m (mg/kg bw/d) sum	PT	TER _{LT}

maize BBCH 12-16	Maize, 90%	0.29	34.5*	0.25	0.19	0.5	0.24	0.40	1	8.3
	Weeds, 10%	0.03	24.5*	0.75	0.53		0.16			

* According to DAR Terbutylazine Vol.3 Annex 9

zRMS comments:

The risk assessment provided by the Applicant was evaluated and verified by zRMS.

Based on the Tier 1 risk assessment for mammals not all of the long-term TER values are greater than the trigger value of 5 indicating potential long-term risk to birds from terbuthylazine for the proposed use. The scenarios which indicate a potential risk and require further consideration are given below:

Pre- emergence application < 10 BBCH:

- at 0.50 kg a.s./ha: Small omnivorous mammal “mouse” Combination (ground invertebrates without interception) 50% weed seeds, 50% ground arthropods

Post -emergence application 10-14 BBCH:

- At 0.50 kg a.s./ha: Small insectivorous mammal “shrew” ground dwelling invertebrates without interception 100% ground arthropods
- At 0.50 kg a.s./ha: Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass
- At 0.50 kg a.s./ha: Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods

Refined Long-term Risk Assessment

The scenarios which did not pass the tier 1 risk assessment and require refinement are detailed in Table below:

Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Bare soil BBCH < 10	Small omnivorous mammal “mouse” Combination (ground invertebrates without interception) 50% weed seeds, 50% ground arthropods	5.7	-	-	2.2
Maize BBCH 10 - 19	Small insectivorous mammal “shrew” ground dwelling invertebrates without interception 100% ground arthropods	4.2	-	-	3.0
Maize BBCH 10 -29	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass	72.3	-	-	0.2
Maize BBCH 10 -29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	-	-	1.6

zRMS provided below the risk assessment in the context of the risk assessment according to EFSA guidance, which requires separate assessment for pre- and post-emergence scenarios.

1. Identification on focal species.

Based on the a generic field data (Wolf, 2005) for mammalian monitoring study for maize the vole and shrew were not species occurring regularly in maize during pre-emergence and early post emergence. It was concluded that that wood mouse is the relevant focal species for these exposure scenarios.

Therefore, wood mouse is presented as the focal species in the refined risk assessment for terbuthylazine.

Pre-emergence application:

RUD refinement:

In case of RUD for seeds only for pre - emergence application we are in the opinion that PEC_{soil} values can be also used alternatively as a surrogate for RUD for seeds max PEC_s=0.667 mg a.s./kg dws for terbuthylazine), since any seeds present at the time of pre-emergence will be buried in the soil and not directly exposed to product application.

The refinement with the mean measured residue value – 1.93 mg a.s./kg insects- from the field study by Bakker, 2006 proposed by the applicant was accepted by the zRMS.

The results of the higher-tier assessment for the omnivorous mammal wood mouse at pre-emergence application of maize indicate acceptable risk, with TER_{LT} well above the trigger, as showed in the tables below.

Higher-tier assessment of the long-term/reproductive risk for wood mouse due to the use of Terbut in maize – BBCH 00-05.

Intended use		Maize – BBCH 00-09								
Active substance/product		Terbuthylazine								
Application rate (g/ha)		1 × 500								
Reprod. toxicity (NOAEL, mg/kg bw/d)		3.3								
TER criterion		5								
Crop scenar- io Growth stage	Generic focal spe- cies	Diet		FIR /bw	Mean RUD	MAF_m × TWA	PT	DDD	DDD_{sum}	TER_{LT}
Bare soil BBCH < 10	Small omnivorous “mouse”	50% weed seeds	PD =0.5	0.24	40.2/0.667**	0.53	1	1.27*/0.021**	1.33* /0.08**	2.48*/ 41.25**
		50% ground arthropods	PD =0.5	0.24	1.93	0.53		0.06		

*Diet from GD for Northern Zone

**RUD refinement

The TER_{LT} value was above 15 for pre-emergence scenario indicated an acceptable risk to mammals.

The risk is considered acceptable for pre-emergence application for wood mouse.

Post-emergence application:

For post emergence application the following approach was considered by zRMS :

1. Focal species - wood mouse
2. PT for wood mouse - 0.44 based on Wolf, 2005 study (90th percentile) as a worst case scenario
3. RUD for maize foliage - 31.9 mg a.s./kg (the value from DAR)
4. RUD for invertebrates - 1.93 mg a.s./kg

ZRMS verified the risk provided by the applicant and presented own risk assessment with consideration of the refined parameter presented above in the Table below:

Higher-tier assessment of the long term risk for mouse due to the terbuthylazine use of PT.

For the intended early post-emergence use (maize), the data for diet in May from Pelz study (1989) appear to be most relevant. The dietary composition of wood mice as reported by Pelz (1989) for these months are summarised in Table below:

Diet composition of wood mice for arable land according to Pelz (1989).

Month	Feed item	Fraction in the diet [%] ^{a)}
April	Insect larvae (considered as ground-dwelling invertebrates)	45
	Earthworms	26
	Vegetative plant tissue (considered as weeds)	24
	Cereal grain (considered as large seeds)	5
May	Earthworms	40
	Cereal grain (considered as large seeds)	30
	Vegetative plant tissue (considered as weeds)	16
	Insect larvae (considered as ground-dwelling invertebrates)	10
	Dicot seeds (considered as weed seeds)	4
June	Earthworms	9
	Cereal grain (considered as large seeds)	32
	Vegetative plant tissue (considered as weeds)	9
	Insect larvae (considered as ground-dwelling invertebrates)	25
	Dicot seeds (considered as weed seeds)	25

a) based on volume of stomach contents

Therefore, zRMS provided further refinement for wood mouse with consideration the diet given in Pelz (1989) study.

Table: Refined reproductive risk assessments for wood mouse by Pelz 1989- terbuthylazine.

Feed item	Fraction in diet (PD _i)	FIR _{i, total} fresh [g fresh weight/d]	Application Rate [kg a.s./ha]	RUD of feed item [mg a.s./kg]	Deposition factor (DF)	MAF	f _{TWA}	PT	Body weight [g]	DDD/ETE [mg/kg bw/day]
Maize – Post-emergence use (May)										
Small omnivorous scenario (BBCH 10-29) – wood mouse										
Inv.	0.10	8.99	0.5	1.93	1	1	0.53	0.44	21.7	0.02
Worms	0.40			0.492			1.0			0.017
Weeds (maize foliage)	0.16			31.9			0.19			0.076
Seeds	0.30			40.2			0.53			0.076
										Sum=0.77*

Weed seeds	0.04									
NOAEL [mg a.s./kg bw/d]		3.3								
TER _{LT}		4.23								

1) FIR/bw=0.41
 2) BCF=0.86 x 21 PEC_{twa}

The TER_{LT} value is below the trigger of 5 for wood mouse when 90% percentile is used, indicated **needs for further refinement for wood mouse.**

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).

With a K(f)_{oc} of 151, terbuthylazine belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for mammals from all other intended uses in groups maize (see 9.1.2).

Effective application rate (g/ha) = 500		
Acute toxicity (mg/kg bw) = 1000	quotient	= 0.5
Reprod. toxicity (mg/kg bw/d) = 3.3	quotient	= 152

With a K(f)_{oc} of 151, Terbuthylazine belongs to the group of less sorptive substances. Since the ratio of effective application rate (500 g/ha) to relevant endpoint (3.3 mg/kg bw/d) exceeds the critical value of 50 for at least one use scenario, a quantitative risk assessment (calculation of TER values) is necessary and presented in Table

Table 9.3-7 Assessment of the risk for mammals due to exposure to Terbuthylazine via contaminated drinking water in puddles

Intended use		Maize			
Active substance		Terbuthylazine			
Application rate (g/ha)		1 × 500			
Reprod. toxicity (mg/kg bw/d)		3.3			
TER criterion		5			
Soil-relevant applic. rate (g/ha)	K _{oc} (L/kg)	PEC _{puddle} (mg/L)	DW uptake (L/kg bw/d)	Daily dose (mg/kg bw/d)	TER _{it}
500	151	0.2028	0.24	0.04867	68

PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below

the relevant trigger.

PEC puddle calculated with equation:

$$PEC_{puddle} = \frac{AR/10}{1000(w + Koc \times s)}$$

where:

- AR = application rate [g/ha]; divisor of 10 to achieve rate in mg/m²
 w = 0.02 (pore water term; volume)
 s = 0.0015 (soil term: volume, density, organic carbon content)

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of terbuthylazine amounts to 3.4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for mammals from all other intended uses in groups maize (see 9.1.2).

Table 9.3-8: Assessment of the risk for earthworm-eating mammals due to exposure to terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

Parameter	terbutylazine	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.5726	
log P _{ow} / P _{ow}	3.41/2512	
Koc	151	Addendum to DAR 2015
foc	0.02	Default
BCF _{worm}	10.26	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw}) = (0.84 + 0.012 × P _{ow}) / foc × Koc
PEC _{worm}	5.87	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	7.51	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	3.3	
TER _{It}	0.43	

TER values shown in bold fall below the relevant trigger.

Risk refinement for risk for earthworm-eating mammals due to exposure to Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

To address this risk, the joint Notifiers have submitted an earthworm bioaccumulation study (Batscher 2007) to measure more realistic body burdens within earthworms from the proposed uses. Therefore, a new refined risk assessment has been performed using this measured BAF (0.86) in place of the BCF calculated in the TIER I assessment.

Table 9.3-9 Risk refinement for risk for earthworm-eating mammals due to exposure to Terbutylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

Parameter	Terbutylazine	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.5726	EFSA Journal 2011; 9(1):1969
log P _{ow} / P _{ow}	3.4/2512	Confirmatory Data Terbutylazine November 2015
Koc	151	Confirmatory Data Terbutylazine November 2015
foc	Organic carbon content of soil (0.02 taken as a default value)	Default
BAF	0.86	DAR Terbutylazine Volume 3 B9 2010
PEC _{worm}	0.4924	PEC _{worm} = PEC _{soil} × BAF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.6303	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	3.3	EFSA Journal 2011; 9(1):1969
TER _{lt}	5.24	Above trigger value 5

zRMS comment:

A bioconcentration factor for earthworms determined experimentally give a BCF of 0.86 value (EU Additional report to the Terbutylazine DAR 2010, reference Bättscher 2007).

The risk for earthworm-eating mammals due to exposure to terbutylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize based on this value is considered an acceptable.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of terbutylazine in water.

Table 9.3-10: Assessment of the risk for fish-eating mammals due to exposure to terbutylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize

Parameter	terbutylazine	comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0001404	FOCUS STEP 4 D4 Pond
BCF _{fish}	34	EFSA Journal 2011; 9(1):1969
BMF	not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.004774	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.0006779	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	3.3	EFSA Journal 2011; 9(1):1969
TER _{lt}	4 868	

TER values shown in bold fall below the relevant trigger.

zRMS comment:

The risk for fish-eating mammals due to exposure to terbuthylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize is considered an acceptable.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

TERBUT 500 SC pose no unacceptable to mammals with according to the label.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

Not required.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, Effects on aquatic organisms of TERBUT 500 SC were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. The selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

Table 9.5.1-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Terbuthylazine and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Terbuthylazine	96 h, static	LC ₅₀ = 2.2 mg a.s./L _{mm}	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	Terbuthylazine	90 d (flow-through)	Early life cycle NOEC = 0.09 mg a.s./L _{mm}	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	Metabolite MT1 (GS 26379, desethyl-terbuthylazine)	96 hr (static)	LC ₅₀ = 18 mg a.s./L _{mm}	EFSA Journal 2011; 9(1):1969
Oncorhynchus	Metabolite MT13	96 hr (static)	LC ₅₀ > 2.5mg a.s./L	EFSA Journal 2011;

Species	Substance	Exposure System	Results	Reference
mykiss	(GS 23158, 2-hydroxy-terbutylazine)		mm	9(1):1969
Daphnia magna	Terbutylazine	48 hr	EC ₅₀ = No definitive endpoint available ²	EFSA Journal 2011; 9(1):1969
Daphnia magna	Terbutylazine	21 d (semi-static)	Reproduction, NOEC = 0.019 mg a.s./L _{mm}	EFSA Journal 2011; 9(1):1969
Daphnia magna	Metabolite MT1 (GS 26379, desethyl-terbutylazine)	48 h (static)	EC ₅₀ =42 mg a.s./L _{mm}	EFSA Journal 2011; 9(1):1969
Daphnia magna	Metabolite MT13 (GS 23158, 2-hydroxy-terbutylazine)	48 h (static)	EC ₅₀ >2.8 mg a.s./L _{mm}	EFSA Journal 2011; 9(1):1969
Chironomus riparius	Terbutylazine	27 d (static)	nomNOEC (water phase)= 0.5 mg a.s./L	EFSA Journal 2011; 9(1):1969
Chironomus riparius	Metabolite MT13 (GS 23158, 2-hydroxy-terbutylazine)	28 d (static)	nomNOEC (sediment phase)= 400 mg/kg (sediment)	EFSA Journal 2011; 9(1):1969
Chironomus riparius	Metabolite MT26 (GS 14260, terbutryn)	28 d (static)	nomNOEC (sediment phase)= 16 mg/kg (sediment)	EFSA Journal 2011; 9(1):1969
Blue green algae (Microcystis aeruginosa)	Terbutylazine	72 h (static)	E _r C ₅₀ = 0.102 mg a.s./L E _b C ₅₀ = 0.016 mg a.s./L	EFSA Journal 2011; 9(1):1969
Pseudokirchneriella subcapitata	Terbutylazine	72 h (static)	E _r C ₅₀ = 0.028 mg a.s./L E _b C ₅₀ = 0.012 mg a.s./L	EFSA Journal 2011; 9(1):1969
Selenastrum capricornutum	Metabolite MT1 (GS 26379, desethyl-terbutylazine)	72 h (static)	E _r C ₅₀ = 0.38 mg a.s./L E _b C ₅₀ = 0.14 mg a.s./L	EFSA Journal 2011; 9(1):1969
Desmodesmus subspicatus	Metabolite MT13 (GS 23158) 2-hydroxy-terbutylazine)	72 h (static)	E _b C ₅₀ > 3.96 mg a.s./L	EFSA Journal 2011; 9(1):1969
Selenastrum capricornutum	Metabolite MT13 (GS 23158) 2-hydroxy-terbutylazine)	72 h (static)	E _r C ₅₀ >3.8 mg/L	EFSA Journal 2011; 9(1):1969
Pseudokirchneriella subcapitata	Metabolite MT26(GS 14260) terbutryn)	72 h (static)	E _r C ₅₀ = 0.0036 mg a.s./L E _b C ₅₀ = 0.0017 mg a.s./L	EFSA Journal 2011; 9(1):1969
Lemna gibba	Terbutylazine	14 d (static)	Fronnd number: nom E _m C ₅₀ = 0.0128 mg	EFSA Journal 2011; 9(1):1969

Species	Substance	Exposure System	Results	Reference
			a.s./L Growth rate: nom ErC50=0.412 mg a.s./L Biomass: nom EbC50= 0.0133 mg a.s./L	
Lemna gibba	Metabolite MT26 (GS 14260, terbutryn)	14 d (static)	FronD density: mm EC50=0.025 mg/L	EFSA Journal 2011; 9(1):1969
Myriophyllum aquaticum	Metabolite MT26 (GS 14260, terbutryn)	14 d (static)	Root fresh weight: nom EC50=2.0 mg/kg (sediment)	EFSA Journal 2011; 9(1):1969
Higher-tier studies (micro- or mesocosm studies)				
Higher tier data are available, but insufficient information is currently available to derive an endpoint.				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations;
 im: based on initial measured concentrations

²As discussed in Section B.9.2.4.3.1 of the DAR no definitive acute toxicity endpoint was derived from the submitted aquatic invertebrate studies as neither of the submitted studies used a suitable method to determine the amount of terbuthylazine in solution. However, the studies were considered to be of adequate quality to clearly demonstrate that terbuthylazine is of less toxicity to aquatic invertebrates than other aquatic species and therefore the risk assessment for fish is deemed to cover the aquatic invertebrate risk assessment.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – TERBUT 500 SC

Species	Substance	Exposure System	Results	Reference
Daphnia magna	TERBUT 500 SC	48 h, s	EC50 = 177.9 mg/L _{nom}	E. Kulec-Płoszczyca, Study code: W/10/18, 2018
Pseudokirchneriella subcapitata	TERBUT 500 SC	72 h, s	ErC50 = 0.1815 mg/L _{nom} EyC50 = 0.0251 mg/L _{nom}	E. Kulec-Płoszczyca, Study code: W/11/18, 2018
Navicula pelliculosa	TERBUT 500 SC	72h, s	ErC50 = 0.02mg/L EyC50 = 0.007mg/L	D. Jenota, Study Code: W/53/19, 2019
Lemna gibba	TERBUT 500 SC	7 d, s	FronD number: ErC50 = 0.1583 mg/L EyC50 = 0.0991 mg/L Dry weight: ErC50= 0.0902 mg/L EyC50= 0.0522 mg/L	E. Kulec-Płoszczyca, Study code:W/12/18, 2018
Higher-tier studies (micro- or mesocosm studies)				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance

with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for aquatic organisms from all other intended uses in groups maize (see 9.1.2).

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 9.5.2-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Terbutylazine for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>	<i>Lemna Gibba</i>	<i>Naviculla sp.</i>
Endpoint (µg/L)		LC ₅₀ 2200	NOEC 90	EC ₅₀ N/A	NOEC 19	EbC50 12	NOEC 500	EfnC50 12.8	ErC50* 10
AF		100	10	100	10	10	10	10	10
RAC (µg/L)		22	9	-	1.9	1.2	50	1.28	1
Exposure	PEC _{gl-max} (µg/L)								
Step 1									
	143.33	6.515	15.93	-	75.44	119.44	2.87		143.33
Step 2									
	28.19	1.28	3.13	-	14.84	23.49	0.5638	22.02	28.19
Step 3									
D3/ditch	2.623	0.1192	0.2914	-	1.38	2.186	0.05246	2.05	2.623
D4/pond	0.1470	0.00668	0.01633	-	0.07737	0.1225	0.00294	0.1148	0.1470
D4/stream	2.250	0.1023	0.25	-	1.1842	1.1875	0.045	1.758	2.250
D5/pond	0.1529	0.00695	0.01699	-	0.08047	0.1274	0.003058	0.1195	0.1529
D5/stream	2.255	0.1025	0.2506	-	1.1868	1.879	0.0451	1.76	2.255

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Algae
D6/ditch	2.631	0.1196	0.292	-	1.385	2.193	0.0526	2.055	2.631
R1/pond	0.2214	0.01006	0.0246	-	0.1165	0.1845	0.004428	0.1730	0.2214
R1/stream	6.948	0.3158	0.772	-	3.657	5.79	0.1390	5.428	6.948
R2/stream	5.317	0.2417	0.5908	-	2.798	4.43	0.1063	4.15	5.317
R3/stream	2.564	0.1165	0.2849	-	1.35	2.14	0.0513	2.00	2.564
R4/stream	17.37	0.7895	1.93	-	9.14	14.48	0.3474	13.57	17.37

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
 *formulation study(expresses in a.s.)

zRMS comment:

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for Daphnia magna (long-term risk), algae and Lemna gibba for several FOCUS Steps 1-3 scenarios. In addition for Navicula sp . the risk was provided by zRMS based on RAC=1.0 µg a.s./L. Therefore, further PEC/RAC ratios were calculated for the most sensitive organism for the a.s. Pseudokirchnella. subcapitata as characterised by an EC₅₀ for species of 12 µg/L in connection with an assessment factor of 10) based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies. In addition for Navicula sp the calculations were provided for RAC of 1.0 µg/L obtained from formulation study (expressed in a.s. units).

For the intended uses not, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for Pseudokirchn. subcapitata as characterised by an EC₅₀ for species of 12 µg/L in connection with an assessment factor of 10) in FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on risk mitigation in FOCUS Step 4 PECSW considering reduced exposure of surface water bodies.

Table 9.5.2-2: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Terbutylazine based on FOCUS Step 4 calculations and toxicity data for most sensitive species Pseudokirchn. Subcapitata and *Navicula sp.* with mitigation of spray drift and run-off for the use of TERBUT 500 SC in crop (maize)

Intended use		maize	maize
Active substance		Terbutylazine	Terbutylazine
Application rate (g/ha)		1 × 500	1 × 500
Nozzle reduction	No-spray buffer (m)	5m VFSmod	5m VFSmod
	Vegetated filter strip (m)	5m VFSmod	5m VFSmod
None	D3/ditch	0.8597	0.8597
None	D4/pond	0.1446	0.1446
None	D4/stream	0.9494	0.9494
None	D5/pond	0.1416	0.1416
None	D5/stream	0.9586	0.9586
None	D6/ditch	0.8669	0.8669
None	R1/pond	0.09461	0.09461
None	R1/stream	0.7632	0.7632
None	R2/stream	1.015	1.015
None	R3/stream	1.080	1.080
None	R4/stream	0.7631	0.7631
RAC (µg/L)			RAC (µg/L)
1.2		PEC/RAC ratio	1.0* PEC/RAC ratio
None	D3/ditch	0.7164	0.7164

None	D4/pond	0.1205	0.1205
None	D4/stream	0.7912	0.7912
None	D5/pond	0.1180	0.1180
None	D5/stream	0.7988	0.7988
None	D6/ditch	0.7224	0.7224
None	R1/pond	0.07884	0.07884
None	R1/stream	0.6360	0.6360
None	R2/stream	0.8458	0.8458
None	R3/stream	0.9000	0.9000
None	R4/stream	0.6359	0.6359

*formulation study for Navicula sp.

zRMS comment:

The PEC/RAC ratios were calculated for the most sensitive organism for a.s.- Pseudokirchn. subcapitata as characterised by an EC₅₀ for species of 12 µg/L in connection with an assessment factor of 10) and for species Navicula sp. with RAC =0.1 µg/L based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies.

Based on FOCUS STEP 4 calculations for scenarios: D3/ditch, D4/stream, D5/stream, D6/ditch, R1/stream, R2/stream, R3/stream

R4/stream to protect aquatic organism **5 meter vegetative buffer zone should be applied to surface water bodies.**

The final risk mitigation measures should be considered at MSs level.

Table 9.5.2-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite (MT1 – desethyl-terbuthylazine) of Terbuthylazine for each organism based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize

MT1 – desethyl-terbuthylazine								
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>	<i>Chironomus riparius</i>	<i>Lemna Gibba</i>
Endpoint (µg/L)		LC ₅₀ 18000	-	EC ₅₀ 42000	-	EbC ₅₀ 140	-	-
AF		100	-	100	-	10	-	-
RAC (µg/L)		180	-	420	-	14	-	-
FOCUS Scenario	PEC ^{gl-max} (µg/L)							
Step 1								
	69.93	0.3885	-	0.1665	-	4.995	-	-
Step 2								
	12.72	0.07067	-	0.03029	-	0.9086	-	-

zRMS comment:

The PEC/RAC ratios were calculated for the most sensitive organism *Selenastrum carpiconantum* risk for metabolite (MT1 – desethyl-terbuthylazine) based on FOCUS Step 2 PEC_{sw} indicated the acceptable risk.

Table 9.5.2.3-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite (MT13 -hydroxy-terbuthylazine) of Terbuthylazine for each organism based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize

MT13 -hydroxy-terbuthylazine								
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Selenestrum capricornutum</i>	<i>Chironomus riparius</i>	<i>Lemna Gibba</i>
Endpoint (µg/L)		LC ₅₀ 2500	-	EC ₅₀ 2800	-	EbC50 3800	NOEC 400mg/kg	- -
AF		100	-	100	-	10	10	-
RAC (µg/L)		25	-	28	-	380	40	-
Exposure	PEC ^{gl-max} (µg/L)							
Step 1								
PEC/RAC	67.72	2.7088	-	2.42	-	0.178	1.693	-
Step 2								
PEC/RAC	13.41	0.5364	-	0.4789	-	0.03529	0.3353	-

zRMS comment:

The PEC/RAC ratios were calculated for the most sensitive organism risk for Daphnia magna for MT14 -hydroxy-terbuthylazine based on FOCUS Step 2 PEC_{sw} indicated the acceptable risk.

Table 9.5.2.3-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite (MT26-terbutyryn) of Terbutylazine for each organism based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize

MT26-terbutyryn								
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Chironomus riparius</i>	<i>Lemna Gibba</i>
Endpoint (µg/L)		LC ₅₀		EC ₅₀	-	EbC50	NOEC	EfnC50
AF		-	-	-	-	10	10	10
RAC (µg/L)		-	-	-	-	0.17	1.6	2.5
Exposure	PEC ^{gl-max} (µg/L)							
STEP 1								
PEC/RAC	8.03	-	-	-	-	47.24	5.02	3.212
STEP 2								
PEC/RAC	1.58	-	-	-	-	9.29	0.9875	0.632
D3/ditch	0.000061	-	-	-	-	0.0003588	0.00003813	0.0000244
D4/pond	0.00038	-	-	-	-	0.002235	0.0002375	0.000152
D4/stream	0.000059	-	-	-	-	0.0003471	0.00003688	0.0000236
D5/pond	0.000898	-	-	-	-	0.005282	0.0005613	0.0003592
D5/stream	0.000091	-	-	-	-	0.0005353	0.00005688	0.0000394

MT26-terbutyryn								
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants
D6/ditch	0.000069	-	-	-	-	0.0004059	0.00004313	0.0000276
R1/pond	0.000730	-	-	-	-	0.004294	0.0004563	0.000292
R1/stream	0.000532	-	-	-	-	0.003129	0.0003325	0.0002128
R2/stream	0.000397	-	-	-	-	0.002335	0.0002481	0.0001588
R3/stream	0.000312	-	-	-	-	0.001835	0.000195	0.0001248
R4/stream	0.002578	-	-	-	-	0.01516	0.001611	0.0010311

zRMS comment:

The PEC/RAC ratios were calculated for the most sensitive organism risk for *Pseudokirchnella subcapitata* for metabolite (MT26-terbutyryn) based on FOCUS Step 3 PEC_{SW} considering reduced exposure of surface water bodies.

Based on FOCUS STEP 3 calculations the risk is considered acceptable.

Table 9.5.2.3-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Desethyl Hydroxy-terbuthylazine (M14) of Terbuthylazine for each organism based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize.

Desethyl Hydroxy-terbuthylazine (M14)	
Group	Algae
Test species	<i>Desmodesmus subspicatus</i>
Endpoint (µg/L)	EC ₅₀
AF	15000
	10

Desethyl Hydroxy-terbuthylazine (M14)		
Group		Algae
RAC (µg/L)		1500
FOCUS Scenario	PEC _{gl-max} (µg/L)	
Step 1	32.05	0.02133

zRMS comment:

The PEC/RAC ratio was calculated for the most sensitive organism risk for Pseudokirchnella subcapitata for metabolite (MT14) based on FOCUS Step 1 PEC_{sw} value. **Based on PEC_{sw} FOCUS STEP 1 calculations the risk is considered acceptable.**

In addition the PEC/RAC was calculated for LM3, LM5 and LM6 metabolites with consideration PEC_{gw} values presented in Section 8 according to Confirmatory data EFSA Journal 2017;15(6):4868.

Table 9.5.2.3-7: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM3 of Terbuthylazine for algae based on FOCUS PEARL PEC_{gw} calculations for the use of TERBUT 500 SC in maize.

LM3		
Group		Algae*
Endpoint (µg/L)		EC ₅₀ 3900
AF		10
RAC (µg/L)		390
FOCUS	PEC _{gw-max} (µg/L)	
	3.56**	0.0091

*Confirmatory data EFSA Journal 2017;15(6):4868

** worst case scenario Thivia

Table 9.5.2.3-8: Aquatic organisms: acceptability of risk ($PEC_{gw}/RAC < 1$) for metabolite LM5 of Terbutylazine for algae based on FOCUS PEARL PECgw calculations for the use of TERBUT 500 SC in maize.

LM5		
Group		Algae*
Endpoint		EC ₅₀
(µg/L)		100 000
AF		10
RAC (µg/L)		10000
FOCUS	PEC_{gw}-max (µg/L)	
	1.692	0.000169

*Confirmatory data EFSA Journal 2017;15(6):4868

**worst case scenario Hamburg

Table 9.5.2.3-9: Aquatic organisms: acceptability of risk ($PEC_{gw}/RAC < 1$) for metabolite LM6 of Terbutylazine for each organism based on FOCUS PEARL PECgw calculations for the use of TERBUT 500 SC in maize.

LM6		
Group		Algae*
Endpoint		EC ₅₀
(µg/L)		100 000
AF		10
RAC (µg/L)		10000
FOCUS	PEC_{gw}max (µg/L)	
	2.937	0.00029

*Confirmatory data EFSA Journal 2017;15(6):4868

** worst case scenario Thivia

zRMS comment:

The PEC/RAC ratios were calculated for algae for metabolites such as: LM3, LM5 and LM6 based on FOCUS PEARL PEC_{gw} values .
 Based on these calculations the risk is considered acceptable for aquatic organism.

9.5.2.1 Risk assessment for formulation to aquatic organisms

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of TERBUT 500 SC for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of TERBUT 500 SC 650 WG in maize

Intended use	maize
Formulation	TERBUT 500 SC
Application rate (g[prod]/ha)	1 X 1105
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	PEC _{sw} [µg prod/L]
1	5.8697
5	1.7415
10	0.9236
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	RAC/PEC ratio Daphnia magna =EC50 177 900 µg/L RAC=17 790 (AF=100)
1	0.0003299

Buffer zone (m)	RAC/PEC ratio Pseudokirchmeirella subcapitata =EC50 181.5 µg/L RAC=18.15 (AF=10)
1	0.3234
Buffer zone (m)	RAC/PEC ratio Naviculla pelliculosa =EC50 20 µg/L RAC=2 (AF=10)
1	2.935
5	0.8708
Buffer zone (m)	RAC/PEC ratio Lemna Gibba =EC50 90.2 µg/L RAC=9.02 (AF=10)
1	0.6507

ZRMS comments:

The risk assessment for TERBUT 500 SC for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations is considered acceptable. Based on the above results 5 meter buffer zone should be applied to surface water bodies to conclude acceptable risk for aquatic organism.

9.5.3 Overall conclusions

Based on the predicted rates of TERBUT 500 SC in aquatic species, the TER values describing the risk for aquatic species following exposure to TERBUT 500 SC according to the GAP of the formulation TERBUT 500 SC **and for active substance** achieve the acceptability criteria PEC/RAC<1 with applying:

- 5 m **vegetative** buffer strip to surface water bodies

Final risk mitigation measures should be considered at MSs level.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related. Effects on bees of CHR/H/TERBI were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed in and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Terbuthylazine	Oral	LD ₅₀ >22.6 µg/bee	EFSA Journal 2011; 9(1):1969
Apis mellifera	Terbuthylazine	Contact	LD ₅₀ >32 µg/bee	EFSA Journal 2011; 9(1):1969
Apis mellifera	TERBUT 500 SC	Oral	LD ₅₀ > 200 µg/bee	P. Parma, Study code: B/87/17, 2017
Apis mellifera	TERBUT 500 SC	Contact	LD ₅₀ > 200 µg/bee	P. Parma, Study code: B/88/17, 2017
Higher-tier studies (tunnel test, field studies)				

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SAN-CO/10329/2002 rev.2 (final), October 17, 2002).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for birds from all other intended uses in groups maize (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of TERBUT 500 SC in maize

Intended use			
Active substance	terbutylazine		
Application rate (g/ha)	1 × 500		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	22.6	500	22.12
Contact toxicity	32		15.63
Product	TERBUT 500 SC		
Application rate (g/ha)	1x 1105		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	200	1105	5.53
Contact toxicity	200		5.53

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger

zRMS comments:

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SAN-CO/10329/2002 rev.2 (final), October 17, 2002).

The submitted risk assessment, based on laboratory studies, has been accepted.

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees) the Applicant should provide the chronic test on bees and chronic test for larvae for formulated product .

Thus, concerned Member States must decide on the consideration of data requirements and the risk assessment at national level.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that TERBUT 500 SC applied at the maximum use rate in maize poses low risk to bees.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on non-target arthropods of TERBUT 500 SC were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. The selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	TERBUT 500 SC	Laboratory test (3 2D) extended	LR ₅₀ = 748.8 g/ha equal to 1654.8 g prod/ha ER ₅₀ = 349.9 g /ha equal to 773.3g prod/ha	P. Parma, Study code: B/90/17, 2018
Aphidius rhopalosiphi (adults)	TERBUT 500 SC	Laboratory test (3D) extended	LR ₅₀ = 748.8 g/ha equal to 1654.8 g prod/ha	P. Parma, Study code: B/89/17
Coccinella septempunctata	TERBUT 500 SC	Laboratory test (3 2D)	LR ₅₀ = 1500 mL prod/ha to 1357.5 g prod/ha	R. Vaughan, Study code: CHR-19-17
Chrysoperla carnea	TERBUT 500 SC	Laboratory test (3 2D)	LR ₅₀ = 1500 mL/ha equal to 1357.5 g prod/ha	R. Vaughan, Study code: CHR-19-18
Field or semi-field tests				
Aged-residue study Typhlodromus pyri	TERBUT 500 SC	1 L/ha	The effects of fresh and aged foliar residues of TERBUT 500 SC on the predatory mite Typhlodromus pyri were evaluated under extended laboratory test conditions. When applied to sweetcorn plants at a rate equivalent to 1 L product/ha, fresh residues, 7-day	L. Fallowfield, Study code: CHR-19-16, 2020

Species	Substance	Exposure System	Results	Reference
			and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).	
Field or semi-field tests				

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for non-target arthropods from all other intended uses in groups maize (see 9.1.2).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of TERBUT 500 SC in maize

Intended use			
Active substance/product	TERBUT 500 SC		
Application rate (g/ha)	1 × 1105		
MAF	1		
Test species Tier I	LR₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i>	1654.8	1105	0.67
<i>Aphidius rhopalosiphi</i>	1654.8		0.67
<i>Coccinella septempunctata</i>	1357.5		0.82
<i>Chrysoperla carnea</i>	1357.5		0.82

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for non-target arthropods from all other intended uses in groups maize (see 9.1.2).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of TERBUT 500 SC in maize

Intended use					
Active substance/product		TERBUT 500 SC			
Application rate (g/ha)		1 x 1105			
MAF		1			
vdf		10			
Test species Tier I	LR₅₀ (lab.) (g/ha)	Drift rate	PER_{off-field}corr (g/ha)	CF	HQ_{off-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i>	1654.8	0.0277	3.06	5	0.009246
<i>Aphidius rhopalosiphi</i>	1654.8				0.009246
<i>Coccinella septempunctata</i>	1357.5		3.06	5	0.01127
<i>Chrysoperla carnea</i>	1357.5				0.01127

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

zRMS comments:

The evaluation of the risk for non-target arthropods provided by the applicant was verified by zRMS with consideration of the recommendations of the guidance document ESCORT 2.

The corrected risk assessment is provided in the Tables below:

Table 9.7-4_{corr}: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of TERBUT 500 SC in maize.

Intended use		Maize		
Active substance/product		TERBUT 500 SC		
Application rate (g/ha)		1 × 1105		
MAF		1		
Test species Tier I	LR₅₀ (lab.) (g/ha)/ER₅₀	PER_{in-field} (g/ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?	
<i>Typhlodromus pyri</i>	1654.8 773.3	1105	Yes No	
<i>Aphidius rhopalosiphi</i>	1654.8		Yes	
<i>Coccinella septempunctata</i>	1357.5		Yes	
<i>Chrysoperla carnea</i>	1357.5		Yes	

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

For *T.pyri* the risk in-field required further refinement when ER₅₀ value was considered for this species.

The effects of fresh and aged foliar residues of TERBUT 500 SC on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions. When applied to sweetcorn plants at a rate equivalent to 1 L product/ha, fresh residues, 7-day and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).

Table 9.7-5corr: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of TERBUT 500 SC in maize.

Intended use	Maize				
Active substance/product	TERBUT 500 SC				
Application rate (g/ha)	1 x 1105				
MAF	1				
vdf	10 for 2D, ** 5 for (2D)*** for 3D - not applicable, CF=5				
Test species Tier I	LR₅₀ (lab.) (g/ha)/ER₅₀	Drift rate	PER_{off-field}corr (g/ha)	CF	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	1654.8 773.3	0.0277	15.30 30.60	5	Yes Yes Yes Yes
<i>Aphidius rhopalosiphi</i>	1654.8		153.04		Yes
<i>Coccinella septempunctata</i>	1357.5		15.30 30.60	5	Yes
<i>Chrysoperla carnea</i>	1357.5				Yes Yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

** according to ESCORT 2

*** according to harmonised approach in the Centrale zone

The risk in-field and off -field is considered acceptable for non-target arthropods.

9.7.2.3 Additional higher-tier risk assessment

According to GLP study of Aged-residue study *L.Fallowfield*, Study code: CHR-19-16, 2020.

The effects of fresh and aged foliar residues of TERBUT 500 SC on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions. When applied to sweetcorn plants at a rate equivalent to 1 L product/ha, fresh residues, 7-day and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).

The results for bioassays initiated at 0, 7 and 14 DAT are summarised below.

Bioassay initiated	Treatment	Test item rate (L/ha)	Mean % mortality at 7 DAI ^{a)}	Corrected % mortality at 7 DAI ^{b)}	Mean number eggs/female (7-14 DAI) ^{c)}	Change in reproduction [%] ^{d)}
0 DAT	Control	-	6.0	-	10.3	-
	TERBUT 500	1	14.0	8.5	9.0 *	12.6
	Toxic reference	-	100 *	100	~	-
7 DAT	Control	-	7.0	-	9.8	-
	TERBUT 500	1	10.0	3.2	11.0	-12.2
14 DAT	Control	-	3	-	9.7	-
	TERBUT 500	1	8	5.2	10.1	-3.7

a) Treatment mortalities were compared using χ^2 2x2 table test ($\alpha = 0.05$, one-sided, > control), a statistically significant effect is denoted by an asterisk (*).

b) Test item treatment mortality corrected for any control treatment deaths using Abbott's formula. A positive value indicates an increase.

c) Treatments were compared by student t-test for homogenous variances ($\alpha = 0.05$, one-sided, < control), a statistically significant effect is denoted by an asterisk (*).

d) Percentage change in numbers of eggs per female, relative to the control. A positive value indicates a decrease and a negative value indicates an increase.

~ indicates no assessments were made for this treatment.

zRMS comment:

We agree with the results.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.
 breach the relevant trigger.

9.7.3 Overall conclusions

All hazard quotients (HQ) are considerably less than 2, indicating that TERBUT 500 SC applied at the maximum use rate in cereals winter poses no risk to non-target arthropods. No risk mitigation needed.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of TERBUT 500 SC were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Terbuthylazine	Mixed into substrate 14 d, acute 10 % peat content	LC50 corr > 141.7 mg tba/kg soil dw OXON	EFSA Journal 2011; 9(1):1969
<i>Eisenia fetida</i>	Terbuthylazine	Mixed into substrate 56 d, chronic 10 % peat content	NOEC corr = 0.500 mg tba/ kg soil	EFSA Journal 2011; 9(1):1969
<i>Folsomia candida</i>	Terbuthylazine	Mixed into substrate 28 d, chronic 10 % peat content	NOECcorr = 21.12 mg tba/ kg soil dw SYN	EFSA Journal 2011; 9(1):1969
<i>Eisenia fetida</i>	Metabolite MT1 (desethyl- terbuthylazine)	Mixed into substrate 14 d, acute 10 % peat content	LC50 corr = 120 mg/kg soil dw SYN	EFSA Journal 2011; 9(1):1969
<i>Eisenia fetida</i>	Metabolite MT1 (desethylterbuthylazine)	Mixed into substrate 56 d, chronic 10 % peat content	NOECcorr 2.8 mg/ kg soil dw	EFSA Journal 2011; 9(1):1969
<i>Eisenia fetida</i>	Metabolite MT13 (hydroxy- terbuthylazine)	Mixed into substrate 14 d, acute 10 % peat content	LC50 > 1000 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
<i>Eisenia fetida</i>	Metabolite MT13 (hydroxyterbuthylazine)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC 7 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
<i>Eisenia fetida</i>	Metabolite MT14 (desethyl-hydroxy- terbuthylazine)	Mixed into substrate 14 d, acute 10 % peat content	LC50 > 1000 mg/kg soil dw	EFSA Journal 2011; 9(1):1969

Species	Substance	Exposure System	Results	Reference
<i>Eisenia andrei</i>	TERBUT 500 SC	Mixed into substrate, 56 d, chronic 10% peat	NOEC 5.6 mg/kg soil dw NOECcorr 2.8 mg/kg soil dw	A. Gierbuszewska, Study code: G/284/17, 2018
<i>Folsomia candida</i>	TERBUT 500 SC	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 18 mg/kg dw NOECcorr 9 mg/kg soil dw	M. Wołany, Study code: G/60/19, 2020
<i>Hypoaspis aculeifer</i>	TERBUT 500 SC	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1000 mg/kg dw NOECcorr 500 mg/kg soil dw	P. Holewik, Study code: G/61/19, 2020
Field studies				
<p>In EFSA Journal 2011; 9(1):1969. was presented field study with Terbutylazine on the representative formulation. With the Preparation -‘Gardoprim’ /‘GS 13529 SC 500’ (‘A-5435 E’) there were presented two studies:</p> <ul style="list-style-type: none"> - Field study – 1 yr (Denmark) and 1 yr in Germany, indicating relevant endpoint No significant ecologically adverse effects at 1.69 L form.n/ha (844 g tba/ha) after 1 yr. <p>With the Preparation -‘Terbutylazine 500 g/L SC was also performed 1 yr study deriving and endpoint No significant ecologically adverse effects at 1.5 L form.n/ha (750 g tba/ha) after 1 yr:</p>				
Litter bag test				
<p>For Terbutylazine was performed litter bag test. According to EFSA Journal 2011; 9(1):1969. The litter bag test was performed with terbutylazine, desethylterbutylazine, 2 hydroxyterbutylazine. Derived endpoint from the study : no significant impact on organic matter breakdown at applications considered to cover an application of terbutylazine of 1 kg/ha, plus any long term accumulation.</p>				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for terbuthylazine.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses in groups maize (see 9.1.2).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of TERBUT 500 SC in maize

Intended use			
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Terbuthylazine	Not required		
Desethyl-terbuthylazine			
Hydroxy-terbuthylazine			
TERBUT 500 SC			
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Terbuthylazine	0.500	0.6667	0.74
Desethyl-terbuthylazine	2.8	0.1276	21.94
Hydroxy-terbuthylazine	7	0.1631	6.13
TERBUT 500 SC	2.8	1.437	1.94
Chronic effects on other soil macro- and mesofauna Folsomia candida			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Terbuthylazine	21.12	0.6667	32.7
TERBUT 500 SC	9	1.437	6.26
Chronic effects on other soil macro- and mesofauna Hypoaspis aculeifer			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
TERBUT 500 SC	500	1.437	348

TER values shown in bold fall below the relevant trigger.

The long-term risk assessment for Terbutylazine and TERBUT 500 SC indicates unacceptable long term risk to earthworms. Therefore, the risk refinement is needed. Such risk refinement is available based on weight of evidence and field studies and presented in point 9.8.2.2.

9.8.2.2 Higher-tier risk assessment

However in EFSA Journal 2011; 9(1):1969 and DAR (2007) Terbutylazine Vol 3 B9 were evaluated several field studies terbuthylazine. In DAR (2007) Terbutylazine Vol 3 B9 is stated an acceptable long-term risk to earthworms from technical terbuthylazine applied at rate of 844 g a.s/ha, based on two field studies submitted in original DAR (2007). As standard, the long-term risk from the active substance as oppose to the formulation is assessed, as the active substance and co-formulants are considered to rapidly disperse after application, therefore long-term exposure to the intact formulation will not occur.

Therefore it is considered that the application of formulation TERBUT 500 SC at rate 1.0 kg prod/ha (which is equal to 500 g tbt/ha) is unlikely to pose a long term risk to earthworms. No new studies are necessary.

zRMS comments:

The long-term risk assessment for Terbutylazine and TERBUT 500 SC indicates unacceptable long term risk to earthworms. Therefore, further refinement was needed. zRMS considered refinement based on the results from two field studies evaluated in the DAR (2007) where technical terbuthylazine was applied at rate of 844 g a.s/ha.

In the DAR (2007) Terbutylazine Vol 3 B9 for dose of 844 g a.s./ha an acceptable long-term risk to earthworms was concluded.

Therefore it is considered that the application of formulation TERBUT 500 SC at rate 1.0 kg prod/ha which is equal to 500 g tbt/ha) is unlikely to pose a long term risk to earthworms.

9.8.3 Overall conclusions

The acute risk to earthworms and other non-target soil organisms (meso- and macrofauna) was assessed as low for TERBUT 500 SC in a first-tier risk assessment. But a potential high risk was indicated on the long-term time scale for earthworms, but based on Risk refinement for terbuthylazine it can be concluded that application of formulation TERBUT 500 SC is unlikely to pose a long term risk to earthworms and other non-target soil organisms (meso- and macrofauna).

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on soil microorganisms of TERBUT 500 SC were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation C-mineralisation	Terbutylazine	28 d/14d, aerobic soil type	No significant effects of > 25 % on carbon mineralisation or nitrogen transformation up to a max tested concentration of 10.9 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
N-mineralisation C-mineralisation	Metabolite MT1 (desethyl-terbutylazine)	28 d/14 d, aerobic soil type	No effects of > 25 % on carbon mineralisation or nitrogen transformation up to a max tested concentration of 1.84 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
N-mineralisation C-mineralisation	Metabolite MT13 (hydroxy-terbutylazine)	28 d/14 d, aerobic soil type	No effects of > 25 % on carbon mineralisation or nitrogen transformation up to a max tested concentration of 3.45 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
N-mineralisation C-mineralisation	Metabolite MT14 (desethyl-hydroxyterbutylazine)	28 d/14 d, aerobic soil type	No effects of > 25 % on carbon mineralisation or nitrogen transformation up to a max tested concentration of 0.52 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
N-mineralisation	TERBUT 500 SC	28 d/ aerobic soil type	The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC (2.2 mg of test item/kg of soil) and 5 x PEC (11.0 mg of test item/kg of soil) did not exceed 25% on 28 day of analysis.	A. Gierbuszewska, Study code: G/285/17, 2018

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group xxx also covers the risk for the soil microorganisms from all other intended uses in groups xxx (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of TERBUT 500 SC in maize

Intended use			
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Terbuthylazine	10.9 mg/kg soil dw/ 28 d	0.6667	YES
Desethyl-terbuthylazine	1.84 mg/kg soil dw /28d	0.1276	YES
Hydroxy-terbuthylazine	3.45 mg/kg soil dw/28 d	0.1631	YES
TERBUT 500 SC	11 mg/kg soil dw/28 d	1.437	YES
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Not required			

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation TERBUT 500 SC and its active substance terbuthylazine in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed after 28 days or more of exposure, indicating that the proposed use of TERBUT 500 SC poses an acceptable risk to soil microorganisms.

zRMS comments:

TERBUT 500 SC has no significant effect on soil micro-organisms at 11 mg product/kg dry soil. Based on it, can be concluded that TERBUT 500 SC under field conditions, use at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with terbuthylazine and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on non-target terrestrial plants of TERBUT 500 SC were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
<i>Sunflower Helianthus annuus</i>	TERBUT 500 SC	21 d Seedling emergence	ER50 > 1500 ml prod//ha equal to 1657.5 g prod/ha	W. Dec, Study code: G/286/17, 2018
<i>Cabbage Brassica oleracea var. capitata</i>	TERBUT 500 SC	21 d Seedling emergence	ER= 49.44 ml prod/ha equal to 54.63 g prod/ha	
<i>Pea Pisum sativum</i>	TERBUT 500 SC	21 d Seedling emergence	ER50 > 1500 ml prod/ha equal to 1657.5 g prod/ha	
<i>Tomato Solanum lycopersicon</i>	TERBUT 500 SC	21 d Seedling emergence	ER50= 63.32 ml prod/ha equal to 69.97 g prod/ha	
<i>Onion Allium cepa</i>	TERBUT 500 SC	21 d Seedling emergence	ER50= 92.17 ml prod/ha equal to 101.85 g prod/ha	
<i>Oats Avena sativa</i>	TERBUT 500 SC	21 d Seedling emergence	ER50= 598.95 ml prod/ha equal to 661.84 g prod/ha	
<i>Sunflower Helianthus annuus</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50 > 212.3ml prod//ha equal to 234.59 g prod/ha	A. Gierbuszewska, Study code: G/287/17, 2018
<i>Cabbage Brassica oleracea var. capitata</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 180 ml prod/ha equal to 198.90 g prod/ha	
<i>Pea Pisum sativum</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50 > 619.6 ml prod/ha equal to 684.66 g prod/ha	
<i>Tomato Solanum lycopersicon</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 57.3 ml prod/ha equal to 63.32 g prod/ha	
<i>Onion Allium cepa</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 51.1 ml prod/ha equal to 56.47 g prod/ha	

Species	Substance	Exposure System	Results	Reference
<i>Oats</i> <i>Avena sativa</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 391.0 ml prod/ha equal to 432.06 g prod/ha	

m: monocotyledonous; d: dicotyledonous

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SAN-CO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group maize also covers the risk for non-target terrestrial plants from all other intended uses in groups maize (see 9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of TERBUT 500 SC in maize

Intended use				
Active substance/product	TERBUT 500 SC			
Application rate (g/ha)	1 × 1105			
MAF	1			
Test species	ER ₅₀ (g/ha)	Drift rate	PER _{off-field} (g/ha)	TER criterion: TER ≥ 5
<i>Sunflower</i> <i>Helianthus annuus</i>	1657.5 g prod/ha	0.0277	30.61	54
<i>Cabbage</i> <i>Brassica oleracea</i> <i>var. capitata</i>	54.63 g prod/ha	0.0277	30.61	1.78
<i>Pea</i> <i>Pisum sativum</i>	1657.5 g prod/ha	0.0277	30.61	54
<i>Tomato</i> <i>Solanum lycopersicon</i>	69.97 g prod/ha	0.0277	30.61	2.29
<i>Onion</i> <i>Allium cepa</i>	101.85 g prod/ha	0.0277	30.61	3.33
<i>Oats</i> <i>Avena sativa</i>	661.84 g prod/ha	0.0277	30.61	21.62
<i>Sunflower</i> <i>Helianthus annuus</i>	234.59 g prod/ha	0.0277	30.61	7.66

<i>Cabbage</i> <i>Brassica oleracea</i> <i>var. capitata</i>	198.90 g prod/ha	0.0277	30.61	6.50
<i>Pea</i> <i>Pisum sativum</i>	684.66 g prod/ha	0.0277	30.61	22.37
<i>Tomato</i> <i>Solanum lycopersicon</i>	63.32 g prod/ha	0.0277	30.61	2.07
<i>Onion</i> <i>Allium cepa</i>	56.47 g prod/ha	0.0277	30.61	1.84
<i>Oats</i> <i>Avena sativa</i>	432.06 g prod/ha	0.0277	30.61	14.11

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of TERBUT 500 SC in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use					
Active substance/product		TERBUT 500 SC			
Application rate (g/ha)		1 × 1105			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g/ha)	PER_{off-field} 50 % drift red. (g/ha)	PER_{off-field} 75 % drift red. (g/ha)	PER_{off-field} 90 % drift red. (g/ha)
1	2.77	30.61	15.3	7.65	3.061
5	0.57	6.30	3.15	1.58	0.63
Toxicity value		TER			
ER ₅₀ = 54.63 g/ha		criterion: TER ≥ 5			
1		1.78	3.57	7.14	17.85
5		8.67	17.34	34.58	86.71

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

zRMS comments:

ZRMS agrees with the calculations of the deterministic risk assessment provided with consideration of the lowest endpoint $ER_{50} = 54.63$ g product/ha and $PER_{off-field}$.

Therefore, the following risk mitigation measures should be applied to non-crop area:

SPe 3:

- 5 m buffer zone or
- 1 m and use of 75% drift reducing nozzles

9.10.3 Overall conclusions

Based on the predicted rates of TERBUT 500 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to TERBUT 500 SC according to the GAP of the formulation TERBUT 500 SC achieve the acceptability criteria $TER \geq 5$ with applying:

- 5 m buffer zone
- 1 m and use of 75% drift reducing nozzles

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not required.

9.12 Monitoring data (KCP 10.8)

9.13 Classification and Labelling

H400 and H410

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1	K. Florynski	2019	TERBU 500 SC - TER Calculations for Terrestrial Vertebrates Chemiroł GLP No Unpublished	N	Chemiroł
KCP 10.1.2	K. Florynski	2019	TERBU 500 SC - TER Calculations for Terrestrial Vertebrates Chemiroł GLP No Unpublished	N	Chemiroł
KCP 10.2/01	E. Kulec-Płoszczyca	2018	Terbut 500 SC Daphnia magna, acute immobilisation test Study code: W/10/18 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from 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
KCP 10.2/02	E. Kulec-Płoszczyca	2018	Terbut 500 SC Pseudokirchneriella subcapitata SAG 61.81 Growth inhibition test Study code: W/11/18 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.2/03	E. Kulec-Płoszczyca	2018	Terbut 500 SC Lemna gibba CPCC 310, Growth inhibition test Study code: W/12/18 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.2/04	D. Jenota	2019	Terbut 500 SC Navicula pelliculosa SAG 1050-3, Growth inhibition test Study code: W/53/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. z o.o. Synthos Agro Sp. z o.o.
KCP 10.3/01	P. Parma	2017	Terbut 500 SC Honeybees (Apis mellifera L.), Acute oral Toxicity Test Study code: B/87/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.3/02	P. Parma	2017	Terbut 500 SC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test Study code: B/88/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from 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
KCP 10.3/03	P. Parma	2018	An extended laboratory test for evaluating Terbut 500 SC on the predatory mite, Typhlodromus pyri (Sch.) Study code: B/90/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.3/04	P. Parma	2018	An extended laboratory test for evaluating the effects of Terbut 500 SC on the parasitic wasp, Aphidius rhopalosiphii (De Stefani-Perez) Study code: B/89/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.3/05	R. Vaughan	2020	TERBUT 50 SC – A rate-response extended laboratory study to determine effects on the ladybird beetle, Coccinella septempunctata (Coleoptera: Coccinellidae) Study code: CHR-19-17 Mambo-Tox A Division of Cawood Scientific Ltd., 2 venture Road, University Science Park, Southampton SO16 7NP, UK GLP Unpublished	N	PUH Chemirol Sp. z o.o. Synthos Agro Sp z o.o.
KCP 10.3/06	R. Vaughan	2020	TERBUT 50 SC – A rate-response extended laboratory study to determine effects on green lacewing, Chrysoperla carnea (Neuroptera, Chrysopidae) Study code: CHR-19-18 Mambo-Tox A Division of Cawood Scientific Ltd., 2 venture Road, University Science Park, Southampton SO16 7NP, UK GLP Unpublished	N	PUH Chemirol Sp. z o.o. Synthos Agro Sp z o.o.
KCP 10.3/07	L. Fallowfield	2020	TERBUT 50 SC – An aged-residue extended laboratory study to determine effects on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae)	N	PUH Chemirol Sp.

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
			Study code: CHR-19-16 Mambo-Tox A Division of Cawood Scientific Ltd., 2 venture Road, University Science Park, Southampton SO16 7NP, UK GLP Unpublished		z o.o. Synthos Agro Sp z o.o.
KCP 10.4/01	A. Gierbuszewska	2018	TERBUT 500 SC Earthworm Reproduction Test (<i>Eisenia andrei</i>) Study code: G/284/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna Department of Ecotoxicological Studies Doświ-adczałna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.4/02	M. Wołany	2020	TERBUT 500 SC Collembolan (<i>Folsomia candida</i>) Reproduction Test Study code: G/60/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna Department of Ecotoxicological Studies Doświ-adczałna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemiroł Sp z o.o. Synthos Agro Sp z o.o.
KCP 10.4/03	P. HOLEWIK	2020	TERBUT 500 SC Predatory mite (<i>Hypoaspis (Geolaelaps) aculeifer</i>) reproduction test in soil Study code: G/61/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna Department of Ecotoxicological Studies Doświ-adczałna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemiroł Sp z o.o. Synthos Agro Sp z o.o.
KCP 10.5	A. Gierbuszewska	2018	TERBUT 500 SC Soil Microorganisms: Nitrogen Transformation Test Study code: G/285/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna Department of Ecotoxicological Studies Doświ-adczałna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from 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
KCP 10.6/01	W. Dec	2018	TERBUT 500 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study code: G/286/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from Synthos Agro Sp. z o.o.
KCP 10.6/02	W. Dev	2018	TERBUT 500 SC Terrestrial Plant Test: Vegetative Vigour Test Study code: G/287/17 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Letter access from 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 10.1.1/01	xxxxxxxxxxxxxxxxxxxx	1994	Acute oral toxicity study with GS 13529 technical in Japanese quail Novartis Crop Protection AG, Basel, Switzerland [REDACTED] Report No 104412 GLP Not Published	Y	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 10.1.1/02	xxxxxxxxxxxxxx	1983	Acute oral LD50 in Mallard duck Novartis Crop Protection AG, Basel, Switzerland [REDACTED] [REDACTED] Report No 108-213 GLP Not Published	Y	Syngenta
KCP 10.1.1/03	Daamen, P.A.M	1994b	5-day Dietary Toxicity Study in Japanese Quail with GS 13529 Technical Novartis Crop Protection AG, Basel, Switzerland [REDACTED] Report No 104434 GLP Not Published	Y	Syngenta
KCP 10.1.1/04	Beavers, J.	1983a	8-day dietary LC50 with Bobwhite quail Novartis Crop Protection AG, Basel, Switzerland [REDACTED] [REDACTED] Report No 108-211 Not GLP Not Published	Y	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 10.1.1/05	xxxxxxxxxxxxxxxxxxxx	1983b	8-day Dietary LC50 with Mallard Duck Novartis Crop Protection AG, Basel, Switzerland [REDACTED] [REDACTED] Report No 108-212 Not GLP Not Published	Y	Syngenta
KCP 10.1.1/06	xxxxxxxxxxxxxxxxxxxx	1995	Reproduction study with GS 13529 technical in the Japanese quail (by dietary admixture) Novartis Crop Protection AG, Basel, Switzerland [REDACTED] Report No 104445 GLP Not Published	Y	Syngenta
KCP 10.2/01	xxxxxxxxxxxxxxxxxxxx	2002	GS13529 (Terbutylamine technical): Acute toxicity to rainbow trout (<i>Oncorhynchus</i> <i>mykiss</i>) Syngenta Crop Protection AG, Basel, Switzerland [REDACTED] [REDACTED] Report No BL7395/B GLP Not Published	Y	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 10.2/02	xxxxxxxxxxxxxxxx	2002	GS13529 (Terbutylazine technical): Acute toxicity to mirror carp (Cyprinus carpio) Syngenta Crop Protection AG, Basel, Switzerland [REDACTED] [REDACTED] Report No BL7396/B GLP Not Published	Y	Syngenta
KCP 10.2/03	xxxxxxxxxxxxxxxxxxxxxxxx	1990	GS 13529, Terbutylazin technical, 21-day prolonged toxicity study in the Rainbow trout under flow-through conditions Novartis Crop Protection AG, Basel, Switzerland [REDACTED] Report No 227248 GLP Not Published	Y	Syngenta
KCP 10.2/04	xxxxxxxxxxxxxxxxxxxxxxxx	1990	Accumulation and elimination of 14C-terbutylazine by Bluegill sunfish in a dynamic flow-through system Novartis Crop Protection AG, Basel, Switzerland [REDACTED] Report No 217451 GLP Not Published	Y	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 10.2/05	An, der Kolk J.	1996	GS 13529, static acute toxicity test with daphnids (Daphnia magna) Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 96-075-1008 GLP Not Published	N	Syngenta
KCP 10.2/06	Shillabeer, N, Maynard, S.J, Woodyer, JM	2002	GS13529 (Terbutylazine technical); Chronic toxicity to Daphnia magna Syngenta Crop Protection AG, Basel, Switzerland Brixham Environmental Laboratory, Brixham, United Kingdom, Report No BL7397/B GLP Not Published	N	Syngenta
KCP 10.2/07	Grade, R.	1993a	Report on the growth inhibition test of GS 13529 tech. to Green algae (Scenedesmus subspicatus) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 928431 GLP Not Published	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 10.2/08	Grade, R.	1993b	Growth inhibition test of GS 13529 tech. to Blue algae (<i>Microcystis aeruginosa</i>) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 928432 GLP Not Published	N	Syngenta
KCP 10.2/09	Grade, R.	1993c	Report on the growth inhibition test of GS 13529 tech. to Diatoms (<i>Navicula pelliculosa</i>) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Basel, Oekotoxikologie, Basel, Switzerland, Report No 928433 GLP Not Published	N	Syngenta
KCP 10.2/10	Palmer, S. Kendall, T, Kreuger, H	2001	A 96-Hour Growth Inhibition Test of GS-26379 (Metabolite of GS-13529) to the Green Alga, <i>Selenastrum capricornutum</i> Syngenta Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 528A-109 GLP Not Published	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 10.2/11	Grade, R.	2000b	Growth inhibition of GS 23158 (metabolite of GS 13529) to green algae (Selenastrum capricornutum) under static conditions Novartis Crop Protection AG, Basel, Switzerland, Report No 2001571 GLP Not Published	N	Syngenta
KCP 10.2/12	Vial, A.	1991g	Report on the growth inhibition test of GS 28620 to Green algae (Scenedesmus subspicatus) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 918160 GLP Not Published	N	Syngenta
KCP 10.2/13	Vial, A.	1991h	Report on the growth inhibition test of G 28273 to Green algae (Scenedesmus subspicatus) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 918140 GLP Not Published	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 10.2/14	Grade,R.	1997	Growth inhibition test of GS 14260 tech. to green algae (<i>Selenastrum capricornutum</i>) under static conditions Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, Report No 961714 GLP Not Published	N	Syngenta
KCP 10.2/15	Mimmert, U.	1998	Effects of 14C-labelled GS 13529 (Terbutylazine tech.) on the development of sediment-dwelling larvae of <i>Chironomus riparius</i> in a water-sediment system Novartis Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 690524 GLP Not Published	N	Syngenta
KCP 10.2/16	Grade, R.	2000c	Toxicity test of GS 23158 (Metabolite of GS 13529) on sediment-dwelling <i>Chironomus riparius</i> (syn. <i>Chironomus thummi</i>) under static conditions Novartis Crop Protection AG, Basel, Switzerland, Report No 2001572 GLP Not Published	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 10.2/17	Hoberg, J.	1993	GS 13529 - Toxicity to Duckweed, Lemna gibba Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No 93-9-4947 GLP Not Published	N	Syngenta
KCP 10.2/18	Douglas M.T., Handley J.W., Macdonald I.A.	1988c	THE ACUTE TOXICITY OF TERBUTHYLAZINE TO DAPHNIA MAGNA Huntingdon Research Centre Ltd., Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 10(a)/88505 GLP: yes published: no	N	Oxon
KCP 10.2/19	Wuntrich V.	1995b	INFLUENCE OF THE SOIL LEACHATES OF THE LYSIMETER STUDY WITH 14C-TERBUTHYLAZINE ON DAPHNIA MAGNA RCC AG., Itingen, Switzerland Oxon Italia S.P.A, Pero, Italy Report-no. 399778 GLP: yes published: no	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
KCP 10.2/20	Bell G.	1995	TERBUTHYLAZINE: PROLONGED TOXICITY TO DAPHNIA MAGNA Huntingdon Research Centre Ltd., Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 18(a)/942069 GLP: yes published: no	N	Oxon
KCP 10.2/21	Kelly C.	1996	TERBUTHYLAZINE TECHNICAL ALGAL GROWTH INHIBITION Huntingdon Life Sciences Limited, Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 180/962297 GLP: yes published: no	N	Oxon
KCP 10.2/22	Wuthrich V.	1995c	INFLUENCE OF THE SOIL LEACHATES OF THE LYSIMETER STUDY WITH 14C-TERABUTHYLAZINE ON THE GROWTH OF SCENEDESMUS SUBSPICATUS RCC AG., Itingen, Switzerland Oxon Italia S.P.A, Pero, Italy Report-no. 399791 GLP: yes published: no	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
KCP 10.2/23	Dengler D.	2004a	TESTING OF TOXIC EFFECTS OF DESETHYL-TERBUTHYLAZINE ON THE SINGLE CELL GREEN ALGA DESMODESMUS SUBSPICATUS (FORMERLY SCENEDESMUS SUBSPICATUS) GAB Biotechnologie GmbH, Niefern- Öschelbron, Germany Oxon Italia S.P.A, Pero, Italy Report-no. 20041034/01-AADs GLP: yes published: no	N	Oxon
KCP 10.2/24	Dengler D.	2004b	TESTING OF TOXIC EFFECTS OF 2- HYDOXY-TERBUTHYLAZINE ON THE SINGLE CELL GREEN ALGA DESMODESMUS SUBSPICATUS (FORMERLY SCENEDESMUS SUBSPICATUS) GAB Biotechnologie GmbH, Niefern- Öschelbron, Germany Oxon Italia S.P.A, Pero, Italy Report-no. 20041035/01-AADs GLP: yes published: no	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
KCP 10.2/25	Migchielsen M.H.J	2002a	120-HOUR FRESH WATER CYANOBACTERIA GROWTH INHIBITION TEST WITH TERBUTHYLAZINE TECHNICAL Notox B.V, 's-Hertogenbosch, The Netherlands Oxon Italia S.P.A, Pero, Italy Report-no. 314055 GLP: yes published: no	N	Oxon
KCP 10.2/26	Migchielsen M.H.J	2002b	FRESH WATER ALGAL GROWTH INHIBITION TEST WITH TERBUTHYLAZINE TECHNICAL Notox B.V, 's-Hertogenbosch, The Netherlands Oxon Italia S.P.A, Pero, Italy Report-no. 346444 GLP: yes published: no	N	Oxon
KCP 10.2/27	Dengler D.	2001	ASSESSMENT OF TOXIC EFFECTS OF TERBUTHYLAZINE TECHNICAL ON THE DUCKWEED LEMNA GIBBA IN A SEMI STATIC TEST AND A RECOVERY PERIOD GAB Biotechnologie GmbH, Niefern- Öschelbron Oxon Italia S.P.A, Pero, Italy Report-no. 20001420/01-ARLg GLP: yes published: no	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
KCP 10.3.1/01	Petto,R., Klepka, S.	1994	Laboratory testing for toxicity (acute contact and oral LD50) of GS 13529 to honey bees (Apis mellifera L.) (Hymenoptera, Apidae) Novartis Crop Protection AG, Basel, Switzerland RCC Umweltchemie GmbH & Co. KG, Rossdorf, Germany, Report No 416902 GLP Not Published	N	Syngenta
KCP 10.3.1/02	Bell G.	1994b	TERBUTHYLAZINE: ACUTE TOXICITY TO HONEY BEES (APIS MELLIFERA) Huntingdon Life Sciences Limited, Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 25/931946 GLP: yes published: no	N	Oxon
KCP 10.3.1/03	Bell G.	1994b	TERBUTHYLAZINE: ACUTE TOXICITY TO HONEY BEES (APIS MELLIFERA) Huntingdon Life Sciences Limited, Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 25/931946 GLP: yes published: no	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
KCP 10.4/01	Rufli, H.	1989	GS 13529, Earthworm, acute toxicity test Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 891347 GLP Not Published	N	Syngenta
KCP 10.4/02	Van, Erp Y.	2000a	Acute toxicity study in the earthworm with GS13529 (terbuthylazine) Novartis Crop Protection AG, Basel, Switzerland NOTOX B.V., 'S Hertogenbosch, Netherlands, Report No 281677 GLP Not Published	N	Syngenta
KCP 10.4/03	Knops, M.	2000	Acute toxicity of GS 26379 to the earthworm Eisenia fetida Novartis Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnorsdorf, Germany, Report No 001048066 GLP Not Published	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 10.4/04	Van, Erp Y.	2000b	Acute toxicity study in the earthworm with GS 26379 (deethylterbuthylazine) Novartis Crop Protection AG, Basel, Switzerland NOTOX B.V., 'S Hertogenbosch, Netherlands, Report No 281699 GLP Not Published	N	Syngenta
KCP 10.4/05	Van, Erp Y.	2000c	Acute toxicity study in the earthworm with GS 23158 (hydroxy-terbuthylazine) Novartis Crop Protection AG, Basel, Switzerland NOTOX B.V., 'S Hertogenbosch, Netherlands, Report No 281688 GLP Not Published	N	Syngenta
KCP 10.4/06	Van, Erp Y.	2000d	Acute toxicity study in the earthworm with GS 28620 (deethylhydroxyterbuthylazine) Novartis Crop Protection AG, Basel, Switzerland NOTOX B.V., 'S Hertogenbosch, Netherlands, Report No 281701 GLP Not Published	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 10.4/07	Gossmann, A.	1998	Effects of GS 13529 / CGA 77102 SC 500 (A-9476 B) on reproduction and growth of earthworms Eisenia fetida (Savigny 1826) in artificial soil Novartis Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 3450022 GLP Not Published	N	Syngenta
KCP 10.4/08	Kleiner, R.	2000	Sublethal toxicity (on reproduction and growth) of GS13529 SC 500 (A5435E) to the earthworm Eisenia fetida Novartis Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 991048021 GLP Not Published	N	Syngenta
KCP 10.4/09	Klein, O.	2006	S-metolachlor (A9396A), terbuthylazine (A5435E) and S-metolachlor + terbuthylazine (A9476C): A field study to evaluate effects on the earthworm fauna in maize in southern Germany. GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20051078/G1-NFEw. GLP: Yes Published: 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
KCP 10.4/10	Pease G., Foster A., Milanesi F.	2006	S-metolachlor (A9396C), terbuthylazine (A5435E) and s-metolachlor + terbuthylazine (A9476C): A field study to evaluate effects on the earthworm fauna of a maize field in Denmark. Ecotox Limited, Devon, UK. Report No. ER-06-KCB 215. Non GLP report from GLP study. Published: No	N	Syngenta
KCP 10.4/11	Meister, A	2002	Effects of GS 13529/CGA77102 SC 500 (A9476 B) on Reproduction of the Collembola Folsomia candida in Artificial Soil Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 11661016 GLP Not Published	N	Syngenta
KCP 10.4/12	Stabler D.	2003	ACUTE TOXICITY OF TERBUTHYLAZINE-DESETHYL ON EARTHWORMS, EISENIA FETIDA USING AN ARTIFICIAL SOIL TEST ArGe GAB Biotech/IFU, D-75223 Niefern-Öschelbronn Oxon Italia S.P.A, Pero, Italy Report-no. 20021389/01-NLEf GLP: yes published: no	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
KCP 10.4/13	Stabler D.	2002	ACUTE TOXICITY OF 2-HYDROXY- TERBUTHYLAZINE ON EARTHWORMS, EISENIA FETIDA USING AN ARTIFICIAL SOIL TEST ArGe GAB Biotech/IFU, D-75223 Niefern- Öschelbronn Oxon Italia S.P.A, Pero, Italy Report-no. 20011377/01-NLEf GLP: yes published: no	N	Oxon
KCP 10.4/14	Luhrs U.	1999	EFFECTS OF CLICK (TERBUTHYLAZINE 500 G/L SC) ON REPRODUCTION AND GROWTH OF EARTHWORMS EISENIA FETIDA (SAVIGNY 1826) IN ARTIFICIAL SOIL IBACON, Rossdorf, Germany Oxon Italia S.P.A, Pero, Italy Report-no. 4580022 GLP: yes published: no	N	Oxon
KCP 10.5/01	Lemnitzer, B.	2001	Effects of terbuthylazine tech. (GS 13529 U) on the activity of soil microflora Syngenta Crop Protection AG, Basel, Switzerland BioChem agrar, Gerichshain, Germany, Report No 0110351004 GLP Not Published	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 10.5/02	Suter, P.	1987	Influence of the herbicide Terbutylazine (GS 13529) on soil microorganisms Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 22-87 GLP Not Published	N	Syngenta
KCP 10.5/03	Van, der Kolk J.	2001	GS23158, GS26379 and GS28620 (metabolites of GS13529 Terbutylazine): Determination of effects on soil microflora activity Syngenta Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 1047.110.747 GLP Not Published	N	Syngenta
KCP 10.5/04	Carter J.N.	1996	TERBUTHYLAZINE TECHNICAL AI EFFECTS ON SOIL NON-TARGET MICRO-ORGANISMS Huntingdon Life Sciences Limited, Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 165/952682 GLP: yes published: no	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
KCP 10.5/05	Kolzer U.	2003	ASSESSMENT OF THE SIDE EFFECTS OF DESETHYL TERBUTHYLAZINE ON THE ACTIVITY OF THE SOIL MICROFLORA ArGe GAB Biotech/IFU, D-75223 Niefern- Öschelbronn Oxon Italia S.P.A, Pero, Italy Report-no. 20021389/01-ABMF GLP: yes published: no	N	Oxon
KCP 10.5/06	Kolzer U.	2002	ASSESSMENT OF THE SIDE EFFECTS OF 2-HYDROXY-TERBUTHYLAZINE ON THE ACTIVITY OF THE SOIL MICROFLORA ArGe GAB Biotech/IFU, D-75223 Niefern- Öschelbronn Oxon Italia S.P.A, Pero, Italy Report-no. 20011377/01-ABMF GLP: yes published: no	N	Oxon
KCP 10.5/07	Carter J.N.	1996	TERBUTHYLAZINE TECHNICAL AI EFFECTS ON SOIL NON-TARGET MICRO- ORGANISMS Huntingdon Life Sciences Limited, Cambridgeshire, UK Oxon Italia S.P.A, Pero, Italy Report-no. OXN 165/952682 GLP: yes published: no	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
KCP 10.5/08	Kolzer U.	2003	ASSESSMENT OF THE SIDE EFFECTS OF DESETHYL TERBUTHYLAZINE ON THE ACTIVITY OF THE SOIL MICROFLORA ArGe GAB Biotech/IFU, D-75223 Niefern- Öschelbronn Oxon Italia S.P.A, Pero, Italy Report-no. 20021389/01-ABMF GLP: yes published: no	N	Oxon
KCP 10.5/09	Kolzer U.	2002	ASSESSMENT OF THE SIDE EFFECTS OF 2-HYDROXY-TERBUTHYLAZINE ON THE ACTIVITY OF THE SOIL MICROFLORA ArGe GAB Biotech/IFU, D-75223 Niefern- Öschelbronn Oxon Italia S.P.A, Pero, Italy Report-no. 20011377/01-ABMF GLP: yes published: no	N	Oxon

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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 the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%).
- The dissolved oxygen concentrations in the test vessels were within the range of 8.6 – 10.1 mg/L (criterion: not less than 3 mg/L).

Agreed endpoints:

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC ₅₀	n.d.	177.9 (57.6 – 1168.6)
EC ₂₀	> 500	88.8 (1.1 – 180.7)
EC ₁₀	341.9 (109.5 – 1628.9)	61.7 (0.07 – 127.6)
LOEC	> 500	250
NOEC	≥ 500	125

Calculations according to [5], [SOP/W/68]
 (-) the 95% confidence interval
 n.d. – not determined

Reference:	KCP 10.2/01
Report	Terbut 500 SC <i>Daphnia magna</i> , acute immobilisation test; E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W/10/18
Guideline(s):	OECD Guideline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item: Terbut 500 SC, batch no. 7/17, content of terbutylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.

Test Species: *Daphnia magna* Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology

Test Design: Static test (exposure: 48 h); four replicates per treatment, five *Daphnia magna* in each replicate

Endpoints: EC₅₀, NOEC and LOEC.

Test Conditions Temperature: 20.6 – 21.8oC; pH of the control: 7.60 – 7.93; dissolved oxygen concentration in the control: 8.6 – 10.1 mg/L; daily cycle: 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration.

Test Concentration:: 15.6, 31.3, 62.5, 125, 250, and 500 mg/L plus the control

Results and discussion:

The effect of the test item on immobilisation of *Daphnia magna* was assessed. The test item concentrations used in the definitive test were determined on the basis of the preliminary test results. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.

In the preliminary test, the recorded temperature was in the range of 18.6 – 20.1°C. The measured pH values were in the ranges of 7.66 – 7.73 at exposure initiation and 7.63 – 7.76 at exposure termination. The measured dissolved oxygen concentrations were in the ranges of 8.8 – 8.9 mg/L at exposure initiation and 8.4 – 8.7 mg/L at exposure termination. In the preliminary test, in the control and in the test item concentration of 0.1 and 1.0 mg/L no immobilisation of *Daphnia magna* was observed during exposure. After 48 hours of exposure, in the test item concentrations of 10 and 100 mg/L, the immobilisation of *Daphnia magna* were 5.0 and 65%, respectively

Nominal test item concentration [mg/L]	Number of <i>Daphnia magna</i>	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
		24 h				48 h					
		Replicates								24 h	48 h
		A	B	C	D	A	B	C	D		
Control	20	0	0	0	0	0	0	0	0	0	0
0.1	20	0	0	0	0	0	0	0	0	0	0
1.0	20	0	0	0	0	0	0	0	0	0	0
10	20	0	0	0	0	0	0	1	0	0	5
100	20	0	0	0	0	3	3	3	4	0	65

Time of exposure: 17.01.2018 – 19.01.2018

Definitive test

The recorded temperature during exposure was in the range of 20.6 – 21.8oC and constant within 1.2oC. The measured pH values at exposure initiation were in the range of 7.85 – 7.96 and at exposure termination were in the range of 7.33 – 7.61. The measured dissolved oxygen concentrations at exposure initiation were in the range of 9.9 – 10.1 mg/L and at exposure termination were in the range of 7.3 – 8.6 mg/L. In the control and in the test item concentration of 15.6 mg/L no immobilisation of *Daphnia magna* was observed during exposure. At exposure

termination in the test item concentrations of 31.3, 62.5, 125, 250, and 500 mg/L immobilisation of *Daphnia magna* was 10, 10, 10, 65 and 100% respectively.

Nominal test item concentration [mg/L]	Number of <i>Daphnia magna</i>	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
		24 h				48 h					
		Replicates								24 h	48 h
		A	B	C	D	A	B	C	D		
Control	20	0	0	0	0	0	0	0	0	0	0
15.6	20	0	0	0	0	0	0	0	0	0	0
31.3	20	0	0	0	0	0	0	1	1	0	10
62.5	20	0	0	0	0	0	0	1	1	0	10
125	20	0	0	0	0	0	1	0	1	0	10
250	20	1	0	0	1	4	4	2	3	10	65
500	20	1	1	1	0	5	5	5	5	15	100

Time of exposure: 14.02.2018 – 16.02.2018

Results of the chemical determinations

The concentrations of the test item were chemically determined using a validated liquid chromatographic method with DAD detection. Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination.

Nominal test item concentration [mg/L]	Mean concentration (n=3) of the test item determined in samples collected [mg/L]			
	at exposure initiation	% of nominal concentration	at exposure termination	% of nominal concentration
Control	<LoD	--	<LoD	--
15.6	15.294	98.0	14.031	89.9
31.3	26.856	85.8	24.532	78.4
62.5	50.049	80.1	49.253	78.8
125	112.747	90.2	107.264	85.8
250	234.410	93.8	215.643	86.3
500	471.994	94.4	449.484	89.9

LoQ = 0.001 mg/L
 LoD = 0.0003 mg/L

At exposure initiation, the determined concentration of the test item was in the range of 80.1 – 98.0% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentration of the test item was in the range of 78.4 – 89.9% of the nominal concentration. Therefore, the concentrations of the test item were stable under test conditions.

Endpoint values

The endpoint values were determined based on the nominal test item concentrations. The endpoint values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyzes. To make calculations and to conduct statistical analyzes, the ToxRat Professional commercial software was used. The median concentration causing 50% immobilisation of *Daphnia magna* after 24 h of exposure, i.e. the EC₅₀/24 h value is not determined. The EC₂₀/24 h value is > 500 mg/L. The EC₁₀/24 h value is 341.9 mg/L (95% confidence interval 109.5 – 1628.9). The median concentration causing 50% immobilisation of *Daphnia magna* after 48 h of exposure, i.e. the EC₅₀/48 h value is 177.9 mg/L (95% confidence interval 57.6 – 1168.6). The EC₂₀/48 h value is 88.8 mg/L (95% confidence interval 1.05 – 180.7). The EC₁₀/48 h value is 61.7 mg/L (95% confidence interval 0.07 – 127.6).

The data on immobilisation of the *Daphnia magna* at exposure termination were analyzed using Fisher's Exact Binominal Test with Bonferroni Correction. The test showed a significant difference between the test item concentrations 250 and 500 mg/L and the control. Therefore, the lowest test item concentration causing immobilisation (LOEC/48 h) is 250 mg/L and the highest test item concentration causing no immobilisation (NOEC/48 h) is 125 mg/L.

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC ₅₀	n.d.	177.9 (57.6 – 1168.6)
EC ₂₀	> 500	88.8 (1.1 – 180.7)
EC ₁₀	341.9 (109.5 – 1628.9)	61.7 (0.07 – 127.6)
LOEC	> 500	250
NOEC	≥ 500	125

Calculations according to [5], [SOP/W/68]
 (-) the 95% confidence interval
 n.d. – not determined

THE VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No. 202 (2004) and EU Method C.2. were met:

- the immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 8.6 – 10.1 mg/L (criterion: not less than 3 mg/L).

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The biomass in the control increased by a factor of 151.0 within the 72-hour test period (criterion: at least a 16-fold growth),
- The coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.2% (criterion: it must not exceed 7%),
- The mean coefficient of variation for the section-by-section growth rate in the control culture was 11.6% (criterion: it must not exceed 35%).

Agreed endpoints:

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _r C ₅₀	0.3506 (0.1470 – 1.3827)	0.1753 (0.0886 – 0.3289)	0.1815 (0.0790 – 0.3778)
E _r C ₂₀	0.0171 (0.0009 – 0.0511)	0.0205 (0.0032 – 0.0477)	0.0231 (0.0020 – 0.0585)
E _r C ₁₀	0.0035 (0.0000 – 0.0164)	0.0067 (0.0005 – 0.0206)	0.0078 (0.0002 – 0.0270)
LOEC	0.0120	0.0120	0.0120
NOEC	0.0041	0.0041	0.0041

Calculations were made according to [8], [SOP/W/88].
 (–) – 95% confidence interval

Table 13. Endpoint values for yield based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _y C ₅₀	0.0891 (0.0316 – 0.1382)	0.0347 (0.0230 – 0.0521)	0.0251 (0.0176 – 0.0471)
E _y C ₂₀	0.0069 (0.0012 – 0.0172)	0.0117 (0.0061 – 0.0182)	0.0117 (0.0071 – 0.0166)
E _y C ₁₀	0.0021 (0.0002 – 0.0067)	0.0068 (0.0029 – 0.0112)	0.0078 (0.0038 – 0.0113)
LOEC	0.0120	0.0120	0.0120
NOEC	0.0041	0.0041	0.0041

Calculations were made according to [8], [SOP/W/88].
 (–) – 95% confidence interval

Reference:

KCP 10.2/02

Report

Terbut 500 SC Pseudokirchneriella subcapitata SAG 61.81 Growth inhibition test; E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W/11/18

Guideline(s):	OECD Guideline No. 201 (2006)/ EU Method C.3
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbutylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	The unicellular freshwater green algae, <i>Pseudokirchneriella subcapitata</i> (Reinsch) Korshikov (syn. <i>Raphidocelis subcapitata</i> , <i>Selenastrum capricornutum</i> Prinz) SAG 61.81 cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology. The algae were obtained from the Culture Collection of Algae at Gottingen University, Germany.
Test Design:	72 hours of exposure; three replicates for each test item concentration and six for the control; a background for the control and each test item concentration; initial algal cell density: 1×10^4 cells/mL
Endpoints:	E _r C ₅₀ , E _y C ₅₀ , LOEC and NOEC..
Test Conditions	Temperature: 21.6 – 22.1oC; pH of the control: 7.29 – 7.97; mean light intensity: 7255 – 7593 lux; constant illumination and shaking; medium: AAP..
Test Concentration::	15.6, 31.3, 62.5, 125, 250 1.0, 0.33, 0.11, 0.037, 0.012, 0.0041, 0.0014 mg/L plus the control.and 500 mg/L plus the control

Results and discussion:

The effect of the test item on the green algal growth was assessed. The range of the test item concentrations used in the definitive test were determined on the basis of the preliminary test results. The growth inhibition was estimated on the basis of the density of the algae cells determined in the definitive test.

The recorded temperature was in the range of 22.1 – 22.5oC, whereas the mean light intensity was in the range of 7450 – 7558 lux. The measured pH values were in the ranges of 7.37 – 7.46 at exposure initiation and 7.46 – 8.99 at exposure termination). The average transmittance values were in the range of 100.0 – 118.4% at exposure initiation and in the range of 100.0 – 116.8% at exposure termination. Hence, the indirect method was adequate to determine the number of algal cells. The growth rate inhibition after 72 hours of exposure was 11.93% in the test item concentration of 0.01 mg/L, 56.27% in the test item concentration of 0.1 mg/L, 82.84% in the test item concentration of 1.0 mg/L and 131.33% in the test item concentration of 10 mg/L when compared to the control. The yield inhibition after 72 hours of exposure was 45.28% in the test item concentration of 0.01 mg/L, 92.64% in the test item concentration of 0.1 mg/L, 92.97% in the test item concentration of 1.0 mg/L and 122.05% in the test item concentration of 10 mg/L when compared to the control.

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.00	0.00
0.01	11.93	45.28
0.1	56.27	92.64
1.0	82.84	92.97
10	131.33 *	122.05 *

* Inhibition is higher than 100.0%, which means that the algal cell density at exposure termination was lower than at exposure initiation

Definitive test

The recorded temperature was in the range of 21.6 – 22.1oC with a variation of up to 0.5oC. This is compliant with the allowed variation during exposure of ± 2.0 oC. The mean light intensity was in the range of 7255 – 7593 lux. The pH values measured at exposure initiation were in the range of 7.26 – 7.36 and at exposure termination were in the range 7.44 – 8.19. Morphology observations of the algae were performed at exposure termination. In test item concentrations of 0.11, 0.037, 0.012, 0.0041, 0.0014 mg/L no differences in shape, size and colour of algae cells were reported as compared to the algae cells in the control. In test item concentration of 1.0 and 0.33 mg/L the algae cells were deformed. The mean transmittance values were in the range of 99.3 – 100.1% at exposure initiation and in the range of 99.3 – 100.0% at exposure termination when compared with the control. Hence, the indirect method was adequate to determine the number of algal cells. The average specific growth rates and yield were calculated based on the numbers of cells recalculated from absorbance values measured at 24, 48 and 72 h for the cell number based on the standard curve. The average sectionby- section specific growth rates and yield calculated for the whole exposure

Results of the chemical determinations

The concentrations of test item were chemically determined using a validated liquid chromatographic method with DAD detection. Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentration of test item was in the range of 82.5 – 107.8% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentration of test item was in the range of 81.1 – 95.7% of the nominal concentration. Therefore, the concentrations of test item were stable under test conditions.

Endpoint values

The endpoint values were determined on the basis of the nominal test item concentrations. The ErCx and the EyCx values were calculated with the probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were determined on the basis of the results of statistical analyses. To make calculations and to conduct statistical analyses, the ToxRat Professional commercial software was used.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
ErC ₅₀	0.3508 (0.1470 – 1.3827)	0.1753 (0.0886 – 0.3289)	0.1815 (0.0790 – 0.3778)
ErC ₂₀	0.0171 (0.0009 – 0.0511)	0.0205 (0.0032 – 0.0477)	0.0231 (0.0020 – 0.0585)
ErC ₁₀	0.0035 (0.0000 – 0.0184)	0.0067 (0.0005 – 0.0208)	0.0078 (0.0002 – 0.0270)
LOEC	0.0120	0.0120	0.0120
NOEC	0.0041	0.0041	0.0041

Calculations were made according to [8], [SOP/W/88].
 (–) – 95% confidence interval

Table 13. Endpoint values for yield based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
EyC ₅₀	0.0891 (0.0318 – 0.1382)	0.0347 (0.0230 – 0.0521)	0.0251 (0.0176 – 0.0471)
EyC ₂₀	0.0089 (0.0012 – 0.0172)	0.0117 (0.0061 – 0.0182)	0.0117 (0.0071 – 0.0166)
EyC ₁₀	0.0021 (0.0002 – 0.0067)	0.0066 (0.0029 – 0.0112)	0.0078 (0.0038 – 0.0113)
LOEC	0.0120	0.0120	0.0120
NOEC	0.0041	0.0041	0.0041

Calculations were made according to [8], [SOP/W/88].
 (–) – 95% confidence interval

The concentration causing a 50% inhibition of the average specific growth rate of *Pseudokirchneriella subcapitata*, i.e. the ErC₅₀/72 h value is 0.1815 mg/L (95% confidence interval: 0.0790 – 0.3778). The ErC₂₀/72 h value is 0.0231 mg/L (95% confidence interval: 0.0020 – 0.0585). The ErC₁₀/72 h value is 0.0078 mg/L (95% confidence interval: 0.0002 – 0.0270). Statistical tests based on the growth rate data were Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) showed that the variances were heterogeneous and Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment which did not showed significant difference between the nominal test item concentrations 0.0014 and 0.0041 mg/L and the control. The lowest test item concentration causing an effect on growth rate inhibition LOEC/72 h value is 0.0120 mg/L. The highest test item concentration not causing any effect on growth rate inhibition NOEC/72 h value is 0.0041 mg/L. The concentration causing a 50% inhibition of yield of *Pseudokirchneriella subcapitata*, i.e. the EyC₅₀/72 h value is 0.0251 mg/L (95% confidence interval: 0.0176 – 0.0471). The EyC₂₀/72 h value is 0.0117 mg/L (95% confidence interval: 0.0071 – 0.0166). The EyC₁₀/72 h value is 0.0078 mg/L (95% confidence interval: 0.0038 – 0.0113). Statistical tests based on the yield data were Shapiro-Wilk's Test on Normal Distribution which did not confirm normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) showed that the variances were homogeneous and Williams Multiple Sequential t-test Procedure which showed significant difference between the nominal test item concentrations in the range of 0.0120 – 1.0 mg/L and the control. The lowest test item concentration causing an effect on yield inhibition LOEC/72 h value is 0.0120 mg/L. The highest test item concentration not causing any effect on yield inhibition NOEC/72 h value is 0.0041 mg/L.

THE VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in OECD Guideline No. 201 (2006) and EU Method C.3. were met:

- the biomass in the control increased by a factor of 151.0 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.2% (criterion: it must not exceed 7%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 11.6% (criterion: it must not exceed 35%).

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The doubling time of frond number in the control was 2.3 days, criterion: less than 2.5 days.
- The average specific growth rate in the control between day 0 and day 7 was 0.304 d-1 (minimum requirement: higher than 0.275 d-1).

Agreed endpoints:

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E _r C ₁₀	0.0433 (0.0256 – 0.0611)	0.0379 (0.0344 – 0.0413)	0.0539 (0.0462 – 0.0611)	0.0345 (0.0292 – 0.0393)
E _r C ₂₀	0.0733 (0.0499 – 0.0955)	0.0576 (0.0536 – 0.0615)	0.0780 (0.0695 – 0.0859)	0.0480 (0.0424 – 0.0530)
E _r C ₅₀	0.2007 (0.1637 – 0.2465)	0.1284 (0.1229 – 0.1342)	0.1583 (0.1477 – 0.1696)	0.0902 (0.0842 – 0.0964)
LOEC	0.33	0.037	0.11	0.037
NOEC	0.11	0.012	0.037	0.012

Calculations were made according to [9], [SOP/W/68]
 (-) - 95% confidence intervals

Table 15. Endpoint values of yield based on nominal test item concentrations, definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E _y C ₁₀	0.0406 (0.0239 – 0.0564)	0.0367 (0.0328 – 0.0403)	0.0530 (0.0376 – 0.0634)	0.0208 (0.0158 – 0.0250)
E _y C ₂₀	0.0639 (0.0434 – 0.0824)	0.0490 (0.0450 – 0.0526)	0.0657 (0.0515 – 0.0748)	0.0285 (0.0234 – 0.0329)
E _y C ₅₀	0.1523 (0.1245 – 0.1864)	0.0850 (0.0811 – 0.0889)	0.0991 (0.0924 – 0.1043)	0.0522 (0.0466 – 0.0587)
LOEC	0.33	0.037	0.11	0.037
NOEC	0.11	0.012	0.037	0.012

Calculations were made according to [9], [SOP/W/68]
 (-) - 95% confidence intervals

Reference:	KCP 10.2/03
Report	Terbut 500 SC <i>Lemna gibba</i> CPCC 310, Growth inhibition test; E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W/12/18
Guideline(s):	OECD Guideline No. 221 (2006)/ EU Method C.26
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	Freshwater aquatic plant <i>Lemna gibba</i> specification CPCC 310, cultured in Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.
Test Design:	Static system (7 days); no renewals; three replicates for each test item concentration and six replicates for the control.
Endpoints:	E _r C ₅₀ , E _y C ₅₀ , LOEC and NOEC..
Test Conditions	Temperature: 25.0 – 25.2°C; pH of the control: 7.40 – 8.90; mean light intensity: 8313 – 8562 lux; constant illumination; the 20X AAP medium glass beakers with a capacity of 600 mL containing 400 mL of each treatment; initial frond number 9, i.e. 3 plants per 3 fronds
Test Concentration::	1.0, 0.33, 0.11, 0.037, 0.012, 0.004 and 0.0013 mg/L plus the control.

Results and discussion:

The effect of the test item, Terbut 500 SC, on growth of *Lemna gibba* was estimated. The test item concentrations used in the definitive test and its design were determined on the basis of the results of the preliminary tests.

Preliminary test (non-GLP)

In the preliminary exposure test, the recorded temperature was in the range of 23.2 – 26.4°C, whereas the mean light intensity was in the range of 7668 – 7988 lux. The pH values measured at exposure initiation were in the range of 7.46 – 7.51 and at exposure termination were in the range of 8.43 – 8.98. In the preliminary test, at exposure termination, the growth rate inhibition based on the frond number was 4.4% in the test item concentration of 0.001 mg/L, 0.0% in the test item concentration of 0.01 mg/L, 25.8% in the test item concentration of 0.1 mg/L, 81.4% in the test item concentration of 1.0 mg/L and 88.1% in the test item concentration of 10 mg/L. The yield inhibition based on the frond number was 8.6% in the test item concentration of 0.001

mg/L, 0.0% in the test item concentration of 0.01 mg/L, 43.1% in the test item concentration of 0.1 mg/L, 91.4% in the test item concentration of 1.0 mg/L and 94.8% in the test item concentration of 10 mg/L. The growth rate inhibition based on the dry weight was 5.2% in the test item concentration of 0.001 mg/L, 8.5% in the test item concentration of 0.01 mg/L, 57.8% in the test item concentration of 0.1 mg/L and 100.0% in the test item concentrations of 1.0 and 10 mg/L. The yield inhibition based on the dry weight was 13.3% in the test item concentration of 0.001 mg/L, 20.8% in the test item concentration of 0.01 mg/L, 82.8% in the test item concentration of 0.1 mg/L and 100.0% in the test item concentrations of 1.0 and 10 mg/L.

Nominal test item concentration [mg/L]	Based on frond number		Based on dry weight	
	% Inhibition of growth rate	% Inhibition of yield	% Inhibition of growth rate	% Inhibition of yield
Control	0.0	0.0	0.0	0.0
0.001	4.4	8.6	5.2	13.3
0.01	0.0	0.0	8.5	20.8
0.1	25.8	43.1	57.8	82.8
1.0	81.4	91.4	100.0	100.0
10	88.1	94.8	100.0	100.0

Results of the chemical determinations

In the stability test at the test initiation, the determined test item concentration was 104.7% of nominal concentration. After 2 days of the test initiation, the determined test item concentration was 106.3% of nominal concentration. After 3 days of the test initiation, the determined test item concentration was 106.4% of nominal concentration. At the test termination, the determined test item concentration was 110.1% of nominal concentration. Therefore, the test item concentration was stable under test conditions. Based on the chemical determination results, the definitive test was performed in a static test design.

Nominal test item concentration [mg/L]	Mean determined test item concentration in samples collected [mg/L]			
	at test initiation	after 2 day of test initiation	after 3 day of test initiation	at test termination
Control	< LoD	< LoD	< LoD	< LoD
10	10.5 (104.7% of nominal concentration)	10.6 (106.3% of nominal concentration)	10.6 (106.4% of nominal concentration)	11.0 (110.1% of nominal concentration)

LoQ = 0.001 mg/L
 LoD = 0.0003 mg/L

Definitive test

The recorded temperature was in the range of 25.0 – 25.2°C with a variation of up to 0.2°C. During exposure, the measured light intensity was in the range of 8313 – 8562 lux. The pH values measured at exposure initiation were in the range of 7.40 – 7.60 and at exposure termination were in the range of 8.34 – 9.10. The number of fronds distinctly visible in each test vessel was counted and recorded as well as changes in plant development on days 2, 4 and after 7 days of exposure. The frond numbers and the dry weight are given in Table 7. The growth rates and yield

were calculated based on frond numbers counted after 2, 4 and 7 days of exposure. The morphology of plants was observed in each test item concentration and the control on days 2, 4 and at exposure termination. The morphological effects were compared with appearance of colonies in the control. After 2 days of exposure, in the test item concentrations in the range of 0.0013 – 0.037 mg/L no distinctive changes from the development of plants in the control were observed. After 2 days of exposure, in the test item concentrations in the range of 0.11 – 1.0 mg/L bending down of frond was observed. After 4 days of exposure, in the test item concentrations in the range of 0.0013 – 0.037 mg/L no distinctive changes from the development of plants in the control were observed. After 4 days of exposure, in the test item concentrations in the range of 0.11 – 1.0 mg/L bending down of frond was observed. At exposure termination, in the test item in the range of 0.0013 – 0.037 mg/L no distinctive changes from the development of plants in the control were observed. At exposure termination, in the test item concentration of 0.11 mg/L smaller fronds and bending down of fronds were observed. At exposure termination, in the test item concentrations of 0.33 and 1.0 mg/L smaller fronds, bending down of fronds and break down of colonies were observed. The inhibition of growth rate and the inhibition of yield estimated in comparison to the control based on frond number and based on dry weight after 7 days of exposure are given below:

Nominal test item concentration [mg/L]	Based on frond number		Based on dry weight	
	% inhibition at exposure termination (day 7) (growth rate)	% inhibition at exposure termination (day 7) (yield)	% inhibition at exposure termination (day 7) (growth rate)	% inhibition at exposure termination (day 7) (yield)
Control	0.0	0.0	0.0	0.0
0.0013	5.3	12.5	-3.6*	-10.4*
0.004	3.8	9.0	-2.0*	-5.8*
0.012	1.5	4.0	1.7	5.1
0.037	-10.7*	-28.5*	12.3	30.5
0.11	34.3	59.0	59.7	85.8
0.33	82.1	93.5	100.6	100.0
1.0	89.2	96.5	119.6	103.0

*calculated inhibition values are lower than 0%, what means that the frond number and measured dry weight at exposure termination were higher than the frond number and measured dry weight in the control

Results of the chemical determinations

The determined test item concentrations in the samples collected at exposure initiation were in the range of 94.9 – 109.1% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined test item concentrations in the samples collected at exposure termination were in the range of 98.1 – 106.8% of the nominal concentration. Therefore, the test item concentrations were stable under the test conditions.

Nominal test item concentration [mg/L]	Average determined test item concentrations (n = 3) [mg/L] in samples collected			
	at exposure initiation	% of the nominal concentration	at exposure termination	% of the nominal concentration
Control	< LoD	---	< LoD	---
0.0013	0.00139	106.7	0.00139	106.8
0.004	0.00430	107.4	0.00394	98.6
0.012	0.01309	109.1	0.01205	100.4
0.037	0.03967	107.2	0.03846	103.9
0.11	0.1116	101.4	0.1081	98.3
0.33	0.3144	95.3	0.3237	98.1
1.0	0.9491	94.9	1.0121	101.2

LoQ = 0.001 mg/L
 LoD = 0.0003 mg/L

Endpoint values

The endpoint values were determined on the basis of the nominal test item concentrations. The ECx values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyzes. To conduct statistical analyses, the ToxRat Professional commercial software was used.

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E_rC₁₀	0.0433 (0.0256 – 0.0611)	0.0379 (0.0344 – 0.0413)	0.0539 (0.0462 – 0.0611)	0.0345 (0.0292 – 0.0393)
E_rC₂₀	0.0733 (0.0499 – 0.0955)	0.0576 (0.0536 – 0.0615)	0.0780 (0.0695 – 0.0859)	0.0480 (0.0424 – 0.0530)
E_rC₅₀	0.2007 (0.1637 – 0.2465)	0.1284 (0.1229 – 0.1342)	0.1583 (0.1477 – 0.1696)	0.0902 (0.0842 – 0.0964)
LOEC	0.33	0.037	0.11	0.037
NOEC	0.11	0.012	0.037	0.012

Calculations were made according to [9], [SOP/W/68]
 (-) - 95% confidence intervals

Table 15. Endpoint values of yield based on nominal test item concentrations, definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E_yC₁₀	0.0406 (0.0239 – 0.0564)	0.0367 (0.0328 – 0.0403)	0.0530 (0.0376 – 0.0634)	0.0208 (0.0158 – 0.0250)
E_yC₂₀	0.0639 (0.0434 – 0.0824)	0.0490 (0.0450 – 0.0526)	0.0657 (0.0515 – 0.0748)	0.0285 (0.0234 – 0.0329)
E_yC₅₀	0.1523 (0.1245 – 0.1864)	0.0850 (0.0811 – 0.0889)	0.0991 (0.0924 – 0.1043)	0.0522 (0.0466 – 0.0587)
LOEC	0.33	0.037	0.11	0.037
NOEC	0.11	0.012	0.037	0.012

Calculations were made according to [9], [SOP/W/68]
 (-) - 95% confidence intervals

The median test item concentration causing 50% inhibition of growth rate of *Lemna gibba* culture based on frond number ErC50/7 d value is 0.1583 mg/L (95% confidence intervals: 0.1477 – 0.1696). The ErC20/7 d value is 0.0780 mg/L (95% confidence intervals: 0.0695 – 0.0859) and the ErC10/7 d value is 0.0539 mg/L (95% confidence intervals: 0.0462 – 0.0611). The statistical tests performed with data on growth rate based on frond number revealed: Shapiro- Wilk’s Test on Normal Distribution confirmed normal distribution of data, Levene’s Test on Variance Homogeneity showed that variances are homogeneous, Williams Multiple Sequential t-test Procedure showed statistically significant difference between nominal test item concentrations in the range of 0.11 – 1.0 mg/L and the control. Therefore, the NOEC/7 d value is 0.037 mg/L and the LOEC/7 d value is 0.11 mg/L. The median test item concentration causing 50% of yield inhibition of *Lemna gibba* culture based on frond number EyC50/7 d value is 0.0991 mg/L (95% confidence intervals: 0.0924 – 0.1043). The EyC20/7 d value is 0.0657 mg/L (95% confidence intervals: 0.0515 – 0.0748) and the EyC10/7 d value is 0.0530 mg/L (95% confidence intervals: 0.0376 – 0.0634). The statistical tests performed with data on yield based on frond number revealed: Shapiro-Wilk’s Test on Normal Distribution confirmed normal distribution of data,

Levene's Test on Variance Homogeneity showed that variances are heterogeneous, Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm showed a significant difference between nominal test item concentrations in the range of 0.11 – 1.0 mg/L and the control. Therefore, the NOEC/7 d value is 0.037 mg/L and the LOEC/7 d value is 0.11 mg/L. The median test item concentration causing 50% inhibition of growth rate of *Lemna gibba* culture based on dry weight ErC50/7 d value is 0.0902 mg/L (95% confidence intervals: 0.0842 – 0.0964). The ErC20/7 d value is 0.0480 mg/L (95% confidence intervals: 0.0424 – 0.0530). The ErC10/7 d value is 0.0345 mg/L (95% confidence intervals: 0.0292 – 0.0393). The statistical tests performed with data on growth rate based on dry weight showed: Shapiro-Wilk's Test on Normal Distribution confirmed normal distribution of data, Levene's Test on Variance Homogeneity showed that variances are homogeneous, Williams Multiple Sequential t-test Procedure showed a significant difference between nominal test item concentrations in the range of 0.037 – 1.0 mg/L and the control. Therefore, the NOEC/7 d value is 0.012 mg/L and the LOEC/7 d value is 0.037 mg/L. The median test item concentration causing 50% of yield inhibition of *Lemna gibba* culture based on dry weight EyC50/7 d value is 0.0522 mg/L (95% confidence intervals: 0.0466 – 0.0587). The ErC20/7 d value is 0.0285 mg/L (95% confidence intervals: 0.0234 – 0.0329). The ErC10/7 d values is 0.0208 mg/L (95% confidence intervals: 0.0158 – 0.0250). The statistical tests performed with data on yield based on dry weight revealed: Shapiro-Wilk's Test on Normal Distribution confirmed normal distribution of data, Levene's Test on Variance Homogeneity showed that variances are homogeneous, Williams Multiple Sequential t-test Procedure showed a significant difference between nominal test item concentrations in the range of 0.037 – 1.0 mg/L and the control. Therefore, the NOEC/7 d value is 0.012 mg/L and the LOEC/7 d value is 0.037 mg/L.

THE VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in OECD Guideline No. 221 (2006) and EU Method C. 26. were met:

- The doubling time of frond number in the control was 2.3 days, criterion: less than 2.5 days;
- The average specific growth rate in the control between day 0 and day 7 was 0.304 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹).

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The biomass in the control increased by a factor of 57.9 within the 72-hour test period (criterion: at least a 16-fold growth).
- The coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.6% (criterion: it must not exceed 7%).
- The mean coefficient of variation for the section-by-section growth rate in the control culture was 29.3% (criterion: it must not exceed 35%).

Agreed endpoints:

Table 11. Growth rate endpoint values based on the nominal test item concentrations, definitive test.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _r C ₅₀	0.007 (0.006 – 0.008)	0.012 (0.008 – 0.017)	0.020 (0.018 – 0.023)
E _r C ₂₀	0.003 (0.002 – 0.004)	0.002 (0.001 – 0.004)	0.007 (0.005 – 0.008)
E _r C ₁₀	0.002 (0.001 – 0.002)	n.d.	0.004 (0.003 – 0.005)
LOEC	<= 0.004	<= 0.004	0.012
NOEC	<0.004	<0.004	0.004

(-) – 95% confidence interval
 Calculations were made according to [8], [SOP/W/68]

Table 12. Yield endpoint values based on the nominal test item concentrations, definitive test.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _y C ₅₀	0.004 (0.004 – 0.005)	0.002 (0.001 – 0.004)	0.007 (0.006 – 0.008)
E _y C ₂₀	0.002 (0.001 – 0.002)	n.d.	0.004 (0.003 – 0.005)
E _y C ₁₀	n.d.	n.d.	0.003 (0.002 – 0.004)
LOEC	<=0.004	<=0.004	0.012
NOEC	<0.004	<0.004	0.004

(-) – 95% confidence interval
 Calculations were made according to [8], [SOP/W/68]

Reference: KCP 10.2/04

Report Terbut 500 SC *Navicula pelliculosa* SAG 1050-3, Growth inhibition test; D. Jenota; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W/53/19

Guideline(s): the OECD Guideline No. 201 (2006)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test Item:	TERBUT 500 SC; batch no: 1/19; content of terbutylazine: 511.9 g/L; production date: April 2019; expiry date: April 2021..
Test Species:	The freshwater diatoms, <i>Navicula pelliculosa</i> (Bréb.) Hilse specification SAG 1050 – 3, cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Toxicology. The diatoms were obtained from the Culture Collection of Algae at Göttingen University, Germany..
Test Design:	72 hours of exposure; three replicates per each test item concentration and six replicates per the control; initial diatom cell density: 1 x 10 ⁴ cells/mL.
Endpoints:	ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.
Test Conditions	Temperature: 21.9 – 22.3°C; pH of the control: 7.53 – 8.29; mean light intensity: 7690 – 7785 lux; constant illumination and shaking; medium: AAP-Si. 1.0, 0.33, 0.11, 0.037, 0.012, 0.004 mg/L plus the control..

Test Concentration::

Results and discussion:

The effect of the test item on the diatoms growth was assessed. The range of the test item concentrations used in the definitive test were determined on the basis of the preliminary exposure test results. The growth inhibition was estimated on the basis of the density of the diatoms cells determined in the definitive test.

Preliminary test (non-GLP)

Table 4. Inhibition of growth rate and yield, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.01	9.1	36.8
0.1	52.8	94.1
1.0	104.3*	100.1*
10	117.6*	100.3*

*Inhibition of growth rate and yield is higher than 100.0% means that the diatoms cell density at exposure termination was lower than diatoms cell density at exposure initiation.

Time of exposure: 12.11.2019 – 15.11.2019

In the preliminary test, the recorded temperature was in the range of 21.5 – 22.5°C, the mean light intensity was in the range of 6390 – 6425 lux. The pH values at exposure initiation were in the range of 7.57 – 7.70 at exposure initiation and in the range of 7.83 – 8.11 at exposure termination. The growth rate inhibition after 72 h of exposure was 9.1% in the test item concentration of 0.01 mg/L, 52.8% in the test item concentration of 0.1 mg/L, 104.3% in the test item concentration of 1.0 mg/L and 117.6% in the test item concentration of 10 mg/L. The yield inhibition after 72 hours of exposure was 36.8% in the test item concentration of 0.01 mg/L, 94.1% in the test item concentration of 0.1 mg/L, 100.1% in the test item concentration of 1.0 mg/L and 100.3% in the test item concentration of 10 mg/L.

Results of the chemical determinations

In the preliminary exposure test, the concentrations of the test item TERBUT 500 SC were not chemically determined.

Definitive test

The definitive test was performed using the test item concentrations of 1.0, 0.33, 0.11, 0.037, 0.012 and 0.004 mg/L.

The mean light intensity was in the range of 7690 – 7785 lux. The pH values measured at exposure initiation were in the range of 7.41 – 7.53 and 7.56 – 8.29 at exposure termination. In all test item concentrations, no differences in shape, size and colour of diatoms cells were reported as compared to the diatoms cells in the control. On the basis of the density of the diatoms cells determined after 24, 48 and 72 hours the average section-by-section specific growth rates and the yield increase during the whole experiment were calculated.

Results of the chemical determinations

The concentrations of the test item TERBUT 500 SC were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/393]. Samples of each treatment were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentrations of the test item were in the range of 95.5 – 108.7% of the nominal concentrations. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of the test item were in the range of 91.3 – 107.8% of the nominal concentration. Therefore, the concentrations of TERBUT 500 SC were stable under test conditions in both series.

Endpoint values

Table 11. Growth rate endpoint values based on the nominal test item concentrations, definitive test.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _r C ₅₀	0.007 (0.006 – 0.008)	0.012 (0.008 – 0.017)	0.020 (0.018 – 0.023)
E _r C ₂₀	0.003 (0.002 – 0.004)	0.002 (0.001 – 0.004)	0.007 (0.005 – 0.008)
E _r C ₁₀	0.002 (0.001 – 0.002)	n.d.	0.004 (0.003 – 0.005)
LOEC	<= 0.004	<= 0.004	0.012
NOEC	<0.004	<0.004	0.004

(-) – 95% confidence interval
 Calculations were made according to [8], [SOP/W/68]

Table 12. Yield endpoint values based on the nominal test item concentrations, definitive test.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _y C ₅₀	0.004 (0.004 – 0.005)	0.002 (0.001 – 0.004)	0.007 (0.006 – 0.008)
E _y C ₂₀	0.002 (0.001 – 0.002)	n.d.	0.004 (0.003 – 0.005)
E _y C ₁₀	n.d.	n.d.	0.003 (0.002 – 0.004)
LOEC	<=0.004	<=0.004	0.012
NOEC	<0.004	<0.004	0.004

(-) – 95% confidence interval
 Calculations were made according to [8], [SOP/W/68]

The endpoint values were determined on the basis of nominal test item concentrations. The EC_x values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyzes. To conduct statistical analyses, the ToxRat Professional commercial software was used.

The median test item concentration causing 50% inhibition of the average specific growth rate of *Navicula pelliculosa*, i.e. the ErC50/72 h is 0.02 mg/L (95% confidence interval: 0.018 – 0.023). The ErC20/72 h value is 0.007 mg/L (95% confidence interval: 0.005 – 0.008), and the ErC10/72 h value is 0.004 mg/L (95% confidence interval: 0.003 – 0.005).

Statistical tests based on the growth rate data were the Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were heterogeneous and the Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm which showed significant difference between the test item concentrations in the range of 0.012 – 1.0 mg/L and the control. Therefore, the LOEC/72 h value is equal to 0.012 mg/L and the NOEC/72 h value is equal to 0.004 mg/L. The median test item concentration causing 50% yield inhibition of *Navicula pelliculosa*, i.e. the EyC50/72 h is 0.007 mg/L (95% confidence interval: 0.006 – 0.008). The EyC20/72 h value is 0.004 mg/L (95% confidence interval: 0.003 – 0.005), and the EyC10/72 h value is 0.003 mg/L (95% confidence interval: 0.002 – 0.004). The determined EyC10/72 value is below the lowest test item concentration used for exposure (i.e. 0.004 mg/L)

Statistical tests based on the yield data were the Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were heterogeneous and the Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm which showed significant difference between the test item concentrations in the range of 0.012 – 1.0 mg/L and the control. Therefore, the LOEC/72 h value is equal to 0.012 mg/L and the NOEC/72 h value is equal to 0.004 mg/L.

THE VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- the biomass in the control increased by a factor of 57.9 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.6% (criterion: it must not exceed 7%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 29.3% (criterion: it must not exceed 35%).

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The average mortality for the total number of controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
- The LD₅₀/24 h of the reference item (dimethoate) was 0.10 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee).

Agreed endpoints:

LD₅₀/24 h and LD₅₀/48 h ≥ 200.0 µg/honeybee (90.7 µg a.i./bee).

No behavioural abnormalities (uncoordinated movement, increased activity, or intensive cleaning) were observed during the 48-hour exposure.

Reference:	KCP 10.3/01
Report	Terbut 500 SC Honeybees (<i>Apis mellifera</i> L.), Acute Oral Toxicity Test;P. Parma; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B/87/17
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	the honeybee, <i>Apis mellifera</i> L., strain: carnica source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna age: approximately 3 weeks.
Test Design:	- the test item: exposure duration: 48 hours number of doses: 4 doses and a control number of replicates: 3 replicates number of bees: 10 bees/replicate - the reference item: exposure duration: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate
Endpoints:	ErC ₅₀ , EyC ₅₀ , LOEC and NOEC..

Test Conditions temperature: 24 – 26°C, relative air humidity: 53 - 56%
 place: a dark room

Test Concentration:: - honeybee mortality 48 hours after dose administration,

Results and discussion:

Preliminary test (non-GLP)

Mortality of the control group after 48 of exposure was 0.0%. The percentages of mortality of the bees treated with the test item at the doses of 4.0, 20.0 and 100.0 µg/honeybee were 0.0, 0.0 and 0.0%, respectively.

Definitive experiment

They contain raw data which were then converted to percentages in order to determine the LD50 values. At the end of the study (after 48 hours) mortality in the control group and the groups with the test item with doses 25.0, 50.0, 100.0 and 200.0 µg/honeybee (11.3, 22.7, 45.4 and 90.7 µg a.i./honeybee) were 0.0%. The median lethal doses (LD50/24 h and LD50/48 h per os) are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee (90.7 µg a.i./bee). No behavioural abnormalities (uncoordinated movement, increased activity, or intensive cleaning) were observed during the 48-hour exposure.

Dose		Exposure replicates	4 h	24 h	48 h
			Number of bees with adverse behaviour*/ number of living bees		
[µg/bee]	[µg a.i./bee]				
0.0 (control)		I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10
25.0	11.3	I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10
50.0	22.7	I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10
100.0	45.4	I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10
200.0	90.7	I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10

- * Sub-lethal toxic effects were:
- a- uncoordinated movements
 - b- increased activity
 - c- intensive cleaning
 - d- paralysis

Food consumption is presented below:

Table 7. Average food consumption [mg] – definitive experiment

Dose [µg/bee]	Time of exposure	
	24 h	48 h
0.0 (control)	124.1	103.9
25.0	94.4	76.3
50.0	123.8	85.8
100.0	91.5	99.4
200.0	122.2	93.3

Table 8. Difference in food consumption between the treated and the control groups [%]

Dose [µg/bee]	Time of exposure	
	24 h	48 h
25.0	23.93	26.56
50.0	0.27	17.42
100.0	26.29	4.36
200.0	1.58	10.26

The reduction ranged from 0.27 to 26.56% when compared to the control. Mortality after 4 and 24 hours and the LD50/24 h of the reference item are presented in Tables 10 and 11. The median lethal dose of dimethoate (LD50/24 h) with 95% confidence limits was determined with the log-probit method. It was 0.10 µg a.i./bee (confidence limits: 0.09 - 0.11) (Table 11). The treated group did not exhibit any abnormal behaviours.

Table 10. Honeybee mortality after 4 hours of exposure – dimethoate

Dose [µg a.i./bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.]			Total	
		replicates				
I	II	III	[no.]	[%]		
0.0 (control)	30	0	0	0	0	0.0
0.03	30	0	0	0	0	0.0
0.06	30	0	0	0	0	0.0
0.12	30	1	0	0	1	3.3

Table 11. Honeybee mortality and the LD₅₀ after 24 hours of exposure – dimethoate

Dose [µg a.i./bee]	Number of tested bees [no.]	Mortality					LD ₅₀ [µg/bee]
		Number of dead bees [no.]			Total		
		replicates					
I	II	III	[no.]	[%]			
0.0 (control)	30	0	0	0	0	0.0	0.10 ^a (0.09-0.11)
0.03	30	0	0	0	0	0.0	
0.06	30	1	0	0	1	3.3	
0.12	30	7	8	7	22	73.3	

^a: oral LD₅₀ value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Professional 3.2.1. statistical software)

DEFINITIONS OF THE ENDPOINTS

The LD₅₀ (median lethal dose) oral, is a statistically derived single dose of a test or reference item that can cause death in 50 per cent of biological test systems when administered by the oral route. The LD₅₀ is expressed in mg test item/bee or µg active ingredient/bee, whereas for the reference item – µg active ingredient/bee. Mortality: a honeybee is dead if it is completely immobile.

THE VALIDITY CRITERIA

The following validity criteria were met during the test:

- the average mortality for the total number of controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%),
- the LD₅₀/24 h of the reference item (dimethoate) was 0.10 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%).
- The 24 hour LD₅₀ of the reference item (dimethoate) was 0.26 µg a.i./bee (criterion: 0.10 -0.30 µg a.i./bee).

Agreed endpoints:

LD₅₀/24 h and LD₅₀/48 h ≥ 200.0 µg/honeybee (90.7 µg a.i./bee).

No behavioural abnormalities (uncoordinated movement, increased activity, or intensive cleaning) were observed during the 48-hour exposure.

Reference:	KCP 10.2/03
Report	Terbut 500 SC Honeybees (<i>Apis mellifera</i> L.), Acute Contact Toxicity Test; P. Parma; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B/88/17
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	the honeybee, <i>Apis mellifera</i> L., strain: carnica source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna age: approximately 3 weeks
Test Design:	- the test item: exposure duration: 48 hours number of doses: 4 doses and a control number of replicates: 3 replicates number of bees: 10 bees/replicate - the reference item: exposure duration: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate

Endpoints: - honeybee mortality after 48 hours of the exposure,
 - the contact LD50 of the test item after 24 and 48 hours of the exposure,
 - the contact LD50/24 h of the reference item (dimethoate).

Test Conditions temperature: 23 – 26°C, relative air humidity: 53 - 57%
 place: a dark room

Test Concentration:: 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee).

Results and discussion:

Preliminary experiment

Mortality of the control group after 48 hours of exposure was 0.0%. The percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/honeybee (3.6, 18.1 and 90.7 µg a.i./honeybee) were 0.0, 10.0 and 10.0%, respectively.

4.2. Definitive experiment

Mortality of the control group after 48 hours of exposure was 0.0%. Mortality of the treated insects is presented in Tables 3 - 5. At the end of the study (after 48 hours) mortality in the groups with the test item with doses 25.0, 50.0, 100.0 and 200.0 µg/honeybee (11.3, 22.7, 45.4 and 90.7 µg a.i./honeybee) were 0.0, 3.3, 3.3 and 0.0% respectively.

Dose		Number of tested bees [no.]	Mortality					LD ₅₀	
			Number of dead bees [no.] replicates			Total			
[µg/bee] ³	[µg a.i./bee]		I	II	III	[no.]	[%]	[µg /bee]	[µg a.i./bee]
0.0 (Control)		30	0	0	0	0	0.0	> 200.0	> 90.7
25.0	11.3	30	0	0	0	0	0.0		
50.0	22.7	30	0	1	0	1	3.3		
100.0	45.4	30	0	1	0	1	3.3		
200.0	90.7	30	0	0	0	0	0.0		

The definitive experiment was conducted between 14 – 16.09.2017.

No behavioural abnormalities (uncoordinated movement, increased activity, or intensive cleaning) were observed during the 48-hour exposure.

Dose [$\mu\text{g}/\text{bee}$]		Exposure replicates	4 h	24 h	48 h
			Number of bees showing adverse behaviour*/ number of living bees		
$[\mu\text{g}/\text{bee}]^a$	$[\mu\text{g a.i./bee}]$				
0.0 (control)		I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10
25.0	11.3	I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10
50.0	22.7	I	0/10	0/10	0/10
		II	0/10	0/9	0/9
		III	0/10	0/10	0/10
100.0	45.4	I	0/10	0/10	0/10
		II	0/10	0/9	0/9
		III	0/10	0/10	0/10
200.0	90.7	I	0/10	0/10	0/10
		II	0/10	0/10	0/10
		III	0/10	0/10	0/10

* Sub-lethal toxic effects were:
 a- uncoordinated movements
 b- increased activity
 c- intensive cleaning
 d- paralysis

The median lethal doses (LD50/24 h and LD50/48 h contact) are higher than the highest dose used in the test, i.e. 200.0 $\mu\text{g}/\text{honeybee}$ (90.7 μg of a.i./bee). The median lethal dose of dimethoate (LD50/24 h) determined with the log-probit method is 0.26 $\mu\text{g}/\text{bee}$ (95% confidence limits: 0.23 - 0.30 $\mu\text{g a.i./bee}$).

5. DEFINITIONS OF THE ENDPOINTS

The LD50 (median lethal dose) contact, is a statistically derived single dose of a substance that can cause death in 50 per cent of animals when administered by contact route. The LD50 is expressed in μg test item/bee or $\mu\text{g a.i./bee}$. It was calculated with the log-probit method. Mortality: a honeybee is dead if it is completely immobile.

THE VALIDITY CRITERIA

The following validity criteria were met during the test:

- the average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%),
- the 24 hour LD50 of the reference item (dimethoate) was 0.26 $\mu\text{g a.i./bee}$ (criterion: 0.10 - 0.30 $\mu\text{g a.i./bee}$).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

ZRMS comments:

The study is considered acceptable. All validity criteria were met.

- Mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%).
- Mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 88.33% on day 7 of exposure (criterion: a minimum of 50%).
- The mean number of eggs per female in the control group was 5.0 (required: ≥ 4 eggs per female).

Agreed endpoints:

LR₅₀ > 1.5 L/ha

NOER_{mortality} = 0.375 L/ha

ER₅₀ = 0.701 L/ha (i.e. 349.9 g a.i./ha)

NOER_{reproduction} ≤ 0.375 L/ha (i.e. < 187.2 g a.i./ha)

Reference:	KCP 10.3/03
Report	An extended laboratory test for evaluating Terbut 500 SC on the predatory mite, Typhlodromus pyri (Sch.); P. Parma; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B/90/17
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The aim of the extended laboratory test was to evaluate the effects of the test item, Terbut 500 SC on mortality and reproduction of the predatory mite, *T. pyri* (Sch.).

On the basis of the preliminary test results, it was decided to use three rates of the test item in the definitive test. These were 0.375, 0.75 and 1.5 L Terbut 500 SC/ha.

The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to bean leaf discs. The mites were fed with pine pollen (*Pinus sp.*). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and one treated group with the test item were made after 8, 11 and 14 days of the treatment.

Mortality of *T.pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints.

To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Danadim 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	the predatory mite, <i>Typhlodromus pyri</i> (Sch.) (Acari: Phytoseiidae) – age: 24-hour-old protonymphs – source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from the Research Institute of Pomology and Floriculture, Skierniewice, Poland and renewed from commercial supplier, Katz Biotech
Test Design:	5 study groups: – a control group (0.0 L/ha) – Terbut 500 SC at the rate of 0.375 L/ha (i.e. 187.2 g a.i./ha), – Terbut 500 SC at the rate of 0.75 L/ha (i.e. 374.4 g a.i./ha), – Terbut 500 SC at the rate of 1.5 L/ha (i.e. 748.8 g a.i./ha), – Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha) number of replicates: 3; number of mites in each replicate: 20
Endpoints:	– mite mortality after 7 days of the treatment – LR50, NOERmortality – reproduction reduction (Pr) after 14 days of the treatment – ER50, NOERreproduction
Test Conditions	Temperature: 25.0 – 25.2°C; pH of the control: 7.40 – 8.90; mean light intensity: 8313 – 8562 lux; constant illumination; the 20X AAP medium glass beakers with a capacity of 600 mL containing 400 mL of each treatment; initial frond number 9, i.e. 3 plants per 3 fronds

Results and discussion:

Mortality of *Typhlodromus pyri*

In the preliminary test, mortality of the control group after 7 days of exposure was 7.5%. After 7 days of exposure to Terbut 500 SC at the rates of 0.17, 0.5 and 1.5 L/ha the percentages of mortality of *T. pyri*, corrected using the formula of Abbott were 0.0, 13.5 and 18.9%, respectively.

Study group /rate		Number of tested mites [no.]	Mortality					
L/ha	g a.i./ha		Number of dead mites [no.]			Total		Corrected
			Replicates			[no.]	[%]	
		I	II					
Control / 0.0		40	2	1		3	7.5	-
0.17	84.9	40	3	0		3	7.5	0.0
0.5	249.6	40	4	4		8	20.0	13.5
1.5	748.8	40	5	5		10	25.0	18.9

The preliminary test was performed between 27.10.2017 - 03.11.2017.

In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to Terbut 500 SC at the rates of 0.375, 0.75 and 1.5 L/ha, the percentages of mortality of *T. pyri*, were 0.0, 6.7 and 8.3%, respectively.

Study group /rate		Number of tested mites [no.]	Mortality					
L/ha	g a.i./ha		Number of dead mites [no.]			Total		Corrected
			Replicates			[no.]	[%]	
		I	II	III				
Control / 0.0		60	0	0	0	0	0.0	-
0.375	187.2	60	0	0	0	0	0.0	-
0.75	374.4	60	1	1	2	4	6.7 ⁺	-
1.5	748.8	60	2	2	1	5	8.3 ⁺	-
LR₅₀		> 1.5 L/ha (>748.8 g/ha)						
NOER_{mortality}		0.375 L/ha (187.2 g/ha)						
mL/ha	g a.i./ha	Danadim 400 EC						
9.0	3.6	60	19	16	18	53	88.3	-

The definitive test was performed between 22.12.2017 – 05.01.2018.

⁺: statistically significant difference

There were no statistically significant differences in mortality between the group treated with the test item at the rate 0.375 L/ha and the control group, statistically significant differences were in

mortality between the groups treated with the test item at the rates 1.75 and 1.5 L/ha and the control group, (step-down Cochran-Armitage test procedure, $p(\text{trend}) > \alpha 0.05$). On the basis of the obtained mortality results, the estimated LR50 value is higher than 1.5 L/ha (> 748.8 g a.i./ha) and NOERmortality value is 0.375 L/ha (187.2 g a.i./ha), respectively. After 7 days of exposure to Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha), mortality of the mites, was 88.3%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

Reproduction of *Typhlodromus pyri*

The sex ratio after 7 days of exposure is presented below:

Study group [rate]		No. of tested mites	Males and females [no.]								
			Replicates								
[L/ha]	[g a.i./ha]		I			II			III		
			♀	♂	SR*	♀	♂	SR*	♀	♂	SR*
Control 0.0		60	11	9	0.55	11	9	0.55	13	7	0.65
0.375	187.2	60	13	7	0.65	12	8	0.60	15	5	0.75
0.75	374.4	56	12	7	0.63	12	7	0.63	11	7	0.61
1.5	748.8	55	10	8	0.56	12	6	0.67	13	6	0.68

SR*: sex ratio – the number of females divided by the total number of females and males per replicate after 7 days of exposure

The sex ratio was correct. Hence, no corrections were made. The mean reproduction rate (Rr) in the control group was 5.0 eggs/female. The mean reproduction rates after 14 days of exposure to Terbut 500 SC at the rates of 0.375, 0.75 and 1.5 L/ha were 3.2, 2.9 and 0.8 eggs/female. The percentages of reproduction reduction (Pr) caused by Terbut 500 SC at the rates of 0.375, 0.75 and 1.5 L/ha were 35.6, 41.6 and 84.5 respectively. At the significance level of 0.05, there were statistically significant differences in reproduction between the groups treated with the test item at the rates of 0.375, 0.75 and 1.5 L/ha (Williams multiple sequential t-test procedure, $|t| > |t^*|$). On the basis of the obtained results, the estimated ER50 value is 0.701 L/ha (i.e. 349.9 g a.i./ha) and NOERreproduction value is lower than 0.375 L/ha (i.e. < 187.2 g a.i./ha), respectively.

Study group [rate]	Replicates (X)	Developmental stages of the mites	Observation period			RrX	Rr	Pr [%]
			DAT 8	DAT 11	DAT 14			
Control 0.0	I	Eggs	6	19	24	5.0	5.0	-
		Larvae	0	3	2			
		Males	9	9	9			
		Females	11	11	10			
	II	Eggs	7	22	30	5.7		
		Larvae	0	0	0			
		Males	9	8	8			
		Females	11	10	10			
	III	Eggs	10	20	24	4.3		
		Larvae	0	1	0			
		Males	7	5	5			
		Females	13	13	12			
Terbut 500 SC [0.375 L/ha]	I	Eggs	2	17	20	3.3	3.2	35.6 *
		Larvae	0	0	0			
		Males	7	6	6			
		Females	13	12	10			
	II	Eggs	5	16	17	3.4		
		Larvae	0	1	0			
		Males	8	8	7			
		Females	12	12	10			
	III	Eggs	4	16	21	3.0		
		Larvae	0	1	2			
		Males	5	5	5			
		Females	15	15	14			
Terbut 500 SC [0.75 L/ha]	I	Eggs	1	15	19	2.9	2.9	41.6 *
		Larvae	0	0	0			
		Males	7	5	5			
		Females	12	12	12			
	II	Eggs	6	18	14	3.2		
		Larvae	0	0	0			
		Males	7	7	6			
		Female	12	12	11			
	III	Eggs	2	12	12	2.7		
		Larvae	0	0	0			
		Males	7	6	6			
		Females	10	10	9			

DAT: days after treatment

RrX: the reproduction rate for each replicate (X) of a given study group after 14 days, calculated according to equation no. 2, point 5.2

Rr: the mean reproduction rate in a given study group after 14 days

Pr: the percentage of reproduction reduction calculated according to equation no. 3, point 5.2

Study group [rate]	Replicates (X)	Developmental stages of the mites	Observation period			RrX	Rr	Pr [%]
			DAT 8	DAT 11	DAT 14			
Terbut 500 SC [1.5 L/ha]	I	Eggs	1	2	5	0.9	0.8	84.5 *
		Larvae	0	0	0			
		Males	7	5	4			
		Females	10	10	8			
	II	Eggs	0	3	5	0.8		
		Larvae	0	0	0			
		Males	6	6	4			
		Females	10	10	9			
	III	Eggs	0	2	4	0.7		
		Larvae	0	0	1			
		Males	6	5	4			
		Females	13	11	9			
ER₅₀		0.701 L/ha (349.9 g a.i./ha)						
NOER_{reproduction}		< 0.375 L/ha (< 187.2 g a.i./ha)						

DAT: days after treatment

RrX: the reproduction rate for each replicate (X) of a given study group after 14 days, calculated according to equation no. 2, point 5.2

Rr: the mean reproduction rate in a given study group after 14 days

Pr: the percentage of reproduction reduction calculated according to equation no. 3, point 5.2

*: statistically significant difference

THE VALIDITY CRITERIA

The following validity criteria were met during the study :

- mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%),
- mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 88.33% on day 7 of exposure (criterion: a minimum of 50%),
- the mean number of eggs per female in the control group was 5.0 (required: ≥ 4 eggs per female).

zRMS comments:

The study is considered acceptable. All validity criteria were met.

Agreed endpoints:

LR₅₀ > 1.5 L/ha

NOER_{mortality} >1.5 L/ha

ER₅₀ > 1.5 L/ha

NOER_{reproduction} >1.5 L/ha

Reference:	KCP 10.3/04
Report	An extended laboratory test for evaluating the effects of Terbut 500 SC on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani - Perez); P. Parma; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B/89/17
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000; Mead-Briggs M.A. et al., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary:

The extended laboratory test involved the evaluation of the effects of the test item, Terbut 500 SC on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphi*. On the basis of the preliminary test results was performed as a limit test on one maximum application rates, i.e. 1.5 L/ha (748.8 g a.i./ha).

Adult female wasps were exposed to the test item applied to barley plants. Observations of settling behaviour were made during initial 3 hours of exposure. The aims were to determine repellent effects of Terbut 500 SC and to check if the test insects had contact with barley plants sprayed with the test item. Settling behaviour of the wasps from each replicate was observed five times. Mortality assessments were made 2, 24, and 48 hours after the introduction of the wasps to the test arenas.

Then, females which survived 48-hour exposure to Terbut 500 SC and the ones from the control group were subjected to fecundity assessments. For the purpose of oviposition, 15 female wasps from the group treated with Terbut 500 SC and the control group were individually introduced into fecundity units containing barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded.

Mortality of the wasps after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints.

To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e. Danadim 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 5.0 mL/ha (2.0 g dimethoate/ha). The control group was treated with distilled water.

Materials and methods

- Test Item:** Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
- Test Species:** the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez);
Hymenoptera: Braconidae, Aphidinae
– age: adult females (24 - 48 hours after emerging from mummies)
– source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from Katz Biotech AG (Baruth, Germany).
- Test Design:** 3 test groups:
– a control group (0.0 L/ha)
– Terbut 500 SC at the rate of 1.5 L/ha (i.e. 748.8 g a.i./ha),
– Danadim 400 EC at the rate of 5.0 mL/ha (2.0 g a.i./ha)
6 replicates/group; 5 females/replicate
- Endpoints:**
– wasp mortality after 48 hours of exposure
– reduction in fecundity (Pr) of surviving female wasps exposed to Terbut 500 SC, recorded 12 days after the oviposition period
- Test Conditions**
– temperature: 19.5 – 21°C
– relative air humidity: 65 - 72%
– photoperiod: 16 hours light (mortality assessment and oviposition: 3323 lx; fecundity assessment: 4287 lx) : 8 hours dark
- Test Concentration::** 1.0, 0.33, 0.11, 0.037, 0.012, 0.004 and 0.0013 mg/L plus the control.

Results and discussion:

Repellent effects of the test item

The mean percentages of wasps settled on the plants were 38.7% in the control group, 39.3% in the group treated with Terbut 500 SC at the rate of 1.5 L/ha (i.e. 748.8 g a.i./ha) and 46.2% in the group treated with the reference item (at the rate of 5.0 mL/ha). Repellent properties of the test item and the reference item were assessed. The results of Shapiro-Wilk's test and Levene's test ($p > 0.05$) confirmed normal data distribution and variances homogeneity in the study groups. At the significance level of 0.05, there were no statistically significant differences in the mean percentages of wasps settled on the plants between the treated and the control groups (one-way analysis of variance, ANOVA, $p > 0.05$). On the basis of the obtained results, it can be concluded that the test item at all the rate 1.5 L/ha and the reference item at the rate of 5.0 mL/ha had no repellent effects on the wasps.

Mortality of *A. rhopalosiphi*

In the preliminary test, after 48 hours, there were no dead wasps in the control group and the groups treated with the test item at the rates of 0.17, 0.5 and 1.5 L/ha (i.e. 83.2, 249.6 and 748.8 g a.i./ha).

Study group [application rate]		Tested wasps [no.]	Mortality			
[L/ha] ^a	[g a.i./ha] ^b		Dead wasps [no.]		Total	
			Replicates			
			I	II	[no.]	[%]
Control 0.0		10	0	0	0	0.0
Terbut 500 SC						
0.17	83.2	10	0	0	0	0.0
0.5	249.6	10	0	0	0	0.0
1.5	748.8	10	0	0	0	0.0

The preliminary test was performed between 30.10 - 01.11.2017

^a: [L of the test item/ha]

^b: [g of the active ingredient/ha]

In the definitive test, there were no dead wasps in the control group. Mortality in the groups treated with the test item at the rate of 1.5 L/ha (i.e. 748.8 g a.i./ha) after 48 hours was 3.3%. On the basis of the obtained mortality results, it was demonstrated that the LR50 of Terbut 500 SC is higher than the rate used in the experiment, i.e. > 1.5 L/ha (> 748.8 g a.i./ha). Mortality of the wasps exposed to Danadim 400 EC at the rate of 5.0 mL/ha was 66.7% after 48 hours. Therefore, the validity criterion specified in the Method description was met. The results showed that the test organisms were sensitive to dimethoate.

Study group [application rate]		Tested wasps [no.]	Mortality								
			Dead wasps [no.]						Total		
[L/ha] ^a	[g a.i./ha] ^b		Replicates								
		I	II	III	IV	V	VI	[no.]	[%]		
Control [0.0]		30	0	0	0	0	0	0	0	0	0.0
Terbut 500 SC											
1.5	748.8	30	0	0	0	0	0	0	1	1	3.3
LR ₅₀		> 1.5 L/ha (> 748.8 g a.i. /ha)									
NOER _{mortality}		≥ 1.5 L/ha (≥ 748.8 g a.i. /ha)									
		Danadim 400 EC									
[mL/ha] ^c	[g a.i./ha] ^b										
5.0	2.0	30	3	4	3	3	3	3	4	20	66.7

The definitive test was performed between 15 – 30.11.2017

^a: [L test item/ha]

^b: [g of the active ingredient/ha]

^c: [mL reference item/ha]

Fecundity of *A. rhopalosiphi*

All wasps survived the 24-hour oviposition period. The fecundity assessment showed that the mean number of mummies per female in the control group was 18.1. As for the wasps treated with Terbut 500 SC at the rate of 1.5 L/ha (748.8 g a.i./ha) the number of mummies/female was 10.7. Fecundity reduction (Pr) in the group treated with Terbut 500 SC at the rate of 1.5 L/ha (748.8 g a.i./ha) was 40.6%. At the significance level of 0.05, there was statistically significant difference in fecundity between the wasps exposed to the test item at the rate 1.5 L/ha and the control group (Two-sample t-test procedure, $p > 0.05$). On the basis of the obtained fecundity results, it was demonstrated that the ER50 is higher than the rate used in the experiment, i.e. > 1.5 L/ha (> 748.8 g a.i./ha).

Replicates (isolator number)	Mummies per female 12 days after oviposition [no.]	
	Control	Terbut 500 SC
	Application rate ^a	
	0.0 [L/ha]	1.5 L/ha ^a (748.8 g a.i./ha) ^b
I	17	7
II	18	8
III	15	9
IV	10	11
V	22	14
VI	17	8
VII	15	9
VIII	21	10
IX	24	14
X	19	18
XI	17	8
XII	18	14
XIII	22	8
XIV	19	13
XV	17	10
Mean number of mummies per female ± SD	18.1 ± 3.4	10.7 ± 3.2 [*]
Fecundity reduction relative to the control (Pr) [%]	-	40.6
ER ₅₀		> 1.5 L/ha ^a > (748.8 g a.i./ha) ^b
NOER _{fecundity}		≥ 1.5 L/ha ^a (≥ 748.8 g a.i./ha) ^b

SD: standard deviation

^a: [L test item/ha]

^b: [g of the active ingredient/ha]

^{*}: statistically significant differences

THE VALIDITY CRITERIA

The following validity criteria were met during the study:

- after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
- after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 66.7% (criterion: a minimum of 50%),
- all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- the mean number of mummies per female in the control group was 18.1 (criterion: a minimum of 5.0 mummies/female),
- all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- a) Pre-imaginal mortality (this includes dead larvae, pupae and adults dying during emergence from their pupae) in the control treatment should not exceed 30%.
- b) The level of mortality in the toxic reference treatment should be $\geq 50\%$.
- c) Mean egg production should be > 2 viable eggs/female/day in the control treatment.

In the current study:

- pre-imaginal mortality -5%
- the level of mortality in the toxic standard – 100%
- mean egg production – 5 viable egg/female/day

Agreed endpoints:

LR₅₀, ER₅₀ > 1500 mL product/ha.

NOER with respect to both pre-imaginal mortality and beetle reproduction was 1500 mL product/ha.

Reference:	KCP 10.3/05
Report	TERBUT 500 SC – A rate-response extended laboratory study to determine effects on the ladybird beetle, <i>Coccinella septempunctata</i> (Coleoptera: Coccinellidae); R. Vaughan; Mambo-Tox A Division of Cawood Scientific Ltd, 2 Venture Road University Science Park Southampton SO16 7NP UK; STUDY CODE: CHR-19-17
Guideline(s):	Schmuck et al. (2000). A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aims

The test item in this study was TERBUT 500 SC, a suspension concentrate formulation containing terbuthylazine (nominally 500 g/L).

An extended laboratory test was carried out to determine the effects of fresh, dry, foliar residues of this test item on the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The aim was to determine any effects on either the incidence of pre-imaginal mortality or the reproductive capacity of surviving insects.

Methods

TERBUT 500 SC was evaluated at five rates, up to a maximum of 1500 mL product/ha. These treatments were compared to a water-treated control. A toxic reference treatment of dimethoate (an EC formulation nominally 400 g a.s./L, applied at a rate of 80 mL/ha) was also included in the bioassay.

For the bioassay, the various treatments were applied to excised leaves of dwarf French bean plants (*Phaseolus vulgaris* L.) at a volume rate equivalent to 200 L spray solution/ha. Once residues had dried, the excised leaves were used to line the floor of the test arenas (n = 40 per treatment), and 3- to 4-day-old larvae of *C. septempunctata* were confined upon them. The larvae were fed with pea aphids (*Acyrtosiphon pisum* (Harris)) and any pre-imaginal mortality of the ladybirds was recorded. A check was then made for sub-lethal effects on the reproductive performance of the surviving adults in the control and the test item treatment. The number of eggs produced by the beetles (i.e. a measure of fecundity) was recorded for a 14-day period and the number that hatched (i.e. a measure of fertility) was also assessed.

Materials and methods

Product code = TERBUT 500 SC

Formulation type = suspension concentrate (SC)

Batch number = 1/19

Active substance = terbuthylazine

Nominal content of a.s. = 500 g/L

Analysed content of a.s. = 511.9 g/L

Appearance = opaque white liquid

Analysed density = 1.106 g/mL

Date of expiry = April 2021

Storage at Test Facility = ambient laboratory conditions (< 30°C)

The test item was evaluated at five application rates, equivalent to 1500, 1000, 667, 444 and 296 mL product/ha (nominally 750, 500, 333.5, 222 and 148 g a.s./ha). Applications were made at a volume rate equivalent to 200 L spray solution/ha.

The test item was diluted in purified water shortly before application and the solutions were thoroughly agitated to ensure their homogeneity. No direct measurement was made of the test item homogeneity in the spray solutions. However, care was taken to combine the test item and diluent thoroughly and a visual inspection of the solutions was made. Dilutions for the test were prepared as follows:

Stock (\equiv 5 L/ha) = 5.530 g * (equivalent to 5 mL) product diluted to 200 mL with purified water

A) 1500 mL/ha = 75 mL Stock diluted to 250 mL with purified water

B) 1000 mL/ha = 50 mL Stock diluted to 250 mL with purified water

C) 667 mL/ha = 33.35 mL Stock diluted to 250 mL with purified water

D) 444 mL/ha = 22.2 mL Stock diluted to 250 mL with purified water

E) 296 mL/ha = 14.8 mL Stock diluted to 250 mL with purified water

Results and discussion:

Pre-imaginal mortality assessments

Overall, there was 5.0% pre-imaginal mortality recorded in the control treatment, which compared with 22.5%, 15.0%, 12.5%, 15.0% and 10.0% mortality in the 1500, 1000, 667, 444 and 296 mL product/ha

treatment rates of TERBUT 500 SC, respectively. The corrected mortality in the respective test-item treatments was therefore 18.4%, 10.5%, 7.9%, 10.5% and 5.3%. This demonstrated that the LR50 value for the test item was > 1500 mL product/ha, the highest rate evaluated.

Statistically, none of the results for the test-item treatment rates differed significantly from the control (Chi² 2 x 2 table test with Bonferroni correction, one sided, > control, $\alpha = 0.05$). The NOER with respect to pre-imaginal mortality was therefore 1500 mL product/ha.

The toxic reference treatment resulted in 100% mortality, which met the validity criterion for this treatment.

Table 1. Summary of pre-imaginal mortality where larvae of *C. septempunctata* were exposed to fresh foliar residues.

Treatment	Test item rate (mL prod./ha)	% larvae pupating	% pupae emerging as adults	Overall % pre-imaginal mortality ^{a)}	Corrected % pre-imaginal mortality ^{b)}
Control	-	97.5	97.4	5.0	-
TERBUT 500 SC	1500	82.5	93.9	22.5	18.4
	1000	87.5	97.1	15.0	10.5
	667	87.5	100	12.5	7.9
	444	87.5	97.1	15.0	10.5
	296	90.0	100	10.0	5.3
Toxic reference	-	0	-	100 *	100

a) Pre-imaginal mortality in individual test item treatments was compared to the control using the Chi² 2 x 2 table test with Bonferroni correction; pre-imaginal mortality in the toxic reference treatment was compared to the control using Fisher's Exact Binomial test (one-sided, > control, $\alpha = 0.05$). An asterisk (*) indicates where differences were significant.

b) Corrected pre-imaginal mortality calculated using Abbott's formula. A positive value indicates an increase in mortality compared to the control.

Reproduction assessments

The numbers of females available for assessments ranged from 14 to 15 in the individual treatments. This was primarily due to differing sex ratios in the specific treatments – a natural phenomenon. According to the guideline of Schmuck *et al.* (2000), the reproductive performance of these beetles can only be evaluated qualitatively and a minimum value of 2.0 viable eggs/female/day is to be taken as being indicative of no harmful effect. All of the test-item treatments included in the reproduction assessments met this performance criterion. Therefore, the NOER with respect to reproduction for TERBUT 500 SC was 1500 mL product/ha.

Table 2. Effects on the reproduction of *C. septempunctata*, for those insects derived from larvae exposed to fresh dry foliar residues.

Treatment	Test item rate (mL product/ha)	Mean eggs/♀/day	Mean % viability	Mean viable eggs/♀/day
Control	-	7.4	68.3	5.0
TERBUT 500 SC	1500	10.1	60.3	6.1
	1000	8.1	71.7	5.8
	667	4.7	52.1	2.4
	444	7.8	61.5	4.8
	296	7.6	66.2	5.1

CONCLUSIONS

In an extended laboratory test to determine the effects of TERBUT 500 SC on the ladybird beetle *Coccinella septempunctata*, the LR50 was > 1500 mL product/ha. The NOER with respect to both pre-imaginal mortality and beetle reproduction was 1500 mL product/ha.

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- a) Pre-imaginal mortality in the control treatment should $\leq 20\%$ (i.e. 8 lacewings from 40).
- b) Corrected pre-imaginal mortality in the toxic reference treatment should be $\geq 50\%$.
- c) For the reproduction assessments, the mean egg production in the control should be ≥ 15 eggs per female per day and mean viability of the eggs should be $\geq 70\%$.

In the current study:

- pre-imaginal mortality was 6.1 %
- the level of mortality in the toxic standard was 100%
- mean egg production – viable egg/female/day; 38.9 eggs/female/day and viability was 92.2%

Agreed endpoints:

LR50 >1500 mL product/ha

NOER_{mortality} = 667 mL product/ha

NOER_{reproduction} = 1500 mL product/ha

Reference:	KCP 10.3/06
Report	TERBUT 500 SC – A rate-response extended laboratory study to determine effects on the green lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae); R.Vaughan; Mambo-Tox A Division of Cawood Scientific Ltd. 2 Venture Road University Science Park Southampton SO16 7NP UK; STUDY CODE: CHR-19-18
Guideline(s):	Vogt et al. (2000). Laboratory method to test effects of plant protection products on larvae of <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim

The test item in this study was TERBUT 500 SC, a suspension concentrate formulation containing terbuthylazine (nominally 500 g/L). The aim of this study was to evaluate the effects of the test item on the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), under extended laboratory test conditions.

Methods

TERBUT 500 SC was evaluated at five application rates, equivalent to 1500, 1000, 667, 444 and 296 mL product/ha. These were compared to a water-treated control. A toxic reference treatment of dimethoate (an EC formulation containing nominally 400 g a.s./L, applied at a rate of 80 mL product/ha) was also included in the bioassay.

Treatments were applied to excised leaves of dwarf French bean plants (*Phaseolus vulgaris* L.) at a volume rate equivalent to 200 L spray solution/ha. Once residues had dried, the excised leaves were used to line the floor of the test arenas (n = 40 per treatment) into which individual larvae of *C. carnea* (2-3 days old) were introduced. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier) until they pupated, with any pre-imaginal mortality of the lacewings being recorded. A check was then made for sub-lethal effects on the reproductive performance of the adults surviving in the control and all test-item treatment rates that resulted in $\leq 50\%$ pre-imaginal mortality. For this assessment, the egg-laying activity of grouped females was monitored for two 24-h periods and the viability of the eggs produced was determined.

The intention was to use the results to determine values for the *median lethal rate* (LR₅₀). Also, to determine the highest test item treatment rate not to have any harmful effects on the insects, and therefore identify the *no-observed effect rate* (NOER) with respect to both survival and reproduction.

Materials and methods

Product code = TERBUT 500 SC

Formulation type = suspension concentrate (SC)

Batch number = 1/19

Active substance = terbuthylazine

Nominal content of a.s. = 500 g/L

Analysed content of a.s. = 511.9 g/L

Appearance = opaque white liquid

Analysed density = 1.106 g/mL

Date of expiry = April 2021

Storage at Test Facility = ambient laboratory conditions (< 30°C)

The test item was evaluated at five application rates, equivalent to 1500, 1000, 667, 444 and 296 mL product/ha (nominally 750, 500, 333.5, 222 and 148 g a.s./ha). Applications were made at a volume rate equivalent to 200 L spray solution/ha.

The test item was diluted in purified water shortly before application and the solutions were thoroughly agitated to ensure their homogeneity. No direct measurement was made of the test item homogeneity in the spray solutions. However, care was taken to combine the test item and diluent thoroughly and a visual inspection of the solutions was made. Dilutions for the test were prepared as follows:

Stock ($\equiv 5$ L/ha) = 5.530 g * (equivalent to 5 mL) product diluted to 200 mL with purified water

A) 1500 mL/ha = 75 mL Stock diluted to 250 mL with purified water

B) 1000 mL/ha = 50 mL Stock diluted to 250 mL with purified water

C) 667 mL/ha = 33.35 mL Stock diluted to 250 mL with purified water

D) 444 mL/ha = 22.2 mL Stock diluted to 250 mL with purified water

E) 296 mL/ha = 14.8 mL Stock diluted to 250 mL with purified water

* based on the analysed density.

No measurement was made of the stability of the test item in its diluted state. However, the test item storage recommendations were followed, the test item was applied soon after dilution and the test system was introduced into each arena as soon as residues had dried.

Results and discussion:

Pre-imaginal mortality assessments

In the control, 17.5% mortality was observed, compared with 22.5%, 42.5%, 25.0%, 32.5% and 30.0% mortality in the 1500, 1000, 667, 444 and 296 mL product/ha treatment rates of TERBUT 500 SC, respectively. The corrected mortalities in the respective test-item treatments were therefore 6.1%, 30.3%, 9.1%, 18.2% and 15.2%. This demonstrated that the LR50 for the test item was > 1500 mL product/ha, the highest rate evaluated.

Only the 1000 mL product/ha test item treatment rate differed significantly from the control (Chi² 2 x 2 test with Bonferroni correction, one sided, > control, $\alpha = 0.05$). From this, the NOER, with respect to pre-imaginal survival was 667 mL product/ha treatment, for the test item.

Mortality in the toxic reference treatment was 100%.

Table 1. Mortality recorded during development of the test insects.

Treatment	Test item rate (mL product/ha)	% pre-imaginal mortality ^{a)}	Corrected % pre-imaginal mortality ^{b)}
Control	-	17.5	-
TERBUT 500 SC	1500	22.5	6.1
	1000	42.5 *	30.3
	667	25.0	9.1
	444	32.5	18.2
	296	30.0	15.2
Toxic reference	-	100 *	100

a) Pre-imaginal mortality in individual test item treatments was compared to the control using the Chi² 2 x 2 table test with Bonferroni correction; pre-imaginal mortality in the toxic reference treatment was compared to the control using Fisher's Exact Binomial test (one-sided, > control, $\alpha = 0.05$). An asterisk (*) indicates where differences were significant.

b) Corrected for any control treatment deaths using Abbott's formula (Abbott, 1925).

Reproduction assessments

The mean number of viable eggs per female per day was 38.9 in the control, compared with 38.4, 36.8, 46.7, 45.5 and 38.8 in the 1500, 1000, 667, 444 and 296 mL product/ha treatment rates of TERBUT 500 SC, respectively.

The reproductive performance in the control and in the test-item treatments exceeded the thresholds of ≥ 15 eggs/female/day and $\geq 70\%$ hatching rate, currently viewed as being indicative of no harmful treatment effects (Vogt *et al.*, 2000). The NOEC for the test item was therefore 1500 mL product/ha, the maximum rate evaluated.

Table 2. The results of the reproduction assessments.

Treatment	Test item rate (mL product/ha)	Mean number eggs/female/day ^{a)}	Mean percentage egg viability ^{b)}	Mean viable eggs/female/day
Control	-	42.2	92.2	38.9
TERBUT 500 SC	1500	41.5	92.7	38.4
	1000	40.9	90.1	36.8
	667	49.5	94.4	46.7
	444	47.8	95.5	45.5
	296	41.4	93.5	38.8

a) Based on two 24-h long assessments made for each oviposition box in each treatment.

b) Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box.

CONCLUSIONS

In an extended laboratory test to determine the effects of fresh residues of TERBUT 500 SC on the green lacewing, *Chrysoperla carnea*, the LR₅₀ was > 1500 mL product/ha, the highest rate tested. Based on statistical comparisons with the control, the NOER with respect to lacewing survival was 667 mL product/ha. In assessments of the reproductive performance of surviving lacewings compared with the control, the NOER for reproduction was 1500 mL product/ha.

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- a) mortality in the control treatment over the initial 7 days of a bioassay should not exceed 20%.
- b) mortality in the toxic reference treatment should be 50-100%.
- c) the mean cumulative number of eggs produced between 7 and 14 days should be equal to or exceed 4.0 per female in the control treatment.

In the current study:

- mortality in the control was 7.5%
- mortality in reference item was 100%
- the mean cumulative number of eggs was 12.6.

Agreed endpoints:

The effects of fresh and aged foliar residues of TERBUT 500 SC on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions. When applied to sweetcorn plants at a rate equivalent to 1 L product/ha, fresh residues, 7-day and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).

Reference:

KCP 10.3/07

Report

TERBUT 500 SC – An aged-residue extended laboratory study to determine effects on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae); L. Fallowfield; Mambo-Tox A Division of Cawood Scientific Ltd. 2 Venture

Road University Science Park Southampton SO16 7NP UK; STUDY CODE:
CHR-19-16

Guideline(s): Blümel et al. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

Deviations: No

GLP: Yes

Acceptability: Yes

**Duplication
(if vertebrate study)** No

Materials and methods

Test item = TERBUT 500 SC

Formulation type = suspension concentrate

Batch No. = 1/19

Common name of a.s. = terbuthylazine

Nominal content of a.s = 500 g/L

Analysed content of a.s. = 511.9 g/L

Analysed density = 1.106 g/mL

Appearance = opaque white liquid

Expiry date = April 2021

Storage at Test Facility = ambient laboratory conditions

TERBUT 500 SC was evaluated at a rate equivalent to 1 L test item/ha and was applied at a volume equivalent to 200 L spray solution/ha.

The test item was diluted in purified water shortly before application and the preparation was thoroughly agitated to ensure its homogeneity. No direct measurement was made of the test item homogeneity in the spray solutions. However, care was taken to combine the test item and diluent thoroughly and a visual inspection of the solutions was made. The dilution prepared was follows:

A) 1 L product/ha = 5.530 g (equivalent to 5 mL*) test item diluted to 1 L with purified water

* Based on analysed density of 1.106 g/mL

No measurement was made of the stability of the test item in its diluted state, however the test item storage recommendations were followed. Additionally, the test item was applied soon after dilution and, in the 0 DAT bioassay the test organism was introduced into each arena as soon as residues had dried.

Results and discussion:

Mortality assessments

1.

In the bioassay initiated at 0 DAT, there was 7.0% mortality in the control treatment at 7 days, compared with 14.0% mortality in the 1 L/ha treatment rate of TERBUT 500 SC. When taking account of the deaths in the control, the corrected mortality was 7.5% in the test-item treatment. Statistically, this result did not differ significantly from the control (chi² 2x2 table test, $\alpha = 0.05$, one-sided, > control). In the toxic reference treatment, 100% mortality (100% corrected mortality) was recorded at 7 days, which met the validity criterion imposed for this treatment. In the bioassay initiated at 7 DAT, there was 7.0% mortality in the control treatment at 7 days, compared with 10.0% mortality in the 1 L/ha treatment rate of TERBUT 500 SC. When taking account of the deaths in the control, the corrected mortality was 3.2% in the test-item treatment.

Statistically, this result did not differ significantly from the control (chi² 2x2 table test, $\alpha = 0.05$, one-sided, > control). In the bioassay initiated at 14 DAT, there was 3.0% mortality in the control treatment at 7 days, compared with 8.0% mortality in the 1 L/ha treatment rate of TERBUT 500 SC. When taking account of the deaths in the control, the corrected mortality was 5.2% in the test-item treatment. Statistically, this result did not differ significantly from the control (chi² 2x2 table test, $\alpha = 0.05$, one-sided, > control).

Table 1. Summary of mite mortality observed in the bioassays initiated 0, 7 and 14 days after treatment (DAT).

Bioassay initiated	Treatment	Test item rate (L/ha)	Mean % mortality ^{a)} at 7 DAI	Corrected % mortality ^{b)} at 7 DAI
0 DAT	Control	-	7.0	-
	TERBUT 500 SC	1	14.0	7.5
	Toxic reference	-	100 *	100
7 DAT	Control	-	7.0	-
	TERBUT 500 SC	1	10.0	3.2
14 DAT	Control	-	3.0	-
	TERBUT 500 SC	1	8.0	5.2

a) Treatment mortalities were compared using chi² 2x2 table test ($\alpha = 0.05$, one-sided, > control), a statistically significant effect is denoted by an asterisk (*).

b) Corrected for any deaths in the control using Abbott's formula. A positive value indicates an increase in mortality.

Reproduction assessments

In the bioassay initiated at 0 DAT, the mean number of eggs per female was 10.3 in the control, compared with 9.0 in the 1 L/ha treatment rate of TERBUT 500 SC. The reduction in reproduction was equivalent to 12.6%, compared to the control. Statistically this result differed significantly compared with the control (student t-test for homogeneous variances, $\alpha = 0.05$, one-sided, < control). In the bioassay initiated at 7 DAT, the mean number of eggs per female was 9.8 in the control, compared with 11.0 in the 1 L/ha treatment rate of TERBUT 500 SC. The increase in reproduction was equivalent to 12.2%, compared to the control. Statistically this result did not differ significantly compared with the control (student t-test for homogeneous variances, $\alpha = 0.05$, one-sided, < control). In the bioassay initiated at 14 DAT, the mean number of eggs per female was 9.7 in the control, compared with 10.1 in the 1 L/ha treatment rate of TERBUT 500 SC. The increase in reproduction was equivalent to 3.7%, compared to the control. Statistically this result did not differ significantly compared with the control (student t-test for homogeneous variances, $\alpha = 0.05$, one-sided, < control).

Table 2. A summary of the reproduction of mites in bioassays initiated 0, 7 and 14 days after treatment (DAT).

Bioassay initiated	Treatment	Test item rate (L/ha)	Mean number of eggs per female ^{a)} (7-14 DAI)	% change in reproduction, relative to the control ^{b)}
0 DAT	Control	-	10.3	-
	TERBUT 500 SC	1	9.0*	12.6
7 DAT	Control	-	9.8	-
	TERBUT 500 SC	1	11.0	-12.2
14 DAT	Control	-	9.7	-
	TERBUT 500 SC	1	10.1	-3.7

a) Treatments were compared by student t-test for homogenous variances ($\alpha = 0.05$, one-sided, < control), a statistically significant effect is denoted by an asterisk (*).

b) A positive value indicates a decrease and a negative value indicates an increase, in egg production.

CONCLUSIONS

The effects of fresh and aged foliar residues of TERBUT 500 SC on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions. When applied to sweetcorn plants at a rate equivalent to 1 L product/ha, fresh residues, 7-day and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- each replicate produced 84.9 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 13.5% (criterion: $\leq 30\%$),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: $\leq 10\%$).

Agreed endpoints:

EC₁₀ = 8.491 mg/kg dry weight of the artificial soil

EC₂₀ = 20.959 mg/kg dry weight of the artificial soil

EC₅₀ = 118.064 mg/kg dry weight of the artificial soil

NOEC= 5.6 mg/kg dry weight of the artificial soil
LOEC= 10 mg/kg dry weight of the artificial soil
LC50 >1000 mg/kg dry weight of the artificial soil

Reference: KCP 10.4/01
Report TERBUT 500 SC Earthworm Reproduction Test (*Eisenia andrei*); A. Gierbuszewska; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/294/17
Guideline(s): OECD Guideline No. 222 (2016)
Deviations: No
GLP: Yes
Acceptability: Yes
Duplication (if vertebrate study) No

Materials and methods

Test Item: Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species: the earthworm, *Eisenia andrei* obtained from a standard laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Soil Toxicology
Test Design: test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate
Endpoints: EC10, EC20, EC50, NOEC
Test Conditions temperature: 18 – 22°C; pH at the beginning of the experiment: 5.64 – 5.69; pH at the end of the experiment: 5.50 – 5.69; soil moisture content at the beginning of the experiment: 20.0 – 23.6% (48.5 – 57.4% of the maximum water holding capacity); soil moisture content at the end of the experiment: 18.4 – 23.0% (44.7 – 55.9% of the maximum water holding capacity); light – dark cycle: 16h : 8h; light intensity: 495 – 623 lux
Test Concentration:: control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg/kg dry soil.

Results and discussion:

Mortality of the adult earthworms

On the basis of the results, it was concluded that after 4 weeks, at the control group there was mortality of adult earthworm noticed. It was equal to 2.5%. At concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality

of the adult earthworms was ranging from 0.0 to 7.5%. However, mortality could not be connected with the test item.

Observations of the earthworms

After 4 weeks of the experiment, the treated earthworms did not exhibit any changes in appearance and behaviour.

Body weights of the living adult earthworms

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry soil, the body weight increase was between -36.6 to 16.9%. As for the control group, it was equal to 9.5%.

4.4. Impact of the test item on reproduction of the earthworms

The obtained results made it possible to conclude that **TERBUT 500 SC** had statistically significant impact on reproduction of the earthworms at concentrations ranging from 10 to 1000 mg/kg dry weight of artificial soil.

The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (**EC10**) is **equal to 8.491 mg/kg dry weight of the artificial soil.**

The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (**EC20**) is **equal to 20.959 mg/kg dry weight of the artificial soil.**

The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (**EC50**) is **equal to 118.064 mg/kg dry weight of the artificial soil.**

The highest concentration used in the experiment at which the test item is observed to have no statistically significant effects on reproduction (**NOEC**) is **equal to 5.6 mg/kg dry weight of the artificial soil.**

The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (**LOEC**) is **equal to 10 mg/kg dry weight of the artificial soil.**

The concentration of the test item causing 50% mortality of the adult earthworms (**LC50**) is **above 1000 mg/kg dry weight of the artificial soil.**

Parameter	Value [mg of test item/kg dry weight of artificial soil]
EC₁₀	8.491 (3.443 – 15.074)
EC₂₀	20.959 (11.053 – 32.178)
EC₅₀	118.064 (86.141 – 164.058)
NOEC	5.600
LOEC	10.000
LC₅₀	> 1000.000

4.5. Observations of the juveniles of earthworms

After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

4.6. Results of the reference test

According to the OECD Guideline No. 222, the NOEC should be between 1 – 5 mg/kg dry soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

THE VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced 84.9 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 13.5% (criterion: $\leq 30\%$),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: $\leq 10\%$).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- mean adult mortality: 12.5% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 705.6 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 13.9 (criterion: $\leq 30\%$).

Agreed endpoints:

- **LC₅₀= 226.4 mg/kg dry weight of the artificial soil (104.8 mg of terbuthylazine / kg dry weight of the artificial soil).**
- **NOEC_{mortality}=32 mg/kg dry weight of the artificial soil.**
- **EC₁₀=19.3 mg/kg dry weight of the artificial soil (8.9 mg of terbuthylazine / kg dry weight of the artificial soil).**
- **EC₂₀ =33.5 mg/kg dry weight of the artificial soil (15.5 mg of terbuthylazine / kg dry weight of the artificial soil).**
- **EC₅₀=95.9 mg/kg dry weight of the artificial soil (44.4 mg of terbuthylazine / kg dry weight of the artificial soil).**
- **NOEC = 18.0 mg/kg dry weight of the artificial soil (8.3 mg of terbuthylazine / kg dry weight of the artificial soil).**

KCP 10.4/02

Report	TERBUT 500 SC Collembolan (<i>Folsomia candida</i>) Reproduction Test; M. Wołany; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/60/19
Guideline(s):	OECD Guideline No. 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 1/19, content of terbutylazine 511.9 g/l
Test Species:	the collembolan, <i>Folsomia candida</i> obtained from a standard laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Laboratory of Soil Toxicology. The collembolans used in the study were 9 – 12 days old
Test Design:	test duration: 28 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate
Endpoints:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Test Conditions	temperature: 17.5 – 22.0°C; pH at the beginning of the test: 6.08 – 6.21; pH at the end of the test: 5.83 – 5.86; soil moisture content at the beginning of the test: 11.9 – 12.8% (41.2 – 44.4% of the maximum water holding capacity); soil moisture content at the end of the test: 11.8 – 13.0% (40.9 – 45.0% of the maximum water holding capacity); lighting: 16 h light and 8h dark;
Test Concentration::	light intensity at the beginning of the experiment: 681.2 – 794.1 lux light intensity at the end of the experiment: 697.2 – 768.3 lux a control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg of the test item/kg of dry weight of the artificial soil.

Results and discussion:

Mortality

Table 6. Endpoint values - the impact of the test item on the mortality of adult collembolans (*Folsomia candida*).

Endpoint	Value [mg test item / kg dry weight of the artificial soil]	Value [mg of terbuthylazine / kg dry weight of the artificial soil]
LC ₁₀	45.0 (15.2 – 99.4)	20.8 (7.0 – 46.0)
LC ₂₀	78.4 (32.7 – 192.4)	36.3 (15.1 – 89.1)
LC ₅₀	226.4 (102.3 – 942.4)	104.8 (47.3 – 436.2)
NOEC	32.0	14.8

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, mortality was between 10.0 to 92.5%. As for the control group, it was equal to 12.5%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is equal to 226.4 mg/kg dry weight of the artificial soil (104.8 mg of terbuthylazine / kg dry weight of the artificial soil).

Impact on reproduction

Table 8. Endpoint values - the impact of the test item on reproduction of collembolans (*Folsomia candida*).

Endpoint	Value [mg test item / kg dry weight of the artificial soil]	Value [mg of terbuthylazine / kg dry weight of the artificial soil]
EC ₁₀	19.3 (11.0 – 29.5)	8.9 (5.1 – 13.6)
EC ₂₀	33.5 (21.1 – 48.9)	15.5 (9.7 – 22.7)
EC ₅₀	95.9 (66.3 – 142.1)	44.4 (30.7 – 65.8)
NOEC	18.0	8.3

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 17.5 – 701.5 per replicate. As for the control group, the number of juveniles was equal to 705.6 per replicate.

The obtained results led to the following conclusions:

- The concentration of TERBUT 500 SC causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 19.3 mg/kg dry weight of the artificial soil (8.9 mg of terbuthylazine / kg dry weight of the artificial soil).
- The concentration of TERBUT 500 SC causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 33.5 mg/kg dry weight of the artificial soil (15.5 mg of terbuthylazine / kg dry weight of the artificial soil).
- The concentration of TERBUT 500 SC causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is equal to 95.9 mg/kg dry weight of the artificial soil (44.4 mg of terbuthylazine / kg dry weight of the artificial soil).
- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is equal to 18.0 mg/kg dry weight of the artificial soil (8.3 mg of terbuthylazine / kg dry weight of the artificial soil).

Results of the reference test

The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 102.3 mg/kg dry weight of the artificial soil.

According to the OECD Guideline No. 232, the EC50 should be about 100 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

The test was conducted between 20.11.2019 – 20.12.2019.

THE VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:

- mean adult mortality: 12.5% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 705.6 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 13.9 (criterion: $\leq 30\%$).

ZRMS comments:

The study is considered acceptable. All validity criteria were met.

- mean adult mortality: 2.5% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 130.9
- (criterion: ≥ 50 juveniles at the end of the test,
- the coefficient of variation for the number of juveniles: 24.6 (criterion: $\leq 30\%$).

Agreed endpoints:

LC₅₀ >1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

NOEC \geq 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

EC₁₀ >1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

EC₂₀ > 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

EC₅₀ >1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

NOEC \geq 1000 mg/kg dry weight of the artificial soil (above or equal to 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

Reference:	KCP 10.4/03
Report	TERBUT 500 SC Predatory mite (<i>Hypoaspis (Geolaelaps) aculeifer</i> reproduction test in soil; P. Holewik; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/61/19
Guideline(s):	OECD Guideline No. 226 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 1/19, content of terbuthylazine 511.9 g/L,
Test Species:	the predatory mites, <i>Hypoaspis (Geolaelaps) aculeifer</i> (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Toxicology. The mites were introduced 7 – 14 days after becoming adult.
Test Design:	test duration: 14 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate
Endpoints:	EC10, EC20, EC50, NOEC
Test Conditions	temperature: 17.5 – 22.0oC pH at the beginning of the test: 5.87 – 5.99 pH at the end of the test: 5.91 – 6.00 soil moisture content at the beginning of the test: 14.9 – 15.6% (46.2 – 48.4% of the maximum water holding capacity) soil moisture content in the middle of the test: 14.4 – 15.7% (44.7 – 48.7% of the maximum water holding capacity) soil moisture content at the end of the test: 14.1 – 15.6% (43.7 – 48.4% of the maximum water holding capacity)
Test Concentration::	light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 767 – 778 lux light intensity at end of the test: 775 – 783 lux a control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry weight of the artificial soil..

Results and discussion: Mortality of adult females

Table 7. Endpoint values – the impact of the test item on survival of adult females (*Hypoaspis aculeifer*).

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of terbuthylazine / kg dry weight of the artificial soil]
LC ₁₀	> 1000.0	> 462.8
LC ₂₀	> 1000.0	> 462.8
LC ₅₀	> 1000.0	> 462.8
NOEC (survival)	≥ 1000.0	≥ 462.8

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil was between 0.0% and 10.0%. Mortality of the control group was equal to 2.5%.

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

Impact on reproduction

Table 9. Endpoint values - the impact of the test item on reproduction of the predatory mites (*Hypoaspis aculeifer*).

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of terbuthylazine / kg dry weight of the artificial soil]
EC ₁₀	> 1000.0	> 462.8
EC ₂₀	> 1000.0	> 462.8
EC ₅₀	> 1000.0	> 462.8
NOEC (reproduction)	≥ 1000.0	≥ 462.8

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 122.8 – 151.8 per replicate. The mean number of juveniles in the control group was equal to 130.9 per replicate.

The obtained results led to the following conclusions:

The concentration of the test item causing a 10% reduction in the number of mites produced within the exposure period (EC10) is above 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

The concentration of the test item causing a 20% reduction in the number of mites produced within the exposure period (EC20) is above 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

The concentration of the test item causing a 50% reduction in the number of mites produced within the exposure period (EC50) is above 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

The highest concentration at which the test item is observed to have no statistically significant effects on mite reproduction (NOEC) is above or equal to 1000 mg/kg dry weight of the artificial soil (above or equal to 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).

Results of the reference test

The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 240.155 mg/kg dry weight of the artificial soil.

Test Species:	Agricultural soil collected from a place belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna..
Test Design:	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 28 days.
Endpoints:	The concentration of nitrate ions [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation. The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28 days
Test Conditions	Temperature: 19 – 23°C, soil moisture: 45.8% – 49.9% of the maximum water holding capacity, incubation in darkness.
Test Concentration::	Control; PEC: 2.2 mg of test item/kg of soil and 5 x PEC: 11.0 mg of test item/kg of soil..

Results and discussion:

Nitrate formation rates [mg nitrate/kg dry weight soil/day] for selected time intervals. i.e. 0 – 7, 0 – 14, 0 – 28 days.

Time interval [d]	Control				PEC 2.2 mg of test item/kg soil				5 x PEC 11.0 mg of test item/kg soil			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	I	II	III		I	II	III		I	II	III	
0 – 7	8.970	5.363	5.513	6.615 ± 2.04	8.285	7.049	6.942	7.425 ± 0.75	8.980	6.415	8.201	7.865 ± 1.31
0 – 14	-4.780	-5.751	-6.058	-5.530 ± 0.67	-1.944	-2.448	-4.030	-2.807* ± 1.09	-2.929	-2.239	-2.750	-2.639* ± 0.36
0 – 28	5.144	4.882	4.950	4.992 ± 0.14	5.053	5.016	6.082	5.384 ± 0.60	5.945	6.202	6.159	6.102 ± 0.14

* statistically significant difference between the control and the treated group ($\alpha=0.05$)

** - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7, 14, 28 d

After 0, 7, 14 and 28 days of incubation, no statistical differences in the nitrate concentration between control soil and soil treated with the test item at the concentrations 2.2 and 11.0 mg of the test item/kg soil were noticed. There were no statistically significant differences between the control and the group treated with test item at both concentrations. i.e. PEC and 5 x PEC in nitrate formation rates at time interval 0 – 7 days. For the time interval 0 – 14, there were statistically significant differences between the control group and the group treated with the test item at both concentrations of the test item, i.e. 2.2 mg of the test item/kg soil and 11.0 mg of the test item/kg soil. At the time interval 0 – 28, there were no statistically significant differences between the control group and the group treated with the test item at both concentrations of the test item, i.e. 2.2 mg of the test item/kg soil and 11.0 mg of the test item/kg soil. The percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at both concentrations (PEC and 5 x PEC) did not exceed 25% on 28 day of the analysis . When the difference in the nitrate formation rate between the lower treatment (i.e. the

maximum predicted concentration) and control is equal to or less than 25% at any sampling time after day 28, the product can be evaluated as having no long-term influence on nitrogen transformation in soils. As regards to the obtained results, it was concluded that **TERBUT 500 SC** at the concentrations corresponding to the PEC (2.2 mg of test item/kg of soil) and 5 x PEC (11.0 mg of test item/kg of soil) can be perceived as having no long-term influence on nitrogen transformations in soil.

THE VALIDITY CRITERIA

The coefficients of variation (CV) in the control group were 2.3, 7.4, 13.4 and 1.3% after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than $\pm 15\%$.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

ZRMS comments:

The study is considered acceptable. All validity criteria were met.

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:

- 95% - sunflower,
- 90% - cabbage,
- 90% – pea,
- 95% – tomato,
- 85% – onion,
- 100% – oats;

- the mean survival of the emerged control seedlings was 100% (validity criterion: at least 90%);

- the control seedlings did not exhibit any visible phytotoxic effects;.

- environmental conditions for all plants of the same species were identical.

Agreed endpoints:

<i>Sunflower</i> <i>Helianthus annuus</i>	TERBUT 500 SC	21 d Seedling emergence	ER50 > 1500 ml prod/ha equal to 1657.5 g prod/ha
<i>Cabbage</i> <i>Brassica oleracea var.</i> <i>capitata</i>	TERBUT 500 SC	21 d Seedling emergence	ER= 49.44 ml prod/ha equal to 54.63 g prod/ha
<i>Pea</i> <i>Pisum sativum</i>	TERBUT 500 SC	21 d Seedling emergence	ER50 > 1500 ml prod/ha equal to 1657.5 g prod/ha
<i>Tomato</i> <i>Solanum lycopersicon</i>	TERBUT 500 SC	21 d Seedling emergence	ER50= 63.32 ml prod/ha equal to 69.97 g prod/ha
<i>Onion</i> <i>Allium cepa</i>	TERBUT 500 SC	21 d Seedling emergence	ER50= 92.17 ml prod/ha equal to 101.85 g prod/ha
<i>Oats</i> <i>Avena sativa</i>	TERBUT 500 SC	21 d Seedling emergence	ER50= 598.95 ml prod/ha equal to 661.84 g prod/ha

Reference:	KCP 10.6/01
Report	TERBUT 500 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; W. Dec; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/286/17
Guideline(s):	OECD Guideline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	sunflower (<i>Helianthus annuus</i>), cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>), pea (<i>Pisum sativum</i>), tomato (<i>Solanum lycopersicon</i>), onion (<i>Allium cepa</i>), oats (<i>Avena sativa</i>).
Test Design:	number of concentrations: 6 application rates + a control for pea, onion and oats; 7 application rates + a control for sunflower and tomato, 8 application rates + a control for cabbage number of replicates: 4 replicates of each application rate and the control number of seeds: 5 seeds/replicate test termination: 14 days after the emergence of 50% of the control seedlings
Endpoints:	ER25, ER50, NOER
Test Conditions	temperature: 16.7 – 30.2°C; humidity: 45.1 – 90.9%; lighting: 16 h light : 8 h dark; light intensity: 53.2 – 135.8 $\mu\text{E}/\text{m}^2/\text{s}$; carbon dioxide concentration: 350 – 375 ppm
Test Concentration::	a control, 15.4, 38.4, 96.0, 240.0, 600.0, and 1500.0 mL of test item/ha for pea, onion and oats; 6.1, 15.4, 38.4, 96.0, 240.0, 600.0, and 1500.0 mL of test item/ha for sunflower and tomato; 2.5, 6.1, 15.4, 38.4, 96.0, 240.0, 600.0, and 1500.0 mL of test item/ha for cabbage

Results and discussion:

Sunflower (*Helianthus annuus*)

After the application of the test item at the rates ranging from 6.1 to 1500 mL/ha, seedling emergence of sunflower was not delayed when compared with the control. The death of sunflower plants was not observed at the all tested rates. At the control group, 95% of plants emerged. At the rates ranging from 6.1 to 1500 mL/ha total number of plants at the end of the experiment ranged from 94.7 to 84.2% in comparison to the control group.

Application rate [mL/ha]	Total number of plants	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	4	5	19	95.0	100.0
6.1	20	3	4	5	5	17	85.0	89.5
15.4	20	4	5	3	4	16	80.0	84.2
38.4	20	5	4	4	5	18	90.0	94.7
96.0	20	5	4	4	4	17	85.0	89.5
240.0	20	5	4	3	5	17	85.0	89.5
600.0	20	3	4	5	5	17	85.0	89.5
1500.0	20	4	5	4	5	18	90.0	94.7

After the application of the test item at the rates ranging from 6.1 to 1500 mL/ha, the sunflower shoot length was between 108.3 – 88.1% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	158.6	204.4	186.0	186.8	184.0	18.9	100.0
6.1	181.3	159.0	168.2	191.4	175.0	14.3	95.1
15.4	187.8	184.2	220.7	204.0	199.2	16.7	108.3
38.4	182.8	180.8	172.5	168.6	176.2	6.7	95.8
96.0	191.2	179.0	177.8	160.8	177.2	12.5	96.3
240.0	191.8	198.5	202.3	188.8	195.4	6.2	106.2
600.0	164.3	163.3	169.2	171.4	167.0	3.9	90.8
1500.0	184.3	177.8	107.5	178.6	162.0	36.5	88.1

After the application of the test item at the rates ranging from 6.1 to 1500 mL/ha, the sunflower shoot weight was between 129.3 – 85.3% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	120.0	165.0	180.8	153.4	154.8	25.8	100.0
6.1	181.7	192.0	127.8	138.2	159.9	31.7	103.3
15.4	191.0	160.0	240.0	209.5	200.1	33.5	129.3
38.4	160.0	194.3	172.8	153.4	170.1	18.0	109.9
96.0	177.0	189.5	186.5	178.3	182.8	6.1	118.1
240.0	157.6	161.3	226.3	154.6	174.9	34.4	113.0
600.0	146.0	110.0	154.0	130.8	135.2	19.4	87.3
1500.0	159.8	130.0	104.0	134.6	132.1	22.8	85.3

After the application of the test item at the rates ranging from 6.1 to 1500 mL/ha, the plant damage was not observed.

Cabbage (*Brassica oleracea* ver. *capitata*)

After the application of the test item at the rates ranging from 2.5 to 1500 mL/ha, seedling emergence of cabbage was not delayed when compared with the control. The death of cabbage plants was not observed at the all tested rates. At the control group, 90% of plants emerged. At the rates ranging from 2.5 to 1500 mL/ha total number of plants at the end of the experiment ranged from 100.0 to 88.9% in comparison to the control group.

Application rate [mL/ha]	Total number of plants	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	4	5	4	5	18	90.0	100.0
2.5	20	4	4	5	4	17	85.0	94.4
6.1	20	5	4	4	4	17	85.0	94.4
15.4	20	4	4	5	4	17	85.0	94.4
38.4	20	4	5	4	5	18	90.0	100.0
96.0	20	4	5	4	4	17	85.0	94.4
240.0	20	5	5	3	5	18	90.0	100.0
600.0	20	5	3	5	3	16	80.0	88.9
1500.0	20	5	4	4	4	17	85.0	94.4

After the application of the test item at the rates ranging from 2.5 to 1500 mL/ha, the cabbage shoot length was between 93.5 – 27.8% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	75.5	76.2	71.5	66.6	72.5	4.4	100.0
2.5	66.5	70.3	63.2	69.8	67.4	3.3	93.1
6.1	62.4	79.0	60.0	69.5	67.7	8.5	93.5
15.4	71.5	72.8	64.8	55.3	66.1	8.0	91.2
38.4	56.3	48.2	49.0	59.6	53.3⁺	5.6	73.5
96.0	47.5	43.6	43.3	38.0	43.1⁺	3.9	59.5
240.0	19.6	30.4	23.7	25.2	24.7⁺	4.5	34.1
600.0	20.6	24.7	24.2	16.0	21.4⁺	4.0	29.5
1500.0	19.2	18.0	17.3	26.3	20.2⁺	4.1	27.8

⁺ - statistically significant difference between the mean shoot dry weight in the control group and in the treated one (Williams Multiple Sequential t – test Procedure, $\alpha = 0.05$)

After the application of the test item at the rates ranging from 2.5 to 1500 mL/ha, the cabbage shoot weight was between 90.9 – 8.4% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	32.0	24.0	32.5	23.2	27.9	5.0	100.0
2.5	24.5	30.0	23.0	24.0	25.4	3.1	90.9
6.1	22.4	14.0	17.3	24.8	19.6⁺	4.9	70.2
15.4	24.8	21.0	16.2	17.5	19.9⁺	3.8	71.1
38.4	17.3	17.4	18.8	22.0	18.9⁺	2.2	67.5
96.0	7.8	9.2	8.8	10.0	8.9⁺	0.9	32.0
240.0	3.4	3.8	2.0	3.4	3.2⁺	0.8	11.3
600.0	2.2	2.7	2.8	1.7	2.3⁺	0.5	8.4
1500.0	2.0	2.8	2.0	3.0	2.4⁺	0.5	8.7

* - statistically significant difference between the mean shoot dry weight in the control group and in the treated one (Williams Multiple Sequential t – test Procedure, $\alpha = 0.05$)

After the application of the test item at the rates ranging from 38.4 to 1500 mL/ha, the plant damage was observed and it was between 10.0 – 87.5% after 14 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis and wilting were observed.

Pea (*Pisum sativum*)

After the application of the test item at the rates from 15.4 to 1500 mL/ha, seedling emergence of pea was not delayed when compared with the control. The death of pea plants was not observed at the all tested rates. At the control group, 90% of plants emerged. At the rates ranging from 15.4 to 1500 mL/ha total number of plants at the end of the experiment ranged from 111.1 to 88.9% in comparison to the control group.

Application rate [mL/ha]	Total number of plants	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	4	5	4	18	90.0	100.0
15.4	20	5	5	5	5	20	100.0	111.1
38.4	20	5	4	4	4	17	85.0	94.4
96.0	20	5	4	5	5	19	95.0	105.6
240.0	20	4	5	3	4	16	80.0	88.9
600.0	20	5	5	3	5	18	90.0	100.0
1500.0	20	5	3	5	5	18	90.0	100.0

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, the pea shoot length was between 106.9 – 95% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	333.6	359.5	333.4	327.3	338.4	14.3	100.0
15.4	335.8	340.6	295.6	357.8	332.5	26.3	98.2
38.4	341.2	360.3	361.8	384.0	361.8	17.5	106.9
96.0	368.0	358.8	322.6	291.2	335.1	35.2	99.0
240.0	358.0	318.8	395.0	330.8	350.6	33.8	103.6
600.0	341.4	323.4	374.0	366.4	351.3	23.2	103.8
1500.0	322.2	331.7	292.0	340.6	321.6	21.1	95.0

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, the pea shoot weight was between 108.9 – 80.6% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	195.6	217.8	218.2	200.3	208.0	11.7	100.0
15.4	179.0	189.2	176.4	216.2	190.2	18.2	91.5
38.4	214.8	236.8	218.5	235.0	226.3	11.2	108.8
96.0	225.8	228.5	184.4	184.2	205.7	24.8	98.9
240.0	247.5	186.4	270.3	201.3	226.4	39.2	108.9
600.0	166.8	190.6	184.7	195.6	184.4	12.6	88.7
1500.0	169.8	181.0	151.6	168.0	167.6⁺	12.1	80.6

⁺ - statistically significant difference between the control and the treated group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, the plant damage was not observed.

Tomato (*Solanum lycopersicon*)

After the application of the test item at the rates ranging from 6.1 to 1500 mL/ha, seedling emergence of tomato was not delayed when compared with the control. The death of tomato plants was observed at the rates ranging from 96 to 1500 mL/ha. At the control group, 95% of plants emerged. At the rates ranging from 6.1 to 1500 mL/ha total number of plants at the end of the experiment ranged from 105.3 to 0.0% in comparison to the control group.

Application rate [mL/ha]	Total number of plants	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	4	5	5	19	95.0	100.0
6.1	20	5	5	5	5	20	100.0	105.3
15.4	20	5	5	5	5	20	100.0	105.3
38.4	20	5	5	5	5	20	100.0	105.3
96.0	20	3	2	3	5	13 ⁺	65.0	68.4
240.0	20	0	0	0	0	0 ⁺	0.0	0.0
600.0	20	0	0	0	0	0 ⁺	0.0	0.0
1500.0	20	0	0	0	0	0 ⁺	0.0	0.0

+ - statistically significant difference between the control and the treated group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 6.1 to 96.0 mL/ha, the tomato shoot length was between 98.2 – 44.8% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	82.2	115.0	112.6	95.4	101.3	15.4	100.0
6.1	104.8	103.4	93.6	91.6	98.4	6.7	97.1
15.4	102.4	100.4	90.2	105.0	99.5	6.5	98.2
38.4	82.8	73.8	83.2	80.4	80.1 ⁺	4.3	79.0
96.0	38.7	50.5	42.0	50.2	45.3 ⁺	5.9	44.8
240.0	-	-	-	-	-	-	-
600.0	-	-	-	-	-	-	-
1500.0	-	-	-	-	-	-	-

+ - statistically significant difference between the control and the treated group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

"-" lack of plants

After the application of the test item at the rates ranging from 6.1 to 96.0 mL/ha, the tomato shoot weight was between 112.1 – 26.8% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	22.2	31.5	36.0	26.6	29.1	6.0	100.0
6.1	32.2	38.8	27.8	31.6	32.6	4.6	112.1
15.4	27.8	29.0	22.8	33.2	28.2	4.3	97.0
38.4	21.2	17.8	22.4	20.8	20.6⁺	2.0	70.7
96.0	6.0	8.0	6.3	10.8	7.8⁺	2.2	26.8
240.0	-	-	-	-	-	-	-
600.0	-	-	-	-	-	-	-
1500.0	-	-	-	-	-	-	-

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 38.4 to 1500 mL/ha, the plant damage was observed and it was between 10.0 – 100.0% after 14 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis, wilting and dead plants were observed.

Onion (*Allium cepa*)

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, seedling emergence of onion was not delayed when compared with the control. The death of onion plants was observed at the rates ranging from 96.0 to 1500 mL/ha. At the control group, 85% of plants emerged. At the rates ranging from 15.4 to 1500 mL/ha total number of plants at the end of the experiment ranged from 111.8 to 0.0% in comparison to the control group.

Application rate [mL/ha]	Total number of plants	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	4	4	4	5	17	85.0	100.0
15.4	20	5	4	5	5	19	95.0	111.8
38.4	20	3	4	4	4	15⁺	75.0	88.2
96.0	20	2	2	2	2	8⁺	40.0	47.1
240.0	20	0	0	0	0	0⁺	0.0	0.0
600.0	20	0	0	0	0	0⁺	0.0	0.0
1500.0	20	0	0	0	0	0⁺	0.0	0.0

+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 15.4 to 96.0 mL/ha, the onion shoot length was between 95.8 – 54.8% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	88.8	53.0	79.8	89.0	77.6	17.0	100.0
15.4	59.6	71.3	86.6	80.0	74.4	11.7	95.8
38.4	69.3	61.0	63.0	87.8	70.3	12.2	90.5
96.0	32.0	40.0	60.0	38.0	42.5⁺	12.2	54.8
240.0	-	-	-	-	-	-	-
600.0	-	-	-	-	-	-	-
1500.0	-	-	-	-	-	-	-

+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 15.4 to 96.0 mL/ha, the onion shoot weight was between 102.9 – 45.5% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	2.5	3.5	2.5	3.6	3.0	0.6	100.0
15.4	2.2	3.3	3.2	3.8	3.1	0.7	102.9
38.4	3.3	2.8	3.0	2.5	2.9	0.4	95.7
96.0	1.0	1.5	2.0	1.0	1.4 ⁺	0.5	45.5
240.0	-	-	-	-	-	-	-
600.0	-	-	-	-	-	-	-
1500.0	-	-	-	-	-	-	-

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 96.0 to 1500 mL/ha, the plant damage was observed and it was between 80.0 – 100.0% after 14 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, wilting and dead plants were observed.

Oats (*Avena sativa*)

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, seedling emergence of oats was not delayed when compared with the control. The death of oats plants was not observed at the all tested rates. At the control group, 100% of plants emerged. At the rates ranging from 15.4 to 1500 mL/ha total number of plants at the end of the experiment ranged from 100.0 to 95.0% in comparison to the control group.

Application rate [mL/ha]	Total number of plants	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
15.4	20	5	5	5	5	20	100.0	100.0
38.4	20	5	5	5	5	20	100.0	100.0
96.0	20	5	4	5	5	19	95.0	95.0
240.0	20	5	5	5	5	20	100.0	100.0
600.0	20	5	5	5	5	20	100.0	100.0
1500.0	20	5	5	5	5	20	100.0	100.0

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, the oats shoot length was between 98.4 – 39.0% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	336.0	313.4	321.8	322.0	323.3	9.4	100.0
15.40	322.8	338.0	295.4	308.0	316.1	18.4	97.8
38.40	306.8	326.8	329.6	309.4	318.2	11.7	98.4
96.00	292.0	300.8	301.4	326.8	305.2	15.0	94.4
240.00	311.8	325.4	332.8	290.0	315.0	18.8	97.4
600.00	241.4	256.0	250.0	295.0	260.6⁺	23.7	80.6
1500.00	83.8	112.4	206.6	101.0	126.0⁺	55.0	39.0

* - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates ranging from 15.4 to 1500 mL/ha, the oats shoot weight was between 106.2 – 21.0% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	73.4	81.8	91.0	79.2	81.4	7.3	100.0
15.40	84.8	90.8	70.4	69.8	79.0	10.5	97.0
38.40	60.6	76.6	78.8	68.0	71.0	8.4	87.3
96.00	73.6	75.5	87.6	109.0	86.4	16.3	106.2
240.00	73.0	77.0	77.8	58.2	71.5	9.1	87.9
600.00	31.8	41.0	33.8	39.2	36.5⁺	4.4	44.8
1500.00	16.0	14.6	23.6	14.0	17.1⁺	4.4	21.0

* - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05)

After the application of the test item at the rates equal to 600 and 1500 mL/ha, the plant damage was observed and it was equal to 22.5% and 67.5%, respectively after 14 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis and necrosis were observed.

THE VALIDITY CRITERIA

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of TERBUT 500 SC on seedling emergence and seedling growth of terrestrial plants were met:

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
 95% - sunflower,
 90% - cabbage,
 90% – pea,
 95% – tomato,
 85% – onion,
 100% – oats;
- the mean survival of the emerged control seedlings was 100% (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;.
- environmental conditions for all plants of the same species were identical.

ZRMS comments:

The study is considered acceptable. All validity criteria were met.

The seedling emergence (validity criterion: at least 70%) was as follows:

- 87.5 – 100.0% – sunflower,
- 85.0 – 95.0% – cabbage,
- 85.0 – 100.0% – pea,
- 90.0 – 97.5% – tomato,
- 80.0 – 90.0% – onion,
- 80.0 – 95.0% – oats.

- the mean survival of the emerged control seedlings was 100% (validity criterion: at least 90%),
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

Agreed endpoints:

<i>Sunflower</i> <i>Helianthus annuus</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50 > 212.3ml prod/ha equal to 234.59 g prod/ha
<i>Cabbage</i> <i>Brassica oleracea var.</i> <i>capitata</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 180 ml prod/ha equal to 198.90 g prod/ha
<i>Pea</i> <i>Pisum sativum</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50 > 619.6 ml prod/ha equal to 684.66 g prod/ha
<i>Tomato</i> <i>Solanum lycopersicon</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 57.3 ml prod/ha equal to 63.32 g prod/ha
<i>Onion</i> <i>Allium cepa</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 51.1 ml prod/ha equal to 56.47 g prod/ha
<i>Oats</i> <i>Avena sativa</i>	TERBUT 500 SC	21 d Vegetative vigour	ER50= 391.0 ml prod/ha equal to 432.06 g prod/ha

Reference:	KCP 10.6/02
Report	TERBUT 500 SC Terrestrial Plant Test: Vegetative Vigour Test; A. Gierbuszewska; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/287/17
Guideline(s):	OECD Guideline No. 227 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Terbut 500 SC, batch no. 7/17, content of terbuthylazine 499.2 g/L, density: 1.102 g/mL at 20°C, production date: 04.2017, expiry date: 04.2020.
Test Species:	sunflower (<i>Helianthus annuus</i>), cabbage (<i>Brassica olerace</i> var. <i>capitata</i>), pea (<i>Pisum sativum</i>), tomato (<i>Solanum lycopersicon</i>), onion (<i>Allium cepa</i>), oats (<i>Avena sativa</i>)..
Test Design:	number of rates: five or seven application rates + control; number of replicates: 4 replicates/rate. The total number of plants per application rate – 20. test termination: 21 days after the spraying.
Endpoints:	ER25, ER50, NOER...
Test Conditions	temperature: 18.2 – 25.8°C, humidity: 40.4 – 90.1%, controlled light – dark cycles (16h:8h), light intensity: 118.5 – 135.4 µE/m ² /s, carbon dioxide concentration: 335 – 364 ppm.
Test Concentration::	a control, 6.14, 15.36, 38.40, 96.00, 240.00, 600.00, and 1500.00 mL/ha in cultivation of sunflower, cabbage, tomato and onion; a control, 38.40, 96.00, 240.00, 600.00, and 1500.00 mL/ha in cultivation of pea and oats; volume of deionised water used to prepare the highest rate: 300 L water/ha

Results and discussion:

Sunflower (*Helianthus annuus*)

After the application of the test item at the rates ranging from 240.00 – 1500.00 mL/ha, plant mortality was observed. After the application of the test item at the rates ranging from 6.14 – 1500.00 mL/ha, the plant number at the end of the experiment was between 40 – 100% in comparison to the control group.

Application rate [mL/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
6.14	20	5	5	5	5	20	100.0	100.0
15.36	20	5	5	5	5	20	100.0	100.0
38.40	20	5	5	5	5	20	100.0	100.0
96.00	20	5	5	5	5	20	100.0	100.0
240.00	20	3	2	5	5	15	75.0	75.0
600.00	20	3	3	4	3	13 ⁺	65.0	65.0
1500.00	20	2	3	1	2	8 ⁺	40.0	40.0

⁺ - statistically significant difference between the control and the treatment group (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater)

After the application of the test item at the rates between 6.14 to 1500.00 mL/ha, the sunflower shoot length was between 41.7 – 108.3% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	223.4	231.8	231.6	207.6	223.6	11.4	100.0
6.14	243.8	223.0	245.0	231.0	235.7	10.6	105.4
15.36	237.4	234.8	242.0	248.0	240.6	5.8	107.6
38.40	238.8	247.2	244.6	237.8	242.1	4.5	108.3
96.00	201.8	197.8	212.2	219.4	207.8 ⁺	9.8	92.9
240.00	143.0	143.5	143.4	147.6	144.4 ⁺	2.2	64.6
600.00	158.0	133.0	148.8	155.0	148.7 ⁺	11.1	66.5
1500.00	88.0	96.0	100.0	89.0	93.3 ⁺	5.7	41.7

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

After the application of the test item at the rates ranging from 6.14 to 1500.00 mL/ha, the sunflower shoot dry weight was between 24.0 – 98.8% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	378.0	356.0	362.0	356.0	363.0	10.4	100.0
6.14	356.0	352.0	366.0	360.0	358.5	6.0	98.8
15.36	332.0	324.0	328.0	348.0	333.0 ⁺	10.5	91.7
38.40	294.0	312.0	298.0	306.0	302.5 ⁺	8.1	83.3
96.00	270.0	264.0	260.0	258.0	263.0 ⁺	5.3	72.5
240.00	120.0	115.0	112.0	122.0	117.3 ⁺	4.6	32.3
600.00	113.3	116.0	122.5	110.0	115.5 ⁺	5.3	31.8
1500.00	90.0	83.3	85.0	90.0	87.1 ⁺	3.4	24.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

After the application of the test item at the rates ranging from 240 to 1500 mL/ha, the plant damage was observed and it was between 15.0 – 87.5% after 21 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis, necrosis, and dead plants were observed.

4.2. Cabbage (*Brassica olerace* var. *capitata*)

After the application of the test item at the rates ranging from 240.00 – 1500.00 mL/ha, plant mortality was observed (on the top concentration plant mortality of all plants was observed). After the application of the test item at the rates ranging from 6.14 – 1500.00 mL/ha, the plant number at the end of the experiment was between 0 – 100% in comparison to the control group.

Application rate [mL/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
6.14	20	5	5	5	5	20	100.0	100.0
15.36	20	5	5	5	5	20	100.0	100.0
38.40	20	5	5	5	5	20	100.0	100.0
96.00	20	5	5	5	5	20	100.0	100.0
240.00	20	4	4	4	5	17	85.0	85.0
600.00	20	3	3	3	4	13 ⁺	65.0	65.0
1500.00	20	0	0	0	0	0 ⁺	0.0	0.0

⁺ - statistically significant difference between the control and the treatment group (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater)

After the application of the test item at the rates between 6.14 to 600.00 mL/ha, the cabbage shoot length was between 58.9 – 99.0% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	109.8	107.2	103.4	111.6	108.0	3.6	100.0
6.14	108.2	101.8	94.2	99.2	100.9	5.8	93.4
15.36	108.0	106.8	99.6	107.0	105.4	3.9	97.5
38.40	111.0	106.8	103.6	106.2	106.9	3.1	99.0
96.00	107.4	103.8	106.2	108.6	106.5	2.0	98.6
240.00	101.3	102.8	100.0	103.8	102.0 ⁺	1.7	94.4
600.00	65.0	64.0	65.0	60.3	63.6 ⁺	2.3	58.9
1500.00	_*	_*	_*	_*	_*	-	0.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

After the application of the test item at the rates ranging from 6.14 to 600.00 mL/ha, the cabbage shoot dry weight was between 23.3 – 100.1% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	424.0	414.0	418.0	428.0	421.0	6.2	100.0
6.14	424.0	420.0	416.0	426.0	421.5	4.4	100.1
15.36	376.0	388.0	364.0	374.0	375.5 ⁺	9.8	89.2
38.40	340.0	344.0	352.0	364.0	350.0 ⁺	10.6	83.1
96.00	252.0	246.0	244.0	238.0	245.0 ⁺	5.8	58.2
240.00	230.0	215.0	186.8	190.0	205.4 ⁺	20.7	48.8
600.00	95.0	86.7	103.0	107.5	98.0 ⁺	9.2	23.3
1500.00	-*	-*	-*	-*	-*	-	0.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

After the application of the test item at the rates ranging from 240 to 1500 mL/ha, the plant damage was observed and it was between 27.5 – 100.0% after 21 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis, and dead plants were observed.

Pea (*Pisum sativum*)

After the application of the test item at the rates ranging from 38.4 – 1500.0 mL/ha, plant mortality was not observed. After the application of the test item at the rates ranging from 38.4 – 1500.00 mL/ha, the plant number at the end of the experiment was equal to 100% in comparison to the control group

Application rate [mL/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
38.40	20	5	5	5	5	20	100.0	100.0
96.00	20	5	5	5	5	20	100.0	100.0
240.00	20	5	5	5	5	20	100.0	100.0
600.00	20	5	5	5	5	20	100.0	100.0
1500.00	20	5	5	5	5	20	100.0	100.0

After the application of the test item at the rates between 38.4 to 1500.0 mL/ha, the pea shoot length was between 45.8 – 99.4% of the control shoot length (Table 18).

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	529.8	531.0	531.6	530.8	530.8	0.7	100.0
38.40	524.0	527.0	528.0	531.0	527.5	2.9	99.4
96.00	529.0	524.0	526.8	529.0	527.2	2.4	99.3
240.00	508.0	513.0	504.0	506.0	507.8 ⁺	3.9	95.7
600.00	457.0	446.0	452.0	450.0	451.3 ⁺	4.6	85.0
1500.00	246.0	243.0	234.0	249.0	243.0 ⁺	6.5	45.8

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

After the application of the test item at the rates ranging from 38.4 to 1500.0 mL/ha, the pea shoot dry weight was between 25.0 – 98.5% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	408.0	410.0	412.0	404.0	408.5	3.4	100.0
38.40	408.0	400.0	396.0	406.0	402.5	5.5	98.5
96.00	368.0	348.0	362.0	364.0	360.5 ⁺	8.7	88.2
240.00	304.0	300.0	312.0	320.0	309.0 ⁺	8.9	75.6
600.00	212.0	226.0	214.0	206.0	214.5 ⁺	8.4	52.5
1500.00	108.0	102.8	98.0	100.0	102.2 ⁺	4.3	25.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

After the application of the test item at the rate 1500 mL/ha, the plant damage was observed and it was equal to 32.5% after 21 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, and chlorosis were observed.

Tomato (*Solanum lycopersicon*)

After the application of the test item at the rates ranging from 6.14 – 96.00 mL/ha, plant mortality was not observed. After the application of the test item at the rates ranging from 240 – 1500 mL/ha, plant mortality of all test plants was observed. After the application of the test item at the rates ranging from 6.14 – 96.00 mL/ha, the plant number at the end of the experiment was equal to 100% in comparison to the control group.

Application rate [mL/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
6.14	20	5	5	5	5	20	100.0	100.0
15.36	20	5	5	5	5	20	100.0	100.0
38.40	20	5	5	5	5	20	100.0	100.0
96.00	20	5	5	5	5	20	100.0	100.0
240.00	20	0	0	0	0	0+	0.0	0.0
600.00	20	0	0	0	0	0+	0.0	0.0
1500.00	20	0	0	0	0	0+	0.0	0.0

* - statistically significant difference between the control and the treatment group (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater)

After the application of the test item at the rates between 6.14 to 96.00 mL/ha, the tomato shoot length was between 71.8 – 109.6% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	182.6	197.2	173.6	197.8	187.8	11.8	100.0
6.14	202.2	195.4	209.2	180.4	196.8	12.3	104.8
15.36	209.4	208.0	205.8	199.8	205.8	4.2	109.6
38.40	198.6	201.8	192.2	201.6	198.6	4.5	105.7
96.00	133.4	132.2	138.4	135.6	134.9 ⁺	2.7	71.8
240.00	_*	_*	_*	_*	_*	-	0.0
600.00	_*	_*	_*	_*	_*	-	0.0
1500.00	_*	_*	_*	_*	_*	-	0.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

* - lack of plants

After the application of the test item at the rates ranging from 6.14 to 96.00 mL/ha, the tomato shoot dry weight was between 31.4 – 99.8% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	278.0	264.0	246.0	238.0	256.5	18.0	100.0
6.14	246.0	244.0	256.0	278.0	256.0	15.6	99.8
15.36	232.0	246.0	261.0	236.0	243.8	12.9	95.0
38.40	158.0	168.0	146.0	172.0	161.0 ⁺	11.6	62.8
96.00	86.0	72.0	90.0	74.0	80.5 ⁺	8.9	31.4
240.00	_*	_*	_*	_*	_*	-	0.0
600.00	_*	_*	_*	_*	_*	-	0.0
1500.00	_*	_*	_*	_*	_*	-	0.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

* - lack of plants

After the application of the test item at the rates ranging from 96 to 1500 mL/ha, the plant damage was observed and it was between 35 – 100% after 21 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis, necrosis, and dead plants were observed.

Onion (*Allium cepa*)

After the application of the test item at the rates ranging from 96 – 1500 mL/ha, plant mortality was observed (at the rates 600 and 1500 mL/ha plant mortality of all test plants was observed). After the application of the test item at the rates ranging from 6.14 – 240.00 mL/ha, the plant number at the end of the experiment was between 50 – 100% in comparison to the control group.

Application rate [mL/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
6.14	20	5	5	5	5	20	100.0	100.0
15.36	20	5	5	5	5	20	100.0	100.0
38.40	20	5	5	5	5	20	100.0	100.0
96.00	20	5	5	4	5	19	95.0	95.0
240.00	20	2	3	2	3	10 ⁺	50.0	50.0
600.00	20	0	0	0	0	0 ⁺	0.0	0.0
1500.00	20	0	0	0	0	0 ⁺	0.0	0.0

⁺ - statistically significant difference between the control and the treatment group (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater)

After the application of the test item at the rates between 6.14 to 240.00 mL/ha, the onion shoot length was between 47.2 – 104.8% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	190.8	197.2	190.4	194.2	193.2	3.2	100.0
6.14	209.2	207.8	193.6	198.8	202.4	7.4	104.8
15.36	182.6	179.0	185.4	175.0	180.5 ⁺	4.5	93.5
38.40	164.6	167.0	167.2	162.4	165.3 ⁺	2.3	85.6
96.00	134.6	143.6	141.0	125.4	136.2 ⁺	8.1	70.5
240.00	87.5	99.0	90.0	88.3	91.2 ⁺	5.3	47.2
600.00	_*	_*	_*	_*	_*	-	0.0
1500.00	_*	_*	_*	_*	_*	-	0.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

* - lack of plants

After the application of the test item at the rates ranging from 6.14 to 240.00 mL/ha, the onion shoot dry weight was between 25.0 – 101.7% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	28.0	34.0	28.0	30.0	30.0	2.8	100.0
6.14	32.0	32.0	26.0	32.0	30.5	3.0	101.7
15.36	16.0	24.0	23.4	18.0	20.4 ⁺	4.0	67.8
38.40	12.0	20.0	16.0	16.0	16.0 ⁺	3.3	53.3
96.00	12.0	12.0	10.0	8.0	10.5 ⁺	1.9	35.0
240.00	5.0	10.0	5.0	10.0	7.5 ⁺	2.9	25.0
600.00	-*	-*	-*	-*	-*	-	0.0
1500.00	-*	-*	-*	-*	-*	-	0.0

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

* - lack of plants

After the application of the test item at the rates ranging from 15.36 to 1500.00 mL/ha, the plant damage was observed and it was between 10 – 100% after 21 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, wiltings, and dead plants were observed.

Oats (*Avena sativa*)

After the application of the test item at the rate 1500.00 mL/ha, plant mortality was observed. After the application of the test item at the rates ranging from 38.4 – 1500.0 mL/ha, the plant number at the end of the experiment was between 25 – 100% in comparison to the control group.

Application rate [mL/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	100.0
38.40	20	5	5	5	5	20	100.0	100.0
96.00	20	5	5	5	5	20	100.0	100.0
240.00	20	5	5	5	5	20	100.0	100.0
600.00	20	5	5	5	5	20	100.0	100.0
1500.00	20	0	0	2	3	5 ⁺	25.0	25.0

⁺ - statistically significant difference between the control and the treatment group (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater)

After the application of the test item at the rates between 38.4 to 1500.0 mL/ha, the oats shoot length was between 22.9 – 103.2% of the control shoot length.

Application rate [mL/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	422.6	418.8	413.4	425.6	420.1	5.3	100.0
38.40	432.6	429.6	433.4	438.4	433.5	3.7	103.2
96.00	429.4	427.2	422.4	431.2	427.6	3.8	101.8
240.00	337.0	338.4	336.4	347.2	339.8 ⁺	5.0	80.9
600.00	307.2	293.8	298.6	294.2	298.5 ⁺	6.2	71.0
1500.00	-*	-*	102.5	90.0	96.3 ⁺	0.8	22.9

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

* - lack of plants

After the application of the test item at the rates ranging from 38.4 to 1500.0 mL/ha, the oats shoot dry weight was between 15.3 – 100.8% of the control shoot weight.

Application rate [mL/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	168.0	186.0	184.0	182.0	180.0	8.2	100.0
38.40	190.0	168.0	172.0	196.0	181.5	13.6	100.8
96.00	176.0	154.0	178.0	178.0	171.5	11.7	95.3
240.00	108.0	116.0	108.0	110.0	110.5 ⁺	3.8	61.4
600.00	72.0	64.0	56.0	62.0	63.5 ⁺	6.6	35.3
1500.00	-*	-*	25.0	30.0	27.5 ⁺	3.5	15.3

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, one-sided smaller)

* - lack of plants

After the application of the test item at the rates ranging from 240 to 1500 mL/ha, the plant damage was observed and it was between 20 – 95% after 21 days of the exposure. Phytotoxic symptoms, i.e. stunted growth, chlorosis, necrosis, and dead plants were observed.

THE VALIDITY CRITERIA

The following validity criteria were met:

- the seedling emergence (validity criterion: at least 70%) was as follows:

87.5 – 100.0% – sunflower,

85.0 – 95.0% – cabbage,

85.0 – 100.0% – pea,

90.0 – 97.5% – tomato,

80.0 – 90.0% – onion,

80.0 – 95.0% – oats.

- the mean survival of the emerged control seedlings was 100% (validity criterion: at least 90%),

- the control seedlings did not exhibit any visible phytotoxic symptoms,

- environmental conditions for all plants belonging to the same species were identical.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data