FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **TERBUT 500 SC**

Product names: **TERBUT 500 SC/**

TAZOPRYM 500 SC / CORNAO 500 SC

Chemical active substance:

Terbuthylazine, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

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

Submission date: 08/2020

MS Finalisation date: 10/2021; 03/2022, 06/2022

Version history

When	What
October 2021	Finalisation of the assessment by zRMS-PL.
March 2022	Final Registration Report
June 2022	zRMS update Registration Report

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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, Tazoprym 500 SC, Cornao 500 SC/ Terbut 500 SC	Formulation type:	suspension concentrate (SC)
Active substance 1:	Terbuthylazine	Conc. of as 1:	500 g/L
Applicant:	Synthos Agro sp. Z o.o.	Professional use:	☒
Zone(s):	Central	Non professional use:	☐
Verified by MS:	No		
Field of use:	herbicide		

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha [f]	**Conclusions**
					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	~~Maize (post-emergence)~~	~~F~~	~~**Sensitive:**~~ ~~*Capsella bursa-pastoris*~~ ~~*Viola arvensis*~~ ~~*Chenopodium album*~~ ~~*Amaranthus retroflexus*~~ ~~*Galium aparine*~~ ~~*Tripleurospermum inodorum*~~ ~~*Veronica arvensis*~~ ~~*Fallopia convolvulus*~~ ~~*Solanum nigrum*~~ ~~*Matricaria Chamomilla*~~ ~~**Medium sensitive:**~~ ~~*Cyanus segetum*~~ ~~*Stellaria media*~~	~~Fine spraying~~	~~BBCH 12-16~~	~~1~~	~~-~~	~~1 l/ha~~	~~500 g as/ha~~	~~200 l/ha~~			
				~~**Sensitive:**~~	~~Fine spraying~~	~~BBCH~~	~~1~~	~~-~~	~~1 l/ha + 0,2~~	~~500 g as/ha~~	~~200-300~~			

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha [f]	**Conclusions**
					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			
				~~*Chenopodium album*~~ ~~*Viola arvensis*~~ ~~*Amaranthus retroflexus*~~ ~~*Galium aparine*~~ ~~*Tripleurospermum inodorum*~~ ~~*Capsella bursa-pastoris*~~ ~~*Veronica arvensis*~~ ~~*Fallopia convolvulus*~~ ~~*Solanum nigrum*~~ ~~*Matricaria Chamomilla*~~ ~~*Stellaria media*~~ ~~**Medium sensitive:**~~ ~~*Cyanus segetum*~~		~~12-16~~			~~(adiuwant)~~		~~l/ha~~			
2	PL	Maize (pre-emergence)	F	**Sensitive:** *Chenopodium album* *Viola arvensis* *Amaranthus retroflexus* *Tripleurospermum inodorum* *Matricaria Chamomilla* **Medium sensitive:** *Stellaria media* *Cyanus segetum*	Fine spraying	BBCH 00	1	-	1 l/ha	500 g as/ha	200-300 l/ha			
				Sensitive: *Viola arvensis* *Amaranthus retroflexus* *Tripleurospermum inodorum* *Capsella bursa-pastoris* *Matricaria Chamomilla* **Medium sensitive:** *Fallopia convolvulus* *Geranium pusillum* *Galium aparine* *Cyanus segetum*	Fine spraying	BBCH 00	1	-	1 l/ha + 0,2 l/ha (adiuwant)	500 g as/ha	200- 300 l/ha			

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	**Conclusions**
					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			
				Stellaria media										

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

*** According to the Regulation (EU) 2021/824 amending Regulations (EU) No 540/2011 and (EU) No 820/2011 the active substance terbuthylazine should be restricted to once every third year on the same field at a maximum rate of 850 g/ha.**

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

9.1.1 Overall conclusions

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

According to the Regulation (EU) 2021/824 amending Regulations (EU) No 540/2011 and (EU) No 820/2011 the active substance terbuthylazine should be restricted to once every third year on the same field at a maximum rate of 850 g/ha.

9.1.1.1 Effects on birds.

An estimation of risk indicate low risk for birds of each range of assessed issues. Calculations conducted due to the influence of TERBUT 500 SC due to the acute, long-term and reproductive toxicity did not indicate any hazardous properties and danger for mammals. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

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

An estimation of risk indicate low risk for terrestrial vertebrates of each range of assessed issues. Calculations conducted due to the influence of TERBUT 500 SC due to the acute, ~~long-term and reproductive~~ toxicity did not indicate any acute hazardous properties and danger for mammals.
However, the long-term risk assessment needs further refinement for focal species -wood mouse.
For pre- emergence application the safe use was demonstrated. For post-emergence use further refinement is required.
There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

9.1.1.3 Effects on aquatic organisms (KCP 10.2)

Taking into consideration risk mitigation calculations for TERBUT 500 SC – pre-emergence use, following risk mitigation measures should be applied:
~~**– 2 m buffer zone with vegetated filter strip and 50 % drift reduction nozzle,**~~
~~**– 5 m buffer zone with vegetated filter strip.**~~

~~For TERBUT 500 SC – post-emergence use, following risk mitigation measures should be applied:~~
~~**– 1 m buffer zone with vegetated filter strip and 75 % drift reduction nozzle,**~~
~~**– 2 m buffer zone with vegetated filter strip and 50 % drift reduction nozzle,**~~
~~**– 4 m buffer zone with vegetated filter strip.**~~

PRE-EMERGENCE and POST –EMERGENCE USES:

-5m buffer non-spray zone with 5 meter vegetated filter strip to surface water bodies

Using the above-mentioned precautions, formulation TERBUT 500 SC can be used and will not have a negative impact on aquatic species.

9.1.1.4 Effects on bees (KCP 10.3.1)

The HQ values are lower than the trigger of 50, indicating low risk to bees from terbuthylazine following application of TERBUT 500 SC. Calculation conducted for TERBUT 500 SC regarding to the oral and contact toxicity also confirm no risk for bees due to the use that formulation: achieved values are lower than 50.
Therefore a low risk to bees is expected from the application of TERBUT 500 SC following application according to the proposed GAP. According to Reg. 284 the chronic test for adult bees and larvae should be provided by the applicant.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

The HQ values based on a result of extended laboratory test is higher than the trigger value, indicating risk to non-target arthropods from terbuthylazine following application of TERBUT 500 SC.
Therefore a higher-tier aged-residue study was performed. According to study, low risk to non-target arthropods is expected from the application of TERBUT 500 SC following application according to the proposed GAP.

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

An estimation of risk indicate low risk for soil mesofauna of each range of assessed issues. Calculations conducted due to the influence TERBUT 500 SC due to the long-term toxicity and reproductive did not indicate any hazardous properties and danger.

9.1.1.7 Effects on soil microbial activity (KCP 10.5)

On the basis of results it was assessed that TERBUT 500 SC in considered applications, does not pose unacceptable risk to soil microorganisms.
The risk to soil micro-organisms is considered to be low for all representative uses.

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

Taking into consideration risk mitigation calculations for TERBUT 500 SC – use in maize, following risk mitigation measures should be applied to non-crop land: ~~for seedlings:~~
~~- 1 m buffer zone with vegetated filter strip and 75 % drift reduction nozzle,~~
~~- 3 m buffer zone with vegetated filter strip.~~
- 3 m buffer zone or
- 1 m and use of 75% drift reducing nozzles

Using the above-mentioned precautions, formulation TERBUT 500 SC can be used and will not have a negative impact on non-target terrestrial plants.

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

Not relevant

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).

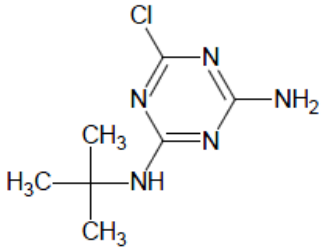
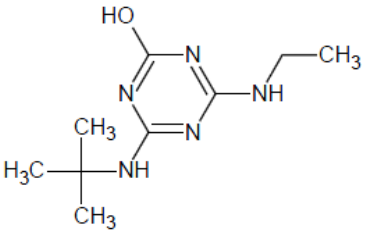
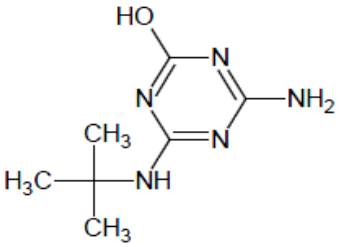
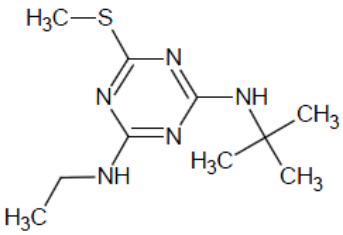
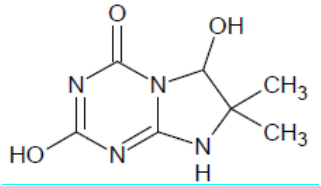
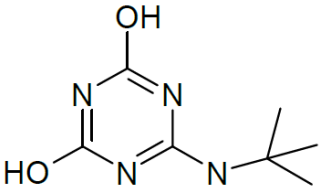
Table 9.1-2: Critical use pattern of TERBUT 500 SC

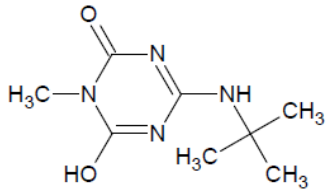
Group	Intended uses	Recommended dose rate
Maize	Maize (pre-emergence)	1.0 L/ha
	Maize (post-emergence)	1.0 L/ha

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 terbuthylazine

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
Desethyl-terbythylazine (MT1) N-tert-butyl-6-chloro-1,3,5-triazine-2,4-diamine	201.7		Soil: 32.9% Water/sediment: 7.3%	PEC_{gw}: leaching potential to groundwater PEC_{soil}: risk for soil organisms $PEC_{sw/sed}$: risk for aquatic organism
Hydroxy-terbythylazine (MT13) 4-(tert-butylamino)-6-(ethylamino)-1,3,5-triazin-2-ol	211.3		Soil: 34.5% Water/sediment: 20.0%	PEC_{gw}: leaching potential to groundwater PEC_{soil}: risk for soil organisms $PEC_{sw/sed}$: risk for aquatic organism
Desethyl hydroxy-terbythylazine (MT14) 4-amino-6-(tert-butylamino)-1,3,5-triazin-2-ol	183.2		Soil: 28%	PEC_{gw}: leaching potential to groundwater PEC_{soil}: risk for soil organisms
Terbutryn (MT26) N^2-tert-butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine	241.4		Soil: 0.001% Water/sediment: 7.4%	PEC_{gw}: leaching potential to groundwater PEC_{soil}: risk for soil organisms $PEC_{sw/sed}$: risk for aquatic organism
LM3 2,6-dihydroxy-7,7-dimethyl-7,8-dihydroimidazo[1,2-*a*][1,3,5]triazin-4(6*H*)-one	198.2		lysimeter metabolite	PEC_{gw}: leaching potential to groundwater
LM5 6-(*tert*-butylamino)-1,3,5-triazine-2,4-diol	184.2		lysimeter metabolite	PEC_{gw}: leaching potential to groundwater

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
LM6 4-(*tert*-butylamino)-6-hydroxy-1-methyl-1,3,5-triazin-2(1*H*)-one	198.2		lysimeter metabolite	PEC_{gw}: leaching potential to groundwater*

* EFSA Journal 2019;17(9):5817, Updated peer review of the pesticide risk assessment for the active substance terbuthylazine in light of confirmatory data.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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).

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Terbuthylazine	Oral 1 d Acute	LD_{50} = 1236 mg/kg bw	EFSA Journal 2011; 9(1):1969
Bobwhite quail	Terbuthylazine	Dietary 5 d Short-term	LD_{50} = 406mg/kg bw/d	EFSA Journal 2011; 9(1):1969
Japanese quail	Terbuthylazine	Dietary Reproductive toxicity	NOEL = 13.85 mg/kg bw/d	EFSA Journal 2011; 9(1):1969

9.2.1.1 Justification for new endpoints

Not relevant

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).

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 SL in maize post-emergence

Intended use	**Maize**				
Active substance/product	Terbuthylazine				
Application rate (g/ha)	1 × 500				
Acute toxicity (mg/kg bw)	1236				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize BBCH 10-19	Herbivorous bird, 'Woodlark' (Lullula arborea)	158.8	1	79.4	16
Reprod. toxicity (mg/kg bw/d)	13.85				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-19 Screening Step	Herbivorous bird, 'Woodlark' (Lullula arborea)	64.8	0.53	17.2	**0.81**
Maize BBCH 10 - 19	Small insectivorous bird "wag-tail" ground invertebrates without interception 50% ground arthro-pods, 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 with-out interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	-	-	**4.8**
Maize Leaf develop-ment BBCH 10 to 19	Small insectivorous/worm feed-ing species "thrush" ground in-vertebrates 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.

TER_a is over the trigger value of 10, no further consideration is needed.

TER_{lt} value for reproductive toxicity is below the trigger value of 5. Therefore, further calculations are needed.

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of TERBUT 500 SL in maize pre-emergence.

Intended use	**Maize**
Active substance/product	Terbuthylazine

Application rate (g/ha)	1 × 500				
Acute toxicity (mg/kg bw)	1236				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize BBCH <10	Herbivorous bird, 'Woodlark' (Lullula arborea)	24.7	1	12.4	100
Reprod. toxicity (mg/kg bw/d)	13.85				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH <10	Small garnivorous bird, 'Linnet' (Carduelis cannabina)	11.4	0.53	3.0	**4.62**
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 inverte-brates without interception) 50% seeds, 50% ground arthropods	8.2	-	-	6.4

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.
In blue added in ZRMS.

TER_a is over the trigger value of 10, no further consideration is needed.

TER_{lt} value for reproductive toxicity is below the trigger value of 5 (~~8% below the trigger~~). However, above calculations are performed for single diet containing weed seeds and using worst case for obtaining whole food from treated field (PT=1). As proved in Draft Risk Assessment (United Kingdom 2007) when using more realistic criterions, the risk for granivorous bird is considered to be low. Therefore no more calculations are needed.

9.2.2.2 Higher-tier risk assessment

Table 9.2-4: Higher-tier assessment of the reproductive risk for birds due to the use of TERBUT 500 SC in maize post-emergence – refined parameters are further described and justified in the text

Intended use	Maize					
Active substance/product	Terbuthylazine					
Application rate (g/ha)	1 × 500					
Reprod. toxicity (mg/kg bw/d)	13.85					
TER criterion	5					
Focal species	**Food category, % in diet**	**FIR**	**Mean RUD_{90} mg/kg**	**F_{TWA}**	**ETE (mg/kg bw/d)**	**TER_{lt}**
Skylark	Maize shoots, 45 %	9.45	34.5	0.19	0.83	
	Weed foliage, 5 %	1.05	24.5	0.53	0.18	

Weed seeds, 20 %	4.20	24.5	0.53	0.73	
Insects, 30%	6.30	1.93	0.53	0.087	
whole diet				1.83	7.6

FIR: Food intake rate; body weight used for calculations was 37,2g based on information obtained from DAR; RUD: residue unit dose;; F_{TWA} time weighed average factor; ETE: Estimated theoretical exposure; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Due to information obtained from Draft Assessment Report (United Kingdom 2007), the focal species for predicting risk for birds is skylark. Calculations based on information obtained from Draft Assessment Report about mixed diet of skylarks, confirm low risk for reproductive toxicity for bird, assuming the worst case scenario with PT = 1.

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 (inver-tebrates 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

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 omnivorouse 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: sklylark, 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 sklylark.

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 xxxxx (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 Terbuthylazine Final Addendum to the Additional Report (September, 2010), the F_{twa} value used for maize is 0.19 derived from a residue trial where the DT_{50} value of 2.8 days was determined (Lucini, 2008).

The mean residue value on insects was also reported in the Terbuthylazine 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.

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										
Inv.	0.81	18.70	0.5	1.93	1	1	0.53	1.0*	40.0	0.33
Weed seeds	0.164			40.2						0.82
Leaves (Maize foliage)	0.026			31.9[a)]						0.10
										Sum 1.25
NOAEL [mg a.s./kg bw/d]			13.85							
TER			11.08							

[a)] according to terbuthylazine Final addendum to the Additional Report (2010), FIR/bw=0.47
*worst case scenario

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.

<u>Wood pigeon:</u>

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 below than the annex VI trigger value of 5 indicating need

to further refinement for the proposed use.

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 TERUT 500 SC is 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 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 151, terbuthylazine belongs to the group of less sorptive substances.

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

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) is below 50, low risk for birds is assumed and no more calculations are needed.

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.

Since pre-emergence use represents the worst case, it is used for calculations.

Table 9.2-5: Assessment of the risk for earthworm-eating birds due to exposure to terbuthylazine bioaccumulation in earthworms (secondary poisoning) for the intended use in maize, pre-emergence use

Parameter	terbuthylazine	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.573	
log P_{ow} / P_{ow}	3.4/2512	
Koc	151	
Foc	0.02	Default
BCF_{worm}	10.3	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	5.9	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	6.2	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	13.85	
TER_{lt}	**2.23**	

TER values shown in bold fall below the relevant trigger.

TER_{lt} is below the trigger value of 5. However, as shown in Draft Assessment Report (United Kingdom 2007) for Terbuthylazine (United Kingdom 2007) and in peer review there is no evidence for bioaccumulation of terbuthylazine in organisms. Therefore no more calculations are needed.

zRMS comments:

Risk refinement for risk for earthworm-eating birds due to exposure to Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize is needed as the TER_{LT} is below trigger of 5. To address this risk an earthworm bioaccumulation study (Batscher 2007, DAR) to measure more realistic body burdens within earthworms from the proposed uses was used by zRMS.

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 9.2-5-1: Risk refinement for 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)	0.573	EFSA Journal 2011; 9(1):1969
log P_{ow} / P_{ow}	3.4/2512	Confirmatory Data Terbuthylazine November 2015
Koc	151	Confirmatory Data Terbuthylazine November 2015
Foc	Organic carbon content of soil (0.02 taken as a default value)	Default
BAF	0.86	DAR Terbuthylazine Volume 3 B9 2010
PEC_{worm}	0.492	$PEC_{worm} = PEC_{soil} \times BAF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.517	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	13.85	EFSA Journal 2011; 9(1):1969
TER_{lt}	26.79	Above trigger value 5

TER_{LT} is above the trigger of 5 indicated acceptable risk to **risk for earthworm-eating-birds due to exposure to Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize.**

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 terbuthylazine in water.

Since pre-emergence use represents the worst case, it is used for calculations

Table 9.2-6: Assessment of the risk for fish-eating birds due to exposure to terbuthylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize, pre-emergence use

Parameter	Terbuthylazine	comments
RAC (mg/L)	0.010	
BCF_{fish}	~~10.3~~ 34	
PEC_{fish}	~~0.20~~ 0.34	$PEC_{fish} = RAC \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	~~0.031~~ 0.054	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	13.85	
TER_{lt}	~~447~~ 256.48	

TER values shown in bold fall below the relevant trigger.

zRMS comments:

ZRMS verified the calculations provided in the Table above .

TER_{LT} is above the trigger of 5 indicated acceptable risk to risk for fish-eating-birds due to exposure to Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize.

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

An estimation of risk indicate low risk for birds of each range of assessed issues. Calculations conducted due to the influence TERBUT 500 SC due to the acute, long-term and reproductive toxicity did not indicate any hazardous properties and danger for birds. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

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 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).

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

Species	Substance	Exposure System	Results	Reference
Rat	terbuthylazine	Oral 1 d, Acute	LD_{50} = 1000 mg a.s./kg bw	EFSA Journal 2011; 9(1):1969
Rat	MT1	Oral 1 d, Acute	LD_{50} = 236 mg a.s./kg bw	EFSA Journal 2011; 9(1):1969
Rat	MT13	Oral 1 d, Acute	LD_{50} > 2000 mg a.s./kg bw	EFSA Journal 2011; 9(1):1969
Rat	MT14	Oral 1 d, Acute	LD_{50} > 2000 mg a.s./kg bw	EFSA Journal 2011; 9(1):1969
Rat	terbuthylazine	Oral 1 d, Long-term	NOAEL = 3.3 mg a.s./kg bw	EFSA Journal 2011; 9(1):1969

9.3.1.1 Justification for new endpoints

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 Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

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

Intended use	Maize				
Active substance/product	terbuthylazine				
Application rate (kg/ha)	1 × 0.500				
Acute toxicity (mg/kg bw)	1000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize BBCH 10 - 29	Small herbivorous mammal (vole)	136.4	1	68.2	15
Reprod. toxicity (mg/kg bw/d)	3.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10 - 29	Small herbivorous mammal (vole)	72.3	0.53	19.2	**0.17**
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.

TER_a is over the trigger value of 10, no further consideration is needed.

For terbuthylazine the resulting TER_{LT} value are below the trigger value of 5 as defined in the EFSA Journal 2009; 7(12):1438. Therefore further calculations are necessary.

In addition, in the Table below the risk for metabolites was provided by the applicant.

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ~~TERBUT 500 SC~~ metabolites in maize post-emergence

Intended use	Maize				
Active substance/product	MT1, MT13 and MT14				
Application rate (kg/ha)	1 × 0.144 / 0.159 / 0.112				
MT1 Acute toxicity (mg/kg bw)	236				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize BBCH 10 - 29	Small herbivorous mammal (vole)	136.4	1	19.6	12
MT 13 Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10 - 29	Small herbivorous mammal (vole)	136.4	1	21.7	92
MT 14 Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10 - 29	Small herbivorous mammal (vole)	136.4	1	15.3	131

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.

TER_a is over the trigger value of 10, no further consideration is needed for metabolites.

Table 9.3-4: Screening and First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of TERBUT 500 SC in maize pre-emergence

Intended use	Maize				
Active substance/product	terbuthylazine				
Application rate (kg/ha)	1 × 0.500				
Acute toxicity (mg/kg bw)	1000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize BBCH <10	Small omnivorous mammal (wood mouse)	14.3	1	7.2	139
Reprod. toxicity (mg/kg bw/d)	3.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small herbivorous mammal	72.3	0.53	19.16	**0.17**
Maize BBCH <10	Small omnivorous mammal (wood mouse)	5.7	0.53	1.5	**2.2**

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.

TER_a is over the trigger value of 10, no further consideration is needed.
TER_{lt} is below the trigger value of 5. However, as shown in Additional Report to the Draft Assessment Report (United Kingdom 2010) for Terbuthylazine, in field studies, wood mice spent an average PT time of 16.6% on treated fields. Taking this value into consideration, the DDD value is 0.25 and the TER_{lt} value is 13. Therefore, it is above the trigger value of 5 and no more calculations are needed.

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of TERBUT 500 SC in maize pre-emergence

Intended use	Maize				
Active substance/product	MT1, MT13 and MT14				
Application rate (kg/ha)	1 × 0.144 / 0.159 / 0.112				
MT1 Acute toxicity (mg/kg bw)	236				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize BBCH 10 - 29	Small omnivorous mammal (wood mouse)	14.3	1	2.1	112
MT 13 Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10 - 29	Small omnivorous mammal (wood mouse)	14.3	1	2.3	870
MT 14 Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10 - 29	Small omnivorous mammal (wood mouse)	14.3	1	1.6	1250

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.

TER_a is over the trigger value of 10, no further consideration is needed.

9.3.2.2 Higher-tier risk assessment

According to Additional Report to the Draft Assessment Report (2010, United Kingdom), the most common mammal on maize fields is wood mouse. Therefore it is used as a focal species for calculations.

To calculate RUD for earthworms, results for PEC_{soil} obtained in Section 8 (Environmental Fate) was used.
PEC_{worm} = $PEC_{soil\ d21}$ * BAF (Bioacumulation Factor)
BAF = 0.86
PEC_{worm} = ~~0.366~~ 0.573*0.86 = ~~0.315 =~~ 0.492

Bodyweight = 18g

As shown in Additional Report to the Draft Assessment Report (United Kingdom 2010) for Terbuthylazine, in field studies, wood mice spent an average PT time of 16.6% on treated fields.

fTWA for maize was based on new study (xxxx2008) which shows lower DT_{50} of terbuthylazine after application in maize.

Diet of wood mouse is based on information obtained from Additional Report to the Draft Assessment Report (United Kingdom 2010) and Peiz 1989

Table 9.3-6: Higher-tier assessment of the reproductive risk for mammals due to the use of TERBUT 500 SC in maize, post-emergence

Intended use	Maize, post-emergence						
Active substance/product	Terbuthylazine						
Application rate (g/ha)	1 × 500						
Reprod. toxicity (mg/kg bw/d)	3.3						
TER criterion	5						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_m (mg/kg food)**	**fTWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Wood mouse	Plant material, 50 %	0.58	34.5	0.19	0.166	0.32	
	Insects, 10 %	0.12	1.93	0.53	0.166	0.010	
	Earthworms, 40 %	0.46	0.315	0.53	0.166	0.006	
	whole diet					0.336	9.8

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DDD: daily dietary dose; TER: toxicity to exposure ratio.
TER values shown in bold fall below the relevant trigger.

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_{lt}**
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 (xxxx 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 scenario Growth stage	**Generic focal species**	**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 TERLT 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 xxxxx , 2005 study (90 the 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 therbuthylazine use of PT.

For the intended early post-emergence use (maize), the data for diet in May from xxxstudy (1989) appear to be most relevant. The dietary composition of wood mice as reported by xxxxx (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 <u>wood mouse</u> with consideration the diet given in xxxxx (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			0.087
Seeds	0.30			40.2			0.53			0.57
Weed seeds	0.04									0.076
										Sum=0.77*
NOAEL [mg a.s./kg bw/d]			3.3							
TER			**4.23***							

***1*) FIR/bw=0.41**
2) BCF=0.86 x 21 PECtwa
***PT-90 percentile**
****PT- mean value**

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 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 151, terbuthylazine belongs to the group of less sorptive substances.

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

Since the obtained value for reproduction toxicity is over trigger value 50, further calculation are needed.

Table 9.3-7: Assessment of the risk for mammals due to exposure to TERBUT 500 SC via contaminated drinking water in puddles

Intended use	Maize (pre an post-emergence)					
Active substance/formulation	terbuthylazine					
Application rate (g/ha)	1 × 500					
Acute toxicity (mg/kg bw)	1000					
TER criterion	10					
Reprod. toxicity (mg/kg bw/d)	3.3					
TER criterion	5					
Soil-relevant applic. rate (g/ha)	**Koc (L/kg)**	**PEC_{puddle} (mg/L)**	**DW uptake (L/kg bw/d)**	**TWA**	**Daily dose (mg/kg bw/d)**	**TER_a** / **TER_{lt}**
500	151	0.20	0.24	1	0.048	20833
				0.53		130

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

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 predicted concentrations in soil.

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 (pre-emergence use)

Parameter	terbuthylazine	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.573*	
log P_{ow} / P_{ow}	3.4/2512	
Koc	151	
foc	0.02	Default
BCF_{worm}	10.3	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	5.9	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	7.55	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	3.3	
TER_{lt}	**0.44**	

TER values shown in bold fall below the relevant trigger.

*pre-emergence

TER_{lt} is below the trigger value of 5. However, as shown in Draft Assessment Report for Terbuthylazine (2007) and in peer review there is no evidence for bioaccumulation of terbuthylazine in organisms. There-

~~fore no more calculations are needed.~~

zRMS comments:

Risk refinement for risk for earthworm-eating birds due to exposure to Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize is needed as the TER_{LT} is below trigger of 5. To address this risk an earthworm bioaccumulation study (xxxxx 2007, DAR) to measure more realistic body burdens within earthworms from the proposed uses was used by zRMS.

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 Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

Parameter	Terbuthylazine	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.573*	EFSA Journal 2011; 9(1):1969
log P_{ow} / P_{ow}	3.4/2512	Confirmatory Data Terbuthylazine November 2015
Koc	151	Confirmatory Data Terbuthylazine November 2015
foc	Organic carbon content of soil (0.02 taken as a default value)	Default
BAF	0.86	DAR Terbuthylazine Volume 3 B9 2010
PEC_{worm}	0.492	$PEC_{worm} = PEC_{soil} \times BAF_{worm/soil\ or}$
Daily dietary dose (mg/kg bw/d)	0.63	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	3.3	EFSA Journal 2011; 9(1):1969
TER_{lt}	5.24	Above trigger value 5

*pre emergence

TER_{LT} is above the trigger of 5 indicated acceptable risk to **risk for earthworm-eating-mammals due to exposure to Terbuthylazine via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize.**

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 terbuthylazine in water.

Table 9.3-10: Assessment of the risk for fish-eating birds due to exposure to terbuthylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize, pre-emergence use

Parameter	terbuthylazine	comments
RAC (mg/L)	0.019	

BCF_{fish}	~~10.3~~ 34	
PEC_{fish}	~~0.20~~ 0.646	$PEC_{fish} = PEC_{water} \times BCF_{fish}$ PEC_{fish}=RACx BCF_{fish}
Daily dietary dose (mg/kg bw/d)	~~0.028~~ 0.09	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	3.3	
TER_{lt}	~~118~~ 36.66	

TER values shown in bold fall below the relevant trigger.

zRMS comments:

The risk for fish-eating mammals due to exposure to terbuthylazine via bioaccumulation in fish (secondary poisoning) for the intended use in maize was corrected by zRMS and considered 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

An estimation of risk indicate low risk for mammals of each range of assessed issues. Calculations conducted due to the influence TERBUT 500 SC due to the acute, long-term and reproductive toxicity did not indicate any hazardous properties and danger for mammals which was confirmed by high risk assessment. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

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

Not relevant

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 Ap-

pendix 2.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – terbuthylazine, relevant metabolites and TERBUT 500 SC

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	terbuthylazine	96 h, s	LC_{50} = 2.2 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	terbuthylazine	90 d f f	NOEC = 0.09 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	MT1	96 h, s	LC_{50} = 18 mg a.s./L $_{nom}$	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	MT13	96 h, s	LC_{50} >2.5 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	MT26	96 h, s	LC_{50} = 1.1 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Daphnia magna	MT1	48 h, s	LC_{50} = 42 mg a.s./L $_{nom}$	EFSA Journal 2011; 9(1):1969
Daphnia magna	MT13	48h, s	LC_{50} = >2.8 mg a.s./L $_{nom}$	EFSA Journal 2011; 9(1):1969
Daphnia magna	terbuthylazine	21 d, ss	NOEC = 0.019 mg a.s./L $_{nom}$	EFSA Journal 2011; 9(1):1969
Chironomus riparius	terbuthylazine	27 d, s	NOEC = 0.5 mg a.s./L $_{nom}$	EFSA Journal 2011; 9(1):1969
Pseudokirchneriella subcapitata	terbuthylazine	72 h, s	E_rC_{50} = 0.028 mg a.s./L $_{mm}$ E_bC_{50} = 0.012 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Selenastrum capricornutum	MT1	72 h, s	E_rC_{50} = 0.38 mg a.s./L $_{mm}$ E_bC_{50} = 0.142 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Desmodesmus subcapitatus	MT13	72 h, s	E_rC_{50} > 3.8 mg a.s./L $_{mm}$ E_bC_{50} >3.96 mg a.s./L $_{nom}$	EFSA Journal 2011; 9(1):1969
Pseudokirchneriella subcapitata	MT26	72 h, s	E_rC_{50} = 0.0036 mg a.s./L $_{mm}$ E_bC_{50} = 0.0017 mg a.s./L $_{mm}$	EFSA Journal 2011; 9(1):1969
Pseudokirchneriella subcapitata	LM3	72 h	Growth rate ErC_{50} nom= 80 Yield: nom E_yC_{50}= 39 Biomass:nomE_bC_{50}= 39	EFSA Journal 2019;17(9):5817)
Pseudokirchneriella subcapitata	LM5	72 h	Growth rate ErC_{50} nom>100 Yield: nom E_yC_{50}>100 Biomass:nomE_bC_{50}>100	EFSA Journal 2019;17(9):5817)
Pseudokirchneriella subcapitata	LM6	72 h	Growth rate ErC_{50} nom>100 Yield: nom E_yC_{50} >100 Biomass:nomE_bC_{50} .100	EFSA Journal 2019;17(9):5817)
Lemna gibba	terbuthylazine	14 d, s	E_rC_{50} = 0.412 mg a.s./L $_{mm}$ E_bC_{50} = 0.0133 mg a.s./L $_{mm}$ Frond number: nom $E_{fn}C_{50}$ = 0.0128 mg a.s./L	EFSA Journal 2011; 9(1):1969
Lemna gibba	MT26	14 d, s	EC_{50} = 0.025mg a.s./L $_{mm}$ (frond density)	EFSA Journal 2011; 9(1):1969
Myriophylum aquaticum	MT26	14 d, s	EC_{50} = 2.0 mg a.s./L $_{nom\ sediment}$ (root fresh weight)	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	TERBUT 500 SC	96 h, s	LC_{50} = 3.779 mg/L $_{nom}$ 1.89 mg a.s./L	xxx 2018, W/13/18
Daphnia magna	TERBUT 500 SC	48 h, s	EC_{50} = 177.9 mg/L $_{nom}$ 88.8 mg a.s./L	Kulec-Płoszczyca E., 2018, W/10/18

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	TERBUT 500 SC	72 h, s	E_rC_{50} = 0.1815 mg/L $_{nom}$ 0.0906mg a.s./L E_yC_{50} = 0.0251 mg/L $_{nom}$ 0.0125 mg a.s./L	Kulec-Płoszczyca E., 2018, W/11/18
Navicula pelliculosa	TERBUT 500 SC	72 h, s	E_rC_{50} = 0.02 mg/L $_{nom}$ 0.01mg a.s./L E_yC_{50} = 0.007 mg/L $_{nom}$ 0.0035 mg a.s./L	Janota D,. 2019, W/53/19
Lemna gibba	TERBUT 500 SC	7 d, ss	$E_rC_{50\ frond\ number}$ = 0.1583 mg/L $_{nom}$ 0.079 mg a.s./L $E_yC_{50\ frond\ number}$ = 0.0991 mg/L $_{nom}$ 0.04955 mg a.s/L $E_rC_{50\ dry\ weight}$ = 0.0902 mg/L $_{nom}$ 0.0451 mg a.s./L $E_yC_{50\ dry\ weight}$ = 0.0522 mg/L $_{nom}$ 0.0261 mg a.s./L	Kulec-Płoszczyca E., 2018, W/11/18

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

9.5.1.1 Justification for new endpoints

Not relevant.

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.

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

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sed. dwell. prolonged**	**Macrophytes**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Navicula pellicu-losa***	***Chironomus riparius***	***Lemna gibba***	**Lemna gibba**
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	*E_rC_{50}	*E_rC_{50}	NOEC	ErC_{50}*	EfnC50
(µg/L)		1890	90	88800	19	28	10	500	79	12.8
AF		100	10	100	10	10	10	10	10	10
RAC (µg/L)		18.9	9	888	1.9	2.8	1.0	50	7.9	1.28
FOCUS Sce-nario	**PEC $_{gl-max}$ (µg/L)**									
Step 1										
	143.3	**7.582**	**15.922**	0.163	**75.421**	**51.179**	**143.300**	**2.866**	**18.139**	**111.953**
Step 2										
N-Europe (Mar-May and Jun – Sep)	22.07	**1.168**	**2.452**	0.025	**11.616**	**7.882**	**22.070**	0.441	**2.794**	
S-Europe (Mar-May)	40.11	**2.122**	**4.457**	0.046	**21.111**	**14.325**	**40.110**	0.802	**5.077**	**31.336**
S-Europe (Jun-Sep)	31.09	**1.645**	**3.454**	0.035	**16.363**	**11.104**	**31.090**	0.622	**3.935**	**24.289**
Step 3										
D3/ditch	2.623	0.139	0.291	0.003	**1.381**	0.937	**2.623**	0.052	0.332	**2.049**
D4/pond	0.153	0.008	0.018	0.000	0.083	0.056	0.158	0.003	0.020	0.120

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae		Sed. dwell. prolonged	Macrophytes	Macrophytes
D4/stream	2.250	0.119	0.250	0.003	**1.185**	0.804	**2.252**	0.045	0.285	**1.758**
R1/pond	0.274	0.014	0.030	0.000	0.144	0.098	0.274	0.005	0.035	0.214
R1/stream	8.703	0.460	0.965	0.010	**4.572**	**3.102**	**8.686**	0.174	**1.099**	**6.799**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*The endpoints from formulation studies

For the intended uses of TERBUT 500 SC, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (diatoms) in most of FOCUS Steps 1-3 scenarios. Therefore, risk mitigation assessment is necessary and PEC/RAC ratios were calculated considering reduced exposure of surface water bodies.

FOCUS Step 4

Table 9.5-3: Global maximum PEC_{sw} values for Terbuthylazine, following single application of TERBUT 500 SC to maize post-emergence use, according to surface water Step 4

PEC_{sw} (µg/L)	Scenario	STEP 4		
Nozzle reduction	Vegetative strip (m)	1	~~2~~	5
	No spray buffer (m)	1	~~2~~	5
None	R1 stream	12.5	~~1.68~~	**0.75**
50 %		6.3	**~~0.84~~**	**0.39**
75 %		3.1	**~~0.42~~**	**0.19**
90 %		1.25	**~~0.17~~**	**0.08**

Values below the RAC are bold
Since the scenario R1 stream represents the worst case, calculations only for this scenario are presented.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for terbuthylazine for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize (pre-emergence use).

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**		**Sed. dwell. prolonged**	**Macrophytes**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Navicula pelliculosa***	***Chironomus riparius***	***Lemna gibba***	**Lemna gibba**
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Er50	EfnC50**
(µg/L)		1890	90	88800	19	28*	10*	500	79*	12.8
AF		100	10	100	10	10	10	10	10	10
RAC (µg/L)		18.9	9	880	1.9	2.8	1.0	50	7.9	1.28
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**									
Step 1										
	143.3	**7.582**	**15.922**	0.163	**75.421**	**51.179**	**143.300**	**2.866**	**18.139**	**111.953**
Step 2										
N-Europe (Mar-May)	28.08	**1.486**	**3.120**	0.032	**14.779**	**10.029**	**28.080**	0.562	**3.554**	**21.938**
S-Europe (Mar-May)	52.13	**2.758**	**5.792**	0.059	**27.437**	**18.618**	**52.130**	**1.043**	**6.599**	**40.727**
Step 3										
D3/ditch	2.619	0.139	0.291	0.003	**1.378**	0.935	**2.619**	0.052	0.332	**2.046**
D4/pond	0.112	0.006	0.012	0.000	0.059	0.040	0.112	0.002	0.014	0.088
D4/stream	2.102	0.111	0.234	0.002	**1.106**	0.751	**2.102**	0.042	0.266	**1.642**
R1/pond	0.233	0.012	0.026	0.000	0.123	0.083	0.233	0.005	0.029	0.182
R1/stream	7.123	0.377	0.791	0.008	**3.749**	**2.544**	**7.123**	0.142	0.902	**5.565**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*the endpoints from formulation studies
**according to EFSA 2011

For the intended uses of TERBUT 500 SC, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (diatoms) in most of FOCUS Steps 1-3 scenarios. Therefore, risk mitigation assessment is necessary and PEC/RAC ratios were calculated considering reduced exposure of surface water bodies.

FOCUS Step 4

Table 9.5-5: Global maximum PEC_{sw} values for Terbuthylazine, following single application of TERBUT 500 SC to maize pre-emergence use, according to surface water Step 4

PEC_{sw} (µg/L)	Scenario	STEP 4		
Nozzle reduction	Vegetative strip (m)	1	~~2~~	~~4~~
	No spray buffer (m)	1	~~2~~	~~4~~
None	R1 stream	4.0	~~1.6~~	**~~0.93~~**
50 %		2.0	**~~0.83~~**	**~~0.47~~**
75 %		**1.0**	**~~0.41~~**	**~~0.23~~**
90 %		**0.4**	**~~0.08~~**	**~~0.09~~**

Values below the RAC are bold

Since the scenario R1 stream represents the worst case, results only for this scenario are presented.

zRMS comments:

For the intended uses pre and post emergence application, calculated PEC/RAC ratios did not indicate an acceptable risk for Daphnia magna (long-term risk) , algae for several FOCUS Steps 3 scenarios and in case of Lemna gibba for R1 scenario FOCUS STEP 3.

It should be noted that in case of Lemna gibba and algae the endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "*... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future*". Such calibration should be extended to algae and shall be performed at EU level. Until relevant information on the level of protection reached is made available, it is recommended to address this uncertainty at Member State level in the National Addendum if considered necessary.

Therefore, further PEC/RAC ratios were calculated by the applicant for the most sensitive organism based on UE endpoints and endpoints from formulation studies for Terbut 500 SC.

For Naviculla peliculosa an E_rC_{50} of 10 µg a.s./L from formulation study in connection with an assessment factor of 10 and the calculations for FOCUS STEP 3 for all scenarios were provided by the applicant. However, in case of FOCUS Step 4 PEC_{SW} the applicant considered only for R1 scenario calcula-

tions without the refined value of PEC_{sw} for D3 and D4 scenarios for all intended uses.

Taking into account agreed endpoint RAC=1.0 microgram/L, further refinement was needed for D3 and D4 scenarios for pre- and post- emergence application.

The applicant provided for request of zRMS the updated calculations in the Section 8 and updated risk assessment with considerations the RAC of 1.0 microgram/L were provided by zRMS below:

PEC_{sw}/ RACvalues for Terbuthylazine, following single application of TERBUT 500 SC to maize pre-emergence use, according to surface water Step 4 calculations and toxicity data for aquatics organisms with mitigation of spray drift and run-off .

PEC_{sw} (µg/L)	**Scenario**		**STEP 4 Terbuthylazine**		
Nozzle reduction	**Vegetative strip (m)**	0	0	5	10
	No spray buffer (m)	5	10	5	10
None	**D3 ditch**	0.8597	0.4558	0.8598	0.4558
50 %		0.4298	0.2280	0.4298	0.2280
None	**D4 pond**	0.1500	0.1445	0.1500	0.1445
50 %		0.1403	0.1376	0.1403	0.1376
None	**D 4 stream**	0.9495	0.5051	0.9495	0.5051
50 %		0.4764	0.2544	0.4764	0.2544
None	**R1 pond**	0.2246	0.2040	0.09458	0.0680
50 %		0.1879	0.1786	0.04729	0.0340
None	**R1 stream**	**7.123**	**7.123**	0.7631	0.4046
50 %		**7.123**	**7.123**	0.3814	0.2023

RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Global maximum PEC_{sw} values for Terbuthylazine, following single application of TERBUT 500 SC to maize post-emergence use, according to surface water Step 4 calculations and toxicity data for aquatics organisms with mitigation of spray drift and run-off .

PEC_{sw} (µg/L)	**Scenario**	**STEP 4 Terbutylezine**		
Nozzle reduction	Vegetative strip (m)	0	5	10
	No spray buffer (m)	10	5	10
None	D3 ditch	0.4559	0.8598	0.4559
50 %		0.2280	0.4299	0.2280
None	D4 pond	0.1503	0.1557	0.1503
50 %		0.1433	0.1460	0.1433
None	D 4 stream	0.5071	0.9515	0.5071
50 %		0.2564	0.4784	0.2564

PEC_{sw} (µg/L)	**Scenario**	**STEP 4 Terbutylezine**		
Nozzle reduction	Vegetative strip (m)	0	5	10
	No spray buffer (m)	10	5	10
50 %		0.2400	0.4415	0.2400
None	R1 pond	0.9034	0.1364	0.0680
50 %		0.8730	0.0938	0.0340
None	R1 stream	**13.85**	0.9773	0.4057

RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Based on the results with consideration FOCUS STEP 4 for scenarios relevant for Poland the following risk mitigation are required:

PRE-EMERGENCE and POST –EMERGENCE USES:

-5m buffer non-spray zone with 5 meter vegetated filter strip to surface water bodies

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT1 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize, post-emergence use

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Selenastrum capricornutum***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		18000	42000	380
AF		100	100	10
RAC (µg/L)		180	420	38
FOCUS Scenario	**PEC_{gl-max} (µg/L)**			
Step 1				
	81.81	0.455	0.195	**2.153**
Step 2				
N-Europe (Mar-May and Jun – Sep)	11.25	0.063	0.027	0.296
S-Europe (Mar-May)	22.22	0.123	0.053	0.585
S-Europe (Jun-Sep)	16.74	0.093	0.040	0.441

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

*PECsw is higer than PECgw.

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT1 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize, pre-emergence use

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Selenastrum capricornutum***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		18000	42000	380
AF		100	100	10
RAC (µg/L)		180	420	38
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	81.81	0.455	0.195	**2.153**
Step 2				
N-Europe	14.91	0.083	0.036	0.392
S-Europe	29.54	0.164	0.070	0.777

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
* PECsw is higer than PECgw.

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT13 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize post-emergence use

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Desmodesmus. subcapitatus***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		2500	2800	3800
AF		100	100	10
RAC (µg/L)		25	28	380
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	67.93	**2.717**	**2.426**	0.179
Step 2				
N-Europe (Mar-May and Jun – Sep)	10.25	0.410	0.366	0.027
S-Europe (Mar-May)	19.77	0.791	0.706	0.052
S-Europe (Jun-Sep)	15.01	0.600	0.536	0.040

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT13 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize pre-emergence use

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Desmodesmus. subcapitatus***

Group		Fish acute	Inverteb. acute	Algae
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		2500	2800	3800
AF		100	100	10
RAC (µg/L)		25	28	380
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	67.93	**2.717**	**2.426**	0.179
Step 2				
N-Europe	13.42	0.537	0.479	0.035
S-Europe	26.12	**1.045**	0.933	0.069

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold. PECsw is higer than PECgw.
* PECsw is higer than PECgw.

Since the result value for S-Europe is just barely over the trigger value and Step 2 is almost the worst case, no further justification for safety use is needed.

Table 9.5-10 : Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Desethyl Hydroxy-terbuthylazine (M14) of Terbuthylazine for aquatic organism based Focus PECgw for the use of TERBUT 500 SC in maize post - emergence.

Desethyl Hydroxy-terbuthylazine (M14)		
Group		Algae
Test species		
Endpoint		EC_{50}
(µg/L)		15000
AF		10
RAC (µg/L)		1500
FOCUS Scenario	PECgw (µg/L)	
	2.884	0.0019

*Hamburg

Table 9.5-11 : Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Desethyl Hydroxy-terbuthylazine (M14) of Terbuthylazine for aquatic organism based Focus PECgw for the use of TERBUT 500 SC in maize pre - emergence.

Desethyl Hydroxy-terbuthylazine (M14)		
Group		Algae
Test species		
Endpoint		EC_{50}

Desethyl Hydroxy-terbuthylazine (M14)		
Group		Algae
(µg/L)		15000
AF		10
RAC (µg/L)		1500
FOCUS Scenario	PECgw (µg/L)	
	2.773	0.0018

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT26 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize post-emergence use

Group		**Fish acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	E_rC_{50}
(µg/L)		1100	3.6
AF		100	10
RAC (µg/L)		11	0.36
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	8.05	0.732	**22.361**
Step 2			
N-Europe (Mar-May and Jun – Sep)	1.24	0.113	**3.444**
S-Europe (Mar-May)	2.24	0.204	**6.222**
S-Europe (Jun-Sep)	1.74	0.158	**4.833**
Step 3			
D3/ditch	0.205	0.019	0.569
D4/pond	0.008	0.001	0.022
D4/stream	0.175	0.016	0.486
R1/pond	0.008	0.001	0.022
R1/stream	0.139	0.013	0.386

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
* PECsw is higer than PECgw.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT26 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of TERBUT 500 SC in maize pre-emergence use

Group		**Fish acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	E_rC_{50}

Group		**Fish acute**	**Algae**
(µg/L)		1100	3.6
AF		100	10
RAC (µg/L)		11	0.36
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**		
Step 1			
	8.05	0.732	**22.361**
Step 2			
N-Europe	1.57	0.143	**4.361**
S-Europe	2.90	0.264	**8.056**
Step 3			
D3/ditch	0.204	0.019	0.567
D4/pond	0.0083	0.001	0.023
D4/stream	0.164	0.015	0.456
R1/pond	0.0083	0.001	0.023
R1/stream	0.141	0.013	0.392

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
* PECsw is higer than PECgw.

zRMS comments:

For the intended uses, calculated PEC/RAC ratios indicated an acceptable risk for aquatic organism from exposure from metabolites such as MT26, M13, M14, MT1.

In addition, the PEC_{gw}/RAC ratio was calculated for LM3, LM5 and LM6 metabolites with consideration PEC_{gw} values presented in Section 8 according to recommendation given in the Confirmatory data EFSA Journal 2019;17(9):5817.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM3 of Terbuthylazine based on FOCUS calculations for the use of TERBUT 500 SC in maize (pre-emergence application)

LM3		
Group		**Algae**
Endpoint		EC_{50}
(µg/L)		3900
AF		10
RAC (µg/L)		390
FOCUS	**PECgw-max (µg/L)**	
	15.334**	0.039

** worst case scenario Thivia

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM5 of Terbuthylazine based on FOCUS PECgw calculations for the use of TER-BUT 500 SC in maize (pre-emergence application)

LM5		
Group		Algae
Endpoint		EC_{50}
(µg/L)		100 000
AF		10
RAC (µg/L)		10000
FOCUS	$PECgw_{1\text{-}max}$ (µg/L)	
	2.319**	0.0002319

**worst case scenario Hamburg

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM6 of Terbuthylazine based on FOCUS PECgw calculations for the use of TER-BUT 500 SC in maize (pre-emergence application)

LM6		
Group		Algae
Endpoint		EC_{50}
(µg/L)		100 000
AF		10
RAC (µg/L)		10000
FOCUS	PEC_{gwmax} (µg/L)	
	6.377 **	0.0001001

** worst case

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM3 of Terbuthylazine based on FOCUS PECgw calculations for the use of TER-BUT 500 SC in maize (post emergence application)

LM3		
Group		Algae
Endpoint		EC_{50}
(µg/L)		3900
AF		10
RAC (µg/L)		390
FOCUS	$PEC_{gw\text{-}max}$ (µg/L)	
	13.741 **	0.352

**worst case scenario Thivia

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM5 of Terbuthylazine based on FOCUS PECgw calculations for the use of TERBUT 500 SC in maize (post-emergence application)

LM5		
Group		**Algae**
Endpoint		EC_{50}
(µg/L)		100 000
AF		10
RAC (µg/L)		10000
FOCUS	**PEC_{gw-max} (µg/L)**	
	2.404**	0.0002404

**worst case scenario Hamburg

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC_{gw}/RAC < 1) for metabolite LM6 of Terbuthylazine based on FOCUS PECgw calculations for the use of TERBUT 500 SC in maize (post -emergence application)

LM6		
Group		**Algae***
Endpoint		EC_{50}
(µg/L)		100 000
AF		10
RAC (µg/L)		10000
FOCUS	**PEC_{gw-max} (µg/L)**	
	5.824	0.0005824

** worst case scenario Thivia

zRMS comment:

The PEC_{gw}/RAC <1 ratios were calculated for algae for metabolites LM3, LM5 and LM6 based on FOCUS PEC_{gw} values. Based on these calculations the risk is considered acceptable.

9.5.3 Overall conclusions

Taking into consideration risk mitigation calculations for TERBUT 500 SC – post-emergence use in maize, following risk mitigation measures should be applied:

- ~~2 m buffer zone with vegetated filter strip and 50 % drift reduction nozzle,~~
- **5 m buffer non-spray zone with 5 meter vegetated filter strip.**

For TERBUT 500 SC – pre-emergence use in non-crop areas in maize, following risk mitigation measures should be applied:

- **1** ~~m buffer zone with vegetated filter strip and 75 % drift reduction nozzle,~~
~~2 m buffer zone with vegetated filter strip and 50 % drift reduction nozzle,~~
- ~~4 m buffer zone with vegetated filter strip.~~
-**5m buffer non-spray zone with 5 meter vegetated filter strip.**

~~Using the above-mentioned precautions, formulation TERBUT 500 SC can be used and will not have a negative impact on non-target terrestrial plants.~~

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. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of TERBUT 500 SC were not evaluated as part of the EU assessment of terbuthylazine. New data submitted with this application are listed 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	Parma P., 2017, W/87/17
Apis mellifera	TERBUT 500 SC	Oral	LD_{50} > 200 µg/bee	Parma P., 2017, W/88/17

9.6.1.1 Justification for new endpoints

Not relevant.

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 (SANCO/10329/2002 rev.2 (final), October 17, 2002).

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 terbuthylazine in maize

Intended use	Maize		
Active substance	terbuthylazine		
Application rate (g/ha)	1 × 500		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**

Oral toxicity	22.6	500	22
Contact toxicity	32		16

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

Table 9.6-3: First-tier assessment of the risk for bees due to the use of TERBUT 500 SC in maize

Intended use	Maize		
Active substance	TERBUT 500 SC		
Application rate (g/ha)	1 × 1000		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 200	1000	5
Contact toxicity	> 200		5

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 (SANCO/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

The HQ values are lower than the trigger of 50, indicating low risk to bees from terbuthylazine following application of TERBUT 500 SC. Calculation conducted for TERBUT 500 SC regarding to the oral and contact toxicity also confirm no risk for bees due to the use that formulation: achieved values are lower than 50.

Therefore a low risk to bees is expected from the application of TERBUT 500 SC following application according to the proposed GAP.

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 not been carried out with terbuthylazine

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.

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	Extended laboratory test barley leafs (2D)	LR_{50} > 1.5 L/ha ER_{50} = 0.741	Parma P., 2018, W/90/17
Aphidius rhopalosiphi (adults)	TERBUT 500 SC	Extended laboratory test barley plants (3D)	LR_{50} > 1.5 L/ha ER_{50} > 1.5 L/ha	Parma P., 2018, W/89/17
Coccinella septempunctata	TERBUT 500 SC	Extended laboratory test bean plants (2D)	LR_{50} > 1.5 L/ha ER_{50} > 1.5 L/ha	Vaughan, R. 2020 CHR-19-17
Chrysoperla carnea	TERBUT 500 SC	Extended laboratory test bean plants (2D)	LR_{50} > 1.5 L/ha ER_{50} > 1.5 L/ha	Vaughan, R. 2020 CHR-19-18
Typhlodromus pyri	TERBUT 500 SC	Aged residue extended laboratory test (2D)	ER_{50} > 1 L/ha after 0, 7 and 14 day after treatment	Fallowfield, L. 2020 CHR-19-16

9.7.1.1 Justification for new endpoints

Not relevant

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

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	Maize
Active substance/product	Terbut 500 SC
Application rate (mL/ha)	1 × 1000

MAF	1		
Test species Extended laboratory test	**LR_{50} (mL/ha)**	**$PER_{in\text{-}field}$ (mL/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1500	1000	0.67
Aphidius rhopalosiphi			
Coccinella septempunctata			
Chrysoperla carnea			
Test species Extended laboratory test	**ER_{50} (mL/ha)**	**$PER_{in\text{-}field}$ (mL/ha)**	**Low risk ER_{50} > $PER_{in\text{-}field}$**
Typhlodromus pyri	0.741	1000	**NO**
Aphidius rhopalosiphi	1500		YES
Coccinella septempunctata			
Chrysoperla carnea			

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.

9.7.2.2 Risk assessment for off-field exposure

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	Maize				
Active substance/product	Terbut 500 SC				
Application rate (g/ha)	1 × 1000				
MAF	1				
vdf	10 (Tier 1) / 5 (Higher-tier)				
Test species Extended laboratory test	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1500	2.77	2.77	10	0.018
Aphidius rhopalosiphi					
Coccinella septempunctata					
Chrysoperla carnea					
Test species Extended laboratory test	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**Low risk ER_{50} > corr. $PER_{off\text{-}field}$**
Typhlodromus pyri	741	2.77	2.77	10	YES
Aphidius rhopalosiphi	1500				
Coccinella septempunctata					
Chrysoperla carnea					

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.

zRMS comments:

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

The results of extended laboratory test is higher than the trigger value only for T.pyri, indicating risk to from terbuthylazine following application of TERBUT 500 SC.

According to the results from this study, low risk to non-target arthropods is expected from the application of TERBUT 500 SC following application according to the proposed GAP.

9.7.2.3 Additional higher-tier risk assessment

Since, using of TERBUT 500 SC according to calculations may indicate risk for *Typhlodromus pyri*, aged-residue test was performed to estimate appropriate time of recolonization.
According to results of higher-tier aged-residue study, low risk for *Typhlodromus pyri* is predicted just after application. Study shows ER_{50} value is higher than 1L/ha, what is proposed dose for TERBUT 500 SC.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The HQ values based on a result of extended laboratory test is higher than the trigger value, indicating risk to non-target arthropods from terbuthylazine following application of TERBUT 500 SC.
Therefore a higher-tier aged-residue study was performed. According to study, low risk to non-target arthropods is expected from the application of TERBUT 500 SC following application according to the proposed GAP.

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 as well as in Appendix 2 of this document (new studies).

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.

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
Eisenia andrei	MT1	Mixed into substrate 56 d, chronic	NOEC = 2.8 mg/kg dw	EFSA Journal 2011; 9(1):1969
Eisenia foetida	MT13	Mixed into substrate 56 d, chronic	NOEC = 7 mg/kg dw	EFSA Journal 2011; 9(1):1969
Eisenia andrei	TERBUT 500 SC	Mixed into substrate 56 d, chronic 10 % peat content	LC_{50} >500* mg/kg dw NOEC = 2.8* mg/kg dw	Gierbuszewska A., 2018, W/284/17
Folsomia candida	TERBUT 500 SC	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 9* mg/kg dw	Wołany M., 2019, G/60/19
Hypoaspis aculeifer	TERBUT 500 SC	Mixed into substrate 14 d, chronic 5 % peat content	NOEC > 500* mg/kg dw	Holewik P., 2019, G/61/19

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

9.8.1.1 Justification for new endpoints

Not relevant.

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 covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) (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	Mazie		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**

TERBUT 500 SC	2.8	1.47 (pre-emergence use)	**1.9**
TERBUT 500 SC	2.8	1.10	**2.5**
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
TERBUT 500 SC	*9 (Folsomia candida)*	~~1.76~~ 1.47	5.2 6.12
TERBUT 500 SC	*> 500 (Hypoaspis aculeifer)*	~~1.76~~ 1.47	284 340.2

TER values shown in bold fall below the relevant trigger.

There is no risk for mesofauna (other than earthworm) from use of TERBUT 500 SC.
Calculations of risk for earthworm show that TER_{lt} for chronic use (pre-emergence use) is below the trigger of 5. However the field study performed in southern Germany and Denmark show that, there is no risk for earthworm after 1 year after application of SC formulation in rate of 844 g a.s./ha, which value is much higher than proposed 500 g a.s./ha. Therefore the risk for earthworm is considered as a low.

zRMS comments:

The long-term risk assessment for Terbuthylazine 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) Terbuthylazine 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.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

An estimation of risk indicate low risk for soil mesofauna of each range of assessed issues. Calculations conducted due to the influence TERBUT 500 SC due to the long-term toxicity and reproductive did not indicate any hazardous properties and danger.

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. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this

document (new studies).

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	terbuthylazine	28 d, aerobic soil type	Nitrate formation rate 10.9 mg/kg soil dw < 25 % inhibition	EFSA Journal 2011; 9(1):1969
N-mineralisation	MT1	28 d, aerobic soil type	Nitrate formation rate 1.84 mg/kg soil dw < 25 % inhibition	EFSA Journal 2011; 9(1):1969
N-mineralisation	MT13	28 d, aerobic soil type	Nitrate formation rate 3.45 mg/kg soil dw < 25 % inhibition	EFSA Journal 2011; 9(1):1969
N-mineralisation	MT14	28 d, aerobic soil type	Nitrate formation rate 0.52 mg/kg soil dw < 25 % inhibition	EFSA Journal 2011; 9(1):1969
N-mineralisation	TERBUT 500 SC	28 d, aerobic soil type	Nitrate formation rate 11 mg/kg soil dw < 25 % inhibition	Gierbuszewska A., 2018, G/285/17

9.9.1.1 Justification for new endpoints

Not relevant

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).

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	Maize		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable**
TERBUT 500 SC	11 (at 28 d)	1.47	yes
MT1	1.84 (at 28 d)	0.193	yes
MT13	3.45 (at 28 d)	0.941	yes

MT14	0.52 (at 28 d)	0.176	yes

Since PEC_{soil} value for pre-emergence use is higher than for post-emergence use, it is used for calculation, to show low risk for soil micro-organisms.

ZRMS comments:

TERBUT 500 SC has no significant effect on soil micro-organisms at 11 mg a.s./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 microorganisms.

9.9.3 Overall conclusions

On the basis of results it was assessed that TERBUT 500 SC in considered applications does not pose unacceptable risk to soil microorganisms.

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

9.10.1 Toxicity data

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.

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

Species	Substance	Exposure System	Results	Reference
Helianthus annuus d *Brassica oleracea* d *Pisum sativum* d *Solanum lycopersicon* d *Allium cepa* m *Avena sativa* m	TERBUT 500 SC	21 d Seedling emergence	1) ER_{50} emergence = 0.097 L/ha 2) ER_{50} plant weight = 0.049 g/ha 3) ER_{50} plant height = 0.086 L/ha	Dec W., 2018, G/286/17
Helianthus annuus d *Brassica oleracea* d *Pisum sativum* d *Solanum lycopersicon* d *Allium cepa* d *Avena sativa* m	TERBUT 500 SC	21 d Vegetative vigour	1) ER_{50} plant weight = 0.051 L/ha 2) ER_{50} plant height = 0.219 L/ha	Gierbuszewska A., 2018, G/287/17

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Not relevant.

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", (SANCO/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.

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

Intended use	Maize pre and post-emergence use			
Active substance/product	TERBUT 500 SC			
Application rate (mL/ha)	1 × 1000			
MAF	1			
Test species	**ER_{50} (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Brassica oleracea	49.44 (seedling dry weight)	2.77	27.7	**1.78**
Allium cepa	51.1 (plant dry weight)	2.77	27.7	**1.84**

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** **Application rate (mL/ha)** **MAF**		Maize pre and post-emergence use TERBUT 500 SC 1 × 1000 1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77	27.7	13.9	6.9	2.8
3	0.95	9.5	4.8	2.4	0.95
Toxicity value ER_{50} = 49.44 g/ha		**TER** **criterion: TER ≥ 5**			
1		1.78	3.6	**7.1**	**17.8**
3		**5.2**	**10.4**	**20.8**	**52**
~~**Toxicity value**~~ ~~ER_{50} = 51.1 g/ha~~		~~**TER**~~ ~~**criterion: TER ≥ 5**~~			
~~1~~		~~1.84~~	~~3.7~~	~~7.4~~	~~**18.4**~~
~~3~~		~~**5.4**~~	~~**10.8**~~	~~**21.5**~~	~~**54**~~

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

9.10.3 Overall conclusions

~~Taking into consideration risk mitigation calculations for TERBUT 500 SC – use in maize, following risk mitigation measures should be applied for seedlings:~~
~~**- 1 m buffer zone with vegetated filter strip and 75 % drift reduction nozzle,**~~
~~**- 3 m buffer zone with vegetated filter strip.**~~

ZRMS comments:

ZRMS verified with the calculations of the deterministic risk assessment provided with consideration of the lowest endpoint ER_{50} = 49.44 g product/ha and $PER_{off\text{-}field}$.

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

SPe 3:

- 3 m buffer zone or

-1 m and use of 75% drift reducing nozzles

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

Not relevant

9.12 Monitoring data (KCP 10.8)

No additional data.

9.13 Classification and Labelling

TERBUT 500 SC was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

For classification of TERBUT 500 SC mixtures classification method was used.

Acute Category 1 (concentration of terbuthylazine is higher than 25%) H400

Chronic Category 1 (concentration of terbuthylazine is higher than 25%) H410

CLASSIFICATION	
Hazard classes, categories:	Aquatic Acute 1, Aquatic Chronic 1
LABELLING	
Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statements:	H410 – Very toxic to aquatic life with long lasting effects
Precautionary statements:	P273 – Avoid release to the environment. P391 - Collect spillage. P501 - Dispose of contents/container to an approved waste disposal plant.

Standard phrases under Regulation (EU) No 547/2011

SP 1	Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
~~SPe3~~	~~To protect aquatic organisms for post-emergence use respect a:~~ **~~- 2 m buffer zone with vegetated filter strip and 50 % drift reduction nozzle,~~** **~~- 5 m buffer zone with vegetated filter strip.~~** ~~To protect aquatic organisms for pre-emergence use respect a:~~ **~~- 1 m buffer zone with vegetated filter strip and 75 % drift reduction nozzle,~~** **~~- 2 m buffer zone with vegetated filter strip and 50 % drift reduction nozzle,~~** **~~- 4 m buffer zone with vegetated filter strip.~~** ~~To protect non-target terrestrial plants respect a:~~ **~~- 1 m buffer zone with vegetated filter strip and 75 % drift reduction nozzle,~~** **~~- 3 m buffer zone with vegetated filter strip.~~**

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 10.2	Janota, D.	2019	Terbut 500 SC, Navicula pelliculosa SAG 1050-3, Growth inhibition test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/53/19 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Kulec-Płoszczyca E.	2018	Terbut 500 SC, Daphnia magna, Acute immobilisation test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/10/18 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Kulec-Płoszczyca E.	2018	Terbut 500 SC, Pseudokirchneriella subcapitata SAG 61.81, Growth inhibition test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/11/18 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Kulec-Płoszczyca E.	2018	Terbut 500 SC, Lemna gibba CPCC 310, Growth inhibition test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/12/18 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	xxxxx	2018	Terbut 500 SC, Rainbow Trout, Acute Toxicity Test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/13/18 GLP; Unpublished	Y	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.10.3.1	Parma P.	2017	Terbut 500 SC, Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/87/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Parma P.	2017	Terbut 500 SC, Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test, Institute of Industrial Organic Chemistry (Pszczyna) Study code: W/88/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
KCP.10.3.2	Parma P.	2018	An extended laboratory test for evaluating the effects of TERBUT 500 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) Institute of Industrial Organic Chemistry (Pszczyna) Study code: B/89/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Parma P.	2018	An extended laboratory test for evaluating the effects of TERBUT 500 SC on the predatory mite, *Typhlodromus pyri* (Sch.) Institute of Industrial Organic Chemistry (Pszczyna) Study code: B/90/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Vaughan, R	2020	TERBUT 500 SC – A rate-response extended laboratory study to determine effects on the ladybird beetle, Coccinella septempunctata (Coleoptera: Coccinellidae) Mambo-Tox UK, Study code CHR-19-17 GLP, Unpublished	N	Synthos Agro Sp. z o.o.
	Vaughan, R	2020	TERBUT 500 SC – A rate-response extended laboratory study to determine effects on the green lacewing, Chrysoperla carnea (Neuroptera, Chrysopidae) Mambo-Tox UK, Study code CHR-19-17 GLP, Unpublished	N	Synthos Agro Sp. z o.o.
	Fallowfield, L.	2020	TERBUT 500 SC – An aged-residue extended laboratory study to determine effects on the	N	Synthos Agro

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			predatory mite Typhlodromus pyri (Acari: Phytoseiidae), Mambo-Tox UK, Study code CHR-19-17 GLP, Unpublished		Sp. z o.o.
KCP.10.4	Gierbuszewka A.	2018	TERBUT 500 SC, Earthworm Reproduction Test (*Eisenia andrei*) Institute of Industrial Organic Chemistry (Pszczyna) Study code: G/284/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Holewik, P.	2019	TERBUT 500 SC, Predatory mite (*Hypoaspis* (Geolaelaps) *aculeifer*) reproduction test in soil Institute of Industrial Organic Chemistry (Pszczyna) Study code: G/61/19 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Wołany, M.	2019	TERBUT 500 SC, Collembolan *(Folsomia candida)* Reproduction Test Institute of Industrial Organic Chemistry (Pszczyna) Study code: G/60/19 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
KCP.10.5	Gierbuszewka A.	2018	TERBUT 500 SC, Soil Microorganisms: Nitrogen Transformation Test Institute of Industrial Organic Chemistry (Pszczyna) Study code: G/285/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
KCP.10.6	Dec W.	2018	TERBUT 500 SC, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Institute of Industrial Organic Chemistry (Pszczyna) Study code: G/286/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.
	Gierbuszewska A.	2018	TERBUT 500 SC, Terrestrial Plant Test: Vegetative Vigour Test Institute of Industrial Organic Chemistry (Pszczyna) Study code: G/287/17 GLP; Unpublished	N	Synthos Agro Sp. z o.o.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.1.1	xxxxx	1989	Bobwhite quail acute oral toxicity study OXON	Y	SYNGENTA
	xxxx	1988	Bobwhite quailshort-term dietary toxicity study OXON	Y	SYNGENTA
	xxxx	1995	Japanese quail reproductive toxicity Syngenta and OXON	Y	SYNGENTA
KCP 10.1.2	xxxx	1991	DACT Technical Ground FL-871776: Acute Oral Toxicity Study in Rats Syngenta Unpublished Report No. 7801-91 GLP	Y	SYNGENTA
	Lucini, L.	2008	Determination of terbuthylazine initial residue and DT50 in maize plant following application of gardo gold^{tm} (187.5 terbuthylazine/L and 312.5 g S-metolachlor/L) Oxon Italia S.P.A, Pero, Italy, Syngenta CP AG, Basel, Switzerland GLP, not published File No A9476C_11126	Y	OXON/ SYNGENTA
	xxxx.	1991a	TK 12 669/1: Test To Evaluate the Acute Toxicity Following a Single Oral administration (LD50) in the Rat Syngenta Unpublished Report No. 012333 GLP	Y	SYNGENTA
	xxxx	2000	GS 28620 Tech. (Metabolite Of GS 13529): Acute Oral Toxicty in the Rat (Limit test Syngenta Unpublished Report No. 20001004 GLP	Y	SYNGENTA
	xxx	2001	GS 23158 Tech (Metabolite of GS 13529): Acute Oral Toxicity in the Rat (Limit test Syngenta Unpublished Report No. 20001014 GLP	Y	SYNGENTA
	xxx	2003	GS 26379: Acute Oral Toxicity Study in the Rat: Up and Down Procedure Syngenta Unpublished Report No. CTL/AR7315	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**
			GLP		
	xxxxxx	1998	Two generation reproduction toxicity studies Syngenta	Y	SYNGENTA
	xxxx	2005	Generic field monitoring of birds and mammals on maize and beet fields in Austria Report no.: WFC/FS 017 GLP	Y	---
	Dengler, D.	2001	Assessment of Toxic Effects of Terbuthylazine Technical on the Duckweed Lemna gibba in a Semi Static Test and a Recovery Period, Oxon Italia S.P.A, Pero, Italy Report-no. 20001420/01-ARLg, GLP, Not published	N	Oxon
KCP 10.2	Grade, A.	2000a	Acute toxicity of GS 23158 (Metabolite of GS 13529) to the cladoceran Daphnia magna Straus in the static system, Novartis Crop Protection AG, Basel, Switzerland, Report No 2001569, GLP, Not Published	N	SYNGENTA
	Grade, A.	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
	Grade, R.	1997	Growth inhibition test of GS 14260 tech. to green algae (Selenastrum capricornutum) under static conditions, Novartis Crop Protection AG, Basel, Switzerland, Report No 961714, GLP, Not Published	N	SYNGENTA
	Kelly, C.	1996	Terbuthylazine Technical Algal Growth Inhibition, Oxon Italia S.P.A, Pero, Italy Report-no. OXN 180/962297, GLP, Not published:	N	Oxon
	Memmert, U.	1998	Effects of 14C-labelled GS 13529 (Terbuthylazine tech.) on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system. Syngenta File No GS13529/1579	N	SYNGENTA

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP		
	Palmer, S, Kendall, T, Krueger, H A	2001	96-Hour Growth Inhibition Test of GS-26379 (Metabolite of GS-13529) to the Green Alga, Selenastrum capricornutum, Syngenta Crop Protection AG, Basel, Switzerland, Report No 528A-109, GLP, Not Published,	N	SYNGENTA
	xxxxx	2000	Acute toxicity of GS 23158 to Rainbow trout (Oncorhynchus mykiss) in a 96-hour static test. Novartis Crop Protection AG, Basel, Switzerland, Report No 765562, GLP Not Published	Y	SYNGENTA
KCP 10.2	Shillabeer, N., Maynard, S.J., Woodyer, J.M.	2002	GS13529 (Terbuthylazine technical) Chronic toxicity to Daphnia magna Syngenta File No GS13529/1783 GLP	N	SYNGENTA
	xxxx	1982	Acute toxicity of Terbutryn technical to Rainbow trout (Salmo Gairdneri) Novartis Crop Protection AG, Basel, Switzerland. , Report No BW-82-8-1241 Not GLP, Not Published,	Y	SYNGENTA
	xxxx	2002	GS13529 (Terbuthylamine technical): Acute toxicity to rainbow trout (Oncorhynchus mykiss) Syngenta Report No BL7395/B GLP Not Published	Y	SYNGENTA
	xxxx	1996	Prolonged Toxicity Test of CGA 293343 tech. to Rainbow Trout (Oncorhynchus mykiss) in the Flow-Through System	Y	SYNGENTA
	xxxx	1991a	Report on the acute toxicity test of GS 26379 to Rainbow trout (Salmo gairdneri) Novartis Crop Protection AG, Basel, Switzerland, Report No 918144 GLP, Not Published	Y	SYNGENTA
	Vial, A.	1991d	Report on the acute toxicity test of GS 26379 to Daphnia (Daphnia magna STRAUS 1820), Novartis Crop Protection AG, Basel, Switzerland,	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**
			Report No 918142, GLP Not Published,		
KCP 10.3	Petto, R., Klepka, S.	1994	Laboratory testing for toxicity (acute contact and oral LD50) of GS 13529 to honey bees (Apis mellifera L.) Syngenta File No GS13529/1239 GLP Not Published	N	SYNGENTA
KCP 10.4	Corboli, M,	2009	Effect on earthworms (Eisenia foetida) reproduction of 2-hydroxy-terbuthylazine (MT13). Flie no. GS13529_10039) GLP	N	SYNGENTA
KCP 10.4	Klein, O.	2006	S-metolachlor (A9396C), terbuthylazine (A5435E) and s-metolachlor + terbuthylazine (A9476C): A field study to evaluate effects on the earthworm fauna a maize field in southern Germany. GAB Biotechnologie GmbH & GAB Analytik GmbH, Germany Report No. 20051078/G1-NFEw. GLP, Not published, Syngenta file no CGA77102/1003	N	SYNGENTA
	Muther	2004a	GS26379 (a Metabolite of GS13529): Sublethal Toxicity to the Earthworm *Eisenia Andrei* in Artificial Soil. Syngenta Unpublished report No: 20041056/01 GLP	N	SYNGENTA
	Pease, G., et al.	2006	S-metolachlor (A9396C), terbuthylazine (A5435E) and s-metolachlor + terbuthylazine (A9476C): A field study to evaluate effects on the earthworm fauna a maize field in Denmark. Ecotox Limited, Devon, UK. Report No. ER-06-KCB 215. Non GLP report from GLP study, Not published, Syngenta file no CGA77102/1002	N	SYNGENTA
KCP 10.5	Kolzer, U.	2002	Assessment of the side effects of 2-hydroxy-terbuthylazine on the Activity of the Soil Microflora Oxon Italia S.P.A, Report No 20011377/01-ABMF GLP, Not published	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
	Kolzer, U.	2003	Assessment of the side effects of desethyl terbuthylazine on the Activity of the Soil Microflora Oxon Italia S.P.A, Report No 20021389/01-ABMF GLP, Not published	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

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

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 10.2 Effects on aquatic organisms

A 2.1.1 KCP 10.2 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Study 1

Report	Terbut 500 SC, Rainbow Trout, Acute Toxicity Test, Study code: W/13/18
Guideline(s):	OECD No. 203
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Oncorhynchus mykiss*
Test design	Semi -static system (96 h of exposure with renewal after 48 h)
Nominal test item concentrations	10, 4.54, 2.07, 0.94, 0.43, 0.19, 0.09, 0.04 mg/L

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations and mortality of fish are given below:
The LC_{50}/96 h values is 3.78 mg/L (95% confidence interval: 2.36 – 6.27).

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

Study 2

Report	Terbut 500 SC, Daphnia magna, Acute immobilisation test, Study code: W/10/18
Guideline(s):	OECD No. 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

~~Materials and methods~~

~~Test organism~~	~~*Daphnia magna*~~
~~Test design~~	~~Static system (48 h of exposure)~~
~~Nominal test item concentrations~~	~~500, 250, 125, 62.5, 31.3, 15.6 mg/L~~

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:	*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:	EC_{50}, 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 *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
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% respectivel.

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
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 Bionominal 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).

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations are given below:
The EC_{50}/96 h values is 177.9 mg/L (95% confidence interval: 57.6 – 1168.6).

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_rC_{60}	0.3506 (0.1470 – 1.3827)	0.1753 (0.0886 – 0.3289)	0.1815 (0.0790 – 0.3778)
E_rC_{20}	0.0171 (0.0009 – 0.0511)	0.0205 (0.0032 – 0.0477)	0.0231 (0.0020 – 0.0585)
E_rC_{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/68].
(–) – 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_yC_{60}	0.0691 (0.0316 – 0.1382)	0.0347 (0.0230 – 0.0521)	0.0251 (0.0176 – 0.0471)
E_yC_{20}	0.0069 (0.0012 – 0.0172)	0.0117 (0.0061 – 0.0182)	0.0117 (0.0071 – 0.0166)
E_yC_{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/68].
(–) – 95% confidence interval

Study 3

Report	Terbut 500 SC, Pseudokirchneriella subcapitata SAG 61.81, Growth inhibition test, W/11/18
Guideline(s):	OECD No. 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

~~**Materials and methods**~~

~~Test organism~~	~~*Pseudokirchneriella subcapitata*~~
~~Test design~~	~~72 h of exposure~~
~~Nominal test item concentrations~~	~~1.0, 0.33, 0.11, 0.037, 0.012, 0.0041, 0.0014 mg/L~~

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 unicellular freshwater green algae, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Raphidocelis subcapitata*, *Selenastrum capricornutum* 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 x 10_4 cells/mL
Endpoints:	E_rC_{50}, E_yC_{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 ± 2.0oC. 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
E_rC_{60}	0.3506 (0.1470 – 1.3827)	0.1753 (0.0886 – 0.3289)	0.1815 (0.0790 – 0.3778)
E_rC_{20}	0.0171 (0.0009 – 0.0511)	0.0205 (0.0032 – 0.0477)	0.0231 (0.0020 – 0.0585)
E_rC_{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/68].
(–) – 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_yC_{60}	0.0691 (0.0316 – 0.1382)	0.0347 (0.0230 – 0.0521)	0.0251 (0.0176 – 0.0471)
E_yC_{20}	0.0069 (0.0012 – 0.0172)	0.0117 (0.0061 – 0.0182)	0.0117 (0.0071 – 0.0166)
E_yC_{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/68].
(–) – 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%).

Conclusion

The endpoint values based on the nominal test item concentrations:
The concentration causing a 50% inhibition of the 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 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).

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_rC_{50}	0.007 (0.006 – 0.008)	0.012 (0.008 – 0.017)	0.020 (0.018 – 0.023)
E_rC_{20}	0.003 (0.002 – 0.004)	0.002 (0.001 – 0.004)	0.007 (0.005 – 0.008)
E_rC_{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_yC_{50}	0.004 (0.004 – 0.005)	0.002 (0.001 – 0.004)	0.007 (0.006 – 0.0008)
E_yC_{20}	0.002 (0.001 – 0.002)	n.d.	0.004 (0.003 – 0.005)
E_yC_{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]

Study 4

Report	Terbut 500 SC, Navicula pelliculosa SAG 1050-3, Growth inhibition test, W/53/19
Guideline(s):	OECD No. 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Navicula pelliculosa*
Test design	72 h of exposure
Nominal test item concentrations	10, 0.33, 0.11, 0.037, 0.012, 0.004 mg/L

Materials and methods

Test Item:	TERBUT 500 SC; batch no: 1/19; content of terbuthylazine: 511.9 g/L; production date: April 2019; expiry date: April 2021..
Test Species:	The freshwater diatoms, Navicula pelliculosa (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 104 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.
Test Concentration::	1.0, 0.33, 0.11, 0.037, 0.012, 0.004 mg/L plus the control..

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_rC_{50}	0.007 (0.006 – 0.008)	0.012 (0.008 – 0.017)	0.020 (0.018 – 0.023)
E_rC_{20}	0.003 (0.002 – 0.004)	0.002 (0.001 – 0.004)	0.007 (0.005 – 0.008)
E_rC_{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_yC_{50}	0.004 (0.004 – 0.005)	0.002 (0.001 – 0.004)	0.007 (0.006 – 0.0008)
E_yC_{20}	0.002 (0.001 – 0.002)	n.d.	0.004 (0.003 – 0.005)
E_yC_{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 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.
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%).

Conclusion

The endpoint values based on the nominal test item concentrations:

The concentration causing a 50% inhibition of the growth rate of *Navicula pelliculosa*,

i.e. the ErC50/72 h value is 0.02 mg/L (95% confidence interval: 0.018 – 0.023).

The concentration causing a 50% inhibition of yield of *Navicula pelliculosa*,

i.e. the EyC50/72 h value is 0.007 mg/L (95% confidence interval: 0.006 – 0.008).

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_rC_{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_rC_{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_rC_{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_yC_{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_yC_{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_yC_{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

Study 5

Report	Terbut 500 SC, Lemna gibba CPCC 310, Growth inhibition test, W/12/18
Guideline(s):	OECD No. 221
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

~~Test organism~~	~~*Lemna gibba*~~
~~Test design~~	~~Semi static system (7 d of exposure with renewal after 48 h)~~
~~Nominal test item concentrations~~	~~1.0, 0.33, 0.11, 0.037, 0.012, 0.004, 0.0013 mg/L~~

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 Lemna gibba 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_rC_{50}, E_yC_{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 con-

trol 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_rC_{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_rC_{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_rC_{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_yC_{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_yC_{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_yC_{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

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).

Conclusion

The endpoint values based on the nominal test item concentrations:
The concentration causing a 50% inhibition of the growth rate of *Lemna gibba* based on frond number, i.e. the ErC50/72 h value is 0.1583 mg/L (95% confidence interval: 0.1477 – 0.1696).
The concentration causing a 50% inhibition of yield of *Lemna gibba* based on frond number, i.e. the EyC50/72 h value is 0.0991 mg/L (95% confidence interval: 0.0924 – 0.1043).
The concentration causing a 50% inhibition of the growth rate of *Lemna gibba* based on dry weight, i.e. the ErC50/72 h value is 0.0902 mg/L (95% confidence interval: 0.0842 – 0.0964).
The concentration causing a 50% inhibition of yield of *Lemna gibba* based on dry weight, i.e. the EyC50/72 h value is 0.0522 mg/L (95% confidence interval: 0.0466 – 0.0587).

A 2.2 KCP 10.3 Effects on arthropods

A 2.2.1 KCP 10.3.1 Effects on bees

A 2.2.1.1 KCP 10.3.1 Acute 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:
LD_{50}/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.

Study 1

Report	TERBUT 500 SC: Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, Parma P., 2017, Study code: B/87/17
Guideline(s):	OECD 213 / EU Method C.16.
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, *Apis mellifera* 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:	E_rC_{50}, E_yC_{50}, LOEC and NOEC..
Test Conditions	temperature: 24 – 26ºC, 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
[µg/bee]	[µg a.i./bee]		Number of bees with adverse behaviour*/ number of living bees		
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.] replicates			Total	
		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 LD_{50} 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.] replicates			Total		
		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 LD_{50} 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).

Results:

The acute oral toxicity study results of the test item CYKLOP 400 SL on honeybees (*Apis mellifera* L.) in the laboratory test after 48 hours, are summarized below.

Dose		**Mortality after 48 h**	**LD_{50} after 48 h**	
TERBUT 500 SC [µg/bee]	**Terbuthylazine[µg/bee]**	**[%] ***	**TERBUT 500 SC [µg/ybee]**	**Active substance [µg/bee]**
0.0 (control)		0.0	above 200.0	above 90.7
25.0	**11.3**	0.0		
50.0	**22.7**	0.0		
100.0	**45.4**	0.0		
200.0	**90.7**	0.0		

The median lethal doses LD_{50}/24 h LD_{50}/48 h are higher than the maximum dose, i.e. 200.0 µg t.i./honeybee, used in the study.
During the definitive test no abnormal behavioural effects were observed after 48 hours of exposition.

Conclusions:
The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than to 200 µg t.i./honeybee.

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 LD_{50} of the reference item (dimethoate) was 0.26 µg a.i./bee (criterion: 0.10 -0.30 µg a.i./bee).

Agreed endpoints:

LD_{50}/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.

Study 2

Report	TERBUT 500 SC: Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test, Parma P., 2017, Study code: B/88/17
Guideline(s):	OECD 214 / EU Method C.17.
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, Apis mellifera 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 – 26ºC, 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][a]	[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 [µg/bee]		Exposure / replicates	4 h	24 h	48 h
			Number of bees showing adverse behaviour*/ number of living bees		
[µg /bee] [a]	[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	0/9	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 µg/honeybee (90.7 µg of a.i./bee). The median lethal dose of dimetho-

ate (LD50/24 h) determined with the log-probit method is 0.26 µg/bee (95% confidence limits: 0.23 - 0.30 µg 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 µg 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 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Results:
The median lethal doses of TERBUT 500 SC (LD_{50} contact) after 24 and 48 h are higher than the highest used dose, i.e. 200 µg/honeybee.

Dose		**Mortality after 48 h**	**LD_{50} after 48 h**	
TERBUT 500 SC [µg/bee]	**Terbuthylazine[µg/bee]**	**[%] ***	**TERBUT 500 SC [µg/ybee]**	**Active substance [µg/bee]**
0.0 (control)		0.0	above 200.0	above 90.7
25.0	**11.3**	0.0		
50.0	**22.7**	3.3		
100.0	**45.4**	3.3		
200.0	**90.7**	0.0		

The median lethal doses LD_{50}/24 h LD_{50}/48 h are higher than the maximum dose, i.e. 200.0 µg t.i./honeybee, used in the study.
During the definitive test no abnormal behavioural effects were observed in doses 25.0, 50.0, 100.0 and 200.0 µg t.i./honeybee.

Conclusions:
The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than 200.0 µg t.i./honeybee. With respect to the test results, it can be concluded that the test item, TERBUT 500 SC, has no adverse effect on mortality of honeybees (*Apis mellifera* L.).

A 2.3 KCP 10.3.2 Effects on arthropods other than bees

ZRMS comments:

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

Agreed endpoints:

LR_{50} > 1.5 L/ha
$NOER_{mortality}$ >1.5 L/ha
ER_{50} =1.5 L/ha
$NOER_{reproduction}$ >1.5 L/ha

Study 1

Report	An extended laboratory test for evaluating the effects of TERBUT 500 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez), Parma P., 2018, 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, Aphiidinae – 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			
			Dead wasps [no.]		Total	
[L/ha] [a]	[g a.i./ha] [b]		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	
			Replicates							
[L/ha] [a]	[g a.i./ha] [b]		I	II	III	IV	V	VI	[no.]	[%]
Control [0.0]		30	0	0	0	0	0	0	0	0.0
Terbut 500 SC										
1.5	748.8	30	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)								
[mL/ha] [c]	[g a.i./ha] [b]	Danadim 400 EC								
5.0	2.0	30	3	4	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*	
	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).

Results:

After 48 hours of exposure TERBUT 500 SC at the rate of 1.5 L/ha percentage of mortality of *A. rhopalosiphi* was 3.3% and reduction of fecundity was 40.6%..
Based on the obtained mortality and fecundity results LR_{50} and ER_{50} could not be estimated. It can be assumed that the LR_{50} and ER_{50} are higher than 1.5 L/ha of TERBUT 500 SC.

Conclusions:
On the basis of the obtained results it can be concluded that TERBUT 500 SC at the rate of 1.5 L/ha has no adverse effect on the mortality and reproduction of the wasps.

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:

LR_{50} > 1.5 L/ha
$NOER_{mortality}$=0.375 L/ha
ER_{50} = 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)

Study 2

Report	An extended laboratory test for evaluating the effects of TERBUT 500 SC on the predatory mite, *Typhlodromus pyri* (Sch.) Parma P., 2018, 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

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, Typhlodromus pyri (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				
			Number of dead mites [no.] Replicates		Total		Corrected
L/ha	g a.i./ha		I	II	[no.]	[%]	[%]
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					
			Number of dead mites [no.] Replicates			Total		Corrected
L/ha	g a.i./ha		I	II	III	[no.]	[%]	[%]
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 +	-
LR_{50}			> 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 con-

trol group, (step-down Cochran-Armitage test procedure, p(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								
			I			II			III		
[L/ha]	[g a.i./ha]		♀	♂	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 DAT 8	DAT 11	DAT 14	RrX	Rr	Pr [%]
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 DAT 8	DAT 11	DAT 14	RrX	Rr	Pr [%]
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			
ER_{50}		**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

Results:

After 7 days of exposure to TERBUT 500 SC at rates of 0.375, 0.75 and 1.5 L/ha, the percentages of *T. pyri*, mortality were 0.0, 6.7 and 8.3%, respectively and reduction of reproduction was 35.6, 41.6 and 84.5%, respectively.

There were statistically significant differences in mortality between groups treated with the test item at rates of 0.75 and 1.5 L/ha and the control group (Step-down Cochran-Armitage test procedure, p>0.05).

On the basis of the obtained mortality results, the LR_{50} is over 1.5 L/ha of TERBUT 500 SC. The $NOER_{mortality}$ is 0.375 L/ha of TERBUT 500 SC.

On the basis of the obtained reproduction results, the ER_{50} is 0.701 L/ha of TERBUT 500 SC.

Conclusions:

Based on the results it can be stated that TERBUT 500 SC, at the rates of 0.75 and 1.5 L/ha has significant adverse effect on mortality of the mites and the LR_{50} is over 1.5 L/ha of TERBUT 500 SC and ER_{50} is 0.701 L/ha of TERBUT 500 SC, what is below of predicted dose used for application.

ZRMS comments:

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

a) Pre-imaginal mortality in the control treatment should ≤ 20% (i.e. 8 lacewings from 40).
b) Corrected pre-imaginal mortality in the toxic reference treatment should be ≥ 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 ≥ 70%.

In the current study:

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

Agreed endpoints:

LR_{50} >1500 mL product/ha
$NOER_{mortality}$ = 667 mL product/ha
$NOER_{reproduction}$ = 1500 mL product/ha

Study 3

Report	TERBUT 500 SC – A rate-response extended laboratory study to determine effects on the green lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae) Vaughan, R., 2020, Study code: CHR-19-18
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 (Vogt *et al*. (2000)
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 ≤ 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* (LR_{50}). 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 (≡ 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 Chi^2 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 ≥ 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.

Results:
After exposure to TERBUT 500 SC at rates of 296, 444, 667, 1000 and 1500 mL/ha, the percentages of *C.carnea* corrected pre-imaginal mortality were 15.2, 18.2, 9.1, 30.3 and 6.1%, respectively and reduction of reproduction was not observed in any of used rate.
There were statistically significant differences in mortality between groups treated with the test item at rates of 1000 mL/ha and the control group (Step-down Cochran-Armitage test procedure, p>0.05).
On the basis of the obtained mortality results, the LR_{50} is over 1.5 L/ha of TERBUT 500 SC. The $NOER_{mortality}$ is 0.667 L/ha of TERBUT 500 SC.
On the basis of the obtained reproduction results, the ER_{50} is over 1.5 L/ha L/ha of TERBUT 500 SC.

Conclusions:
Based on the results it can be stated that for TERBUT 500 SC the LR_{50} is over 1.5 L/ha and ER_{50} is over 1.5 L/ha of TERBUT 500 SC, what indicate safe use of product.

Study 4

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 ≥ 50%.
c) Mean egg production should be > 2 viable eggs/female/day in the control treatment.

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

Agreed endpoints:
LR_{50}, ER_{50} > 1500 mL product/ha.
NOER with respect to both pre-imaginal mortality and beetle reproduction was 1500 mL product/ha.

Report	TERBUT 500 SC – A rate-response extended laboratory study to determine effects on the green lacewing, *Coccinella septempunctata* (Coleoptera: Coccinellidae) Vaughan, R., 2020, Study code: CHR-19-17
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 (Schmuck *et al*. (2000)
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 (*Acyrthosiphon 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 (≡ 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, α = 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, α = 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

Results:
After exposure to TERBUT 500 SC at rates of 296, 444, 667, 1000 and 1500 mL/ha, the percentages of *C.septapunctata* corrected pre-imaginal mortality were 5.3, 10.5, 7.9, 10.5 and 18.4%, respectively and reduction of reproduction was not observed in any of used rate.
On the basis of the obtained mortality results, the LR_{50} is over 1.5 L/ha of TERBUT 500 SC. The $NOER_{mortality}$ is 1.5 L/ha of TERBUT 500 SC.
On the basis of the obtained reproduction results, the ER_{50} is over 1.5 L/ha L/ha of TERBUT 500 SC.

Conclusions:
Based on the results it can be stated that for TERBUT 500 SC the LR_{50} is over 1.5 L/ha and ER_{50} is over 1.5 L/ha of TERBUT 500 SC, what indicate safe use of product.

Study 5

Report	TERBUT 500 SC – An aged-residue extended laboratory study to determine effects on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae), Fallowfield, L., 2020, Study code: CHR-19-16
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 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Results:
After exposure to TERBUT 500 SC at rates of 1000 mL/ha, the percentages of *T.pyri* corrected pre-imaginal mortality were 8.5, 3.2 and 5.2%, for samples applied 0, 7 and 14 days before introduction organisms on treated plants, respectively and reduction of reproduction was 12.6, -12.2 and -3.7, respectively.
On the basis of the obtained mortality and reproduction results, the LR_{50} and ER_{50} are over 1.0 L/ha of TERBUT 500 SC.
Result show low risk for organisms and short time of possible recolonization after treatment.

Conclusions:

Based on the results it can be stated that for TERBUT 500 SC the LR_{50} is over 1.0 L/ha and ER_{50} is over 1.0 L/ha of TERBUT 500 SC, what indicate safe use of product.

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

A 2.4.1.1 KCP 10.4 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: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

Agreed endpoints:
EC_{10}= 8.491 mg/kg dry weight of the artificial soil
EC_{20}= 20.959 mg/kg dry weight of the artificial soil
EC_{50}= 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
LC_{50} >1000 mg/kg dry weight of the artificial soil

Report	TERBUT 500 SC: Earthworm Reproduction Test (*Eisenia andrei*), Gierbuszewska A., 2018, Study code: G/284/17
Guideline(s):	OECD No. 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Eisenia andrei*
Test design	8 weeks of exposure
Nominal test item concentrations	5.6, 10, 18, 32, 56, 100, 180, 320, 560, 1000 mg/L

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]
EC_{10}	8.491 (3.443 – 15.074)
EC_{20}	20.959 (11.053 – 32.178)
EC_{50}	118.064 (86.141 – 164.058)
NOEC	5.600
LOEC	10.000
LC_{50}	> 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: ≤ 30%),
· adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

Results:

On the basis of the results, it was concluded that after 4 weeks, at the control group the mortality of adult earthworm was 2.5%. At concentrations ranging from 5.6 to 1000 mg of the test item/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%. There was no statistically significant differences between the test item concentrations and the control.

The concentration of the test item causing 50% mortality of the adult earthworms (LC_{50}) is higher than 1000 mg/kg dry weight of artificial soil. No changes in the appearance (morphology) and behaviour of the earthworms were noticed.

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of artificial soil, the body weight increase was between -36.6 to 16.9%. As for the control group, it was equal to 9.5%.

After 8 weeks of the experiment, the obtained results led to the following conclusions:

After 8 weeks of the experiment, it was concluded that TERBUT 500 SC had statistically significant impact on reproduction of the earthworms at the concentrations between 100 - 1000 mg/kg dry weight of artificial soil.

Conclusions

	TERBUT 500 SC [mg/kg dry weight of artificial soil]
EC_{10}	**8.491** (3.443 – 15.074)
EC_{20}	**20.959** (11.053 – 32.178)
EC_{50}	**118.064** (86.141 – 164.058)
NOEC (reproduction)	**5.6**
LOEC (reproduction)	**10**
LC_{50}	**>1000**
NOEC (survival)	**≥1000**
LOEC (survival)	**>1000**

A 2.4.2 KCP 10.4 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: 2.5% (criterion: ≤ 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: ≤ 30%).

Agreed endpoints:
LC_{50} >1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).
NOEC≥1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).
EC_{10} >1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).
EC_{20} > 1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).
EC_{50} >1000 mg/kg dry weight of the artificial soil (above 462.8 mg of terbuthylazine / kg dry weight of the artificial soil).
NOEC ≥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).

Study 1

Report	TERBUT 500 SC: Predatory mite *(Hypoaspis (Geolaelaps) aculeifer)* reproduction test in soil, Holewik, P., 2019, Study code: G/61/19
Guideline(s):	OECD No. 226
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Hypoaspis (Geolaelaps) aculeifer*
Test design	14 days of exposure
Nominal test item concentrations	5.6, 10, 18, 32, 56, 100, 180, 320, 560, 1000 mg/L

Test Item:	Terbut 500 SC, batch no. 1/19, content of terbuthylazine 511.9 g/L, .
Test Species:	the predatory mites, Hypoaspis (Geolaelaps) aculeifer (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) 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
Test Concentration::	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.
According to the OECD Guideline No. 226, the EC50 should be between 100 and 500 mg/kg dry weight of the artificial 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 control:
 mean adult mortality: 2.5% (criterion: ≤ 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: ≤ 30%).

Results:
On the basis of the results, it was concluded that after 14 days, at the control group the mortality of adult mites was 2.5%. At concentrations ranging from 5.6 to 1000 mg of the test item/kg dry weight of artificial soil, after 14 days of exposure to the test item, mortality of the adult mites was ranging from 0.0 to 10.0%. There was no statistically significant differences between the test item concentrations and the control.
The concentration of the test item causing 50% mortality of the adult mites (LC_{50}) is higher than 1000 mg/kg dry weight of artificial soil.
After 14 days of the experiment, it was concluded that TERBUT 500 SC had no statistically significant impact on reproduction of the mites at the concentrations up to 1000 mg/kg dry weight of artificial soil.

Conclusions

	TERBUT 500 SC [mg/kg dry weight of artificial soil]
EC_{50}	**>1000**
NOEC (reproduction)	**>1000**
LOEC (reproduction)	**>1000**
LC_{50}	**>1000**
NOEC (survival)	**≥1000**
LOEC (survival)	**>1000**

Study 2

ZRMS comments:

The study is considered acceptable. All validity criteria were met.
• mean adult mortality: 12.5% (criterion: ≤ 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: ≤ 30%).

Agreed endpoints:
- **LC_{50}= 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.**

- **EC_{10}=19.3 mg/kg dry weight of the artificial soil (8.9 mg of terbuthylazine / kg dry weight of the artificial soil).**
- **EC_{20} =33.5 mg/kg dry weight of the artificial soil (15.5 mg of terbuthylazine / kg dry weight of the artificial soil).**
- **EC_{50}=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).**

Report	TERBUT 500 SC: Collembolan *(Folsomia candida)* Reproduction Test, Wołany, M.., 2019, Study code: G/60/19
Guideline(s):	OECD No. 232
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Folsomia candida*
Test design	28 days of exposure
Nominal test item concentrations	5.6, 10, 18, 32, 56, 100, 180, 320, 560, 1000 mg/L

Materials and methods

Test Item:	Terbut 500 SC, batch no. 1/19, content of terbuthylazine 511.9 g/l
Test Species:	the collembolan, Folsomia candida 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; 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
Test Concentration::	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: ≤ 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: ≤ 30%).

Results:
On the basis of the results, it was concluded that after 28 days, at the control group the mortality of adult collembolans was 2.5%. At concentrations ranging from 5.6 to 1000 mg of the test item/kg dry weight of artificial soil, after 28 days of exposure to the test item, mortality of the adult collembolans was ranging from 10.0 to 92.5%.
The concentration of the test item causing 50% mortality of the adult collembolans (LC_{50}) is 226.4 mg/kg dry weight of artificial soil.
After 28 days of the experiment, it was concluded that TERBUT 500 SC had statistically significant impact on reproduction of the collembolans at the concentrations in the range from 32 - 1000 mg/kg dry weight of artificial soil.

Conclusions

	TERBUT 500 SC [mg/kg dry weight of artificial soil]
EC_{50}	**95.9**
NOEC (reproduction)	**18**
LOEC (reproduction)	**32**
LC_{50}	**226.4**
NOEC (survival)	**32**
LOEC (survival)	**56**

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

ZRMS comments:

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

- 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 ± 15%.

Agreed endpoints:

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.

Study 13

Report	TERBUT 500 SC: Soil Microorganisms: Nitrogen Transformation Test, Gierbuszewska A., 2018, Study code: G/285/17
Guideline(s):	OECD 216 / EU Method C.21.
Deviations:	Yes (concerning soil extraction)
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:	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 (α=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 ± 15%.

Results:
The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentration corresponding to the PEC: 2.2 mg test item/kg dry weight soil and 5 x PEC: 11 mg the test item/kg dry soil did not exceed 25% on 56 day of analysis.

Conclusions:
On the basis of the results, it was concluded that TERBUT 500 SC at the concentration corresponding to the PEC: 2.2 mg test item/kg dry weight soil and 5 x PEC: 11 mg the test item/kg dry did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

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

Study 1

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:

Sunflower *Helianthus annuus*	TERBUT 500 SC	21 d Seedling emergence	ER50 > 1500 ml prod//ha equal to 1657.5 g prod/ha
Cabbage *Brassica oleracea var. capitata*	TERBUT 500 SC	21 d Seedling emergence	ER= 49.44 ml prod/ha equal to 54.63 g prod/ha
Pea *Pisum sativum*	TERBUT 500 SC	21 d Seedling emergence	ER50 > 1500 ml prod/ha equal to 1657.5 g prod/ha
Tomato *Solanum lycopersicon*	TERBUT 500 SC	21 d Seedling emergence	ER50= 63.32 ml prod/ha equal to 69.97 g prod/ha
Onion *Allium cepa*	TERBUT 500 SC	21 d Seedling emergence	ER50= 92.17 ml prod/ha equal to 101.85 g prod/ha
Oats *Avena sativa*	TERBUT 500 SC	21 d Seedling emergence	ER50= 598.95 ml prod/ha equal to 661.84 g prod/ha

Report	TERBUT 500 S.C. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. Dec W., 2018, G/286/17
Guideline(s):	OECD 208
Deviations:	Yes (changed light intensity 53.2 – 135.8 µE/m2/s instead of recommended 350 ± 50µE/m2/s.)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Helianthus annuus, Brassica oleracea, Pisum sativum, Solanum lycopersicon, Allium cepa, Avena sativa*,
Test design	14 d after emergence of 50% of the control seedlings
Nominal test item concentrations	1500, 600, 240, 96, 38.4, 15.4, 6.1, 2.5 mL/ha

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)

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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. Phyto-toxic 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. Phyto-toxic 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**

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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.

Results:

After the application of the test item at the all tested rates test plant species such as sunflower, cabbage, pea, tomato, onion and oats emerged.
After the application of the test item at the all tested rates the emergence of plants was not delayed in comparison to the control group.
The test item caused mortality of tomato and onion at rates ranging from 96.0 to 1500 mL/ha. There was no mortality observed for sunflower, cabbage, pea and oats.

Some phototoxic symptoms were observed after 14 days of the exposure:

- stunted growth (cabbage, tomato, onion, oats),

- wilting (cabbage, tomato, onion),

- chlorosis (cabbage, tomato, oats)

- necrosis (oats)

- mortality of plants (tomato, onion).
The plant damage for sunflower and pea was not observed.

Based on shoot length, the test item did not inhibit the process of growth of sunflower and pea. The shoot length was inhibited for cabbage, tomato, onion and oats.
Based on shoot dry weight, test item did not inhibit the process of growth of sunflower. The test item had a little impact on the growth of pea. The shoot dry weight of cabbage, tomato, onion and oats was inhibited.

Conclusions:
On the basis of the results, it was concluded that TERBUT 500 SC at the concentration of 1.5 L/ha may affects the seedling of non-target plants and risk mitigation options must be considered.

Study 2

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:

Sunflower *Helianthus annuus*	TERBUT 500 SC	21 d Vegetative vigour	ER50 > 212.3ml prod//ha equal to 234.59 g prod/ha
Cabbage *Brassica oleracea var. capitata*	TERBUT 500 SC	21 d Vegetative vigour	ER50= 180 ml prod/ha equal to 198.90 g prod/ha
Pea *Pisum sativum*	TERBUT 500 SC	21 d Vegetative vigour	ER50 > 619.6 ml prod/ha equal to 684.66 g prod/ha
Tomato *Solanum lycopersicon*	TERBUT 500 SC	21 d Vegetative vigour	ER50= 57.3 ml prod/ha equal to 63.32 g prod/ha
Onion *Allium cepa*	TERBUT 500 SC	21 d Vegetative vigour	ER50= 51.1 ml prod/ha equal to 56.47 g prod/ha
Oats *Avena sativa*	TERBUT 500 SC	21 d Vegetative vigour	ER50= 391.0 ml prod/ha equal to 432.06 g prod/ha

Report	TERBUT 500 SC: Terrestrial Plant Test: Vegetative Vigour Test. Gierbuszewska A., 2018 Study code: G/287/17
Guideline(s):	OECD 227
Deviations:	Yes (changed light intensity 118.5 – 135.4 µE/m^2/s instead of recommended 350 ± 50µE/m2/s.)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test organism	*Helianthus annuus, Brassica oleracea, Pisum sativum, Solanum lycopersicon, Allium cepa, Avena sativa,*
Test design	21 d after exposure
Nominal test item concentrations	1500, 600, 240, 96, 38.4, 15.4, 6.1 mL/ha

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 (Helianthus annuus), cabbage (Brassica olerace var. capitata), pea (Pisum sativum), tomato (Solanum lycopersicon), onion (Allium cepa), oats (Avena sativa)..
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/m2/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.

Results:
The test item caused mortality of sunflower, cabbage, and tomato at rates ranging from 240 to 1500 mL/ha, onion at the rates ranging from 96 to 1500 mL/ha, and oats at the rate equal to 1500 mL/ha. On the basis of shoot length and shoot dry weight, it was observed that the test item caused inhibition of growth of all tested species.
Some phototoxic symptoms were observed after 21 days of the exposure:
- stunted growth (all tested species),
- wilting (onion),
- chlorosis (sunflower, cabbage, pea, oats),
- necrosis (sunflower, oats),
- mortality of plants (sunflower, cabbage, tomato, oats).

Conclusions:
On the basis of the results, it was concluded that TERBUT 500 SC at the concentrations of 1.5 L/ha may affects the growth of non-target plants and risk mitigation options must be considered.