

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB1913H

Product name: Roxy XL

Chemical active substances:

Prosulfocarb, 900 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Globachem NV

Submission date: September 2022

MS Finalisation date: 04/08/2023

After commenting period: 15/11/2023

Version history

When	What
September 2022	Initial dossier submission by applicant for new product authorization.
August 2023	zRMS assessment
November 2023	After commenting period

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPS

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destina- tion / 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 saf- ener/ syner- gist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val 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			Birds	Mammals	Aquatic organ-	Bees	Non-target ar-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	PL, IE, BE, HU, SK	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre-emergence	a) 1 b) 1	/	a) 4.0 b) 4.0	a) Prosulfocarb: 3.6 b) Prosulfocarb: 3.6	155-300	/	/							
2	PL, IE, BE, HU, SK	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre-emergence	a) 1 b) 1	/	a) 3.5 b) 3.5	a) Prosulfocarb: 3.15 b) Prosulfocarb: 3.15	155-300	/	/							
3	PL, IE, BE, HU, SK	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	BBCH10-29	a) 1 b) 1	/	a) 4.0 b) 4.0	a) Prosulfocarb: 3.6 b) Prosulfocarb: 3.6	155-300	/	/							
4	PL, IE, BE, HU, SK	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	BBCH10-29	a) 1 b) 1	/	a) 3.5 b) 3.5	a) Prosulfocarb: 3.15 b) Prosulfocarb: 3.15	155-300	/	/							
5	PL, IE, BE, HU, SK	Potato (SOL-TU)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre-emergence	a) 1 b) 1	/	a) 4.4 b) 4.4	a) Prosulfocarb: 3.96 b) Prosulfocarb: 3.96	155-300	/	/							
6	PL, IE, BE, HU, SK	Potato (SOL-TU)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre-emergence	a) 1 b) 1	/	a) 3.5 b) 3.5	a) Prosulfocarb: 3.15 b) Prosulfocarb: 3.15	155-300	/	/							
7	PL, IE, BE,	Winter durum wheat	F	Annual broad leaved weeds	Downward spraying	Pre-emergence	a) 1 b) 1	/	a) 2.6 b) 2.6	a) Prosulfocarb: 2.34 b) Prosulfocarb: 2.34	155-300	/	/							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	HU, SK	(TRZDW)		(BBBAN) & grass-es (GGGAN)																
8	PL, IE, BE, HU, SK	Winter durum wheat (TRZDW)	F	Annual broad leaved weeds (BBBAN) & grass-es (GGGAN)	Downward spraying	BBCH10-29	a) 1 b) 1	/	a) 2.6 b) 2.6	a) Prosulfocarb: 2.34 b) Prosulfocarb: 2.34	155-300	/	/							

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** 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

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

Remarks table:

- Numeration necessary to allow references
- Use official codes/nomenclatures of EU
- For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
- 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
- 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
- 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
- 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
- The maximum number of application possible under practical conditions of use must be provided
- Minimum interval (in days) between applications of the same product.
- 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
- The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
- PHI - minimum pre-harvest interval
- Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2),

<p>9.1.1.2</p> <p>zRMS</p> <p>Comments:</p>	<p>The toxicity data for acute and long-term risk were agreed at the EU level.</p> <p>The risk assessment to mammals was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).</p> <p>The results of the ‘screening phase’ acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TERA and TERLT) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects with the exception of the TERLT values for the lagomorph and the mouse.</p> <p>Lagomorph</p> <p>In the higher tier risk assessment applicant proposed to consider the brown hare (<i>Lepus europaeus</i>) as a more appropriate focal species consider the brown hare (<i>Lepus europaeus</i>) in spite of rabbit (<i>Oryctolagus cuniculus</i>). In the evaluator opinions is justified to use the brown hare as the focal species to represent the large herbivorous mammal in winter early cereal fields.</p> <p>Additionally for higher tier applicant proposed refinement of DT50 value. The DT50 of prosulfocarb in young cereal plants were estimated in 5 residue trials after a single application of Prosulfocarb 800 g/L EC in autumn. These trials are summarized and accepted by the zRMS in the Part B section 7. The highest DT50 value of 1.82 days was used for the refinement of the exposure to prosulfocarb, which leads to a TWA of 0.125.</p> <p>Mouse</p> <p>Higher tier for long term risk assessment for mouse is based on the refinement of focal specie - the wood mouse (<i>Apodemus sylvaticus</i>) and foliar DT50 and/or PT values (Prosser (2010).</p> <p>Even using the more conservative PT values the refined TERLT values for small omnivorous mammals and the lagomorphs in bare soils and cereals is greater than the trigger of 5, indicating that the long-term risk to mammals is acceptable following use of Roxy XL (GLOB1913H) according to the proposed use pattern.</p>
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A quantitative drinking water risk assessment is not triggered for the proposed use pattern of GLOB1913H according to EFSA/2009/1438 criteria and therefore the risk to mammals via drinking water is acceptable.

Secondary poisoning.

The risk assessment for earthworm-eating mammals needed to be revised due to the rejection of the study conducted by Sacker in 2008 during the zonal evaluation of the product Roxy in the UK. As a result, the initial risk assessment, as shown in Table 9.3-4, was deemed unacceptable, requiring further refinement. To address this issue, the study conducted by Bätischer in 2006 was considered. The findings of this study were summarized in the Addendum to the Draft Assessment Report in July 2007. Based on the information from Bätischer's study, new bioaccumulation factors were calculated for the two treatments, resulting in values of 0.59 and 0.77, respectively.

In the refined risk assessment presented below, a conservative approach was adopted, using the higher value of 0.77. This cautious approach ensures a thorough evaluation of the potential risk for earthworm-eating mammals in relation to the assessed factors.

Table 9.3-15: Assessment of the risk for earthworm-eating mammals due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes – refined BCF

Parameter	Prosulfocarb	comments
PECsoil (twa = 21 d) (mg/kg soil)	3.1765	dRR B8 Table 8.7-3
BCFworm	0.77	Bätischer, 2006
PECworm	2.45	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	3.13	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	
TERlt	16	

TER values shown in bold fall below the relevant trigger.

Since the acceptability criterion of $TER \geq 5$ is achieved for active substance, an acceptable risk to earthworm-eating and fish-eating mammals via secondary poisoning can be concluded for all intended uses.

No risk mitigation measures are required.

Conclusion

	According to the performed risk assessment there is no potential of risk to mammals resulting from exposure to active substances following use of Roxy XL (GLOB1913H) in compliance with proposed GAP.
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9.1.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

GLOB1913H poses a low risk to birds, mammals and other terrestrial vertebrate wildlife when applied according to the proposed use.

9.1.1.4 Effects on aquatic organisms (KCP 10.2)

A low risk to aquatic organisms is expected from the application of GLOB1913H taking into account the mitigation measures where necessary.

9.1.1.5 Effects on bees (KCP 10.3.1)

A low risk to bees is expected from the application of GLOB1913H.

9.1.1.6 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field risks of GLOB1913H for arthropods other than bees are acceptable for the intended use.

9.1.1.7 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

GLOB1913H poses low risk to earthworms and other non-target soil organisms when applied according to the proposed use rate.

There is no unacceptable risk on soil microbial activity for GLOB1913H.

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

GLOB1913H poses low risk to non-target plants taking into account the proposed mitigation measures: a buffer zone of 1 m in combination with 90% drift reducing techniques or a buffer zone of 3 m in combination with 75% drift reducing techniques or a buffer zone of 5 m in combination with 50% drift reducing techniques or a buffer zone of 10 m without drift reduction.

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

Not required.

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 0-1: Critical use pattern of GLOB1913H grouped according to application timing

Grouping according to application timing			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
1	Cereals BBCH 0-09	Application timing	Pre-emergence
2	Cereals BBCH 10-29		Post-emergence
3	Potato BBCH 0-09		Pre-emergence

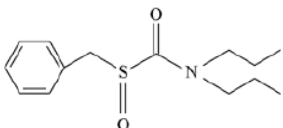
Table 0-2: Critical use pattern of GLOB1913H grouped according to dose rate

Grouping according to dose rate			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
4	Cereals BBCH 0-09 and BBCH 10-29	Dose rate	4 L/ha
5	Cereals BBCH 0-09 and BBCH 10-29 Potato BBCH 0-09		3.5 L/ha (covering also 2.6 L/ha)
6	Potato BBCH 0-09		4.4 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 GLOB1913H is indicated in the table.

Table 0-3 Metabolites of prosulfocarb

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Prosulfocarb sulfoxide		267.4	Soil: 6.8%	Yes, soil and aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of GLOB1913H were not evaluated as part of the EU assessment of prosulfocarb.

However, the provision of further data on the GLOB1913H is not considered essential, because the risk for birds from GLOB1913H can be adequately assessed from the risk assessment for the active substance.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail, <i>Colinus virginianus</i>	Prosulfocarb	Oral 1 d Acute	LD ₅₀ > 2250 mg/kg bw	EFSA, 2007
Mallard duck, <i>Anas platyrhynchos</i>	Prosulfocarb	Dietary 8 d Short-term	LD ₅₀ > 1505.6 mg/kg bw/d	EFSA, 2007
Mallard duck, <i>Anas platyrhynchos</i>	Prosulfocarb	Dietary Reproductive toxicity	NOEL = 131 mg/kg bw/d	EFSA, 2007

9.2.1.1 Justification for new endpoints

In accordance with the EFSA Guidance Document on Birds and Mammals, the acute risk assessment of prosulfocarb will be performed using the LD₅₀ from the dietary toxicity study since this endpoint is lower than the acute LD₅₀ from the acute toxicity study. The reproductive risk assessment will be performed using the NOEL from the reproduction study since this value is lower than the LD₅₀/10.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 3 also covers the risk for birds from all other intended uses in group 1 (see 9.1.2).

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

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

Table 9.2-2: Screening step and first-tier assessment of the acute and long-term/reproductive risk for birds due to the pre-emergence use of GLOB1913H in winter cereals and potato

Intended use		Bare soil				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 3.96				
Acute toxicity (mg/kg bw)		1505.6				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Bare soil	Small granivorous bird	25.3	1	100.2	15.0	

Reprod. toxicity (mg/kg bw/d)	131				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Bare soil	Small granivorous bird	11.4	0.53	23.9	5.5

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.

Table 9.2-3: Screening step and first-tier assessment of the acute and long-term/reproductive risk for birds due to the post-emergence use of GLOB1913H in winter cereals

Intended use		Winter cereals			
Active substance/product		Prosulfocarb			
Application rate (kg/ha)		1 × 3.6			
Acute toxicity (mg/kg bw)		1505.6			
TER criterion		10			
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals	Small omnivorous bird	158.8	1	571.7	2.63
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	30.5	1	109.8	13.7
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24.0	1	86.4	17.43
Reprod. toxicity (mg/kg bw/d)		131			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals	Small omnivorous bird	64.8	0.53	123.6	1.06
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	30.9	4.24
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	20.8	6.30

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.

9.2.2.2 Higher-tier risk assessment

The reproductive first-tier risk assessment for prosulfocarb did not indicate an acceptable risk for the goose. Therefore, a higher-tier risk assessment is provided here.

Table 9.2-4: Higher-tier assessment of the long-term/reproductive risk for birds due to the post-emergence use of GLOB1913H in winter cereals – refined parameters (*) are further described and justified in the text

Intended use	Winter cereals
Active substance/product	Prosulfocarb

Application rate (kg/ha)		1 × 3.6			
Reprod. toxicity (mg/kg bw/d)		131			
TER criterion		5			
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA*	DDD_m (mg/kg bw/d)	TER_{it}
Cereals, Early (shoots) autumn- winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.125	7.29	18.0

*Refined parameters:

For the reproductive risk assessment of the goose, the exposure was refined using the DT₅₀ of prosulfocarb on young cereal plants since the goose feeds on 100% cereal shoots. The DT₅₀ of prosulfocarb in young cereal plants were estimated in 5 residue trials after a single application of Prosulfocarb 800 g/L EC in autumn as shortly summarized in the table below. These trials are thoroughly summarized in the Part B section 7. In order to comply with the recommendations of the FOCUS workgroup on degradation kinetics (FOCUS 2006, 2014), a kinetic analysis of these residue decline data is provided. The resulting highest DT₅₀ value of 1.82 days was used for the refinement of the exposure to prosulfocarb, which leads to a TWA of 0.125.

It should be noted that this assessment still assumes that an animal obtains 100% of its diet from the treated area over a prolonged period of time, and as such still represents a conservative and protective approach to risk evaluation.

Country Year Trial No.	Application					Average T	Rainfall	Residues (prosulfocarb)			DT50
	Formu- lation	N°	kg a.i./ha	L/ha	Growth stage (BBCH)			Commodity and growth stage (BBCH)	PHI (days)	mg/kg	
North France	800 EC	1	4.093	307	12	9.6°C	0 mm	Whole plant (12)	0	454.41	1.43 days
2009						9.9°C	1.1 mm	Whole plant (12)	1	316.95	
A9051 AN1						8.5°C	12.9 mm	Whole plant (12)	2	92.47	
						11.9°C	4.3 mm	Whole plant (12)	4	20.85	
						9°C	0 mm	Whole plant (12/13)	7	10.72	
						11.9°C	0 mm	Whole plant (13)	13	1.59	
Germany	800 EC	1	4.013	301	12	5°C	0 mm	Whole plant (12)	0	714.54	1.75 days
2009						4.6°C	0 mm	Whole plant (12)	1	452.58	
A9051 GE1						6.2°C	2 mm	Whole plant (12)	2	327.9	
						3.8°C	0 mm	Whole plant (12)	4	123.23	
						8.1°C	0 mm	Whole plant (12)	6	38.85	
						-0.7°C	0 mm	Whole plant (12-13)	13	5.7	
North France	800 EC	1	3.84	288	12	6.2°C	0 mm	Whole plant (12)	0	286.5	2.2 days
2011						7.1°C	0 mm	Whole plant (12)	1	233.9	
B1234 AN1						8°C	0 mm	Whole plant (12-13)	2	135.6	
						3.1°C	0.3 mm	Whole plant (12-13)	4	42.5	
						4.4°C	0 mm	Whole plant (12-13)	7	29.4	
						0.2°C	0.3 mm	Whole plant (12-13)	14	4.4	
North France	800 EC	1	4.227	317	12	13.5°C	0.3 mm	Whole plant (12)	0	443.6	1.93 days
2011						10.8°C	0.1 mm	Whole plant (12)	1	280.2	
B1234 BM1						11°C	0.3 mm	Whole plant (12)	2	158.3	

						11.5°C	0.1 mm	Whole plant (12)	4	59.8	
						8.8°C	0 mm	Whole plant (12-13)	7	28	
						9.5°C	1.8 mm	Whole plant (12-13)	14	4.1	
North France	800 EC	1	3.827	287	12	8.3°C	0.3 mm	Whole plant (12)	0	278.3	1.92 days
2011						6.8°C	0.2 mm	Whole plant (12)	1	122.4	
B1234 BP1						6°C	0.1 mm	Whole plant (12)	2	74.2	
						11°C	0.2 mm	Whole plant (12)	4	57.5	
						12.3°C	0.1 mm	Whole plant (13)	7	13.7	
						8.3°C	0.2 mm	Whole plant (13)	14	2.7	

Kinetic analysis

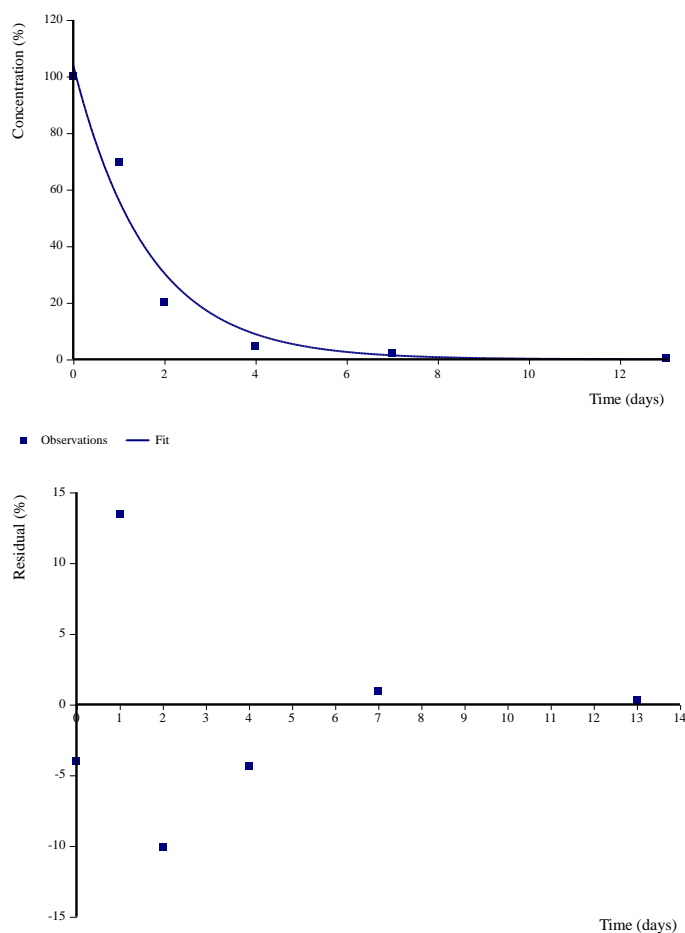
In order to comply with the recommendations of the FOCUS workgroup on degradation kinetics (FOCUS 2006, 2014), a kinetic analysis of the above residue decline data is provided here below.

Residue data were fitted using CAKE v3.3 to determine first order half-lives. The data were directly fitted un-weighted with the complete data set and unconstrained initial concentration (M0) for the parent. The acceptability of the kinetic fits was judged as follows:

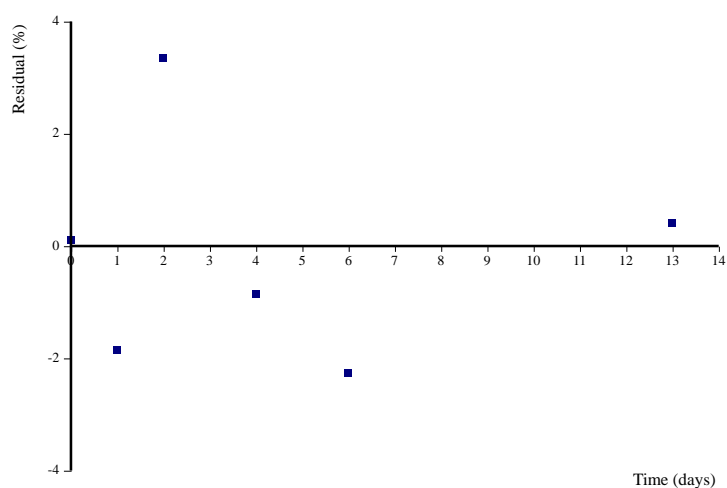
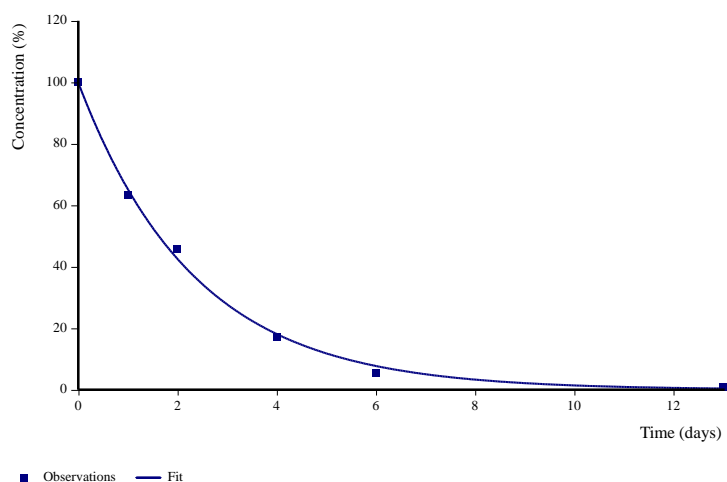
- Visually using a three point scale:
Poor = an unacceptable fit, the fitted curve does not represent the trend of the data points and residuals show strong deviations from random distribution;
Acceptable = the fitted curve describes the trend of the data points, residuals may show some deviation from random distribution but it is not significant;
Good = the fitted curve closely follows all the data points, residuals are randomly distributed.
- Fit to the data points (χ^2 error%):
It is recommended that a χ^2 error% of 15% or less indicates acceptable fits, although for data that may include intrinsically variable data, higher values can be tolerated if the visual fit is acceptable or good. Where two or more models are acceptable fits to the data, the χ^2 error% parameter has been used to assess goodness of fit. In these cases, the model with the lowest value of this parameter has been chosen as the best fit.

Graphs with the measured residues plotted versus time and with calculated minus measured data (residuals) of the different residue trials are shown in the figures below.

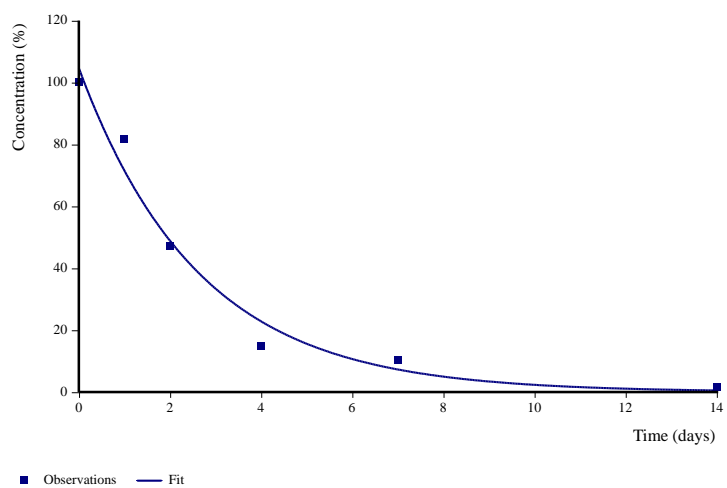
Graphs for trial A9051 AN1

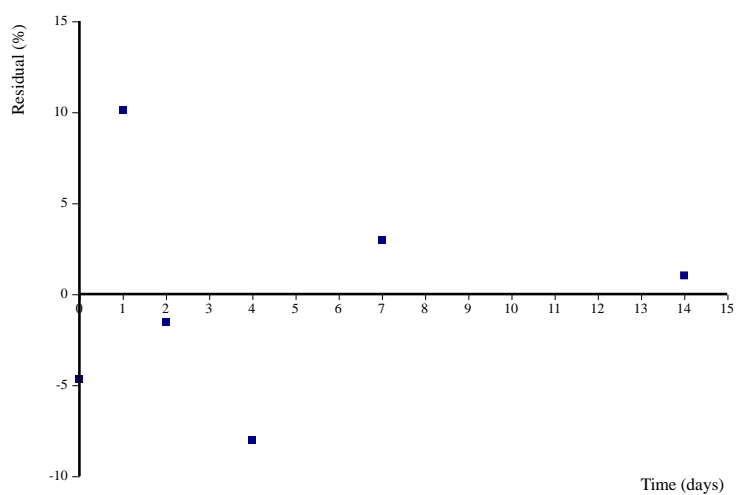


Graphs for trial A9051 GE1

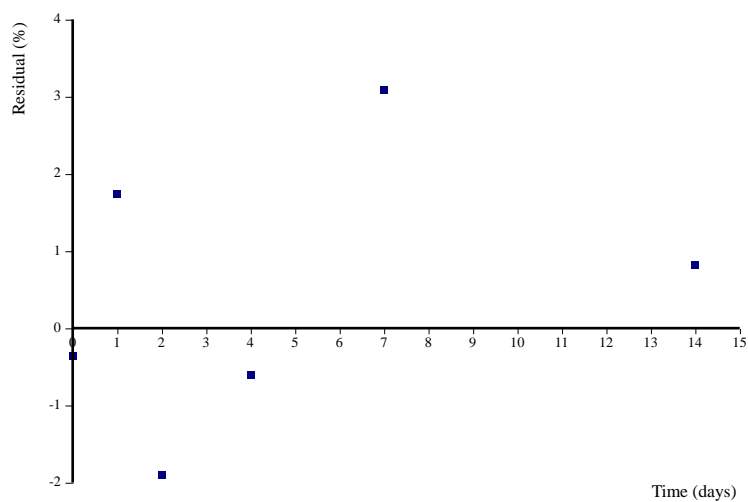
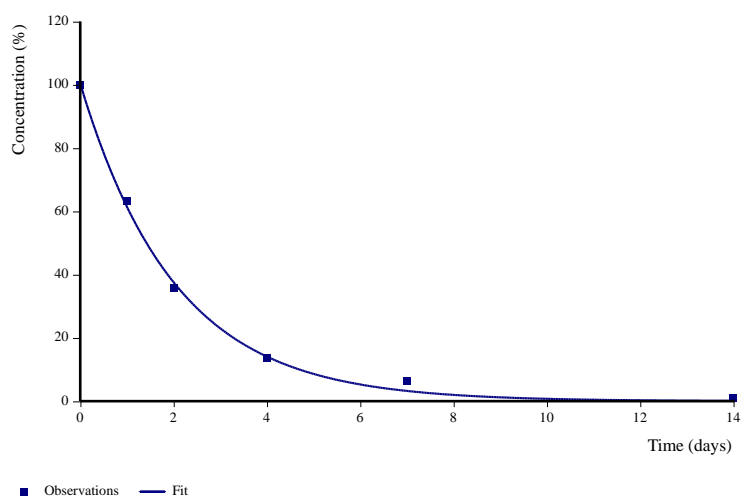


Graphs for trial B1234 AN1

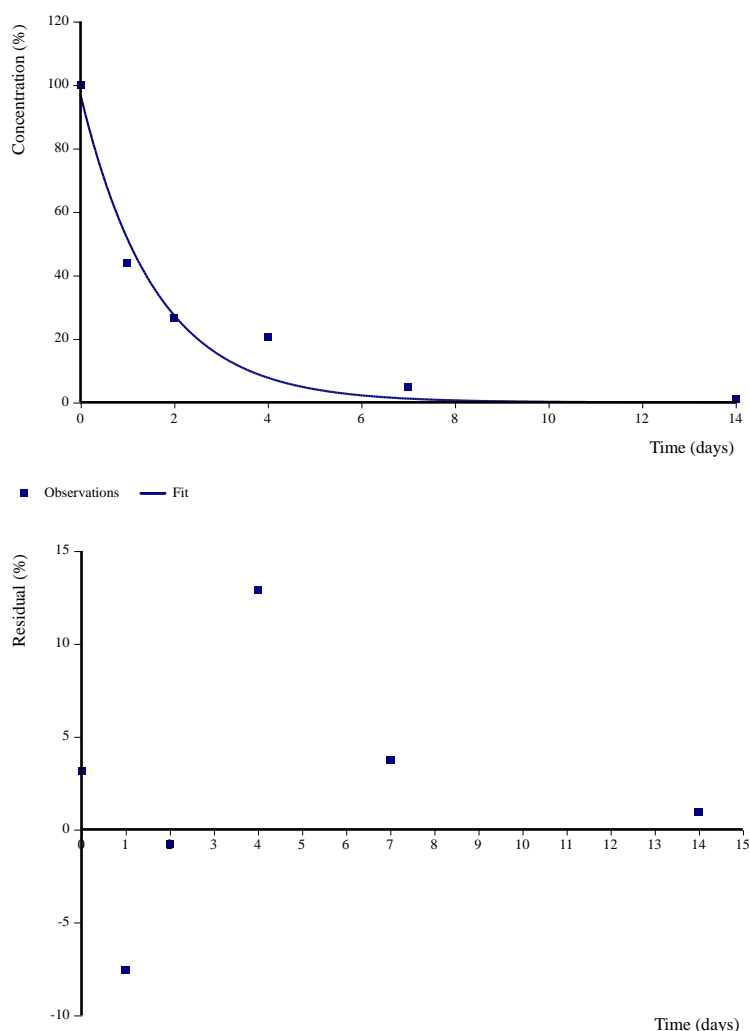




Graphs for trial B1234 BM1



Graphs for trial B1234 BP1



Foliar DT₅₀ values determined by kinetic fitting are summarized in the table below.

Summary of foliar DT₅₀ values

Trial No.	Foliar DT ₅₀ (days)	Foliar DT ₉₀ (days)	Error % (χ^2)	Kinetic model	Reference
A9051 AN1	1.13	3.75	17.6	SFO	Jonchère, 2010
A9051 GE1	1.62	5.39	3.81	SFO	
B1234 AN1	1.82	6.05	10.8	SFO	Perny, 2012
B1234 BM1	1.41	4.69	3.69	SFO	
B1234 BP1	1.10	3.65	15.6	SFO	

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 GLOB1913H 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 1799 L/kg, prosulfocarb belongs to the group of more sorptive substances.

Effective application rate (g/ha)=	3960			
Acute toxicity (mg/kg bw) =	1505.6	quotient	=	2.63
Reprod. toxicity (mg/kg bw/d) =	131	quotient	=	30.2

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of prosulfocarb amounts to 4.48 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 predicted concentrations in soil.

Table 9.2-5: Assessment of the risk for earthworm-eating birds due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes

Parameter	Prosulfocarb	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	3.1765	dRR B8 Table 8.7-3
$\log P_{ow} / P_{ow}$	4.48/30199	
K_{oc}	1799	Geomean (n = 6)
foc	0.02	Default
BCF_{worm}	10.10	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times K_{oc}$
PEC_{worm}	32.07	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	33.67	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	131	
TER_{lt}	3.89	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} is under the threshold value, but a bioaccumulation study in earthworms (Sacker D., 2008a) is available for the formulation Prosulfocarb 800 EC, which is considered representative for effects of the active substance. A BCF of 1.39 was concluded based on this study.

In addition, a bioaccumulation study (Bätscher R., 2006) is provided in the Addendum to the Draft Assessment Report of prosulfocarb (July 2007) leading to a BCF of 0.59 and 0.77 for the test concentrations of respectively 0.60 and 6.0 mg a.s./kg dry soil.

In the refined risk assessment, presented below, a conservative approach was used by applying the BCF of 1.39 instead of the calculated value.

Table 9.2-6: Assessment of the risk for earthworm-eating mammals due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes – refined BCF

Parameter	Prosulfocarb	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	3.1765	dRR B8 Table 8.7-3
BCF _{worm}	1.39	Sacker D., 2008a
PEC _{worm}	4.42	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	4.64	DDD = PEC _{worm} × 1.05
NOEL (mg/kg bw/d)	131	
TER _{lt}	28.3	

TER values shown in bold fall below the relevant trigger.

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.

Table 9.2-7: Assessment of the risk for fish-eating birds due to exposure to prosulfocarb via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals and potatoes

Parameter	Prosulfocarb	comments
PEC _{sw} (twa = 21 d) (mg/L)	0.01529	dRR B8 Table 8.9-5
BCF _{fish}	700	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	10.7	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	1.70	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	131	
TER _{lt}	77.0	

TER values shown in bold fall below the relevant trigger.

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

The risk to birds is acceptable after exposure to GLOB1913H when applied according to the intended

uses.

<p>zRMS Comments:</p>	<p>The toxicity data for acute and long-term risk were agreed at the EU level.</p> <p>The risk assessment to birds was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).</p> <p>The results of the ‘screening phase’ acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed endpoints for the most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects with the exception of the TER_{LT} value for large herbivorous bird “goose”.</p> <p>For large herbivorous bird higher tier risk assessment was proposed by applicant based on refinement of DT50 value. The DT50 of prosulfocarb in young cereal plants were estimated in 5 residue trials after a single application of Prosulfocarb 800 g/L EC in autumn. These trials are summarized and accepted by the zRMS in the Part B section 7 and also accepted in refinement of other products containing prosulfocarb. The highest DT50 value of 1.82 days was used for the refinement of the exposure to prosulfocarb, which leads to a TWA of 0.125.</p> <p>Comparing proposed DT50 with DT50 values available in the DAR and draft RAR of prosulfocarb, the value of 1.82 d as used by the applicant is still the worst-case.</p> <ul style="list-style-type: none"> - DAR: Devine, 2004: 2 trials in winter barley in UK, resulting in DT50 values between 0.51 and 0.59 days. - dRAR: North, 2015: 4 trials on spring barley in north of France and UK, resulting in DT50 values between 0.62 and 1.60 days. <p>Based on all the above arguments, the value of 1.82 days as used by the applicant is regarded as the worst-case and relevant for the whole central zone. Taking into account the highest DT50 (1.82 days) refined TER_{LT} value for the GLOB1913H are greater than the Annex VI triggers of 5, indicating that product present no unacceptable long-term risk to large herbivorous bird “goose” following their application to winter cereals.</p> <p>A quantitative drinking water risk assessment is not triggered for the proposed use pattern of GLOB1913H according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.</p> <p>Secondary poisoning.</p> <p>The risk assessment for earthworm-eating birds needed to be revised due to the rejection of the study conducted by Sacker in 2008 during the zonal evaluation of the product Roxy in the UK. As a result, the initial risk assessment, as shown in Table 9.3-4, was deemed unacceptable, requiring further refinement. To address this issue, the study conducted by Bätischer in 2006 was considered. The findings of this study were summarized in the Ad-</p>
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<p>dendum to the Draft Assessment Report in July 2007. Based on the information from Bätischer's study, new bioaccumulation factors were calculated for the two treatments, resulting in values of 0.59 and 0.77, respectively.</p> <p>In the refined risk assessment presented below, a conservative approach was adopted, using the higher value of 0.77. This cautious approach ensures a thorough evaluation of the potential risk for earthworm-eating birds in relation to the assessed factors.</p> <p>Table 9.2-8: Assessment of the risk for earthworm-eating birds due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes – refined BCF</p>		
Parameter	Prosulfocarb	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	3.1765	dRR B8 Table 8.7-3
BCF _{worm}	0.77	Bätischer, 2006
PEC _{worm}	2.45	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	2.57	DDD = PEC _{worm} × 1.05
NOEL (mg/kg bw/d)	131	
TER _{lt}	51	
<p>Since the acceptability criterion of $TER \geq 5$ is achieved for active substance, an acceptable risk to earthworm-eating and fish-eating birds via secondary poisoning can be concluded for all intended uses.</p> <p>No risk mitigation measures are required.</p> <p>Conclusion</p> <p>According to the performed risk assessment there is no potential of risk to birds resulting from exposure to active substance following use of Roxy XL (GLOB1913H) in compliance with proposed GAP.</p>		

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

Effects on mammals of GLOB1913H were not evaluated as part of the EU assessment of prosulfocarb. However, the provision of further data on the formulation GLOB1913H is not considered essential, because the risk for mammals from GLOB1913H can adequately be assessed from the risk assessment for the active substance.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Prosulfocarb	Oral 1 d Acute	LD₅₀ = 1820 mg/kg bw	EFSA, 2007
Rat	Prosulfocarb	Dietary Reproductive toxicity	NOAEL = 50 mg/kg bw/d	EFSA, 2007

9.3.1.1 Justification for new endpoints

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9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the pre-emergence use of GLOB1913H in winter cereals, 4 L/ha

Intended use		Bare soil				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 3.6				
Acute toxicity (mg/kg bw)		1820				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Bare soil	Small granivorous mammal	14.4	1	51.8	35.11	
Reprod. toxicity (mg/kg bw/d)		50				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Bare soil	Small granivorous mammal	6.6	0.53	12.6	4.0	
Bare soil	Small omnivorous mammal “mouse”	5.7	0.53	10.9	4.6	

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.

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the pre-emergence use of GLOB1913H in winter cereals and potatoes 3.5 L/ha

Intended use		Bare soil				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 3.15				
Acute toxicity (mg/kg bw)		1820				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Bare soil	Small granivorous mammal	14.4	1	45.4	40.1	
Reprod. toxicity (mg/kg bw/d)		50				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Bare soil	Small granivorous mammal	6.6	0.53	11.02	4.5	
Bare soil	Small omnivorous mammal “mouse”	5.7	0.53	12.0	5.3	

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.

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the post-emergence use of GLOB1913H in winter cereals, 4 L/ha

Intended use		Winter cereals				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 3.6				
Acute toxicity (mg/kg bw)		1820				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Cereals	Small herbivorous mammal	118.4	1	426.2	4.3	
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	7.6	1	27.4	66.5	
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	42.1	1	151.6	12.0	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1	61.9	29.4	
Cereals, BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1	19.4	93.6	
Reprod. toxicity (mg/kg bw/d)		50				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						

Cereals	Small herbivorous mammal	48.3	0.53	92.2	0.5
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	8.0	6.2
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.53	42.6	1.2
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	14.9	3.4
Cereals, BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1	3.62	13.8

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.

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the post-emergence use of GLOB1913H in winter cereals, 3.5 L/ha

Intended use		Winter cereals				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 3.15				
Acute toxicity (mg/kg bw)		1820				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Cereals	Small herbivorous mammal	118.4	1	373	4.9	
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	7.6	1	23.9	76.0	
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	42.1	1	132.6	13.7	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1	54.2	33.6	
Cereals, BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1	17.0	107	
Reprod. toxicity (mg/kg bw/d)		50				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Cereals	Small herbivorous mammal	48.3	0.53	80.6	0.6	
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	4.2	0.53	7.01	7.1	
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	22.3	0.53	37.2	1.3	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	0.53	13.02	3.8	
Cereals, BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1	3.165	15.8	

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.

Table 9.3-6: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the pre-emergence use of GLOB1913H in potato

Intended use	Bare soil				
Active substance/product	Prosulfocarb				
Application rate (kg/ha)	1 × 3.96				
Acute toxicity (mg/kg bw)	1820				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Bare soil	Small granivorous mammal	14.4	1	57.0	31.9
Reprod. toxicity (mg/kg bw/d)	50				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Bare soil	Small granivorous mammal	6.6	0.53	13.9	3.6
Bare soil	Small omnivorous mammal “mouse”	5.7	0.53	12.0	4.2

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.

9.3.2.2 Higher-tier risk assessment

The reproductive first-tier risk assessment for prosulfocarb did not indicate an acceptable risk for the lagomorph and the mouse, except for the pre-emergence use in winter cereals and potato at a dose rate of 3.5 L/ha. Therefore, a higher-tier risk assessment is provided here.

Large herbivorous mammal “lagomorph”

Based on the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) the large herbivorous mammal is represented by the focal species rabbit (*Oryctolagus cuniculus*). There is good reason to consider the brown hare (*Lepus europaeus*) as a more appropriate focal species, as rabbits have their dens outside the field and do not frequent the open field for feeding as regularly as hares do. This can be demonstrated in a study (Schroeer and Grimm, 2011; see summary in Appendix 2) which had been performed to investigate the frequency of occurrence and abundance of both species – rabbits and brown hares in 90 winter cereal fields (BBCH <30) at two study sites in Northern Germany.

Based on this study, it is considered as justified to use the brown hare as the focal species to represent the large herbivorous mammal in winter early cereal fields. Based on this, the shortcut value of 17.3 for the brown hare is used for refined calculations as this is the highest value available for the brown hare in the Guidance Document on Risk Assessment for Birds and Mammals (2009).

Refinement of TWA value

For the reproductive risk assessment of the lagomorph, the exposure was refined using the DT₅₀ of prosulfocarb on young cereal plants since the lagomorph feeds on 100% crop leaves. The DT₅₀ of prosulfocarb in young cereal plants were estimated in 5 residue trials after a single application of Prosulfocarb 800 g/L EC in autumn as summarized in the higher-tier risk assessment for birds here above. This highest DT₅₀ value of 1.82 days was used for the refinement of the exposure to prosulfocarb, which leads to a TWA of 0.125.

Proportion of diet obtained in treated area (PT)

Based on the proposed use pattern at early post-emergence it is highly questionable whether the large herbivorous hares foraging on the cereal fields during this early growth stage will find a sufficient amount of food to feed 100% on the field. The small cereal plants at this growth stage have only a few leaves and thus mammals need to look for another food sources to cover their daily diet. Thus, the use of a PT value of 1.0 is considered to be not justified during the application of GLOB1913H at early post-emergence.

A generic field monitoring study was conducted in the north of Germany (Voigt and Zaccaroni, 2013; see summary in Appendix 2). The aim was to investigate the habitat selection, activity pattern and home range distribution of the brown hare (*Lepus europaeus*) in a landscape that is mainly composed of agricultural crops (around 83% of the total study area of 1,125 ha). The field monitoring was performed in spring, thus in order to cover the uncertainties related to the extrapolation from data on cereals in spring to early shoots of cereals in autumn, it is proposed to use the maximum mean PT value rounded at 0.8.

The use of a PT value of 0.8 is also supported by the historical data in the publication of Prosser P. (2010¹), where a 90th percentile PT value of 0.69 for consumers only (15 individuals) and a 90th percentile PT value of 0.63 for all animals (23 individuals) in winter cereals at early stages is reported.

Table 9.3-7: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of GLOB1913H in winter cereals

Intended use	Winter cereals					
Active substance/product	Prosulfocarb					
Application rate (kg/ha)	1 × 3.6					
Reprod. toxicity (mg/kg bw/d)	50					
TER criterion	5					
Crop scenario	Focal species	SV_m	MAF_m × TWA*	PT	DDD_m (mg/kg bw/d)	TER_{It}
Growth stage						
Cereals, early (shoots)	Brown hare	17.3	0.125	0.8 1	6.23 7.79	8.0 6.42
Intended use	Winter cereals					
Active substance/product	Prosulfocarb					
Application rate (kg/ha)	1 × 3.15					
Reprod. toxicity (mg/kg bw/d)	50					
TER criterion	5					
Crop scenario	Focal species	SV_m	MAF_m × TWA*	PT	DDD_m (mg/kg bw/d)	TER_{It}
Growth stage						
Cereals, early (shoots)	Brown hare	17.3	0.125	0.8 1	5.45 6.81	9.2 7.34

Small omnivorous mammal “mouse”

Justification of the wood mouse as relevant focal species in higher tier refinement

¹ Prosser P. (2010). Consolidation of bird and mammal PT data for use in risk assessment. Food and Environment Research Agency, UK.

Following the recommendations in Appendix A of EFSA (2009), the wood mouse (*Apodemus sylvaticus*) is the only small mammal to be addressed as representative tier 1 species on bare soil (corresponding to pre-emergence) fields as well as the only small omnivorous mammal to be addressed on cereal fields from BBCH 10-29. The wood mouse is widely distributed throughout all GAP relevant countries (see Figure A below) and occurs in variable and diverse habitats, including agricultural land (Mitchell-Jones et al. 1999).

Apodemus sylvaticus

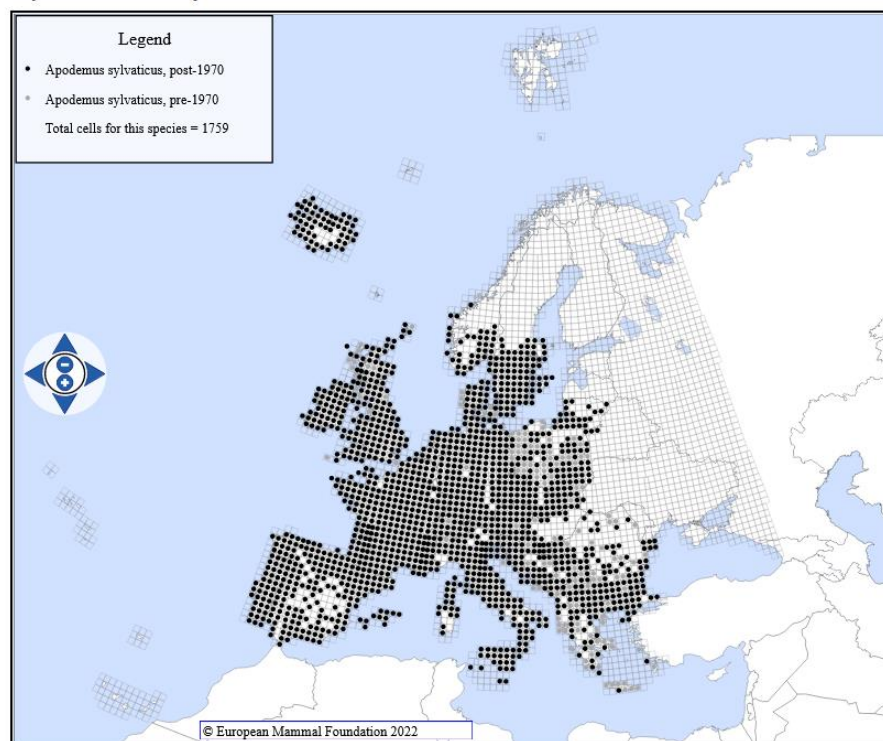


Figure A: Distribution of the wood mouse (*Apodemus sylvaticus*) in Europe <https://www.european-mammals.org/php/showmap.php?latname=Apodemus+sylvaticus&latname2=>

Due to the characteristic lack of sheltering vegetation as protection against predators, pre-emergence fields as well as early-staged cereal fields are generally considered unattractive habitats for small mammals and are rarely frequented as foraging habitat. However, the wood mouse was the small mammal caught in highest number within crop fields (including wheat and barley) during spring by Janova et al. (2011). During spring, none or only slight vegetation cover is available in crop-fields, representing a situation comparable to bare soil and/or early staged potato/cereal fields. In addition, referring to DEFRA (2009), the wood mouse was the most abundant small omnivorous mammal species in UK potato and cereal fields. Also, Heroldová et al. (2007) found the wood mouse as one of three dominant mice species within cereal fields. Further, a field study was conducted to track wood mice on winter cereals and potatoes by Crocker and colleagues (Crocker and Irving, 2003), where most animals were caught in the hedgerow, but a special effort was made to track animals caught within the crop resulting in a median contact time of more than 6 hours (measured based on 58 individuals). The study shows that wood mice are found within crop fields, even though off crop structures are generally preferred (as for all small mammals). Considering that the studies above were conducted in the central zone, they verify the wood mouse as relevant focal species in the central zone. Furthermore, it is also inhabiting agricultural land in the southern zone (Torre et al., 2010; Rodriguez and Peris, 2007; Balestrieri, 2017; Gentili et al., 2014; Ouin et al., 2000).

In conclusion, the wood mouse, compared to other small omnivorous mammals of agricultural landscapes, appears to be the most relevant for the purpose of the presented risk assessment, due to its comparatively high frequency of occurrence and abundance in crop fields as well as its low body weight.

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Food intake rate (FIR/bw)

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) the diet of the wood mouse in bare soil consists of 50% weed seeds and 50% ground arthropods, which results in a FIR/bw of 0.24. The diet of the wood mouse in cereals consists of 25% weeds, 50% weed seeds and 25% ground arthropods, which results in a FIR/bw of 0.27.

Refinement of TWA value

Data from residue decline studies are available as presented in the refined risk assessment of the lagonorm above. Considering a foliar DT₅₀ of 1.82 days, the refined TWA is 0.125.

Proportion of diet obtained in treated area (PT)

PT data is taken from the DEFRA project PS2328 (Prosser, 2010¹), containing tables listing all the PT data from FERA's (formerly CSL's) previous projects in a single document. Radio tracking data is available for wood mice in newly-drilled cereals. The 90th percentile PT values for wood mice in newly-drilled cereals are 0.51 (consumers only, based on 12 individuals) and 0.37 (all tracked mice included, based on 21 individuals). The 90th percentile PT values for wood mice in established cereals (BBCH 10-29) are 0.81 (consumers only, based on 10 individuals) and 0.70 (all tracked individuals, based on 36 individuals).

In this case, the data for all consumers might be considered more relevant, since the wood mice in the radiotracking study described in Prosser (2010¹) were caught either within the crop or very close (i.e. within two meters) to the fields. In the study, those animals caught within the crop were always tagged in

preference to those caught in close proximity to it. The mice were tracked from dusk to dawn to reflect the nocturnal habits of the species.

Because wood mice are highly mobile, it is unlikely that any of them had no access to the newly drilled crop during the time they were observed in which the feeding places were determined. Therefore, animals that do not enter the field represent only animals with the decision not to enter that habitat even though it was within reach. The reasons why small rodents avoid open fields have often been mentioned and are largely due to the fact that small rodents avoid the risk of predation. The lack of protection from, mainly avian, predators is the reason that wood mice will not forage extensively in fields without vegetation cover when safe habitats, such as hedgerows and woodland edge characterized by good vegetation cover, are in reach.

It is therefore concluded that the PT for consumers only is actually not more relevant in this case, but a value that just has a greater bias towards those animals that spend a larger proportion of their time in the newly sown cereals. Since the 90th percentile instead of the mean PT is already used for the risk assessment, only using the consumer only data and disregarding the remaining individuals (which were also potential consumers as determined by the study design described above) is considered overly conservative and is not reflecting the realistic long-term exposure of wood mice in the field. Therefore, and because animals were trapped in or adjacent to newly drilled cereal fields, it is recommended to use data for all animals.

Moreover, this follows a recent recommendation in the Northern zone guidance (2021²) where it is recommended that for focal species caught within (or in close proximity to) the crop, PT should be estimated from all individuals – whether they used the crop of concern or not. For the focal species caught in the general farmland, PT should be estimated from only those individuals proved by radio-tracking to visit the crop of concern. This is in line with the recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)

However, for completeness, the long-term risk assessment for small omnivorous mammals is presented below using both set of PT figures: for all tracked individuals, and more conservatively using only consumers.

Further refinement of the PT is provided by the study of Katzschner (2022) where the use of freshly drilled cereal fields as a foraging habitat for small omnivorous mammals was investigated in Central Europe. The focus was the determination of PT values of wood mice during the pre-emergence period of cereals via radio-tracking. PT data from 18 different individuals and 22 radio tracking sessions were collected. The 90th percentile PT value was 0.082 for all tracked individuals and 0.136 for confirmed consumers. Since all radio tracked mouse were captured directly adjacent to the cereal fields, they all had access to the study fields and could be determined as potential consumers. In line with the argumentation provided for the PT value from Prosser (2010) above, the PT value of all tracked individuals is regarded as the relevant one. However, for completeness, the long-term risk assessment for small omnivorous mammals for the pre-emergence use (bare soil) is presented below using both PT values.

The very low PT values indicate that freshly drilled cereal fields are no attractive foraging habitat for wood mice. The data obtained in the study of Katzschner (2022) therefore also support the use of PT values of 0.81 (consumers only) and 0.70 (all tracked individuals) based on Prosser (2010) for the early post-emergence use in winter cereals.

Residues in the food

Residue values for weeds, weed seeds and ground arthropods were taken from Appendix F of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Table 9.3-8: Refined long-term risk assessment of small omnivorous mammal mouse exposed to prosulfocarb in bare soils (risk assessment performed at 4.4 L/ha covering both the pre-emergence use in winter cereals and in potatoes) – PT from Prosser (2010)

Parameter	Long-term exposure
Crop	Bare soil (BBCH < 10)
Generic focal species	Small omnivorous mammal “mouse”
Representative species	Wood mouse

FIR/bw	0.24			
Application rate	3.96			
Diet	Weed seeds	Ground arthropods	Weed seeds	Ground arthropods
PD	0.5	0.5	0.5	0.5
Mean RUD (mg/kg)	40.2	7.5	40.2	7.5
MAF _m	1	1	1	1
TWA	0.53	0.53	0.53	0.53
PT	0.51 ^a	0.51 ^a	0.37 ^b	0.37 ^b
DDD (mg/kg bw/d)	5.16	0.96	3.75	0.70
DDD sum (mg/kg bw/d)	6.13		4.45	
NOAEL (mg/kg bw/d)	50		50	
TER	8.2		11.3	

^a PT value for consumers only

^b PT value for all tracked individuals

Table 9.3-9: Refined long-term risk assessment of small omnivorous mammal mouse exposed to prosulfocarb in bare soils (risk assessment performed at 4.4 L/ha covering both the pre-emergence use in winter cereals and in potatoes) – PT from Katzschner (2022)

Parameter	Long-term exposure			
Crop	Bare soil (BBCH < 10)			
Generic focal species	Small omnivorous mammal “mouse”			
Representative species	Wood mouse			
FIR/bw	0.24			
Application rate	3.96			
Diet	Weed seeds	Ground arthropods	Weed seeds	Ground arthropods
PD	0.5	0.5	0.5	0.5
Mean RUD (mg/kg)	40.2	7.5	40.2	7.5
MAF _m	1	1	1	1
TWA	0.53	0.53	0.53	0.53
PT	0.136 ^a	0.136 ^a	0.082 ^b	0.082 ^b
DDD (mg/kg bw/d)	5.16	0.96	3.75	0.70
DDD sum (mg/kg bw/d)	6.13		4.45	
NOAEL (mg/kg bw/d)	50		50	
TER	30.6		50.8	

^a PT value for consumers only

^b PT value for all tracked individuals

Table 9.3-10: Refined long-term risk assessment of small omnivorous mammal mouse exposed to prosulfocarb in cereals (4 L/ha)

Parameter	Long-term exposure					
Crop	Cereals (BBCH 10-29)					
Generic focal species	Small omnivorous mammal “mouse”					
Representative species	Wood mouse					
FIR/bw	0.27					
Application rate	3.6					
Diet	Weeds	Weed seeds	Ground arthropods	Weeds	Weed seeds	Ground arthropods
PD	0.25	0.5	0.25	0.25	0.5	0.25
Mean RUD (mg/kg)	28.7	40.2	7.5	28.7	40.2	7.5
MAF _m	1	1	1	1	1	1
TWA	0.125	0.53	0.53	0.125	0.53	0.53
PT	0.81 ^a	0.81 ^a	0.81 ^a	0.70 ^b	0.70 ^b	0.70 ^b
DDD (mg/kg bw/d)	0.71	8.39	0.78	0.62	7.25	0.68
DDD sum (mg/kg bw/d)	9.88			8.53		
NOAEL (mg/kg bw/d)	50			50		
TER	5.1			5.9		

^a PT value for consumers only

^b PT value for all tracked individuals

Table 9.3-11: Refined long-term risk assessment of small omnivorous mammal mouse exposed to prosulfocarb in cereals (3.5 L/ha)

Parameter	Long-term exposure					
Crop	Cereals (BBCH 10-29)					
Generic focal species	Small omnivorous mammal “mouse”					
Representative species	Wood mouse					
FIR/bw	0.27					
Application rate	3.15					
Diet	Weeds	Weed seeds	Ground arthropods	Weeds	Weed seeds	Ground arthropods
PD	0.25	0.5	0.25	0.25	0.5	0.25
Mean RUD (mg/kg)	28.7	40.2	7.5	28.7	40.2	7.5
MAF _m	1	1	1	1	1	1
TWA	0.125	0.53	0.53	0.125	0.53	0.53
PT	0.81 ^a	0.81 ^a	0.81 ^a	0.70 ^b	0.70 ^b	0.70 ^b
DDD (mg/kg bw/d)	0.62	7.34	0.68	0.53	6.34	0.59
DDD sum (mg/kg bw/d)	8.64			7.47		
NOAEL (mg/kg bw/d)	50			50		
TER	5.8			6.7		

^a PT value for consumers only

^b PT value for all tracked individuals

Even using the more conservative PT value derived from use of consumers only in radio tracking studies, the refined TER_{LT} value for small omnivorous mammals in bare soils and cereals is greater than the trigger of 5, indicating that the long-term risk to mammals is acceptable following use of GLOB1913H according to the proposed use pattern.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a $K(f)_{oc}$ of 1799 L/kg, prosulfocarb belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	3960			
Acute toxicity (mg/kg bw) =	1820	quotient =		2.18
Reprod. toxicity (mg/kg bw/d) =	50	quotient =		79.2

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of prosulfocarb amounts to 4.48 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 9.3-12: Assessment of the risk for earthworm-eating mammals due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes

Parameter	Prosulfocarb	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	3.1765	dRR B8 Table 8.7-3
log P _{ow} / P _{ow}	4.48/30199	
K _{oc}	1799	Geomean (n = 6)
f _{oc}	0.02	Default
BCF _{worm}	10.10	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	32.07	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	41.05	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	
TER _{lt}	1.22	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} is under the threshold value, but the refined BCF of 1.39 from the bioaccumulation study in earthworms (Sacker D., 2008a) can be used. A refined assessment is provided in the table below.

Table 9.3-13: Assessment of the risk for earthworm-eating mammals due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes – refined BCF

Parameter	Prosulfocarb	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	5.2800	dRR B8 Table 8.7-3
BCF _{worm}	1.39	Sacker D., 2008a
PEC _{worm}	4.42	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	5.65	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	
TER _{lt}	8.85	

TER values shown in bold fall below the relevant trigger.

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.

Table 9.3-14: Assessment of the risk for fish-eating mammals due to exposure to prosulfocarb via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals and potatoes

Parameter	Prosulfocarb	comments
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PEC _{sw} (twa = 21 d) (mg/L)	0.01529	dRR B8 Table 8.9-5
BCF _{fish}	700	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	10.7	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	1.52	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	50	
TER _{lt}	32.9	

TER values shown in bold fall below the relevant trigger.

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

The risk to mammals is acceptable after exposure to GLOB1913H when applied according to the intended uses.

zRMS Comments:	<p>The toxicity data for acute and long-term risk were agreed at the EU level.</p> <p>The risk assessment to mammals was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).</p> <p>The results of the ‘screening phase’ acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects with the exception of the TER_{LT} values for the lagomorph and the mouse.</p> <p>Lagomorph</p> <p>In the higher tier risk assessment applicant proposed to consider the brown hare (<i>Lepus europaeus</i>) as a more appropriate focal species consider the brown hare (<i>Lepus europaeus</i>) in spite of rabbit (<i>Oryctolagus cuniculus</i>). In the evaluator opinions is justified to use the brown hare as the focal species to represent the large herbivorous mammal in winter early cereal fields.</p> <p>Additionally for higher tier applicant proposed refinement of DT50 value. The DT50 of prosulfocarb in young cereal plants were estimated in 5 residue trials after a single application of Prosulfocarb 800 g/L EC in autumn. These trials are summarized and accepted</p>
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by the zRMS in the Part B section 7. The highest DT50 value of 1.82 days was used for the refinement of the exposure to prosulfocarb, which leads to a TWA of 0.125.

Mouse

Higher tier for long term risk assessment for mouse is based on the refinement of focal specie - the wood mouse (*Apodemus sylvaticus*) and foliar DT50 and/or PT values (Prosser (2010).

Even using the more conservative PT values the refined TER_{LT} values for small omnivorous mammals and the lagomorphs in bare soils and cereals is greater than the trigger of 5, indicating that the long-term risk to mammals is acceptable following use of Roxy XL (GLOB1913H) according to the proposed use pattern.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of GLOB1913H according to EFSA/2009/1438 criteria and therefore the risk to mammals via drinking water is acceptable.

Secondary poisoning.

The risk assessment for earthworm-eating mammals needed to be revised due to the rejection of the study conducted by Sacker in 2008 during the zonal evaluation of the product Roxy in the UK. As a result, the initial risk assessment, as shown in Table 9.3-4, was deemed unacceptable, requiring further refinement. To address this issue, the study conducted by Bätischer in 2006 was considered. The findings of this study were summarized in the Addendum to the Draft Assessment Report in July 2007. Based on the information from Bätischer's study, new bioaccumulation factors were calculated for the two treatments, resulting in values of 0.59 and 0.77, respectively.

In the refined risk assessment presented below, a conservative approach was adopted, using the higher value of 0.77. This cautious approach ensures a thorough evaluation of the potential risk for earthworm-eating mammals in relation to the assessed factors.

Table 9.3-15: Assessment of the risk for earthworm-eating mammals due to exposure to prosulfocarb via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals and potatoes – refined BCF

Parameter	Prosulfocarb	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	3.1765	dRR B8 Table 8.7-3
BCF_{worm}	0.77	Bätischer, 2006
PEC_{worm}	2.45	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	3.13	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	

	TER _{lt}	16	
	<p>TER values shown in bold fall below the relevant trigger.</p> <p>Since the acceptability criterion of $TER \geq 5$ is achieved for active substance, an acceptable risk to earthworm-eating and fish-eating mammals via secondary poisoning can be concluded for all intended uses.</p> <p>No risk mitigation measures are required.</p> <p>Conclusion</p> <p>According to the performed risk assessment there is no potential of risk to mammals resulting from exposure to active substances following use of Roxy XL (GLOB1913H) in compliance with proposed GAP.</p>		

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

Birds and mammals are regarded as adequate surrogates for terrestrial stages of amphibians and reptiles. For the aquatic stages of amphibians, please refer to the risk assessment for fish presented in KCP 10.2.

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 prosulfocarb and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on aquatic organisms of GLOB1913H were not evaluated as part of the EU assessment of prosulfocarb. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Prosulfocarb and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Rainbow trout, <i>Oncorhynchus mykiss</i>	Prosulfocarb	96 h, f	LC ₅₀ = 0.84 mg/L_{nom}	EFSA, 2007 (Douglas and Pell, 1986)
Fathead minnow, <i>Pimephales promelas</i>	Prosulfocarb	96 h	LC ₅₀ = 2.4 mg/L	DAR, 2006 (Peter, 2001)

Species	Substance	Exposure System	Results	Reference
Fish	Prosulfocarb	Acute	LC ₅₀ = 1420 µg a.s./L	Geomean
Rainbow trout, <i>Oncorhynchus mykiss</i>	Prosulfocarb	Acute	24h LC ₅₀ = 4.3 mg/L	EFSA, 2007 (Behsen, 2001)
Rainbow trout, <i>Oncorhynchus mykiss</i>	Prosulfocarb	21 d, f	NOEC = 0.31 mg/L _{mm}	EFSA, 2007 (Tapp, 1989)
Aquatic invertebrate				
Water flea, <i>Daphnia magna</i>	Prosulfocarb	48 h, s	EC ₅₀ = 0.51 mg/L _{mm}	EFSA, 2007 (Bätscher, 2004)
<i>Chaoborus sp.</i>			EC ₅₀ = 790 µg/L	DAR, 2006 (Ashwell, 2001)
<i>Cleon sp.</i>			EC ₅₀ = 1410 µg/L	
<i>Asellus sp.</i>			EC ₅₀ = 810 µg/L	
<i>Hylalella azteca</i>			EC ₅₀ = 1080 µg/L	
Aquatic invertebrates	Prosulfocarb	Acute	EC ₅₀ = 869.5 µg/L	Geomean
Water flea, <i>Daphnia magna</i>	Prosulfocarb	21 d, ss	NOEC = 0.047 mg/L	DAR, 2006 (Stewart K.M. et al, 1989)
			NOEC = 0.045 mg/L _{mm}	EFSA, 2007 (Bätscher, 2004)
Sediment dwelling organisms				
Freshwater midge, <i>Chironomus riparius</i>	Prosulfocarb	25 d	NOEC = 1.25 mg/L	EFSA, 2007 (Schmidt, 2004)
Algae				
<i>Pseudokirchneriella subcapitata</i>	Prosulfocarb	72 h, s	E _b C ₅₀ = 49 µg/L E _r C ₅₀ = 120 µg/L	EFSA, 2007 (Volz, 2006)
<i>Desmodesmus subspicatus</i>	Prosulfocarb	72 h	72h E _r C ₅₀ = 86 µg/L	Sacker, 2008b
<i>Anabaena flos-aquae</i>	Prosulfocarb	72 h	E _b C ₅₀ = 3770 µg/L E _r C ₅₀ = 7480 µg/L	DAR, 2006 (Wallace, 2001)
<i>Chlorella vulgaris</i>	Prosulfocarb	72 h/96 h	72h E _b C ₅₀ = 1540 µg/L 96h E _r C ₅₀ = 8340 µg/L	DAR, 2006 (Wallace, 2001)
<i>Chlamydomonas reinhardtii</i>	Prosulfocarb	72 h/96 h	72h E _b C ₅₀ = 3690 µg/L 96h E _r C ₅₀ = 7720 µg/L	DAR, 2006 (Swarbrick, 2001)
<i>Navicula pelliculosa</i>	Prosulfocarb	72 h	E _b C ₅₀ = 330 µg/L E _r C ₅₀ = 680 µg/L	DAR, 2006 (Smyth, 1998)
<i>Pseudokirchneriella subcapitata</i>	Prosulfocarb sulfoxide	72 h	E _b C ₅₀ = 1.28 µg/L E _r C ₅₀ = 4.33 µg/L	DAR (Volz, 2004)
<i>Desmodesmus subspicatus</i>	Prosulfocarb sulfoxide	72 h	E _r C ₅₀ = 85 µg/L	DAR (Liedtke, 2012)
<i>Chlamydomonas reinhardtii</i>	Prosulfocarb sulfoxide	72 h	E _b C ₅₀ = 97.1 µg/L E _r C ₅₀ = 253.9 µg/L	Juckeland D., 2012a

Species	Substance	Exposure System	Results	Reference
		72 h	E _r C ₅₀ = 410 µg/L	DAR (Liedtke, 2012)
<i>Chlorella vulgaris</i>	Prosulfocarb sulfoxide	72 h	E _b C ₅₀ = 730 µg/L E _r C ₅₀ = 1320 µg/L	Juckeland D., 2012b
		72 h	E _r C ₅₀ = 2860 µg/L	DAR (Liedtke, 2012)
<i>Anabaena flosaquae</i>	Prosulfocarb sulfoxide	72 h	E _b C ₅₀ = 19500 µg/L E _r C ₅₀ = 42500 µg/L	Juckeland D., 2012c
		72 h	E _r C ₅₀ = 43000 µg/L	DAR (Liedtke, 2012)
<i>Navicula pelliculosa</i>	Prosulfocarb sulfoxide	72 h	E _b C ₅₀ = 1400 µg/L E _r C ₅₀ = 7650 µg/L	Juckeland D., 2012d
		72 h	E _r C ₅₀ = 2700 µg/L	DAR (Liedtke, 2012)
<i>Skeletonema costatum</i>	Prosulfocarb sulfoxide	72 h	E _b C ₅₀ = 53.8 µg/L E _r C ₅₀ = 134.8 µg/L	Juckeland D., 2012e
Higher plants				
<i>Lemna gibba</i>	Prosulfocarb	14 d	E _y C ₅₀ = 690 µg/L E _r C ₅₀ = 2230 µg/L	EFSA, 2007 (Smyth, 1999) + re-evaluation for E _r C ₅₀
<i>Myriophyllum spicatum</i>	Prosulfocarb (based on Prosulfocarb 800 EC)	14 d	E_rC₅₀ = 381 µg/L	Juckeland, 2013a
<i>Lemna gibba</i>	Prosulfocarb sulfoxide	7 d	E_rC₅₀ = 13 µg/L E _b C ₅₀ = 2.8 µg/L	DAR (Liedtke, 2012)
<i>Myriophyllum spicatum</i>	Prosulfocarb sulfoxide	14 d	E _r C ₅₀ = 31.1 µg/L	Juckeland, 2013b
Primary producers				
Algae & higher plants	Prosulfocarb	-	E_rC₅₀ = 381 µg/L	Geomean
Algae & higher plants	Prosulfocarb	-	HC₅ = 45.5 µg/L	HC ₅
Algae & higher plants	Prosulfocarb sulfoxide	-	HC₅ = 1.524 µg/L	HC ₅
Higher-tier studies (micro- or mesocosm studies)				
<i>Mesocosm</i>	Prosulfocarb	NOEC = 15 µg a.i./L => ETO-RAC = 7.5 µg/L with a safety factor 2)		EFSA 2007 DAR, 2006 (van Wijngaarden 2006) + Deneer J., Roessink I. & Rico A. (2015)
	Prosulfocarb sulfoxide	NOEC = 30 µg/L => ETO-RAC = 15 µg/L with a safety factor 2		EFSA 2007 DAR, 2006 (Taylor 2013) + Taylor & Dark. (2015)

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB1913H

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	GLOB1913H	48 h, s	EC ₅₀ = 0.421 mg/L_{nom} (equivalent to 0.3652 mg prosulfocarb/L)	Siche O., Wydra V., 2021a
<i>Pseudokirchneriella subcapitata</i>	GLOB1913H	72 h, s	ErC ₅₀ = 0.342 mg/L_{nom} (equivalent to 0.2967 mg prosulfocarb/L) EyC ₅₀ = 0.087 mg/L _{nom}	Siche O., Wydra V., 2021b
<i>Lemna gibba</i>	GLOB1913H	7 d, s	ErC ₅₀ = 0.944 mg/L_{nom} (equivalent to 0.8189 mg prosulfocarb/L) EyC ₅₀ = 0.376 mg/L _{nom}	Siche O., Wydra V., 2021c
<i>Myriophyllum spicatum</i>	GLOB1913H	14 d, s	ErC ₅₀ = 1.00 mg/L_{nom} (equivalent to 0.8675 mg prosulfocarb/L) EyC ₅₀ = 0.437 mg/L _{nom}	Siche O., Wydra V., 2021d
Higher-tier studies (micro- or mesocosm studies)				
-				

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

9.5.1.1 Justification for new endpoints

According to 7.5.3.1 in the Aquatic Guidance Document (EFSA Journal 2013;11(7):3290), when the comparison of toxicity data between the active substance and the formulated product reveals differences, the risk assessment should be based on the lower of the endpoints, i.e. either active substance data or formulation data (calculated as a.s. content) are used.

Therefore, all formulation endpoints to be considered for the risk assessment were recalculated to active substance content in Table 9.5-2. According to 2.3.1 in the Recurring Issues in Ecotoxicology (EFSA Supporting publication 2019:EN-1673), the formulation is considered more toxic than the active substance if the endpoint of the formulated product is at least three times lower than the equivalent endpoint for the active substance. Comparing the data in Table 9.5-1 and 9.5-2, GLOB1913H should not be considered more toxic than the active substance.

Nevertheless, a formulation risk assessment is provided using all endpoints of the formulated product and the PEC_{sw} based on spray drift of the formulation.

Following refinements of the endpoints of the active substance and the metabolite were used in the risk assessment:

Prosulfocarb

Acute fish – geomean approach

A geomean acute RAC for fish of 14.2 µg/L was calculated based on additional estimates of toxicity of prosulfocarb to fish in single species laboratory tests as shown in the table below.

Summary of the toxicity values of prosulfocarb used for the acute risk assessment to fish

Organism	Test substance	Endpoint	Value	Reference	Value (µg a.s./L)
Fish					
Rainbow trout <i>Oncorhynchus mykiss</i>	Prosulfocarb	96h LC ₅₀	840 µg/L	EFSA, 2007	840
Fathead minnow <i>Pimephales promelas</i>	Prosulfocarb	96h LC ₅₀	2400 µg/L	DAR, 2006 (Peter P., 2001)	2400
Geometric mean					1420
RAC					14.2

This geomean RAC is based on only 2 fish species, but further support for this geomean acute RAC of 14.2 µg/L is provided by the study of Behsen (2001) evaluated in the DAR of prosulfocarb and providing an estimate of the acute toxicity of prosulfocarb to rainbow trout in a more realistic exposure scenario, as per Tier 2C of the Aquatic Guidance Document ((EFSA Journal 2013;11(7):3290). Concentrations of the test item were decreased over 96 h at a rate based on a DT₅₀ in water of 1.5 days, to mimic dissipation from the water phase under static field conditions. This higher tier study reported no lethality following an initial exposure of 4.5 mg/L and an LC₅₀ value of 6.4 mg/L, with all the fish dying in the first 24 hours. In the DAR, a revised LC₅₀ value was calculated by the RMS Sweden to take into account the measured prosulfocarb concentration after 24 hours to give an LC₅₀ value of 4.3 mg/L. This recalculation also accounts for the DT₅₀ of 6.2 days reported for the prosulfocarb mesocosm study (van Wijngaarden, 2006). This more conservative endpoint, derived from the Behsen study by the calculations of RMS Sweden and subject to standard Tier 1 acute assessment factor for fish of 100, generates a refined Tier 2C RAC of 43 µg/L, which is about 3 times higher than the geomean RAC of 14.2 µg/L proposed above.

Daphnia – geomean approach

A geomean acute RAC for aquatic invertebrates of 8.7 µg/L was calculated based on four additional species. Estimates of toxicity of prosulfocarb to aquatic invertebrates in single species laboratory tests as submitted in the Annex I application are shown in the table below.

Summary of the toxicity values of prosulfocarb used for the acute risk assessment to Daphnia

Organism	Test substance	Endpoint	Value	Reference
Aquatic invertebrates				
<i>Daphnia magna</i>	Prosulfocarb	48h EC ₅₀	510 µg /L	EFSA, 2007 (Bätscher, 2004)
<i>Chaoborus sp.</i>	Prosulfocarb		790 µg/L	DAR, 2006 (Ashwell, 2001)
<i>Cleon sp.</i>	Prosulfocarb		1410 µg/L	
<i>Asellus sp.</i>	Prosulfocarb		810 µg/L	
<i>Hylalella azteca</i>	Prosulfocarb		1080 µg/L	
Geometric mean			869.5 µg/L	
RAC			8.7	

However a further refinement is based on RAC issued from the cosm study (van Wijngaarden (2006)) to conclude on aquatic invertebrates, algae and macrophyte (see primary producers - Mesocosm study).

Primary producers – geomean approach

To address the uncertainty inherent in the estimate of toxicity of prosulfocarb to primary producers, toxicity studies in additional species of freshwater phytoplankton as well as the *Lemna* and *Myriophyllum* value were used. Estimates of toxicity of prosulfocarb to green algae, blue-green algae, and freshwater diatoms and monocot and dicot macrophytes in single species laboratory tests are shown in the table below.

Primary producer endpoints for prosulfocarb (technical)

Taxonomic group	Organism	Endpoint*	Value	Reference	Geomean (µg a.s./L)
Green algae	<i>Pseudokirchneriella subcapitata</i>	72h E _r C ₅₀	120 µg a.i./L	EFSA, 2007	903
	<i>Desmodesmus subspicatus</i>	72h E _r C ₅₀	86 µg a.i./L	Sacker, 2008b	
	<i>Chlorella vulgaris</i>	96h E _r C ₅₀	8340 µg a.i./L	DAR, 2006 (Wallace, 2001)	
	<i>Chlamydomonas reinhardtii</i>	96h E _r C ₅₀	7720 µg a.i./L	DAR, 2006 (Swarbrick, 2001)	
Blue-green algae	<i>Anabaena flos-aquae</i>	72h E _r C ₅₀	7480 µg a.i./L	DAR, 2006 (Wallace, 2001)	7480 ¹
Freshwater diatom	<i>Navicula pelliculosa</i>	72h E _r C ₅₀	680 µg a.i./L	DAR, 2006 (Smyth, 1998)	680 ¹
Monocot macrophyte	<i>Lemna gibba</i>	14d E _r C ₅₀	2230 µg a.i./L	DAR, 2006 (Smyth, 1999)	2230 ¹
Dicot macrophyte	<i>Myriophyllum spicatum</i>	14d E _r C ₅₀	381 µg/L	Juckeland, 2013a	381 ¹
Lowest geomean E_rC₅₀					381²

* The updated AGD recommends the use 72 h E_rC₅₀ values for algal toxicity, but acknowledges that 96 h endpoints from non-OECD algal toxicity test guidelines are acceptable.

¹ the single species value is taken where there is only one species in that taxonomic group

² the lowest of the geomean/single values among the represented taxonomic groups is taken as the overall “Geomean” EC₅₀ for generation of the RAC_{geomean}.

Following the EFSA guidance and recognizing the taxonomic differences among these phytoplankton species a geomean has been generated and then the lowest geomean value has been selected from these four groups as the “Geomean EC₅₀” (381 µg/L).

Primary producers – HC₅ approach

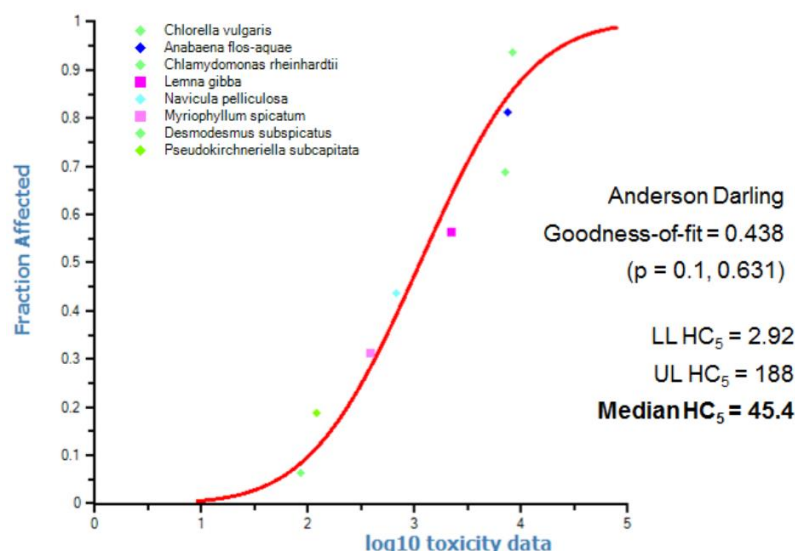
The toxicity assessment for primary producers can also be refined by calculating the HC₅ from a species sensitivity distribution (SSD). The AGD (EFSA, 2013) recommends that SSDs should be constructed with at least 8 representative toxicity endpoints from different primary producers. A primary producer SSD for prosulfocarb can therefore be generated with the E_rC₅₀ values from toxicity studies on 6 species of algae and from 2 studies with aquatic macrophytes.

Summary of the toxicity values of prosulfocarb used for the risk assessment to primary producers (algae and aquatic plants)

Taxonomic group	Organism	Endpoint*	Value	Reference
Green algae	<i>Pseudokirchneriella subcapitata</i>	72h E _r C ₅₀	120 µg a.i./L	EFSA, 2007 (Volz, 2006)
	<i>Desmodesmus subspicatus</i>	72h E _r C ₅₀	86 µg a.i./L	Sacker, 2008b
	<i>Chlorella vulgaris</i>	96h E _r C ₅₀	8340 µg a.i./L	DAR (Wallace, 2001)
	<i>Chlamydomonas reinhardtii</i>	72h E _r C ₅₀	7720 µg a.i./L	DAR (Swarbrick, 2001)
Blue-green algae	<i>Anabaena flos-aquae</i>	96h E _r C ₅₀	7480 µg a.i./L	DAR (Wallace, 2001)
Freshwater diatom	<i>Navicula pelliculosa</i>	72h E _r C ₅₀	680 µg a.i./L	DAR (Smyth, 1998)
Monocot macrophyte	<i>Lemna gibba</i>	14d E _r C ₅₀	2230 µg a.i./L	DAR (Smyth, 1999)
Dicot macrophyte	<i>Myriophyllum spicatum</i>	14d E _r C ₅₀	381 µg/L	Juckeland, 2013a

* The updated AGD recommends the use 72 h E_rC₅₀ values for algal toxicity, but acknowledges that 96 h endpoints from non-OECD algal toxicity test guidelines are acceptable.

The endpoints from the studies listed above are used for constructing the primary producer SSD as shown below.



In accordance with the AGD (EFSA, 2013) for refined risk assessment using an SSD for primary producers, an assessment factor of 3 should be applied to the median HC₅ to generate the SSD-RAC. Therefore the SSD-RAC for primary producers for prosulfocarb is $45.4/3 = 15.1 \mu\text{g/L}$.

Primary producers - Mesocosm study

EFSA conclusion (2007)

The experts discussed the endpoints derived from the new mesocosm study. Only statistically significant effects in two consecutive sampling time points were taken into account to derive the NOEC population for zooplankton. For cladocera (*Daphnia longispina*) the NOEC population was determined as $76 \mu\text{g a.s./L}$. The lowest NOEC population for zooplankton was $15 \mu\text{g a.s./L}$ based on effects on the rotifer *Pol-yarthra remata*. The zooplankton community NOEC was estimated as $76 \mu\text{g a.s./L}$. No agreement was reached on the NOEC population for periphytic algae. The algae *Tetradon trigonum* was affected at all tested concentrations and on day 28 it was not present in the samples from the mesocosms at all treatment rates. However the abundance of this algae species in the mesocosms was generally very low and therefore it was difficult to detect statistically significant differences. Significant long-term effects on other periphyton green algae species were observed at $76 \mu\text{g a.s./L}$ and concerns were raised by some experts with regard to potential indirect effects on sediment dwelling invertebrates and species feeding on periphyton which were not investigated in the study.

The experts' meeting agreed to the suggested NOEC phytoplankton community of $15 \mu\text{g a.s./L}$. The overall conclusion of the meeting was that a NOEC of $15 \mu\text{g a.s./L}$ could be derived from the mesocosm study. No conclusion was reached on the safety factor which should be applied to the endpoint. It was acknowledged that the study is of high quality but it was considered by the meeting that one mesocosm with its specific composition of species and environmental conditions can only be representative for some types of aquatic ecosystems but not for all aquatic ecosystems in the vicinity of agricultural landscapes in Europe. Therefore it was suggested to use a safety factor at Member State level according to the representativeness of the mesocosm for their aquatic ecosystems. If Member States apply a safety factor of >3 then the endpoint of $15 \mu\text{g a.s./L}$ would become the critical endpoint driving the aquatic risk assessment.

Summary of effects observed in enclosures treated with prosulfocarb (formulation A8545C). Within each endpoint category the most sensitive measurement endpoints (individual taxa) and the endpoints for the whole group in each case are presented.

Endpoint	Treatment. $\mu\text{g a.s./L}$			
	3	15	76	380
Phytoplankton				
PRC phytoplankton	1	1	3	5
Desmids	1	1	3↓ ¹	5↓ ²

Endpoint	Treatment. µg a.s./L			
	3	15	76	380
Greens	1	1	3↓ ³	5↓ ⁴
Diatoms	1	1	1	3↓ ⁵
Yellow-greens	1	1	1	1
Blue-greens	1	1	1	1
Flagellates	1	1	1	1
Chlorophyll-a	1	1	1	1
Periphyton				
PRC periphyton	1	1	1	3
Desmids	1	1	1	1
Greens	1	(2-3↓)? ⁶	5↓ ⁷	5↓ ⁷
Diatoms	1	1	1	1
Yellow-greens	1	1	1	1
Blue-greens	1	1	1	3↑ ⁸
Flagellates	1	1	1	1
Chlorophyll-a	1	1	3↑	3↑
Zooplankton				
PRC zooplankton	1	1	1	1 ⁹
Cladocera	1	1	1	3↓ ¹⁰
Rotifera	1	1	3↑ ¹¹	3↑ ¹¹
Copepoda	1	1	1	1
Macrophytes				
Biomass	1	1	1	1
Coverage	1	-- ¹²	-- ¹²	-- ¹²
Community metabolism ¹³	1	1	1	1

1 *S. cuspidatus*. reduction days 14-28. and *S. alternans* increase days 21-28.

2 *Euastrum* sp.. reduction day 3-56.

3 *A. spiralis*. reduction on day 14 and day 21.

4 *A. spiralis*. reduction on day 14 till the end of the experiment.

5 *F. ulna*. slight reductions in the time period days 3-28.

6 *T. trigonum*. reduction on day 28. Low abundance. also in controls.

7 *T. trigonum*. reductions directly (day 7/14) after application till the end of the experiment. Low abundance. also in controls. *A. spiralis*. reduction on day 14 till the end of the experiment though statistically not significant (i.e. trend).

8 *P. vulgaris*. increase days 28-42.

9 One statistical hit at the end of the experiment. causality with treatment unclear.

10 *D. longispina*. reduction day 3-21.

11 *P. remata*. increase day 3 and day 7.

12 Data for macrophyte species *Myriophyllum spicatum* coverage were inconclusive since this taxon was not present in the enclosures prior to application for the treatment rates of 15 µg a.s./L and above.

13 Alkalinity lower than in controls. overall community response not affected.

In generating a RAC from these data, Anses considered that the concentration of 15 µg a.s./L (which is an Effect Class 1 for phytoplankton, macrophyte, zooplankton, periphyton and Effect Class 2 for green algae, except for *T. trigonum* for which the Effect Class could be classified as 3 (reduction on day 21 and 28, but this short-term though difficult to interpret because of low abundance, also in controls), can be considered as an overall ETO-RAC and then the appropriate AF would be 2-3.

A statistical re-analysis of the mesocosm study is available (MDD report). As Globachem NV has access to this modelling performed by Syngenta, an AF of 1 can be used.

Prosulfocarb sulfoxide

Primary producers – HC₅ approach

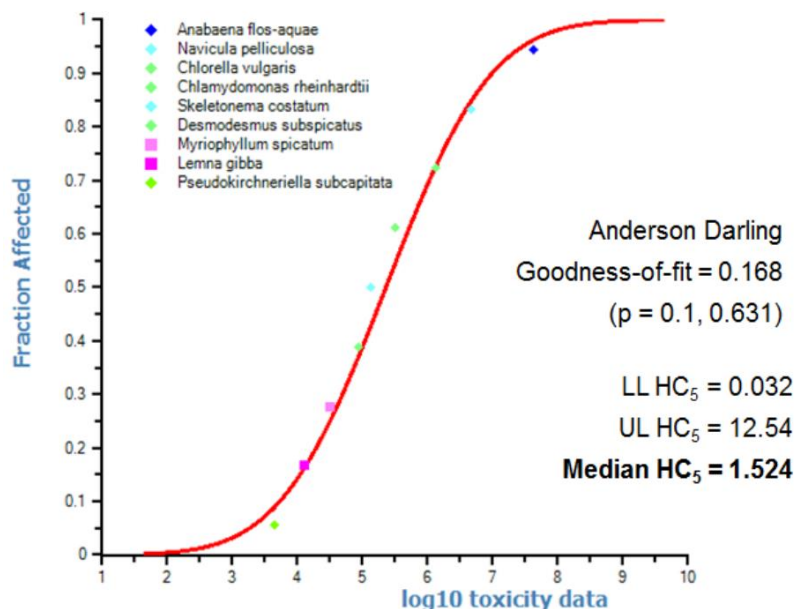
The toxicity assessment for primary producers can be refined by calculating the HC₅ from a species sensitivity distribution (SSD). The AGD (EFSA, 2013) recommends that SSDs should be constructed with at least 8 representative toxicity endpoints from different primary producers. A primary producer SSD for

prosulfocarb sulfoxide can therefore be generated with the E_rC_{50} values from toxicity studies on 7 species of algae and from 2 studies with aquatic macrophytes.

Summary of the toxicity values of prosulfocarb sulfoxide used for the risk assessment to primary producers (algae and aquatic plants)

Taxonomic group	Organism	Endpoint ($\mu\text{g/L}$)		Reference
Green algae	<i>Pseudokirchneriella subcapitata</i>	E_rC_{50}	4.33	DAR (Volz, 2004)
	<i>Desmodesmus subspicatus</i>	E_rC_{50}	85	DAR (Liedtke, 2012)
	<i>Chlorella vulgaris</i>	E_rC_{50}	1320	Juckeland, 2012b
	<i>Chlamydomonas reinhardtii</i>	E_rC_{50}	410	DAR (Liedtke, 2012)
		E_rC_{50}	253.9	Juckeland, 2012a
		Geomean	322.64	
Blue-green algae	<i>Anabaena flos-aquae</i>	E_rC_{50}	43000	DAR (Liedtke, 2012)
		E_rC_{50}	42500	Juckeland, 2012c
		Geomean	42749	
Freshwater diatom	<i>Navicula pelliculosa</i>	E_rC_{50}	2700	DAR (Liedtke, 2012)
		E_rC_{50}	7650	Juckeland, 2012d
		Geomean	4545	
	<i>Skeletonema costatum</i>	E_rC_{50}	134.8	Juckeland, 2012e
Monocot macrophyte	<i>Lemna gibba</i>	7 d E_rC_{50}	13	DAR (Liedtke, 2012)
Dicot macrophyte	<i>Myriophyllum spicatum</i>	14 d E_rC_{50}	31.1	Juckeland, 2013b

The endpoints from the studies listed above are used for constructing the primary producer SSD as shown below.



In accordance with the AGD (EFSA, 2013) for refined risk assessment using an SSD for primary producers, an assessment factor of 3 should be applied to the median HC_5 to generate the SSD-RAC. Therefore the SSD-RAC for primary producers for prosulfocarb sulfoxide is $1.524/3 = 0.508 \mu\text{g/L}$.

Mesocosm study

A mesocosm study is available for prosulfocarb sulfoxide (Taylor, 2013), as well as a statistical re-analysis of this study (MDD report). Based on these elements an overall NOEC of 30 µg/L can be derived from this study from which an ETO-RAC of 15 µg/L can be derived (assessment factor of 2) for use in the higher tier aquatic risk assessment for prosulfocarb sulfoxide.

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 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, pre-emergence, 3.5 L/ha

Group		Fish acute		Fish pro-longed	Inverteb. acute		Inverteb. pro-longed	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>			Higher tier for Daphnia and primary producers	
End-point (µg/L)		LC ₅₀	Geomean LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	NOEC	E _r C ₅₀	NOEC	E _r C ₅₀	Geomean E _r C ₅₀	HC ₅	NOEC _{community}	NOEC _{community}
AF		840	1420	310	510	869.5	45	113	1250	381	381	45.4	15	15
RAC (µg/L)		100	100	10	100	100	10	10	10	10	10	3	2	1
RAC (µg/L)		8.40	14.2	31.0	5.10	8.695	4.5	11.3	125.0	38.1	38.1	15.1	7.50	15
FOCUS Scenario	PEC _{gl-max} (µg/L)													
Step 1														
	337.91	40.227	23.796	10.900	66.257	38.863	75.091	29.904	2.703	8.869	8.869	22.378	45.055	22.527
Step 2														
N-Europe	133.9	15.940	9.430	4.319	26.255	15.400	29.756	11.850	1.071	3.514	3.514	8.868	17.853	8.927
S-Europe	109.42	13.026	7.706	3.530	21.455	12.584	24.316	9.683	0.875	2.872	2.872	7.246	14.589	7.295
Step 3														
D1/ditch	20.17	2.401	1.420	0.651	3.955	2.320	4.482	1.785	0.161	0.529	0.529	1.336	2.689	1.345
D1/stream	17.64	2.100	1.242	0.569	3.459	2.029	3.920	1.561	0.141	0.463	0.463	1.168	2.352	1.176

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D2/ditch	20.20	2.405	1.423	0.652	3.961	2.323	4.489	1.788	0.162	0.530	0.530	1.338	2.693	1.347
D2/stream	17.96	2.138	1.265	0.579	3.522	2.066	3.991	1.589	0.144	0.471	0.471	1.189	2.395	1.197
D3/ditch	19.88	2.367	1.400	0.641	3.898	2.286	4.418	1.759	0.159	0.522	0.522	1.317	2.651	1.325
D4/pond	0.6878	0.082	0.048	0.022	0.135	0.079	0.153	0.061	0.006	0.018	0.018	0.046	0.092	0.046
D4/stream	17.24	2.052	1.214	0.556	3.380	1.983	3.831	1.526	0.138	0.452	0.452	1.142	2.299	1.149
D5/pond	0.6892	0.082	0.049	0.022	0.135	0.079	0.153	0.061	0.006	0.018	0.018	0.046	0.092	0.046
D5/stream	18.60	2.214	1.310	0.600	3.647	2.139	4.133	1.646	0.149	0.488	0.488	1.232	2.480	1.240
D6/ditch	20.10	2.393	1.415	0.648	3.941	2.312	4.467	1.779	0.161	0.528	0.528	1.331	2.680	1.340
R1/pond	1.960	0.233	0.138	0.063	0.384	0.225	0.436	0.173	0.016	0.051	0.051	0.130	0.261	0.131
R1/stream	15.59	1.856	1.098	0.503	3.057	1.793	3.464	1.380	0.125	0.409	0.409	1.032	2.079	1.039
R3/stream	21.12	2.514	1.487	0.681	4.141	2.429	4.693	1.869	0.169	0.554	0.554	1.399	2.816	1.408
R4/stream	13.18	1.569	0.928	0.425	2.584	1.516	2.929	1.166	0.105	0.346	0.346	0.873	1.757	0.879

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, pre-emergence, 4 L/ha

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>			Higher tier for Daphnia and primary producers	

Group		Fish acute		Fish pro- longed	Inverteb. acute		Inverteb. pro- longed	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
End- point (µg/L)		LC ₅₀	Geomea n LC ₅₀	NOEC	EC ₅₀	Geomea n EC ₅₀	NOEC	ErC ₅₀	NOEC	ErC ₅₀	Geomean ErC ₅₀	HC ₅	NOEC _{community}	NOEC _{commu nity}
		840	1420	310	510	869.5	45	113	1250	381	381	45.4	15	15
AF		100	100	10	100	100	10	10	10	10	10	3	2	1
RAC (µg/L)		8.40	14.2	31.0	5.10	8.695	4.5	11.3	125.0	38.1	38.1	15.1	7.50	15
FOCUS Scenario	PEC gl-max (µg/L)													
Step 1														
	386.1 9	45.975	27.196	12.458	75.724	44.415	85.820	34.176	3.090	10.136	10.136	25.575	51.492	25.746
Step 2														
N- Europe	153.1 3	18.230	10.784	4.940	30.025	17.611	34.029	13.551	1.225	4.019	4.019	10.141	20.417	10.209
S-Europe	125.0 5	14.887	8.806	4.034	24.520	14.382	27.789	11.066	1.000	3.282	3.282	8.281	16.673	8.337
Step 3														
D1/ditch	23.05	2.744	1.623	0.744	4.520	2.651	5.122	2.040	0.184	0.605	0.605	1.526	3.073	1.537
D1/strea m	20.16	2.400	1.420	0.650	3.953	2.319	4.480	1.784	0.161	0.529	0.529	1.335	2.688	1.344
D2/ditch	23.08	2.748	1.625	0.745	4.525	2.654	5.129	2.042	0.185	0.606	0.606	1.528	3.077	1.539
D2/strea m	20.53	2.444	1.446	0.662	4.025	2.361	4.562	1.817	0.164	0.539	0.539	1.360	2.737	1.369
D3/ditch	22.72	2.705	1.600	0.733	4.455	2.613	5.049	2.011	0.182	0.596	0.596	1.505	3.029	1.515
D4/pond	0.786 0	0.094	0.055	0.025	0.154	0.090	0.175	0.070	0.006	0.021	0.021	0.052	0.105	0.052

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D4/stream	19.70	2.345	1.387	0.635	3.863	2.266	4.378	1.743	0.158	0.517	0.517	1.305	2.627	1.313
D5/pond	0.7877	0.094	0.055	0.025	0.154	0.091	0.175	0.070	0.006	0.021	0.021	0.052	0.105	0.053
D5/stream	21.25	2.530	1.496	0.685	4.167	2.444	4.722	1.881	0.170	0.558	0.558	1.407	2.833	1.417
D6/ditch	22.97	2.735	1.618	0.741	4.504	2.642	5.104	2.033	0.184	0.603	0.603	1.521	3.063	1.531
R1/pond	2.251	0.268	0.159	0.073	0.441	0.259	0.500	0.199	0.018	0.059	0.059	0.149	0.300	0.150
R1/stream	17.98	2.140	1.266	0.580	3.525	2.068	3.996	1.591	0.144	0.472	0.472	1.191	2.397	1.199
R3/stream	24.36	2.900	1.715	0.786	4.776	2.802	5.413	2.156	0.195	0.639	0.639	1.613	3.248	1.624
R4/stream	15.07	1.794	1.061	0.486	2.955	1.733	3.349	1.334	0.121	0.396	0.396	0.998	2.009	1.005

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

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, post-emergence, 3.5 L/ha

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>			Higher tier for <i>Daphnia</i> and primary producers	
End-point (µg/L)		LC ₅₀	Geomean LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	NOEC	ErC ₅₀	NOEC	ErC ₅₀	Geomean ErC ₅₀	HC ₅	NOEC _{community}	NOEC _{community}
		840	1420	310	510	869.5	45	113	1250	381	381	45.4	15	15
AF		100	100	10	100	100	10	10	10	10	10	3	2	1
RAC (µg/L)		8.40	14.2	31.0	5.10	8.695	4.5	11.3	125.0	38.1	38.1	15.1	7.50	15

Group		Fish acute		Fish pro- longed	Inverteb. acute		Inverteb. pro- longed	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
FOCUS Scenario	PEC gl-max (µg/L)													
Step 1														
	337.9 1	40.227	23.796	10.900	66.257	38.863	75.091	29.904	2.703	8.869	8.869	22.378	45.055	22.527
Step 2														
N- Europe	133.9	15.940	9.430	4.319	26.255	15.400	29.756	11.850	1.071	3.514	3.514	8.868	17.853	8.927
S-Europe	109.4 2	13.026	7.706	3.530	21.455	12.584	24.316	9.683	0.875	2.872	2.872	7.246	14.589	7.295
Step 3														
D1/ditch	20.17	2.401	1.420	0.651	3.955	2.320	4.482	1.785	0.161	0.529	0.529	1.336	2.689	1.345
D1/strea m	17.64	2.100	1.242	0.569	3.459	2.029	3.920	1.561	0.141	0.463	0.463	1.168	2.352	1.176
D2/ditch	20.05	2.387	1.412	0.647	3.9314	2.306	4.456	1.774	0.160	0.526	0.526	1.328	2.673	1.337
D2/strea m	16.27	1.937	1.146	0.525	3.1902	1.871	3.616	1.440	0.130	0.427	0.427	1.077	2.169	1.085
D3/ditch	19.87	2.365	1.399	0.641	3.896	2.285	4.416	1.758	0.159	0.522	0.522	1.316	2.649	1.325
D4/pond	0.687 8	0.082	0.048	0.022	0.135	0.079	0.153	0.061	0.006	0.018	0.018	0.046	0.092	0.046
D4/strea m	17.24	2.052	1.214	0.556	3.380	1.983	3.831	1.526	0.138	0.452	0.452	1.142	2.299	1.149
D5/pond	0.690 0	0.082	0.049	0.022	0.1353	0.079	0.153	0.061	0.006	0.018	0.018	0.046	0.092	0.046
D5/strea m	18.60	2.214	1.310	0.600	3.647	2.139	4.133	1.646	0.149	0.488	0.488	1.232	2.480	1.240
D6/ditch	20.10	2.393	1.415	0.648	3.941	2.312	4.467	1.779	0.161	0.528	0.528	1.331	2.680	1.340

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
R1/pond	1.949	0.232	0.137	0.063	0.3822	0.224	0.433	0.172	0.016	0.051	0.051	0.129	0.260	0.130
R1/stream	15.46	1.840	1.089	0.499	3.0314	1.778	3.436	1.368	0.124	0.406	0.406	1.024	2.061	1.031
R3/stream	19.67	2.342	1.385	0.635	3.857	2.262	4.371	1.741	0.157	0.516	0.516	1.303	2.623	1.311
R4/stream	22.60	2.690	1.592	0.729	4.431	2.599	5.022	2.000	0.181	0.593	0.593	1.497	3.013	1.507

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, post-emergence, 4 L/ha

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>			Higher tier for Daphnia and primary producers	
End-point (µg/L)		LC ₅₀	Geomean LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	NOEC	E _r C ₅₀	NOEC	E _r C ₅₀	Geomean E _r C ₅₀	HC ₅	NOEC _{community}	NOEC _{community}
AF		840	1420	310	510	869.5	45	113	1250	381	381	45.4	15	15
RAC (µg/L)		100	100	10	100	100	10	10	10	10	10	3	2	1
RAC (µg/L)		8.40	14.2	31.0	5.10	8.695	4.5	11.3	125.0	38.1	38.1	15.1	7.50	15
FOCUS Scenario	PEC _{gl-max} (µg/L)													
Step 1														
	386.19	45.975	27.196	12.458	75.724	44.415	85.820	34.176	3.090	10.136	10.136	25.575	51.492	25.746

Group		Fish acute	Fish pro- longed	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers	Mesocosm				
Step 2														
N-Europe	153.13	18.230	10.784	4.940	30.025	17.611	34.029	13.551	1.225	4.019	4.019	10.141	20.417	10.209
S-Europe	125.05	14.887	8.806	4.034	24.520	14.382	27.789	11.066	1.000	3.282	3.282	8.281	16.673	8.337
Step 3														
D1/ditch	23.05	2.744	1.623	0.744	4.520	2.651	5.122	2.040	0.184	0.605	0.605	1.526	3.073	1.537
D1/stream	20.16	2.400	1.420	0.650	3.953	2.319	4.480	1.784	0.161	0.529	0.529	1.335	2.688	1.344
D2/ditch	22.92	2.729	1.614	0.739	4.494	2.636	5.093	2.028	0.183	0.602	0.602	1.518	3.056	1.528
D2/stream	18.60	2.214	1.310	0.600	3.647	2.139	4.133	1.646	0.149	0.488	0.488	1.232	2.480	1.240
D3/ditch	22.70	2.702	1.599	0.732	4.451	2.611	5.044	2.009	0.182	0.596	0.596	1.503	3.027	1.513
D4/pond	0.7860	0.094	0.055	0.025	0.154	0.090	0.175	0.070	0.006	0.021	0.021	0.052	0.105	0.052
D4/stream	19.70	2.345	1.387	0.635	3.863	2.266	4.378	1.743	0.158	0.517	0.517	1.305	2.627	1.313
D5/pond	0.7887	0.094	0.056	0.025	0.155	0.091	0.175	0.070	0.006	0.021	0.021	0.052	0.105	0.053
D5/stream	21.25	2.530	1.496	0.685	4.167	2.444	4.722	1.881	0.170	0.558	0.558	1.407	2.833	1.417
D6/ditch	22.97	2.735	1.618	0.741	4.504	2.642	5.104	2.033	0.184	0.603	0.603	1.521	3.063	1.531
R1/pond	2.238	0.266	0.158	0.072	0.439	0.257	0.497	0.198	0.018	0.059	0.059	0.148	0.298	0.149
R1/stream	17.83	2.123	1.256	0.575	3.496	2.051	3.962	1.578	0.143	0.468	0.468	1.181	2.377	1.189
R3/stream	22.69	2.701	1.598	0.732	4.449	2.610	5.042	2.008	0.182	0.596	0.596	1.503	3.025	1.513
R4/stream	26.04	3.100	1.834	0.840	5.106	2.995	5.787	2.304	0.208	0.683	0.683	1.725	3.472	1.736

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

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in potato

Group		Fish acute		Fish pro- longed	Inverteb. acute		Inverteb. pro- longed	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhyn- chus mykiss</i>		<i>Oncorhyn- chus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chirono- mus ripar- ius</i>	<i>Myriophyl- lum spi- catum</i>			Higher tier for Daphnia and primary producers	
End- point (µg/L)		LC ₅₀	Geomea n LC ₅₀	NOEC	EC ₅₀	Geomea n EC ₅₀	NOEC	ErC ₅₀	NOEC	ErC ₅₀	Geomean ErC ₅₀	HC ₅	NOEC _{community}	NOEC _{commu nity}
AF		840	1420	310	510	869.5	45	113	1250	381	381	45.4	15	15
RAC (µg/L)		100	100	10	100	100	10	10	10	10	10	3	2	1
RAC (µg/L)		8.40	14.2	31.0	5.10	8.695	4.5	11.3	125.0	38.1	38.1	15.1	7.50	15
FOCUS Scenario	PEC gl-max (µg/L)													
Step 1														
	424.8 1	50.573	29.916	13.704	83.296	48.857	94.402	37.594	3.398	11.150	11.150	28.133	56.641	28.321
Step 2														
N- Europe	75.78	9.021	5.337	2.445	14.859	8.715	16.840	6.706	0.606	1.989	1.989	5.019	10.104	5.052
S-Europe	137.5 5	16.375	9.687	4.437	26.971	15.819	30.567	12.173	1.100	3.610	3.610	9.109	18.340	9.170
Step 3														
D3/ditch	20.74	2.469	1.461	0.669	4.067	2.385	4.609	1.835	0.166	0.544	0.544	1.374	2.765	1.383
D4/pond	0.837 0	0.100	0.059	0.027	0.164	0.096	0.186	0.074	0.007	0.022	0.022	0.055	0.112	0.056

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D4/stream	17.12	2.038	1.206	0.552	3.357	1.969	3.804	1.515	0.137	0.449	0.449	1.134	2.283	1.141
D6/ditch, 1 st	20.51	2.442	1.444	0.662	4.022	2.359	4.558	1.815	0.164	0.538	0.538	1.358	2.735	1.367
D6/ditch, 2 nd	20.86	2.483	1.469	0.673	4.090	2.399	4.636	1.846	0.167	0.548	0.548	1.381	2.781	1.391
R1/pond	1.448	0.172	0.102	0.047	0.284	0.167	0.322	0.128	0.012	0.038	0.038	0.096	0.193	0.097
R1/stream	14.33	1.706	1.009	0.462	2.810	1.648	3.184	1.268	0.115	0.376	0.376	0.949	1.911	0.955
R2/stream	18.97	2.258	1.336	0.612	3.720	2.182	4.216	1.679	0.152	0.498	0.498	1.256	2.529	1.265
R3/stream	20.23	2.408	1.425	0.653	3.967	2.327	4.496	1.790	0.162	0.531	0.531	1.340	2.697	1.349

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, pre-emergence, 3.5 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		E _r C ₅₀ 13	E _r C ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Step 1					
	81.36	62.585	189.209	160.157	5.424
Step 2					
N-Europe	15.92	12.246	37.023	31.339	1.061
S-Europe	12.77	9.823	29.698	25.138	0.851
Step 3					
D1/ditch	65.73	50.562	152.860	129.390	4.382
D1/stream	43.24	33.262	100.558	85.118	2.883
D2/ditch	123.6	95.077	287.442	243.307	8.240
D2/stream	78.77	60.592	183.186	155.059	5.251
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	2.715	2.088	6.314	5.344	0.181
D4/stream	5.033	3.872	11.705	9.907	0.336
D5/pond	8.886	6.835	20.665	17.492	0.592
D5/stream	13.59	10.454	31.605	26.752	0.906
D6/ditch	28.61	22.008	66.535	56.319	1.907
R1/pond	0.3793	0.292	0.882	0.747	0.025
R1/stream	13.93	10.715	32.395	27.421	0.929
R3/stream	11.35	8.731	26.395	22.343	0.757
R4/stream	10.60	8.154	24.651	20.866	0.707

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

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, pre-emergence, 4 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		ErC ₅₀ 13	ErC ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	92.98	71.523	216.233	183.031	6.199
Step 2					
N-Europe	18.20	14.000	42.326	35.827	1.213
S-Europe	14.59	11.223	33.930	28.720	0.973
Step 3					
D1/ditch	75.75	58.269	176.163	149.114	5.050
D1/stream	49.87	38.362	115.977	98.169	3.325
D2/ditch	142.9	109.923	332.326	281.299	9.527
D2/stream	90.98	69.985	211.581	179.094	6.065
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	3.110	2.392	7.233	6.122	0.207
D4/stream	5.772	4.440	13.423	11.362	0.385

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D5/pond	10.19	7.838	23.698	20.059	0.679
D5/stream	15.63	12.023	36.349	30.768	1.042
D6/ditch	32.59	25.069	75.791	64.154	2.173
R1/pond	0.4308	0.331	1.002	0.848	0.029
R1/stream	15.96	12.277	37.116	31.417	1.064
R3/stream	13.01	10.008	30.256	25.610	0.867
R4/stream	12.08	9.292	28.093	23.780	0.805

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

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, post-emergence, 3.5 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		ErC ₅₀ 13	ErC ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	81.36	62.585	189.209	160.157	5.424
Step 2					

Group		Aquatic plants	Algae	Primary producers	Mesocosm
N-Europe	15.92	12.246	37.023	31.339	1.061
S-Europe	12.77	9.823	29.698	25.138	0.851
Step 3					
D1/ditch	74.22	57.092	172.605	146.102	4.948
D1/stream	46.51	35.777	108.163	91.555	3.101
D2/ditch	110.2	84.769	256.279	216.929	7.347
D2/stream	69.78	53.677	162.279	137.362	4.652
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	4.184	3.218	9.730	8.236	0.279
D4/stream	7.814	6.011	18.172	15.382	0.521
D5/pond	8.887	6.836	20.667	17.494	0.592
D5/stream	13.59	10.454	31.605	26.752	0.906
D6/ditch	28.43	21.869	66.116	55.965	1.895
R1/pond	0.3768	0.290	0.876	0.742	0.025
R1/stream	13.77	10.592	32.023	27.106	0.918
R3/stream	12.76	9.815	29.674	25.118	0.851
R4/stream	15.03	11.562	34.953	29.587	1.002

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

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in winter cereals, post-emergence, 4 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Endpoint (µg/L)		ErC ₅₀ 13	ErC ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	92.98	71.523	216.233	183.031	6.199
Step 2					
N-Europe	18.20	14.000	42.326	35.827	1.213
S-Europe	14.59	11.223	33.930	28.720	0.973
Step 3					
D1/ditch	85.34	65.646	198.465	167.992	5.689
D1/stream	53.48	41.138	124.372	105.276	3.565
D2/ditch	126.6	97.385	294.419	249.213	8.440
D2/stream	80.18	61.677	186.465	157.835	5.345
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	4.793	3.687	11.147	9.435	0.320
D4/stream	8.961	6.893	20.840	17.640	0.597
D5/pond	10.19	7.838	23.698	20.059	0.679
D5/stream	15.63	12.023	36.349	30.768	1.042
D6/ditch	32.39	24.915	75.326	63.760	2.159
R1/pond	0.4280	0.329	0.995	0.843	0.029

Group		Aquatic plants	Algae	Primary producers	Mesocosm
R1/stream	15.78	12.138	36.698	31.063	1.052
R3/stream	14.60	11.231	33.953	28.740	0.973
R4/stream	17.17	13.208	39.930	33.799	1.145

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

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1913H in potato

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		E _r C ₅₀ 13	E _r C ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	102.28	78.677	237.860	201.339	6.819
Step 2					
N-Europe	8.1	6.231	18.837	15.945	0.540
S-Europe	16.05	12.346	37.326	31.594	1.070
Step 3					
D3/ditch	< 0.000001	< 0.00001	< 0.00001	0.099	< 0.00001

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D4/pond	0.05040	0.039	0.117	0.173	0.003
D4/stream	0.08796	0.068	0.205	18.094	0.006
D6/ditch, 1st	9.192	7.071	21.377	20.315	0.613
D6/ditch, 2nd	10.32	7.938	24.000	1.351	0.688
R1/pond	0.6865	0.528	1.597	26.024	0.046
R1/stream	13.22	10.169	30.744	29.075	0.881
R2/stream	14.77	11.362	34.349	43.051	0.985
R3/stream	21.87	16.823	50.860	0.099	1.458

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

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1913H for each organism group based on FOCUS Drift Swash Tool calculations for the use in winter cereals, 3.5 L/ha

Group		Inverteb. acute	Algae	Aquatic plants	
Test species		<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		EC ₅₀ 421	E _r C ₅₀ 342	E _r C ₅₀ 944	E _r C ₅₀ 1000
AF		100	10	10	10
RAC (µg/L)		4.21	34.2	94.4	100
FOCUS Scenario	PEC _{gl-max} (µg/L)				
1 m					
	27.9935	6.6493	0.8185	0.2965	0.2799

Group		Inverteb. acute	Algae	Aquatic plants		
5 m						
	7.5878	1.8023	-	-	-	
10 m						
	4.0242	0.9559	-	-	-	

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1913H for each organism group based on FOCUS Drift Swash
Tool calculations for the use in winter cereals, 4 L/ha

Group		Inverteb. acute	Algae	Aquatic plants	
Test species		<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		EC ₅₀ 421	E _r C ₅₀ 342	E _r C ₅₀ 944	E _r C ₅₀ 1000
AF		100	10	10	10
RAC (µg/L)		4.21	34.2	94.4	100
FOCUS Scenario	PEC _{gl-max} (µg/L)				
1 m					
	31.9948	7.5997	0.9355	0.3389	0.3199
5 m					
	8.6724	2.0600	-	-	-
10 m					
	4.5995	1.0925	-	-	-
12 m					
	3.8777	0.9211	-	-	-

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1913H for each organism group based on FOCUS Drift Swash Tool calculations for the use in potato

Group		Inverteb. acute	Algae	Aquatic plants	
Test species		<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		EC ₅₀ 421	E _r C ₅₀ 342	E _r C ₅₀ 944	E _r C ₅₀ 1000
AF		100	10	10	10
RAC (µg/L)		4.21	34.2	94.4	100
FOCUS Scenario	PEC _{gl-max} (µg/L)				
1 m					
	35.1942	8.3597	1.0291	0.3728	0.3519
5 m					
	9.5396	2.2659	0.2789	-	-
10 m					
	5.0594	1.2018	-	-	-
12 m					
	4.2654	1.0132	-	-	-
14 m					
	3.6888	0.8762	-	-	-

For the intended use, calculated PEC/RAC ratios for prosulfocarb did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish as characterised by a geomean LC₅₀ for of 1420 µg/L in connection with an assessment factor of 100) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prosulfocarb based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB1913H in winter cereals, pre-emergence, 3.5 L/ha

Intended use		Winter cereals (pre-emergence; 3.5 L/ha)				
Active substance		Prosulfocarb				
Application rate (g/ha)		1 × 3150				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D1 ditch	5.610	-	-	-	
None	D1 stream	6.451	-	-	-	
None	D2 ditch	5.624	-	-	-	
None	D2 stream	6.570	-	-	-	
None	D3 ditch	5.389	-	-	-	
None	D4 stream	6.324	-	-	-	
None	D5 stream	6.802	-	-	-	
None	D6 ditch	11.60	-	-	-	
None	R1 stream	15.59	15.59	15.59	6.980	
None	R3 stream	21.12	21.12	21.12	9.639	5.05
None	R4 stream	12.6	12.6	12.6	5.49	
RAC (µg/L)		PEC/RAC ratio				
14.20						
None	D1 ditch	0.395	-	-	-	-
None	D1 stream	0.454	-	-	-	-
None	D2 ditch	0.396	-	-	-	-
None	D2 stream	0.463	-	-	-	-
None	D3 ditch	0.380	-	-	-	-
None	D4 stream	0.445	-	-	-	-
None	D5 stream	0.479	-	-	-	-
None	D6 ditch	0.817	-	-	-	-
None	R1 stream	1.098	1.098	1.098	0.492	-
None	R3 stream	1.487	1.487	1.487	0.679	-
None	R4 stream	0.856	0.856	0.856	0.387	-
RAC (µg/L)		PEC/RAC ratio				
8.4						

None	D1 ditch	0.668				
None	D1 stream	0,768				
None	D2 ditch	0.670				
None	D2 stream	0.782				
None	D3 ditch	0.641				
None	D4 stream	0.810				
None	D5 stream	0.810				
None	D6 ditch	1.381				
None	R1 stream	1.856	1.856	1.856	0.831	
None	R3 stream	2.514	2.514	2.514	1.148	0.601
None	R4 stream	1.448	1.448	1.448	0.654	

Table 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prosulfocarb based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB1913H in winter cereals, pre-emergence, 4 L/ha

Intended use		Winter cereals (pre-emergence; 4 L/ha)				
Active substance		Prosulfocarb				
Application rate (g/ha)		1 × 3600				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D1 ditch	6.410	-	-	-	-
None	D1 stream	7.373	-	-	-	-
None	D2 ditch	6.428	-	-	-	-
None	D2 stream	7.509	-	-	-	-
None	D3 ditch	6.158	-	-	-	-
None	D4 stream	7.227	-	-	-	-
None	D5 stream	7.774	-	-	-	-
None	D6 ditch	13.53	-	-	-	-
None	R1 stream	17.98	17.98	17.98	8.051	-
None	R3 stream	24.36	24.36	24.36	11.12	5.83
None	R4 stream	14.01	-	-	6.32	-
RAC (µg/L)		PEC/RAC ratio				
14.20						
None	D1 ditch	0.451	-	-	-	--
None	D1 stream	0.519	-	-	-	--
None	D2 ditch	0.453	-	-	-	-
None	D2 stream	0.529	-	-	-	-

None	D3 ditch	0.434	-	-	-	-
None	D4 stream	0.509	-	-	-	-
None	D5 stream	0.547	-	-	-	-
None	D6 ditch	0.953	-	-	-	-
None	R1 stream	1.266	1.266	1.266	0.567	-
None	R3 stream	1.715	1.715	1.715	0.783	-
None	R4 stream	0.987	-	-	-	-
RAC (µg/L) 8.4		PEC/RAC ratio				
None	D1 ditch	0.763	-	-	-	-
None	D1 stream	0.878	-	-	-	-
None	D2 ditch	0.765	-	-	-	-
None	D2 stream	0.894	-	-	-	-
None	D3 ditch	0.733	-	-	-	-
None	D4 stream	0.860	-	-	-	-
None	D5 stream	0.925	-	-	-	-
None	D6 ditch	1.611	-	-	-	-
None	R1 stream	2.140	2.140	2.140	0.958	-
None	R3 stream	2.900	2.900	2.900	1.324	0.694
None	R4 stream	1.668	-	-	0.752	-

Table 9.5-18: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prosulfocarb based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB1913H in winter cereals, post-emergence, 3.5 L/ha

Intended use		Winter cereals (post-emergence; 3.5 L/ha)				
Active substance		Prosulfocarb				
Application rate (g/ha)		1 × 3150				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D1 ditch	5.612	-	-	-	
None	D1 stream	6.451	-	-	-	
None	D2 ditch	5.438	-	-	-	
None	D2 stream	6.038	-	-	-	
None	D3 ditch	5.386	-	-	-	
None	D4 stream	6.324	-	-	-	
None	D5 stream	6.802	-	-	-	
None	D6 ditch	11.60	-	-	-	

None	R1 stream	15.46	15.46	15.46	6.923	
None	R3 stream	19.67	19.67	19.67	8.861	4.63
None	R4 stream	22.60	22.60	22.60	10.20	5.33
RAC (µg/L) 14.20		PEC/RAC ratio				
None	D1 ditch	0.395	-	-	-	
None	D1 stream	0.454	-	-	-	
None	D2 ditch	0.383	-	-	-	
None	D2 stream	0.425	-	-	-	
None	D3 ditch	0.379	-	-	-	
None	D4 stream	0.445	-	-	-	
None	D5 stream	0.479	-	-	-	
None	D6 ditch	0.817	-	-	-	
None	R1 stream	1.089	1.089	1.089	0.488	
None	R3 stream	1.385	1.385	1.385	0.624	
None	R4 stream	1.592	1.592	1.592	0.718	
RAC (µg/L) 8.4		PEC/RAC ratio				
None	D1 ditch	0.668				
None	D1 stream	0.768				
None	D2 ditch	0.647				
None	D2 stream	0.719				
None	D3 ditch	0.641				
None	D4 stream	0.753				
None	D5 stream	0.810				
None	D6 ditch	1.381				
None	R1 stream	1.840	1.840	1.840	0.824	
None	R3 stream	2.342	2.342	2.342	1.055	0.551
None	R4 stream	2.690	2.690	2.690	1.214	0.635

Table 9.5-19: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prosulfocarb based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB1913H in winter cereals, post-emergence, 4 L/ha

Intended use Active substance Application rate (g/ha)		Winter cereals (post-emergence; 4 L/ha) Prosulfocarb 1 × 3600				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20

None	D1 ditch	6.413	-	-	-	
None	D1 stream	7.373	-	-	-	
None	D2 ditch	6.214	-	-	-	
None	D2 stream	6.900	-	-	-	
None	D3 ditch	6.155	-	-	-	
None	D4 stream	7.227	-	-	-	
None	D5 stream	7.774	-	-	-	
None	D6 ditch	13.53	-	-	-	
None	R1 stream	17.83	17.83	17.83	7.983	
None	R3 stream	22.69	22.69	22.69	10.22	5.34
None	R4 stream	26.04	26.04	26.04	11.76	6.14
RAC (µg/L) 14.20		PEC/RAC ratio				
None	D1 ditch	0.452	-	-	-	-
None	D1 stream	0.519	-	-	-	-
None	D2 ditch	0.438	-	-	-	-
None	D2 stream	0.486	-	-	-	-
None	D3 ditch	0.433	-	-	-	-
None	D4 stream	0.509	-	-	-	-
None	D5 stream	0.547	-	-	-	-
None	D6 ditch	0.953	-	-	-	-
None	R1 stream	1.256	1.256	1.256	0.562	-
None	R3 stream	1.598	1.598	1.598	0.720	-
None	R4 stream	1.834	1.834	1.834	0.828	-
RAC (µg/L) 8.4		PEC/RAC ratio				
None	D1 ditch	0.763				
None	D1 stream	0.878				
None	D2 ditch	0.740				
None	D2 stream	0.821				
None	D3 ditch	0.733				
None	D4 stream	0.860				
None	D5 stream	0.925				
None	D6 ditch	1.611				
None	R1 stream	2.123	2.123	2.123	0.950	
None	R3 stream	2.701	2.701	2.701	1.217	0.636
None	R4 stream	3.100	3.100	3.100	1.400	0.731

Table 9.5-20: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prosulfocarb based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB1913H in potato

Intended use		Potato		
Active substance		Prosulfocarb		
Application rate (g/ha)		1 × 3960		
Nozzle reduction	No-spray buffer (m)	5	10	10
	Vegetated filter strip (m)	None	None	10
None	D3 ditch	6.797	-	-
None	D4 stream	7.306	-	-
None	D6 ditch, 1 st	6.721	-	-
None	D6 ditch, 2nd	6.889	-	-
None	R1 stream	10.80	-	4.89
None	R2 stream	8.143	-	-
None	R3 stream	15.52	15.52	7.081
RAC (µg/L)		PEC/RAC ratio		
14.20				
None	D3 ditch	0.479	-	-
None	D4 stream	0.515	-	-
None	D6 ditch, 1 st	0.473	-	-
None	D6 ditch, 2nd	0.485	-	-
None	R1 stream	0.761	-	-
None	R2 stream	0.573	-	-
None	R3 stream	1.093	1.093	0.499
RAC (µg/L)		PEC/RAC ratio		
8.4				
None	D3 ditch	0.809	-	-
None	D4 stream	0.870	-	-
None	D6 ditch, 1 st	0.800	-	-
None	D6 ditch, 2nd	0.820	-	-
None	R1 stream	1.286	-	0.582
None	R2 stream	0.969	-	-
None	R3 stream	1.848	1.848	0.843

For the intended use, calculated PEC/RAC ratios for prosulfocarb sulfoxide did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for primary producers as characterised by a NOEC_{community} of 30 µg/L in connection with an assessment factor of 2) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on Tier 2 PEC_{sw} calculations for prosulfocarb sulfoxide (please refer to Table 8.9-27 – 8.9-31 in dRR Section B8).

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1913H in winter cereals, pre-emergence, 3.5 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		E _r C ₅₀ 13	E _r C ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
D1/ditch	3.297	2.536	7.667	6.490	0.220
D1/stream	2.086	1.605	4.851	4.106	0.139
D2/ditch	14.77	11.362	34.349	29.075	0.985
D2/stream	9.446	7.266	21.967	18.594	0.630
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	0.01378	0.011	0.032	0.027	0.001
D4/stream	0.02423	0.019	0.056	0.048	0.002
D5/pond	0.04959	0.038	0.115	0.098	0.003
D5/stream	0.3066	0.236	0.713	0.604	0.020
D6/ditch	3.205	2.465	7.453	6.309	0.214
R1/pond	0.1110	0.085	0.258	0.219	0.007
R1/stream	10.38	7.985	24.140	20.433	0.692
R3/stream	14.18	10.908	32.977	27.913	0.945
R4/stream	2.241	1.724	5.212	4.411	0.149

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

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1913H in winter cereals, pre-emergence, 4 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		E _r C ₅₀ 13	E _r C ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15

Group		Aquatic plants	Algae	Primary producers	Mesocosm
FOCUS Scenario	PEC _{gl-max} (µg/L)				
D1/ditch	3.766	2.897	8.758	7.413	0.251
D1/stream	2.383	1.833	5.542	4.691	0.159
D2/ditch	16.95	13.038	39.419	33.366	1.130
D2/stream	10.83	8.331	25.186	21.319	0.722
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	0.01576	0.012	0.037	0.031	0.001
D4/stream	0.02755	0.021	0.064	0.054	0.002
D5/pond	0.05651	0.043	0.131	0.111	0.004
D5/stream	0.3501	0.269	0.814	0.689	0.023
D6/ditch	3.656	2.812	8.502	7.197	0.244
R1/pond	0.1274	0.098	0.296	0.251	0.008
R1/stream	11.92	9.169	27.721	23.465	0.795
R3/stream	16.26	12.508	37.814	32.008	1.084
R4/stream	2.551	1.962	5.933	5.022	0.170

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

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1913H in winter cereals, post-emergence, 3.5 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		ErC ₅₀ 13	ErC ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
D1/ditch	12.45	9.577	28.953	24.508	0.830
D1/stream	8.200	6.308	19.070	16.142	0.547
D2/ditch	10.13	7.792	23.558	19.941	0.675
D2/stream	6.531	5.024	15.188	12.856	0.435
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	0.3181	0.245	0.740	0.626	0.021
D4/stream	0.5705	0.439	1.327	1.123	0.038

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D5/pond	0.05295	0.041	0.123	0.104	0.004
D5/stream	0.3277	0.252	0.762	0.645	0.022
D6/ditch	1.099	0.845	2.556	2.163	0.073
R1/pond	0.001579	0.001	0.004	0.003	0.000
R1/stream	1.362	1.048	3.167	2.681	0.091
R3/stream	11.40	8.769	26.512	22.441	0.760
R4/stream	2.241	1.724	5.212	4.411	0.149

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

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfide for each organism group based on Tier 2 calculations for the use of GLOB1913H in winter cereals, post-emergence, 4 L/ha

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		E _r C ₅₀ 13	E _r C ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
D1/ditch	14.43	11.100	33.558	28.406	0.962
D1/stream	9.515	7.319	22.128	18.730	0.634
D2/ditch	11.71	9.008	27.233	23.051	0.781
D2/stream	7.529	5.792	17.509	14.821	0.502
D3/ditch	< 0.000001	< 0.000001	< 0.000001	< 0.000001	< 0.000001
D4/pond	0.3640	0.280	0.847	0.717	0.024
D4/stream	0.6538	0.503	1.520	1.287	0.044
D5/pond	0.06033	0.046	0.140	0.119	0.004
D5/stream	0.3741	0.288	0.870	0.736	0.025
D6/ditch	1.254	0.965	2.916	2.469	0.084
R1/pond	0.001814	0.001	0.004	0.004	0.000
R1/stream	1.564	1.203	3.637	3.079	0.104
R3/stream	13.07	10.054	30.395	25.728	0.871
R4/stream	2.551	1.962	5.933	5.022	0.170

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

Table 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1913H in potato

Group		Aquatic plants	Algae	Primary producers	Mesocosm
Test species		<i>Lemna gibba</i>	<i>Anabaena flosaquae</i>		Higher tier for primary producers
Endpoint (µg/L)		E _r C ₅₀ 13	E _r C ₅₀ 4.3	HC ₅ 1.524	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	0.508	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
D3/ditch	< 0.000001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
D4/pond	0.000014	< 0.0001	< 0.0001	< 0.0001	< 0.0001
D4/stream	0.000039	< 0.0001	< 0.0001	< 0.0001	< 0.0001
D6/ditch, 1st	2.109	1.622	4.905	4.152	0.141
D6/ditch, 2nd	0.07144	0.055	0.166	0.141	0.005
R1/pond	0.2113	0.163	0.491	0.416	0.014
R1/stream	5.186	3.989	12.060	10.209	0.346
R2/stream	1.087	0.836	2.528	2.140	0.072
R3/stream	0.007077	0.005	0.016	0.014	0.0005

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

For the intended use, calculated PEC/RAC ratios for prosulfocarb sulfoxide did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for primary producers as characterised by a NOEC_{community} of 30 µg/L in connection with an assessment factor of 2) in a few FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-26: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide based on FOCUS Step 4 calculations and toxicity data for primary producers with mitigation of spray drift and run-off for the use of GLOB1913H in winter cereals, pre-emergence, 4 L/ha

Intended use		Winter cereals			
Active substance		Prosulfocarb			
Application rate (g/ha)		1 × 3600			
Nozzle reduction	No-spray buffer (m)	5	10	20	10
	Vegetated filter strip (m)	None	None	None	10
None		D2 ditch	16.95	16.95	16.95
					-

None	R3 stream	16.26	16.26	16.26	7.340
RAC (µg/L)					
15		PEC/RAC ratio			
None	D2 ditch	1.130	1.130	1.130	-
None	R3 stream	1.084	1.084	1.084	0.489

9.5.3 Overall conclusions

An acceptable risk is concluded for prosulfocarb at Step 3 for the D4 pond, D5 pond, R1 pond and R4 stream scenario for the dose rate of 3.5 L/ha in winter cereals, the D4 pond, D5 pond and R1 pond scenario for the dose rate of 4 L/ha in winter cereals and the D4 pond and R1 pond scenarios in potatoes. The risk in all other scenarios was acceptable using a 5 m no spray buffer zone, except for the R1 and R3 scenario for the pre-emergence use in winter cereals, the R1, R3 and R4 scenarios for the post-emergence use in winter cereals and the R3 scenario for the use in potatoes where a 10 m no spray buffer zone including a 10 m vegetated buffer strip is required to obtain an acceptable risk.

The risk for the metabolite prosulfocarb sulfoxide is acceptable at Step 3, except for the D2 ditch and R3 stream scenario for the pre-emergence use in winter cereals at a dose rate of 4 L/ha. The risk in the R3 stream scenario can be resolved by using a 10 m no spray buffer zone including a 10 m vegetated filter strip. The risk in D2 ditch remains unresolved, but it represents <1% of the drained cereal growing land in Europe and it is mainly located in areas of the UK. The D2 scenario is therefore of very limited relevance in Member States and, if relevant, can be addressed with a label restriction for heavy clay soils.

An acceptable risk for the formulation GLOB1913H following spray drift is concluded using a 10 m no spray buffer zone for the use in winter cereals at 3.5 L/ha, a 12 m no spray buffer zone for the use in winter cereals at 4 L/ha and a 14 m no spray buffer zone for the use in potatoes.

Based on the relevant FOCUS scenario's in each Member State, the following mitigation measures are proposed:

zRMS comments:

Prosulfocarb

For the first tier risk assessment for active substance prosulfocarb the EU agreed endpoints were used except daphnia. For daphnia, based on the EFSA Scientific Report (2007) 111, 1-81, Conclusion on the peer review of prosulfocarb, Appendix 1 – list of end points: previously used $EC_{50} = 510 \mu\text{g/L}$ was replaced by data from a new formulation study $EC_{50} = 419 \mu\text{g/L}$ (see Addendum), and this endpoint should be used for the risk assessment. However the refinement of risk was performed for daphnia, therefore no new PEC/RAC ratios were calculated.

For the refinements of acute risk to fish the geomean approach, based on the only 2 fish species, was proposed by Applicant. Because the use of only two species of fish can be questioned, therefore the acceptability of the geomean approach should be considered on the MSs level.

If the geomean approach is not accepted the acute risk assessment to fish should be based on the standard 96h LC_{50} at 840 $\mu\text{g/L}$, agreed at expert meeting as UE agreed endpoint.

The refinement of risk to invertebrates and primary producers was conducted in accordance with EFSA Journal 2013; 11(7): 3290, 268 pp using the geo-mean approach. For primary producers the HC5 from SSD was also presented.

The further refinement of risk for aquatic invertebrates, algae and macrophyte was based on the result of mesocosm study (Wijngaarden, 2006) accepted on the UE level and AF of 1 (proposed by the Applicant)

In the next step the PEC/RAC ratios were calculated based on the FOCUS Step 4 PEC_{sw} values and the geomean endpoint for fish, as the lowest endpoint.

Additionally the PEC/RAC ratios were calculated by zRMS with the EU agreed acute endpoint for fish. The calculations are highlighted in grey.

Prosulfocarb metabolite

The higher tier risk assessment for metabolite prosulfocarb sulfoxide was based on the ETO-RAC at 15 µg/L.

The risk is not acceptable for scenarios D2 ditch and R3 stream for winter cereals, pre-emergence at dose rate 4 L/ha. When FOCUS Step 4 PEC_{sw} values with 10 m no-spray buffer and 10 m vegetated filter strip were used the risk was acceptable.

Formulation

The risk assessment was performed based on the drift PEC_{sw} values
For use:

- in winter cereals at dose rate 3,5 L/ha 10 m buffer zone is required,
- in winter cereals at dose rate 4 L/ha 12 m buffer zone is required,
- in potato 14 m buffer zone is required.

Conclusion:

According to the performed risk assessment there is no potential of risk for aquatic organisms resulting from acute and long-term exposure to active substances following use of Roxy XL (GLOB1913H) in compliance with proposed GAP when the risk mitigation measures were applied.

The appropriate risk mitigation measures should be considered at national level. If it is necessary Member states will need to further consider the risk to aquatic organisms based on national requirements.

Member state	Relevant scenarios	Crop	Mitigation measure
Poland	D3, D4, R1	Winter cereals	10 m no spray buffer zone including a 10 m vegetated filter strip
		Potato	5 m no spray buffer zone
Belgium	D3, D4, R1	Winter cereals	10 m no spray buffer zone including a 10 m vegetated filter strip
		Potato	5 m no spray buffer zone
Hungary	D3, D5, R1, R3, R4	Winter cereals	10 m no spray buffer zone including a 10 m vegetated filter strip
		Potato	10 m no spray buffer zone including a 10 m vegetated filter strip
Ireland	D4	Winter cereals	5 m no spray buffer zone
		Potato	5 m no spray buffer zone
Slovakia	D4, D5, R1	Winter cereals	10 m no spray buffer zone including a 10 m vegetated filter strip
		Potato	5 m no spray buffer zone

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

Effects on bees of the formulation were not evaluated as part of the EU assessment of prosulfocarb. New data submitted with this application are listed in Table 9.6-1, **Błąd! Nie można odnaleźć źródła odwołania.**Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Prosulfocarb	Oral, acute	LD ₅₀ > 103.4 µg/bee	EFSA, 2007
<i>Apis mellifera</i>	Prosulfocarb	Contact, acute	LD ₅₀ = 79.3 µg/bee	EFSA, 2007
<i>Apis mellifera</i>	GLOB1913H	Oral, acute, 48 h	LD ₅₀ = 464.1 µg/bee	Sekine T., 2020
<i>Apis mellifera</i>	GLOB1913H	Contact, acute, 96 h	LD ₅₀ = 149.5 µg/bee	Sekine T., 2020
<i>Bombus terrestris</i>	GLOB1913H	Oral, acute	LD ₅₀ > 334.0 µg/bee NOED ≥ 206.3 µg/bee	Chwiesko D., 2021
<i>Bombus terrestris</i>	GLOB1913H	Contact, acute	LD ₅₀ > 453.3 µg/bee NOED ≥ 453.3 µg/bee	Chwiesko D., 2021
<i>Apis mellifera</i>	GLOB1913H	Adult, chronic	NOEDD = 128.1 µg/bee/d LDD50 = 206.1 µg/bee/d	Berg C., 2021a
<i>Apis mellifera</i>	GLOB1913H	Larvae, chronic	NOED = 50.0 µg/larva	Colli M., 2021
Higher-tier studies (tunnel test, field studies)				
-				

9.6.1.1 Justification for new endpoints

-

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 6 also covers the risk for bees from all other intended uses in groups 4 and 5 (see 9.1.2).

Table 9.6-2: First-tier assessment of the risk for bees due to the pre-emergence use of GLOB1913H in winter cereals and potatoes and the post-emergence use of GLOB1913H in winter cereals

Intended use	Winter cereals (pre-+post-emergence), potatoes		
Active substance	Prosulfocarb		
Application rate (g/ha)	1 × 3960		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	103.4	3960	38.30
Contact toxicity	79.3		49.94
Product	GLOB1913H		
Application rate (g/ha)	1 × 4565		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	464.1	4565	9.84
Contact toxicity	149.5		30.5

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

9.6.3 Chronic risk assessment (KCP 10.3.1.2)

9.6.3.1 Larval chronic risk assessment

A chronic larval study is available and the potential acceptable risk can be further demonstrated by carrying out a worst-case risk assessment through the calculation of a TER value as set out in the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028).

A worst-case of potential exposure via residues in pollen and nectar can be estimated based on the default worst-case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts, as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from *Rortais et al., 2005*², as proposed in the EPPO scheme, have been used to estimate the consumption by bee larvae:

Worker larvae consuming 59.4 mg sugar in 5 days Assuming 30% sugar content of nectar the worst-case consumption with worker larvae is:

$$59.4/0.30 = 198 \text{ mg nectar in 5 days.}$$

² Agnès RORTAIS, Gérard ARNOLD, Marie-Pierre HALM, Frédérique TOUFFET-BRIENS (2005). Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36 (2005) 71–83

In addition worker larvae are considered to consume 2 mg pollen during their development phase (EFSA 2013).

Thus considering the mean RUD values for nectar and pollen in EFSA 2013 exposure can be estimated for the whole development period.

Nectar dose: $4.565 \times 2.9 \times 198/1000 = 2.6212 \mu\text{g/larva}$

Pollen dose: $4.565 \times 6.1 \times 2/1000 = 0.0557 \mu\text{g/larva}$

Total exposure ETE = $2.6769 \mu\text{g/larvae}$ (as a default worst-case residue at 4.565 kg a.s./ha)

This can be compared to the larval NOED of $50.0 \mu\text{g/larva}$.

$\text{TER} = \text{NOEDD} (\mu\text{g/larva}) / \text{ETE} (\mu\text{g/larva}) = 50.0/2.6769 = 18.7$

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the chronic risk to honey bees. It is clear that with a TER value of 18.7, the proposed uses of GLOB1913H pose an acceptable risk to bee larval development.

The risk assessment was also conducted according to the “EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)” (EFSA Journal 2013;11(7):3295).

Chronic oral exposure larvae (liquid formulations):

Screening step assessment for spray applications:

$\text{ETR} = \text{AR} \times \text{SV} / \text{NOEL} = 4.565 \times 4.4 / 50.0 = 0.40$

The protection goal is not met as the calculated value is above the trigger value of 0.2. Therefore, refined risk assessment is needed.

Cereals & Potatoes, BBCH < 10

Treated crop:

$$\text{ETR}_{\text{larvae}} = \text{AR} \times \text{Ef} \times \text{SV} \times \text{TWA} / \text{NOEL}_{\text{larvae}} = 4.565 \times 1 \times 0.002 \times 0.85 / 50.0 = 0.0002$$

Adjacent crop:

$$\text{ETR}_{\text{larvae}} = \text{AR} \times \text{Ef} \times \text{SV} \times \text{TWA} / \text{NOEL}_{\text{larvae}} = 4.565 \times 0.0033 \times 4.4 \times 0.85 / 50.0 = 0.0011$$

Weeds in the treated field:

$$\text{ETR}_{\text{larvae}} = \text{AR} \times \text{Ef} \times \text{SV} \times \text{TWA} / \text{NOEL}_{\text{larvae}} = 4.565 \times 1 \times 2.2 \times 0.85 / 50.0 = 0.17$$

Plants in the field margin:

$$\text{ETR}_{\text{larvae}} = \text{AR} \times \text{Ef} \times \text{SV} \times \text{TWA} / \text{NOEL}_{\text{larvae}} = 4.565 \times 0.0092 \times 2.2 \times 0.85 / 50.0 = 0.002$$

Succeeding crops:

$$\text{ETR}_{\text{larvae}} = \text{AR} \times \text{Ef} \times \text{SV} \times \text{TWA} / \text{NOEL}_{\text{larvae}} = 4.565 \times 1 \times 0.4 \times 0.85 / 50.0 = 0.03$$

Cereals, BBCH 10-29

Treated crop:

$$\text{ETR}_{\text{larvae}} = \text{AR} \times \text{Ef} \times \text{SV} \times \text{TWA} / \text{NOEL}_{\text{larvae}} = 4.565 \times 1 \times 0.15 \times 0.85 / 50.0 = 0.01$$

Adjacent crop:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 4.565 * 0.0033 * 4.4 * 0.85 / 50.0 = 0.0011$$

Weeds in the treated field:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 4.565 * 1 * 2.2 * 0.85 / 50.0 = 0.17$$

Plants in the field margin:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 4.565 * 0.0092 * 2.2 * 0.85 / 50.0 = 0.002$$

Succeeding crops:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 4.565 * 1 * 0.4 * 0.85 / 50.0 = 0.03$$

The protection goal is met as the calculated value is below the trigger value of 0.2.

9.6.3.2 Adult chronic risk assessment

The adult chronic risk assessment is performed using the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028).

This is based upon the method of EPPO 2010 risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. It uses NOEDD values for the endpoint so avoids the issues associated with the generation of LDD₅₀ values for substances of low toxicity, and calculates exposure in a similar way to EFSA 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals). EPPO 2010 recommended the calculation of a TER using the following equation:

$$TER = NOEDD / \text{daily dose}$$

Where daily dose (DD) is based on the worst case a sugar need of 128 mg/bee/day (Rortais et al 2005) of a bee feeding exclusively from nectar containing 30% sugar using the following equation:

$$\text{Daily dose } (\mu\text{g a.i./bee}) = A.R. \times [128 \text{ mg}/(1000 \times 0.3)] \times RUD = 4.565 \times [128/(1000 \times 0.3)] \times 2.9 = 5.6484 \mu\text{g/bee}$$

A.R. = application rate in kg a.i./ha

RUD = residue per unit dose from the EFSA bee guidance. Mean RUD_{nectar} = 2.9 mg a.i./kg (foliar sprays).

$$TER = NOEDD / \text{daily dose} = 128.1 / 5.6484 = 22.7$$

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the chronic risk to honey bees. It is clear that with a TER value of 22.7, the proposed uses of GLOB1913H pose an acceptable chronic risk to adult bees.

The risk assessment was also conducted according to the “EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)” (EFSA Journal 2013;11(7):3295).

Chronic oral exposure adult bees (liquid formulations):

Screening step assessment for spray applications:

$$ETR = AR * SV / 10d \text{ LDD}_{50} = 4.565 * 7.6 / 206.1 = 0.168$$

The protection goal is not met as the calculated value is greater than the trigger value of 0.03. Therefore, a refined risk assessment is needed.

Cereals & Potatoes, BBCH < 10:

Treated crop:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 1 * 0.012 * 0.72 / 206.1 = 0.0002$$

Adjacent crop:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 0.0033 * 5.8 * 0.72 / 206.1 = 0.0003$$

Weeds in the treated field:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 1 * 2.9 * 0.72 / 206.1 = 0.046$$

Plants in the field margin:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 0.0092 * 2.9 * 0.72 / 206.1 = 0.0004$$

Succeeding crops:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 1 * 0.54 * 0.72 / 206.1 = 0.009$$

Cereals, BBCH 10-29:

Treated crop:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 1 * 0.92 * 0.72 / 206.1 = 0.015$$

Adjacent crop:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 0.0033 * 5.8 * 0.72 / 206.1 = 0.0003$$

Weeds in the treated field:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 1 * 2.9 * 0.72 / 206.1 = 0.046$$

Plants in the field margin:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 0.0092 * 2.9 * 0.72 / 206.1 = 0.0004$$

Succeeding crops:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 4.565 * 1 * 0.54 * 0.72 / 206.1 = 0.009$$

The protection goal is met, except for weeds in the treated fields, as the calculated value is below the trigger value of 0.03.

However, with regard to weeds in the treated field, if realistic farming practices (e.g. tilling and herbicide applications) are considered, weeds are usually not prevalent in arable fields. It has been demonstrated by Maynard *et al.* (2015)³ that less than 2% of all weeds recorded in arable crop trials are at a flowering stage. The EFSA Guidance (2013) states that if less than 10% of the area of use is flowering weeds, then the exposure route is not relevant in the 90th percentile case. Therefore, this scenario does not need to be considered here.

Also taking into account the application timing, only very limited exposure of bees is to be expected.

³ Maynard S.K., Albuquerque R., Weber C., von Mérey G., Geiger M.F., Becker R., Keppler J., Masche J., Brougham K., Coulson M., 1.8 Weeds in the treated field – a realistic scenario for pollinator risk assessment? Hazards of pesticides to bees – 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium, September 15-17, 2014, Julius-Kühn-Archiv, 450, 2015.

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

Not relevant.

9.6.4 Effects on bumble bees

The effect of GLOB1913H on bumble bees was assessed according to the “EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)” (EFSA Journal 2013;11(7):3295).

Contact exposure assessment for spray applications

Screening step assessment for spray applications:

$$HQ_{\text{contact}} = AR/LD_{50\text{contact}} = 4565/453.3 = 10.1$$

The protection goal is not met as the calculated value is above the trigger value of 7. Therefore, refined risk assessment is needed.

Cereals, BBCH < 30:

Treated crop:

$$ETR_{\text{acute adult contact}} = f_{\text{dep}}/100 \cdot AR/LD_{50\text{contact}} = 0/100 \cdot 4565/453.3 = 0.0$$

Weeds in the treated field:

$$ETR_{\text{acute adult contact}} = f_{\text{dep}}/100 \cdot AR/LD_{50\text{contact}} = 100/100 \cdot 4565/453.3 = 10.1$$

Plants in the field margin:

$$ETR_{\text{acute adult contact}} = f_{\text{dep}}/100 \cdot AR/LD_{50\text{contact}} = 2.8/100 \cdot 4565/453.3 = 0.3$$

Potato, BBCH < 40:

Treated crop:

$$ETR_{\text{acute adult contact}} = f_{\text{dep}}/100 \cdot AR/LD_{50\text{contact}} = 0/100 \cdot 4565/453.3 = 0.0$$

Weeds in the treated field:

$$ETR_{\text{acute adult contact}} = f_{\text{dep}}/100 \cdot AR/LD_{50\text{contact}} = 100/100 \cdot 4565/453.3 = 10.1$$

Plants in the field margin:

$$ETR_{\text{acute adult contact}} = f_{\text{dep}}/100 \cdot AR/LD_{50\text{contact}} = 2.8/100 \cdot 4565/453.3 = 0.3$$

The protection goal is met for all scenarios, except weeds in the treated field, as the calculated value is below the trigger value of 7.

However, with regard to weeds in the treated field, if realistic farming practices (e.g. tilling and herbicide applications) are considered, weeds are usually not prevalent in arable fields. It has been demonstrated by Maynard *et al.* (2015)⁴ that less than 2% of all weeds recorded in arable crop trials are at a flowering

⁴ Maynard S.K., Albuquerque R., Weber C., von Mérey G., Geiger M.F., Becker R., Keppler J., Masche J., Brougham K., Coulson M., 1.8 Weeds in the treated field – a realistic scenario for pollinator risk assessment? Hazards of pesticides to bees – 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium, September 15-17, 2014, Juliuus-Kühn-Archiv, 450, 2015.

stage. The EFSA Guidance (2013) states that if less than 10% of the area of use is flowering weeds, then the exposure route is not relevant in the 90th percentile case. Therefore, this scenario does not need to be considered here.

Also taking into account the application timing, only very limited exposure of bees is to be expected.

Oral exposure assessment for spray applications

Screening step assessment for spray applications:

$$ETR_{\text{acute adult oral}} = AR * SV / LD_{50\text{oral}} = 4.565 * 11.2 / 334 = 0.15$$

The protection goal is not met as the calculated value is greater than the trigger value of 0.036. Therefore, refined risk assessment is needed.

Cereals & potato, BBCH < 10:

Treated crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 1 * 0.03 / 334 = 0.0004$$

Adjacent crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 0.0033 * 11.2 / 334 = 0.0005$$

Weeds in the treated field:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 1 * 6.5 / 334 = 0.0888$$

Plants in the field margin:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 0.0092 * 6.5 / 334 = 0.0008$$

Succeeding crops:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 1 * 0.9 / 334 = 0.0123$$

Cereals, BBCH 10-29:

Treated crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 1 * 2.3 / 334 = 0.0314$$

Adjacent crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 0.0033 * 11.2 / 334 = 0.0005$$

Weeds in the treated field:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 1 * 6.5 / 334 = 0.0902$$

Plants in the field margin:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 0.0092 * 6.5 / 334 = 0.0008$$

Succeeding crops:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 4.565 * 1 * 0.9 / 334 = 0.0123$$

The protection goal is met, except for weeds in the treated field, as the calculated value is below the trigger value of 0.036.

However, with regard to weeds in the treated field, if realistic farming practices (e.g. tilling and herbicide applications) are considered, weeds are usually not prevalent in arable fields. It has been demonstrated by Maynard *et al.* (2015)⁵ that less than 2% of all weeds recorded in arable crop trials are at a flowering stage. The EFSA Guidance (2013) states that if less than 10% of the area of use is flowering weeds, then the exposure route is not relevant in the 90th percentile case. Therefore, this scenario does not need to be considered here.

Also taking into account the application timing, only very limited exposure of bees is to be expected.

9.6.5 Effects on solitary bees

Not required.

9.6.6 Overall conclusions

GLOB1913H does not pose unacceptable risk to bees when applied according to the intended uses.

⁵ Maynard S.K., Albuquerque R., Weber C., von Mérey G., Geiger M.F., Becker R., Keppler J., Masche J., Brougham K, Coulson M., 1.8 Weeds in the treated field – a realistic scenario for pollinator risk assessment? Hazards of pesticides to bees – 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium, September 15-17, 2014, Juliuis-Kühn-Archiv, 450, 2015.

<p>zRMS Comments:</p>	<p>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).</p> <p>The required study on oral and contact toxicity of the formulated product GLOB1913H to honey bees was conducted and considered to be valid.</p> <p>The hazard quotients are below the trigger value, indicating that the formulation GLOB1913H poses an acceptable acute risk to bees.</p> <p>New studies for acute toxicity of bumble bees were submitted and accepted.</p> <p>The specific requirements of the Regulation (EU) 546/2011 regarding effects on bee brood development and possible chronic effects on adults were included by the Applicant and accepted.</p> <p>The EPPO 2010 (ECPA proposal of 9 June 2017) scheme proposes a trigger of 1 for assessment of the risk to honey bees. All TER values for chronic risk assessment for adult bees and bee larvae were above a trigger of 1, indicating that the proposed uses of GLOB1913H poses an acceptable chronic risk to adult bees and bee larvae.</p> <p>Moreover, the evaluator provided the risk assessment according to the new bee guidance “EFSA Guidance Document on the risk assessment of plant protection products on bees (<i>Apis mellifera</i>, <i>Bombus</i> spp.) and solitary bees”, EFSA Journal 2013; 11(7):3295. The risk assessment performed in accordance with EFSA guidance (2013) was also submitted but as this guidance has not been agreed yet its relevance will be decided at the Member State level. For chronic oral exposure of adult bees and acute contact and oral of bumble bees an unacceptable risk was indicated for bees foraging on weeds in the treated field as the ETR are above the trigger values. However taking into account realistic farming practices (e.g. tilling and herbicide applications) and the application timing weeds are usually not prevalent in arable fields. The EFSA Guidance (2013) states that if less than 10% of the area of use is flowering weeds, then the exposure route is not relevant in the 90th percentile case. Therefore, this scenario does not need to be considered here.</p>
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9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with prosulfocarb and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of formulation were not evaluated as part of the EU assessment of prosulfocarb. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	GLOB1913H	Extended laboratory test Bean leaf discs (2D)	LR ₅₀ = 0.6013 L/ha ER ₅₀ > 0.6921 L/ha NOER _{mortality} < 0.2772 L/ha NOER _{reproduction} ≥ 0.6921 L/ha	Leopold J., 2020a
<i>Aphidius rhopalosiphi</i> (adults)	GLOB1913H	Extended laboratory test Barley plants (3D)	LR ₅₀ = 1.501 L/ha ER ₅₀ > 0.6921 L/ha NOER _{mortality} = 0.6921 L/ha NOER _{reproduction} ≥ 0.6921 L/ha	Leopold J., 2020b
<i>Aleochara bilineata</i>	GLOB1913H	Extended laboratory test Sandy soil (2D)	ER ₅₀ > 10.814 L/ha NOER _{reproduction} = 1.730 L/ha	Berg C., 2021b
<i>Poecilus cupreus</i>	GLOB1913H	Extended laboratory test Sandy soil (2D)	LR ₅₀ > 10.814 L/ha NOER _{mortality} ≥ 10.814 L/ha	Berg C., 2021c
<i>Aphidius rhopalosiphi</i> (adults)	GLOB1913H	Extended laboratory test Aged residues Barley plants (3D)	Effects < 50% on survival and reproduction at DAT14 and DAT28 at 4.4 L/ha.	Röhlig U., 2022a
<i>Typhlodromus pyri</i> (protonymphs)	GLOB1913H	Extended laboratory test Aged residues Bean plants (3D)	Effects < 50% on survival and reproduction at DAT14 and DAT28 at 4.4 L/ha.	Röhlig U., 2022b
Field or semi-field tests				
-				

9.7.1.1 Justification for new endpoints

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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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 6 also covers the risk for non-target arthropods from all other intended uses in groups 4 and 5 (see 9.1.2).

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 pre-emergence use of GLOB1913H in winter cereals and potato and the post-emergence use in winter cereals

Intended use	Winter cereals (pre-+post-emergence), potato		
Active substance/product	GLOB1913H		
Application rate (L/ha)	1 × 4.4		
MAF	/		
Test species Higher-tier	LR₅₀ (lab.) (L/ha)	PER_{in-field} (L/ha)	HQ_{in-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i>	0.6013	4.4	7.32
<i>Aphidius rhopalosiphi</i>	1.501		2.93
<i>Aleochara bilineata</i>	10.814		0.41
<i>Poecilus cupreus</i>	10.814		0.41
Test species Higher-tier	Rate with ≤ 50 % effect (L/ha) at 14 DAT	PER_{in-field} (L/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	4.4	4.4	yes
<i>Aphidius rhopalosiphi</i>	4.4		yes

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 GLOB1913H in winter cereals and potato and the post-emergence use in winter cereals

Intended use	Winter cereals (pre-+post-emergence), potato				
Active substance/product	GLOB1913H				
Application rate (L/ha)	1 × 4.4				
MAF	/				
vdf	5*				
Test species Higher-tier	LR₅₀ (lab.) (L/ha)	Drift rate	PER_{off-field} (L/ha)	CF	HQ_{off-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i>	0.6013	0.0277	0.02438	5	0.20
<i>Aphidius rhopalosiphi</i>	1.501				0.081
<i>Aleochara bilineata</i>	10.814				0.011
<i>Poecilus cupreus</i>	10.814				0.011

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.

* A vdf of 5 was used in accordance with the proposal made in the EFSA Recurring Issues in Ecotoxicology (EFSA Supporting publication 2019: EN-1673).

9.7.2.3 Additional higher-tier risk assessment

Not required.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

As evidenced by the off-field risk assessment, no adverse effects on off-field arthropods are likely and therefore rapid recolonization can take place. ESCORT 2 states as a general acceptability criterion for in-field effects, that the potential for recolonisation should be demonstrated within a year, so potential for recovery within 14 days is well within this timeframe. Therefore, no unacceptable risk to non-target arthropods is expected when GLOB1913H is applied according to the intended use.

<p>zRMS Comments:</p>	<p>The submitted risk assessment based on the “Guidance Document on Terrestrial Ecotoxicology” (2002) was accepted.</p> <p>The laboratory studies 2D and 3D for <i>Aphidius rhopalosiphi</i>, <i>Typhlodromus pyri</i>, <i>Aleochara bilineata</i> and <i>Poecilus cupreus</i>. are submitted and accepted for risk assessment.</p> <p>In field risk The hazard quotients are below the trigger value ($HQ \leq 1$) for <i>Aleochara bilineata</i> and <i>Poecilus cupreus</i>. For <i>Typhlodromus pyri</i> and <i>Aphidius rhopalosiphi</i> the $HQ > 1$ therefore the higher tier assessment was required. Effects at the highest application rate x MAF are below 50% at DAT14 since the resulting HQ value would be < 2 for <i>Typhlodromus pyri</i> and <i>Aphidius rhopalosiphi</i>. The potential for recolonisation within a year has been demonstrated.</p> <p>Off-field risk The hazard quotients are below the trigger value ($HQ \leq 1$) for all species indicating that the formulation GLOB1913H poses an acceptable risk to arthropods other than bees.</p> <p>The risk from GLOB1913H to non-target arthropods in the in-crop area is considered acceptable for all the proposed uses of GLOB1913H.</p>
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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 prosulfocarb and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GLOB1913H were not evaluated as part of the EU assessment of prosulfocarb. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Prosulfocarb (Based on GLOB1913H)	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 53.6 mg/kg dw NOEC _{corr} = 26.8 mg/kg dw*	Straube D., 2020a
<i>Eisenia fetida</i>	Prosulfocarb sulfoxide	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 5.36 mg/kg dw	Worst case assumption: 10x more toxic than parent.
<i>Folsomia candida</i>	Prosulfocarb (Based on GLOB1913H)	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 16.94 mg a.s./kg dw NOEC _{corr} = 8.47 mg/kg dw*	Straube D., 2020b
<i>Folsomia candida</i>	Prosulfocarb sulfoxide	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 1.694 mg a.s./kg dw	Worst case assumption: 10x more toxic than parent.
<i>Hypoaspis aculeifer</i>	Prosulfocarb (Based on GLOB1913H)	Mixed into substrate 14 d, chronic 5% peat content	NOEC = 83.6 NOEC _{corr} = 41.8 mg/kg dw*	Straube D., 2020c
<i>Hypoaspis aculeifer</i>	Prosulfocarb sulfoxide	Mixed into substrate 14 d, chronic 5% peat content	NOEC = 8.36 mg a.s./kg dw	Worst case assumption: 10x more toxic than parent.
<i>Eisenia fetida</i>	GLOB1913H	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 62.6 mg/kg dw NOEC _{corr} = 31.3 mg/kg dw* EC ₁₀ = 49.4 mg/kg dw (95% CI : 0.36-86.2) EC ₂₀ = 75.3 mg/kg dw (95% CI: 3.49-113.4) EC ₅₀ = 142.6 mg/kg dw (95% CI : 73.27-249.9)	Straube D., 2020a
<i>Folsomia candida</i>	GLOB1913H	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 26.3 mg/kg dw NOEC _{corr} = 13.15 mg/kg dw* EC ₁₀ = 35.5 mg/kg dw (95% CI : 21.4-40.1) EC ₂₀ = 38.9	Straube D., 2020b

Species	Substance	Exposure System	Results	Reference
			mg/kg dw (95% CI: 28.3-43.3) EC ₅₀ = 46.4 mg/kg dw (95% CI: 41.5-58.9)	
<i>Hypoaspis aculeifer</i>	GLOB1913H	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 98.0 mg/kg dw NOEC _{corr} = 49.0 mg/kg dw* EC ₁₀ = 169.4 mg/kg dw (95% CI : 129.0-196.2) EC ₂₀ = 202.7 mg/kg dw (95% CI: 167.3-226.6) EC ₅₀ = 285.7 mg/kg dw (95% CI : 261.1-314.6)	Straube D., 2020c
Field studies				
In an earthworm field study with Prosulfocarb 800 EC, no adverse effects were observed at 4000 g prosulfocarb/ha on bare soil. In a Collembola field study with GLOB1913H, no adverse effects were observed at 3960 g prosulfocarb/ha.				
Litter bag test				
-				

* 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

As there is no chronic endpoint for earthworms, *Folsomia candida* and *Hypoaspis aculeifer* for the active substance in the EFSA review report of prosulfocarb, the endpoint of the formulated product GLOB1913H were converted to active ingredient.

For the metabolite no EU data are available so the worst case assumption was made by dividing the endpoint of the parent by 10.

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 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 pre-emergence use of GLOB1913H in winter cereals and potato and the post-emergence use in winter cereals

Intended use	Potato, 4.4 L/ha		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Prosulfocarb	26.8	5.2800	5.10
Prosulfocarb sulfoxide	5.36	0.3816	14.1
GLOB1913H	31.3	6.0867	5.14
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Folsomia candida			
Prosulfocarb	8.47	5.2800	1.60
Prosulfocarb sulfoxide	1.694	0.3816	4.44
GLOB1913H	13.15	6.0867	2.16
Hypoaspis aculeifer			
Prosulfocarb	41.8	5.2800	7.92
Prosulfocarb sulfoxide	8.36	0.3816	21.9
GLOB1913H	49.0	6.0867	8.05
Intended use	Winter cereals (pre-+ post-emergence), 4 L/ha		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Prosulfocarb	26.8	4.800	5.58
Prosulfocarb sulfoxide	5.36	0.3469	15.5
GLOB1913H	31.3	5.5333	5.66
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Folsomia candida			
Prosulfocarb	8.47	4.800	1.76
Prosulfocarb sulfoxide	1.694	0.3469	4.88
GLOB1913H	13.15	5.5333	2.38
Hypoaspis aculeifer			
Prosulfocarb	41.8	4.800	8.71
Prosulfocarb sulfoxide	8.36	0.3469	24.1
GLOB1913H	49.0	5.5333	8.86
Intended use	Winter cereals (pre-+ post-emergence) + potato, 3.5 L/ha		

Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prosulfocarb	26.8	4.200	6.38
Prosulfocarb sulfoxide	5.36	0.3035	17.7
GLOB1913H	31.3	4.8413	6.47
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)
<i>Folsomia candida</i>			
Prosulfocarb	8.47	4.200	2.02
Prosulfocarb sulfoxide	1.694	0.3035	5.58
GLOB1913H	13.15	4.8413	2.72
<i>Hypoaspis aculeifer</i>			
Prosulfocarb	41.8	4.200	9.95
Prosulfocarb sulfoxide	8.36	0.3035	27.6
GLOB1913H	49.0	4.8413	10.12

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

The TER_{lt} for *Folsomia candida* due to exposure to prosulfocarb is below the trigger of 5. However, a field study is available showing that the risk for *Folsomia candida* is acceptable when using GLOB1913H as intended. A full study summary is provided in Appendix 2.

In addition, an earthworm field study with Prosulfocarb 800 EC is available where no adverse effects on earthworm abundance or biomass over a period of one year were observed at an application rate of 4000 g prosulfocarb/ha on bare soil. A full study summary is provided in Appendix 2.

The results of these studies, which lasted for one year, should also be considered in context of the environmental fate and behaviour properties of prosulfocarb, which has a short persistence in soil under field conditions with DT₅₀ values ranging from 6.5 to 13 days (EFSA, 2007). GLOB1913H is only applied once per season, so prolonged exposure of soil organisms to prosulfocarb is highly unlikely.

9.8.3 Overall conclusions

The risk for soil meso- and macro-organisms is acceptable when using GLOB1913H as intended.

zRMS comments:

PEC_{soil} values were calculated considering GAP of Roxy XL (GLOB1913H). The highest predicted environmental concentrations (PEC_{soil}) of the active substances and formulation were taken into account for the risk assessment.

For earthworms, *Folsomia candida* and *Hypoaspis aculeifer* the NOEC for active substance was assessed based on the formulation studies as there are no endpoints agreed at EU level.

For metabolite the worst case assumption (the metabolite toxicity is 10 x more toxic than parent) was proposed by Applicant and this approach was accepted.

All TER values for earthworms and *Hypoaspis aculeifer* are above the trigger value of 5 indicating acceptable risk.

The TER values for *Folsomia candida* are below the trigger value of 5.

However, the results of field study with *Folsomia candida* shows that the of GLOB1913H at an application rate of 4.4 L/ha on bare soil had no adverse effects on Collembola about one year after test item application.

Additionally, an earthworm field study with Prosulfocarb 800 EC shows no adverse effects on earthworm abundance or biomass over a period of one year at an application rate of 4000 g prosulfocarb/ha on bare soil.

Conclusion:

According to the performed risk assessment there is low chronic risk to earthworms and other non-target organisms resulting from long-term exposure to active substance following use of Roxy XL (GLOB1913H) in compliance with proposed GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on the toxicity to soil microorganisms have been carried out with prosulfocarb and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of GLOB1913H were not evaluated as part of the EU assessment of prosulfocarb. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prosulfocarb	42 d, aerobic loamy sand and clay-clay loam	No effects > 25% effect at day 42 at 5.33 and 53.3 mg/kg d.w. soil (4 and 40 kg/ha)	EFSA, 2007
N-mineralisation	Prosulfocarb sulfoxide	42 d, aerobic loamy sand and clay-clay loam	No effects > 25% effect at day 42 at 5.33 mg/kg d.w. soil	Worst case assumption: 10x more toxic than parent.
N-mineralisation	GLOB1913H	28 d, aerobic loamy sand	No effects > 25% at 59.8 mg/kg d.w. soil	Hammersfahr U., 2020

* Based on standard assumptions of soil bulk density 1.5 g/cm³ and incorporation depth of 5 cm.

9.9.1.1 Justification for new endpoints

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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 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 6 also covers the risk for soil organisms from all other intended uses in groups 4 and 5 (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the pre-emergence use of GLOB1913H in winter cereals and potato and the post-emergence use in winter cereals

Intended use	Winter cereals (pre + post-emergence), potato		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Prosulfocarb	53.3 (at 42 d)	5.2800	yes
Prosulfocarb sulfoxide	5.33 (at 42 d)	0.3816	yes
GLOB1913H	59.8 (at 28 d)	6.0867	yes

9.9.3 Overall conclusions

The risk for soil microorganisms is acceptable when applying GLOB1913H according to the intended uses.

zRMS comments:

The predicted environmental concentrations in soil (PEC_{soil}) of the active substance, metabolite and formulation were taken into account for the risk assessment.

For the assessment of risk to micro-organisms for formulation the endpoint from study presented in Appendix 2 was used. For active substances the EU agreed endpoint was used. For metabolite the worst case assumption (the metabolite toxicity is 10 x more toxic than parent) was proposed and accepted.

Conclusion:

Since no effects (> 25%) were seen at application rates far higher than the values of PEC_{soil} for active substances, metabolite and formulation it can be concluded that application of Roxy XL (GLOB1913H), according to the GAP, will not cause any detrimental effect to soil micro-organisms

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with prosulfocarb and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of GLOB1913H were not evaluated as part of the EU assessment of prosulfocarb. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Brassica napus</i> (oilseed rape) _d ¹⁾ <i>Phaseolus vulgaris</i> (French bean) _d ²⁾ <i>Daucus carota</i> (carrot) _d ³⁾ <i>Solanum lycopersicum</i> (tomato) _d ⁴⁾ <i>Echinochloa crus-galli</i> (cockspur grass) _m ⁵⁾ <i>Lolium perenne</i> (perennial ryegrass) _m ⁶⁾	GLOB1913H	21 d Seedling emergence	¹⁾ ER ₅₀ = 1127 mL/ha ²⁾ ER ₅₀ > 4400 mL/ha ³⁾ ER ₅₀ > 1467 mL/ha ⁴⁾ ER ₅₀ > 1325 mL/ha ⁵⁾ ER ₅₀ = 960 mL/ha ⁶⁾ ER ₅₀ = 72.7 mL/ha	Bützler R., 2021a
<i>Brassica napus</i> (oilseed rape) _d ¹⁾ <i>Phaseolus vulgaris</i> (French bean) _d ²⁾ <i>Daucus carota</i> (carrot) _d ³⁾ <i>Amaranthus retroflexus</i> (red-root amaranth) _d ⁴⁾ <i>Avena sativa</i> (oat) _m ⁵⁾ <i>Lolium perenne</i> (perennial ryegrass) _m ⁶⁾	GLOB1913H	21 d Vegetative vigour	¹⁾ ER ₅₀ > 4400 mL/ha ²⁾ ER ₅₀ > 4400 mL/ha ³⁾ ER ₅₀ > 4400 mL/ha ⁴⁾ ER ₅₀ > 4400 mL/ha ⁵⁾ ER ₅₀ > 4400 mL/ha ⁶⁾ ER ₅₀ = 3749 mL/ha	Bützler R., 2021b

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

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9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Since prosulfocarb is volatile, dry deposition at the edge of the field was included in the calculation of the PER calculations using deposition rates calculated with the UBA tool EVA 3.0 rev2h (see table below). These deposition rates were converted to mL product/ha and added to the PER_{off-field} that was calculated based on the application rate and the drift rate.

Table 9.10-2: Dry deposition rates for prosulfocarb (from EVA 3.0 rev2h)

Application pattern	Spray drift scenario/interception	Time after application (hours)	Deposition rates (g/ha)				
			1 m	3 m	5 m	10 m	20 m
1 x 3960 g a.s./ha	Arable crops/0%	0-24	0.2910	0.2613*	0.2340	0.1782	0.1034
1 x 3600 g a.s./ha	Arable crops/0%	0-24	0.2645	0.2375	0.2128	0.1620	0.0940
1 x 3150 g a.s./ha	Arable crops/0%	0-24	0.2315	0.2078	0.1862	0.1418	0.0822

*intrapolated

Table 9.10-3: Assessment of the risk for non-target plants due to the use of GLOB1913H in winter cereals and potato

Intended use		Potato				
Active substance/product		GLOB1913H				
Application rate (mL/ha)		1 × 4400				
MAF		-				
Test species	ER ₅₀ (mL/ha)		Drift rate	PER _{off-field} * (mL/ha)	TER criterion: TER ≥ 5	
<i>Lolium perenne</i>	72.7		2.77%	122.2 (=121.88 + 0.291)	0.60	
Intended use		Winter cereals (pre + post-emergence)				
Active substance/product		GLOB1913H				
Application rate (mL/ha)		1 × 4000				
MAF		-				
Test species	ER ₅₀ (mL/ha)		Drift rate	PER _{off-field} * (mL/ha)	TER criterion: TER ≥ 5	
<i>Lolium perenne</i>	72.7		2.77%	111.1 (=110.8 + 0.265)	0.65	
Intended use		Winter cereals (pre + post-emergence), potato				
Active substance/product		GLOB1913H				
Application rate (mL/ha)		1 × 3500				
MAF		-				
Test species	ER ₅₀ (mL/ha)		Drift rate	PER _{off-field} * (mL/ha)	TER criterion: TER ≥ 5	
<i>Lolium perenne</i>	72.7		2.77%	97.2 (=96.95 + 0.232)	0.75	

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

fall below the relevant trigger.
*including dry deposition

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 9.10-4: Risk assessment for non-target terrestrial plants due to the use of GLOB1913H in winter cereals and potato considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Potato			
Active substance/product		GLOB1913H			
Application rate (mL/ha)		1 × 4400			
MAF		-			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (mL/ha)	PER_{off-field} 50 % drift red. (mL /ha)	PER_{off-field} 75 % drift red. (mL /ha)	PER_{off-field} 90 % drift red. (mL /ha)
1	2.77	122.2 (=121.88 + 0.29)	61.1	30.5	12.2
3	1	44.26 (= 44.0 + 0.26)	22.1	11.1	4.43
5	0.57	25.3 (= 25.08 + 0.23)	12.7	6.33	2.53
10	0.29	12.9 (= 12.76 + 0.18)	6.48	3.23	1.29
Toxicity value		TER			
ER ₅₀ = 72.7 mL/ha		criterion: TER ≥ 5			
1		0.60	1.19	2.38	5.95
3		1.64	3.29	6.57	-
5		2.87	5.74	-	-
10		5.62	-	-	-
Intended use		Winter cereals (pre + post-emergence)			
Active substance/product		GLOB1913H			
Application rate (mL/ha)		1 × 4000			
MAF		-			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (mL/ha)	PER_{off-field} 50 % drift red. (mL /ha)	PER_{off-field} 75 % drift red. (mL /ha)	PER_{off-field} 90 % drift red. (mL /ha)

1	2.77	111.1 (=110.8 + 0.26)	55.5	27.8	11.1
3	1	44.3 40.24 (= 40.0 + 0.24)	20.12	10.06	4.02
5	0.57	25.3 23.01 (= 22.8 + 0.21)	11.51	5.75	2.30
10	0.29	12.9 11.76 (= 11.6 + 0.16)	5.88	2.94	1.18
Toxicity value ER ₅₀ = 72.7 mL/ha		TER criterion: TER ≥ 5			
1		0.65	1.31	2.62	6.55
3		1.81	3.61	7.23	-
5		3.16	6.32	-	-
10		6.18	-	-	-
Intended use Active substance/product Application rate (mL/ha) MAF		Winter cereals (pre + post-emergence), potato GLOB1913H 1 × 3500 -			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (mL/ha)	PER_{off-field} 50 % drift red. (mL /ha)	PER_{off-field} 75 % drift red. (mL /ha)	PER_{off-field} 90 % drift red. (mL /ha)
1	2.77	97.2 (= 96.95 + 0.23)	48.6	24.3	9.72
3	1	35.2 (= 35.0 + 0.21)	17.6	8.80	3.52
5	0.57	20.1 (= 19.95 + 0.19)	10.1	5.03	2.01
10	0.29	10.3 (= 10.15 + 0.14)	5.15	2.57	1.03
Toxicity value ER ₅₀ = 72.7 mL/ha		TER criterion: TER ≥ 5			
1		0.75	1.50	2.99	7.48
3		2.06	4.13	8.26	-
5		3.61	7.22	-	-
10		7.06	-	-	-

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

A buffer zone of 1 m in combination with 90% drift reducing techniques or a buffer zone of 3 m in combination with 75% drift reducing techniques or a buffer zone of 5 m in combination with 50% drift reducing techniques or a buffer zone of 10 m without drift reduction is needed to protect non-target plants after application of GLOB1913H according to the intended uses.

zRMS Comments:

The risk assessment was based on the results of studies for formulation presented in Appendix 2 (vegetative vigour and on seedling emergence). *Lolium perenne* (perennial ryegrass) was the most sensitive species in both tests. In the deterministic approach, the risk assessment was based on the most sensitive species.

The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA (2000) from the spray-drift predictions of Ganzelmeier & Rautmann (2000) to calculate maximum off-field predicted environmental rates (PER_{off-field}).

Since prosulfocarb is volatile, Applicant proposed to add dry deposition at the edge of the field. The calculation of the PER was performed using deposition rates calculated with the UBA tool EVA 3.0 rev2h. These PER_{off-field} values represent the worst-case and can be used in the risk assessment.

The TER value is above the trigger of 5 when:

- **in potato:** 10 m buffer strip or 5 m buffer strip with 50% drift reduction or 3 m buffer zone with 75% drift reduction or 90% drift reduction is applied;
- **in winter cereals** (pre and post emergence, **4000 mL/ha**): 10 m buffer strip or 5 m buffer strip with 50% drift reduction or 3 m buffer zone with 75% drift reduction or 90% drift reduction is applied;
- **in winter cereals** (pre and post emergence, **3500 mL/ha**): 10 m buffer strip or 5 m buffer strip with 50% drift reduction or 3 m buffer zone with 75% drift reduction or 90% drift reduction is applied;

Conclusion:

The risk for the terrestrial plants following use of Roxy XL (GLOB1913H) could be considered as low when the following risk mitigation measures are applied:

- 10 m buffer strip or
- 5 m buffer strip with 50% drift reduction or
- 3 m buffer zone with 75% drift reduction or
- 90% drift.

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

Not available, not required.

9.12 Monitoring data (KCP 10.8)

Not required.

9.13 Classification and Labelling

Classification of GLOB1913H was performed according to the EU Regulation 1272/2008 (CLP labelling).

Acute toxicity tests were performed with the formulation GLOB1913H. Reference is made to the table 9.5-2 provided under point 9.5 of section B9 for a summary table of the acute toxicity studies to daphnia, algae and aquatic plants (*Lemna* and *Myriophyllum*) performed with GLOB1913H. No chronic toxicity

data with the formulation is available. As all EC₅₀ values were ≤ 1 mg/L, GLOB1913H must be classified as Acute Aquatic Toxicity Category 1; **H400**.

For chronic classification, the summation method was applied. The product GLOB1913H should **be classified** as category 2 for chronic aquatic toxicity; **H411**. For more details, reference is made to the Part C.

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 respect an unsprayed buffer zone of 5 m to surface water bodies for the use in winter cereals. (*taking into account the relevant scenarios for IE*)

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies for the use in winter cereals. (*taking into account the relevant scenarios for CZ, BE, HU, IE, ~~PL~~, SK*)

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 5 m to surface water bodies for the use in potatoes. (*taking into account the relevant scenarios for CZ, BE, IE, ~~PL~~, SK*)

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies for the use in potatoes. (*taking into account the relevant scenarios for HU*)

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies for the use in winter cereals (3,5 L/ha) (*taking into account the relevant scenarios for PL*)

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 12 m including a 10 m vegetated filter strip to surface water bodies for the use in winter cereals (4 L/ha) (*taking into account the relevant scenarios for PL*)

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 14 m including a 10 m vegetated filter strip to surface water bodies for the use in potatoes (*taking into account the relevant scenarios for PL*)

SPe3: To protect non-target plants respect an unsprayed buffer zone of 10 m or an unsprayed buffer zone of 3 m in combination with 75% drift reducing nozzles or an unsprayed buffer zone of 5 m in combination with 50% drift reducing nozzles or ~~an unsprayed buffer zone of 1 m in combination with~~ 90% drift reducing nozzles to non-agricultural land.

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
KCA 8.1.3	Sacker, D.	2008a	The bioaccumulation potential of prosulfocarb in earthworm (<i>Eisenia foetida foetida</i>). ENV8333/040822 Chemex Environmental International Ltd GLP Unpublished	N	Globachem NV
KCA 8.2.6.1	Sacker D.	2008b	The growth inhibition of Prosulfocarb Technical to the algae <i>Scenedesmus subspicatus</i> over a 72 hour exposure period Chemex Environmental International Ltd GLP Unpublished	N	Syngenta Globachem access
KCA 8.2.6.1	Juckeland, D.	2012a	Effects of Prosulfocarb sulfoxide on <i>Chlamydomonas reinhardtii</i> in an algal growth inhibition test 12 10 48 057 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.6.1	Juckeland, D.	2012b	Effects of Prosulfocarb sulfoxide on <i>Chlorella vulgaris</i> in an algal growth inhibition test 12 10 48 059 W Biochem Agrar GmbH	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Unpublished		
KCA 8.2.6.2	Juckeland, D.	2012c	Effects of Prosulfocarb sulfoxide on <i>Anabaena flos-aquae</i> in an algal growth inhibition test 12 10 48 058 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.6.2	Juckeland, D.	2012d	Effects of Prosulfocarb sulfoxide on <i>Navicula pelliculosa</i> in an algal growth inhibition test 12 10 48 053 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.6.2	Juckeland, D.	2012e	Effects of Prosulfocarb sulfoxide on <i>Skeletonema costatum</i> in an algal growth inhibition test 12 10 48 060 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.7	Juckeland, D.	2013b	Effects of prosulfocarb sulfoxide on <i>Myriophyllum spicatum</i> in a growth inhibition test under semi-static conditions 13 10 48 017 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.1.2.2	Schroeer, A., Grimm, T.	2011	Field monitoring of hares and rabbits in cereal fields Rifcon GmbH, Heidelberg, Germany BASF Report No.: M-442679-02-1 Amended: 2012-01-09 Unpublished	N	BASF <i>Globachem access</i>

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 10.1.2.2	Voigt, U., Zaccaroni, M.	2013	Generic field monitoring of hares in a mixed landscape in Germany University of Veterinary Medicine Hannover, Hannover, Germany Bayer Report No.: BAR/FS069 Not GLP Unpublished	N	Bayer <i>Globachem access</i>
KCP 10.1.2.2	Katzschner, I.	2022	Small omnivorous mammal PT study on freshly drilled cereal fields in Central Europe (Germany) Report No.: R2140054 Rifcon GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Siche, O., Wydra V.	2021a	GLOB1913H: Acute toxicity to <i>Daphnia magna</i> in a static 48-hour immobilization test 155401220 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Siche, O., Wydra V.	2021b	GLOB1913H: Acute toxicity to <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test 155401210 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Siche, O., Wydra V.	2021c	GLOB1913H: Toxicity to the aquatic plant <i>Lemna gibba</i> in a static growth inhibition test 155401240 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Juckeland, D.	2013a	Effects of Prosulfocarb 800 EC on <i>Myriophyllum spicatum</i> in a growth inhibition test under semi-static test conditions 13 10 48 018 W Biochem Agrar GmbH	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Unpublished		
KCP 10.2.1	Siche, O., Wydra V.	2021d	GLOB1913H: Toxicity to the aquatic plant <i>Myriophyllum spicatum</i> in a static growth inhibition test with a prior rooting phase 155401215 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1	Sekine, T.	2020	GLOB1913H: Effects (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory. 155401035 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP10.3.1.1.1	Chwiesko, D.	2021	GLOB1913H: Acute contact and oral toxicity to bumblebees (<i>Bombus terrestris</i> L.) in the laboratory. 155401105 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.2	Berg, C.	2021a	GLOB1913H: Chronic oral toxicity test on the honey bee (<i>Apis mellifera</i> L.) in the laboratory. 155401136 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.3	Colli, M.	2021	Effects of GLOB1913H on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure BT273/20 Biotechnologie BT S.r.l. GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2	Leopold, J.	2020a	GLOB1913H: Effects on the predatory mite <i>Typhlodromus Pyri</i> (Acari: Phytoseiidae), extended laboratory study –dose response test- 155401062 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Leopold, J.	2020b	GLOB1913H: Effects on the parasitoid <i>Aphidius rhopalosiphi</i>) extended laboratory study –dose response test- 155401002 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Berg, C.	2021b	GLOB1913H: Effects on the reproduction of rove beetles <i>Aleochara bilineata</i> -extended laboratory study- –dose response test- 155401071 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Berg, C.	2021c	GLOB1913H: Effects on the carabid beetle <i>Poecilus cupreus</i> -extended laboratory study- –dose response study- 155401007 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Röhlig, U.	2022a	Effects of GLOB1913H on the parasitic wasp <i>Aphidius rhopalosiphi</i> DeStephani-Perez under extended laboratory conditions (under semi-field conditions aged residues on potted barley plants) 22 48 NAR 0003 BioChem agrar GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2	Röhlig, U.	2022b	Effects of GLOB1913H on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in an extended laboratory test (under semi-field conditions aged-residues on potted bean plants) 22 48 NTR 0002 BioChem agrar GLP Unpublished	N	Globachem NV
KCP 10.4.1.1	Straube, D.	2020	GLOB1913H: Effects on reproduction and growth of earthworms <i>Eisenia Andrei</i> in artificial soil. 155401022 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.1.2	Schulz, L.	2015	Effects of prosulfocarb 800 g/L EC on earthworms under field conditions. Biochem Agrar Report Number 14 10 48 008 F GLP Unpublished	N	Globachem NV
KCP 10.4.2.1	Straube, D.	2021	GLOB1913H: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil. 155401089 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2.1	Straube, D.	2020	GLOB1913H: Effects on reproduction of the collembola <i>Folsomia candida</i> in artificial soil. 155401016 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2.2	Henkes, G.	2022	Prosulfocarb: GLP-compliant Collembola field study in Germany 2140003 Rifcon GmbH GLP	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.5	Hammesfahr, U.	2020	GLOB1913H: Effects on the activity of the soil microflora in the laboratory (Nitrogen transformation) 155401080 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.6	Bützler, R.	2021a	GLOB1913H: Effects on terrestrial (non-target) plants: seedling emergence and seedling growth test 155401086 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 10.6	Bützler, R.	2021b	GLOB1913H: Effects on terrestrial (non-target) plants: vegetative vigour test 155401087 Ibacon GmbH GLP Unpublished	N	Globachem NV

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			None		

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No new studies submitted.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

Comments of zRMS:	The submitted study was evaluated in Central Zone and was not accepted. Please refer to p. 9.3
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Reference: KCA 8.1.3

Report The bioaccumulation potential of prosulfocarb in earthworm (*Eisenia foetida*), Sacker D., 2008a, ENV8333/040822

Guideline(s): Yes, OECD Guideline for Testing of Chemicals 207: Earthworm acute toxicity tests (1984), OECD Guideline for Testing of Chemicals 222: Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*) (2004), OECD Guidelines for Testing of Chemicals 305, Bioconcentration: Flow-through Fish Test. (2006), OECD Guidelines for Testing of Chemicals, Bioaccumulation in sediment-dwelling Benthic Oligochaetes (Proposed December 2007)

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive Summary

The study was undertaken to determine the bioconcentration and subsequent depuration of prosulfocarb in earthworms (*Eisenia foetida*). Calculated bioconcentration factors (BCF) were based on analyses of sediment and worm tissues for prosulfocarb. The study was run with concentrations of 0.75 (low) and 7.50 (high) mg prosulfocarb/kg, and a control.

Based on the results from the data generated in the high test concentration (7.5 mg prosulfocarb/kg) the steady-state BCF was determined to be 1.26. The calculated uptake rate constant (K_1) was 8.60, and the depuration rate constant (K_2) was calculated to be 6.19. The kinetic BCF was 1.39.

It was concluded that prosulfocarb has very little potential for bioaccumulation in earthworms.

Materials

Test material:	Prosulfocarb 800 EC
Description:	Yellow liquid
Lot/Batch#:	DNA0259
Purity:	796.4 g/L

Stability of test compound:	Stable under standard conditions
Expiry date:	June 2009
Density:	1.026 g/mL
Test concentrations:	Controls and two concentrations of 0.75 (low) and 7.5 (high) mg prosulfocarb/kg (0.6 and 6.0 mg a.i./kg)
Analysis of test concentration:	Yes, analysis of prosulfocarb in sediment and earthworm tissue on days 1, 2, 4, 7, 10 and 14 (uptake phase) and days 1, 2, 5, 7 and 14 (depuration phase) using HPLC-UV analysis
Test organisms	
Species:	Earthworms (<i>Eisenia foetida foetida</i>)
Age:	Not reported
Wet weight range at test starts:	mean wet weight: 194 mg
Source:	Obtained from Blades Biological Ltd., Kent, UK
Acclimatisation period:	6 weeks
Feeding:	Air dried, ground and sieved horse manure, weekly
Test design	
Test vessels:	2 L plastic containers, each with 750 g of wet artificial sediment
Artificial sediment:	75% quartz sand, 20% kaolin and 5% sphagnum peat moss, adjusted to pH of 5.5 to 6.5 using calcium carbonate
Replicates:	12 per treatment and control
No of worms/arena:	5
Environmental conditions	
Test temperature:	22.5 – 23.0°C
Soil pH:	6.3 - 7.3
Soil moisture content:	Not reported. Water content adjusted with deionised water
Lightning:	16 h light (daylight fluorescent tubes) and 8 h dark at approx. 400 to 800 lux
Length of the test:	Uptake: 14 days, Depuration: 14 days

Study Design and Methods

Experimental dates: 28 May 2008 to 26 June 2008

Exposure phase

The formulated sediment (according OECD 207) was prepared 8 days prior to the addition of the test material, and the test material was added 2 days before the addition of the worms.

An initial stock solution of 100 mg prosulfocarb/L was prepared in deionised water. Appropriate volumes of this solution were diluted to 1000 mL with deionised water and mixed with 7500 g dry weight of the prepared sediment to give final test concentrations of 0.75 and 7.5 mg prosulfocarb/kg. The control sediment was prepared with deionised water only.

Five earthworms were placed in each of the control and test vessels, containing 750 g of the wet artificial sediment. Observations and records of mortalities and abnormal behaviour were made on days 1, 2, 4, 7, 10 and 14, and sediment and earthworm samples were taken at the same time.

Depuration phase

The 14-day uptake phase was followed by a 14-day depuration phase. Observations and records of mortalities and abnormal behaviour during this phase were made on days 1, 2, 5, 7 and 14, and sediment and earthworm samples were taken at the same time.

Sampling and analysis

In both the uptake and depuration phase, 50 g samples of sediment were taken from each appropriate container and refrigerated until extraction could be conducted. Five earthworms were removed from a replicate test container, rinsed in deionised water, blotted dry and weighed, before being humanely killed and ground with a pestle and mortar prior to extraction. The concentration of prosulfocarb in extracted samples was determined using HPLC-UV analysis.

Physical and chemical parameters

The temperatures of the test vessels were measured daily. Sediment pH was measured on days 0, 7 and 14 in both the uptake and depuration phase.

Calculation of Bioconcentration Factors (BCF)

BCF_{ss} (steady-state)

Steady-state was defined as three successive analyses of the test substance in/on earthworms made on samples taken at intervals of at least two days that are within 20% of each other, and was determined as days 4, 7 and 10.

BCF_{ss} was calculated from:

$$C_w \text{ at steady-state (mean)}/C_s \text{ at steady-state (mean)}$$

Where C_s is the average concentration of prosulfocarb in sediment (3.85 mg/kg) and C_w is the average concentration of prosulfocarb in worms (4.85 µg/g) in these samples.

BCF_k (kinetic)

The kinetic bioconcentration factor was calculated from:

$$BCF_k = k_1/k_2$$

Where k_1 is the uptake rate constant and k_2 is the depuration constant

The uptake rate constant (k_1) was calculated from:

$$k_1 = c_w k_2 / c_s \times [1 - e^{(-k_2 t)}]$$

$k_2 t$ = depuration constant at time t

The depuration constant was calculated from:

$$k_2 = \ln(c_{w1}/c_{w2})/t_2 - t_1$$

t = time in days

Results and Discussion

The results of this study were based on the data generated in the highest test concentrations, as the concentration of prosulfocarb accumulated in the earthworms in the low test concentration were below the limit of detection (0.01 µg prosulfocarb/g) of the analytical method. No mortalities were observed in the control replicate at the end of the test period, and no abnormal behaviour was recorded in either of the test concentration or the control.

Steady-state was determined as days 4, 7 and 10. The BCF_{ss} for prosulfocarb in the high concentration was calculated to be 1.26. The BCF_k for prosulfocarb in the high concentration was calculated to be 1.39. Uptake (k_1) and depuration (k_2) rate constants for prosulfocarb in the high concentration were calculated to be 8.60 and 6.19, respectively.

Although the depuration phase lasted for 14 days, analysis of the earthworms demonstrated a reduction to 0.00 mg prosulfocarb/kg within 2 days and therefore analysis of further extracts was not reported.

The concentrations of prosulfocarb in earthworm tissue and sediment during the 14-day exposure phase followed by the 14-day depuration phase are given in the table below:

Uptake and depuration of prosulfocarb in the earthworm

Day		Mean concentration of prosulfocarb					
		Sediment ^a (mg prosulfocarb/kg)			Earthworm (µg prosulfocarb/g)		
		Control	0.75 mg prosulfocarb/kg	7.5 mg prosulfocarb/kg	Control	0.75 mg prosulfocarb/kg	7.5 mg prosulfocarb/kg
Uptake phase	0	-	-	-	0.00	-	-
	1	0.00	0.46	5.33	0.00	0.00	2.03
	2	0.00	0.52	4.79	0.00	0.00	7.34
	4	0.00	0.34	4.13	0.00	0.00	4.28
	7	0.00	0.30	4.18	0.00	0.12	5.89
	10	0.00	0.27	4.17	0.00	0.00	4.39
	14	0.00	0.26	4.17	0.00	0.00	0.97
Depuration phase	1	0.00	0.00	0.00	0.00	0.00	0.07
	2 ^b	0.00	0.00	0.00	0.00	0.00	0.00

^aGeometric means

^bFurther extract analysis not reported since concentrations of prosulfocarb had reduced to 0.00 mg/kg within 2 days

The steady-state and kinetic BCF values, and uptake and depuration rate constants are given in the table below.

Steady-state and kinetic BCF, and uptake (k_1) and depuration (k_2) constants of prosulfocarb during the 28-day bioconcentration/depuration study exposing earthworms to prosulfocarb

	0.75 mg prosulfocarb/kg (0.6 mg a.s./kg)	7.5 mg prosulfocarb/kg (6 mg a.s./kg)
BCF_{ss}	Unable to determine*	1.26
k_1		8.60

k₂		6.19
BCF_k		1.39

*Prosulfocarb concentrations were below the limit of detection (0.01 µg/g) of the method

Conclusions

Based on the results from the data generated in the high test concentration (7.5 mg prosulfocarb/kg) the steady-state BCF was determined to be 1.26. The calculated uptake rate constant (k_1) was 8.60, and the depuration rate constant (k_2) was calculated to be 6.19. The kinetic BCF was 1.39.

It was concluded that prosulfocarb has very little potential for bioaccumulation in earthworms.

Comments of zRMS:	Has been used as a supporting study
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Reference:	KCP 10.1.2.2
Report	Field monitoring of hares and rabbits in cereals fields. Schroeer A., Grimm, T. 2011. Amended 2012-01-09. Study No. 442679-02-1
Guideline(s):	No, no official test guidelines available at present
Deviations:	NA
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

According to the EU directive 91/414/EEC and Regulation (EC) 1107/2009 the effects of crop protection products on wild vertebrates have to be assessed. Effects are depending on the inherent toxicity of those products and their exposure to wild vertebrates. This study aimed at assessing the frequency of occurrence and abundance of brown hares (*Lepus europaeus*) and European rabbits (*Oryctolagus cuniculus*) in cereal fields (BBCH <30) and consequently their potential of being exposed to plant protection products applied

Materials and Methods

In order to assess the potential exposure risk of hares and rabbits to plant production products in cereal fields, a total number of 90 fields were monitored in two regions of Northern Germany. Monitoring of hares and rabbits was conducted via spotlight scanning at night. For this purpose alongside of each study field a transect was defined. Two persons drove slowly (mean speed 12.4 km/h) along these transects in a car and thereby the study field or a part of the study field was surveyed by means of a hand-held spotlight. In this illuminated area (approximate depth of 150 m) all observed hares and rabbits were recorded. Spotlight scanning was carried out at four different sets of study fields, which were monitored on two consecutive nights each, resulting in a total of eight night surveys. Spotlight scanning started approximately 1.5 hours after sun set and was finished before dawn. The abundance of hares and rabbits per 100 hectare surveyed area was calculated as well as the abundance per hectare. Additionally, the frequency of occurrence (FO) was determined based on the total number of study fields (field approach; FO_{field} n=90) and on the total number of surveys (survey approach; FO_{survey} n=180).

Study Sites

Two study sites each containing two sets of individual study fields were selected in Germany (Lower Saxony (Set 1/2) and Schleswig-Holstein (Set 3/4)). The presence of hares and rabbits in these regions was confirmed through recordings in previous years (e.g. by information given in WTE 2009, Landesjagdverband Schleswig-Holstein 2007) and/or information gathered from local game keepers. In total 90 study fields were chosen, in which the BBCH development stage of winter cereals was less than

30, thus providing appropriate conditions for spotting lagomorphs in the crops. Every set of study fields comprised between 20 and 26 cereal fields, on which hares and rabbits were counted during two consecutive nights.

Study field details

Observation period	10 April – 21 April 2011		
BBCH growth stage	23-29		
Number of cereal fields	90		
Study fields	Number of study fields monitored	Surveyed area [ha]	Mean size of study fields [ha] \pm SD
Set 1 (I-26)	26	91.96	6.23 \pm 3.57
Set 2 (B1-B22)	22	68.69	5.20 \pm 5.69
Set 3 (G1-G20)	20	126.83	17.39 \pm 11.58
Set 4 (H1-H22)	22	144.84	20.76 \pm 14.35
Total area	90	432.32	12.01 \pm 11.62

All data were collated and analysed using standard spread sheet applications. The surveyed area for each study field was calculated by multiplying the transect length with the range of the hand-held spotlight (=150 m) and thus quadratic dimensions were assumed. If the latter was not true marginal subareas were identified by the means of Google Earth Pro and either subtracted or added to the calculated surveyed area. In addition, if the study field itself or parts of the study field were less than 150 m deep, exact measures were taken and used for calculating the illuminated area instead.

Results

Frequency of occurrence and abundance of hares in cereal fields

Hares were counted in 67.78% (61 out of 90) of the cereal fields surveyed (in 47.92% of the fields surveyed in Lower Saxony and in 90.48% of the fields surveyed in Schleswig-Holstein). Hares were present in 86 out of 180 field surveys (FOsurvey = 47.78%). The mean abundance of hares in the cereal fields was 22.22 hares per 100 ha.

Frequency of occurrence and abundance of rabbits in cereal fields

Rabbits were counted in 8.89% (8 out of 90) of the cereal fields surveyed (in 4.17% of the fields surveyed in Lower Saxony and in 14.29% of the fields surveyed in Schleswig-Holstein). Rabbits were observed in 12 out of 180 fields surveyed (FOsurvey = 6.67%). The mean abundance of rabbits in the cereal fields was 2.27 rabbits per 100 ha.

Frequency of occurrence and abundance of hares in cereal fields in Germany

Observation period	10 April – 21 April 2011			
BBCH growth stage	23-29			
Number of cereal fields	90			
Number of surveys	180			
	FO _{field} [%]	FO _{survey} [%]	Abundance [hares / 100 ha surveyed area] \pm SD	Abundance [hares / ha surveyed area]
Total	67.78	47.78	22.22 \pm 9.44	0.22
Set 1	57.69	-	19.58 \pm 10.77	0.19
Set 2	36.36	-	12.38 \pm 7.21	0.12
Set 3	95.00	-	33.12 \pm 2.23	0.33
Set 4	86.36	-	23.82 \pm 0.49	0.23

Frequency of occurrence and abundance of rabbits in cereal fields in Germany

Observation period	10 April – 21 April 2011			
BBCH growth stage	23-29			
Number of cereal fields	90			
Number of surveys	180			
	FO _{field} [%]	FO _{survey} [%]	Abundance [rabbits / 100 ha surveyed area]	Abundance [rabbits / ha surveyed area]
			± SD	
Total	8.89	6.67	2.27 ± 1.51	<0.1
Set 1	0.00	-	0.00 ± 0.00	0.0
Set 2	9.09	-	2.91 ± 0.00	<0.1
Set 3	5.00	-	2.37 ± 0.00	<0.1
Set 4	22.73	-	3.80 ± 0.49	<0.1

Conclusion

Both hares and rabbits use cereal fields at BBCH growth stages below 30 as a night time refuge e.g. for foraging and probably reproduction (in hares). However, the mean abundance of hares detected in cereal fields was about 10 times higher than the mean abundance of rabbits. This study supports the conclusion that the Brown hare is the adequate focal species to represent the large herbivorous mammal in winter cereal fields instead of the rabbit.

Comments of zRMS:	Has been used as a supporting study
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Reference:	KCP 10.1.2.2
Report	Generic Field Monitoring of hares in a mixed landscape in Germany. Voigt, U., Zaccaroni, M. 2013. BAR/FS069
Guideline(s):	No, no official test guidelines available at present
Deviations:	NA
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

According to EU directive 91/414/EEC and EC/2009/1107, the effects of crop protection products on wild vertebrates have to be assessed. This includes the medium herbivorous mammal, such as the hare. The Brown hare (*Lepus europaeus*) is a mammalian herbivore with wide distribution in the agricultural land-scape of Europe. The aim of this study was to investigate the habitat selection, activity pattern and home range distribution of the Brown hare in a plain area characterized by intensive agriculture in an intensive agriculture landscape characterized by cereal, sugar beet and maize growing. Specifically for risk assessments of plant protection products, this study provides quantitative data on time budgets (including proportion of foraging time, PT, in different habitats) of hares in an intensive agricultural landscape in Northern Germany.

Material and Methods

The study was conducted in a study area of 1,125 hectares located in the Börde landscape which is part of the Central European loess zone at the north of the German Central uplands and is directly adjacent to the

composition. From March to June the land use and growth stages of each field were mapped weekly by direct observations.

In the study area the density of hare was 22.6 hares/100 ha. In March 2012, 24 hares (11 females, 13 males) were captured and tagged with GPS collars. Collars were scheduled to collect an Extensive Tracking (ET) of 3 positions every 24 hours for 15 weeks, and 10 intensive tracking (IT) sessions from the second half of March to the end of June 2012. IT sessions occurred every 5 days for the first 4 weeks, and from 1. May every 15 and 10 days resp. till the end of June.

To analyse the proportion of foraging time in different habitats, the GPS fixes collected during the IT were used. The IT sessions covered the night-time from sunset to sunrise. During these sessions one position fix was recorded every ten minutes by GPS, additionally, the activity of the hares was recorded for each position. By identifying positions where the animals were resting a more precise estimate of the proportion of time spent foraging inside the crops was made.

Results

The landscape was composed mainly of agricultural crops, which occupied around 83% of the total study area of 1,125 ha. The landscape composition changes throughout the study period, according to the growth of the crops. Over the study period, a maximum of 43.6% of the fields were cereals, sugar beets occupied at maximum 25.6% of the whole surface, and maize occupied up to 16.5% of the studied area. Other annual crops, such as potatoes, carrot, and meadow constituted between 2.6% and 5.5% of the total area. Bare soils and the off-crop area ranged together from 40.2% to 11.5% of the total surveyed area depending on the month considered.

Monthly habitat availability (% of total surface – 1,125 ha)

Dates (from-to)	19/03-15/04	16/04-13/05	14/05-10/06	11/06-02/07	Mean
Month (weeks)	3 (12-15)	4 (16-19)	5 (20-23)	6 (24-26)	
Cereals	43.6	39.4	37.2	37.1	39.3
Sugar beet	8.8	25.5	25.6	25.6	21.4
Maize	1.3	10.9	16.4	16.5	11.3
Oilseed rape	3.6	3.7	3.7	3.7	3.6
Others	2.6	4.1	5.2	5.5	4.3
Bare soil	23.0	4.2	0.3	0.0	6.9
Non-crop Off-crop	17.2	12.3	11.5	11.5	13.1

On a crop basis, consolidated by month, cereals were used at an average rate of 49 %, varying from 73 % (March/April) to 6.7 % (June). Sugar beets were used at an average rate of 35 %, varying from 4.8 % (March/April) to 82.2 % (June). Thus, from March to June a decrease of use of cereals corresponded to an increase of use of sugar beets. Maize was used, on average, only 0.9 % of the foraging time with the use being only between March and May. Oilseed rape was also used at low intensity (0.9 %) this crop being used only in March and April. Bare soil was used exclusively at the beginning of the study period.

Monthly habitat use by hares (percent of active potential feeding time → PT_{foraging})

Dates (from-to)	19/03-15/04	16/04-13/05	14/05-10/06	11/06-02/07	Mean
Month (weeks)	3 (12-15)	4 (16-19)	5 (20-23)	6 (24-26)	
Cereals	73.1	79.3	37.5	6.7	49.1
Sugar beet	4.8	12.1	41.6	82.2	35.2
Maize	0.0	0.6	2.9	0.0	0.9
Oilseed rape	3.7	0.0	0.0	0.0	0.9
Others	1.3	3.4	12.0	1.1	4.5
Bare soil	7.8	0.0	0.0	0.0	2.0
Non-crop Off-crop	9.2	4.6	6.0	10.0	7.5
Number of hares	12	6	5	2	6.25

Conclusion

The utilization of crops in agricultural landscapes by hares depends on the landscape composition. Based on this study in an intensive agriculture landscape, hares spend almost 90% of their foraging time inside crops between March and June. Usage of the crops varied over time according to the type and the growth stage of each crop. In particular from March to June there was a decrease in the use of cereals and an increase in the use of sugar beet.

Comments of zRMS:	Has been used as a supporting information.
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Reference:	KCP 10.1.2.2
Report	Small omnivorous mammal PT study on freshly drilled cereal fields in Central Europe (Germany). Katzschner I. 2022. Study No. R2140054.
Guideline(s):	No, no official test guidelines available at present
Deviations:	NA
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

Aim

The aim of this generic study was to investigate the use of freshly drilled winter cereal fields as foraging habitat by small omnivorous mammals in Central Europe. The focus was the determination of PT values (i.e. proportion of diet obtained in treated area, calculated as proportion of potentially foraging time spent in freshly drilled cereal fields) of wood mice (*Apodemus sylvaticus*) during the pre-emergence period of cereals via radio tracking.

Materials and methods

Study site

The study was conducted in the region of Sohren, district of Rhine-Hunsrück, in RhinelandPalatinate, Germany. Eight cereal fields were selected as study fields based on non-GLP pre-trapping in order to select most suitable study fields regarding the presence of wood mice.

Trapping

From 18 September 2021 to 06 October 2021, GLP-compliant live trapping was conducted to identify suitable individuals for radio tagging and subsequent radio tracking. Forty-five to eighty 'Ugglan' multiple capture live traps (Grahnb, Sweden) were installed in- and off-crop and baited with crimped oat. Live trapping followed a Capture-Mark-Recapture design, which allowed identification of individually marked animals upon recapture (via Passive Integrated Transponders).

Radio-tracking

During live trapping, 19 suitable wood mouse individuals (with a body weight of ≥ 20 g) were equipped with radio tags that were designed as collars with a radio transmitter attached to a cable tie. Tags were fitted around the animal's neck. After radio tracking, the tags of recaptured individuals were removed. Twenty-two radio tracking sessions from 18 wood mouse individuals were successfully performed after drilling and before the emergence of the cereal plants. Animals were not tracked on the day of tagging to exclude any bias during the initial adaptation process. The radio tracking sessions were conducted from 24 September 2021 until 11 October 2021. During the radio tracking sessions, wood mice were tracked continuously, in order to record their location and any behavioural changes for a complete activity period within one night. Each location of the radio tracked individual was recorded on a map. The exact coordi-

notes of the location were calculated afterwards, from the information documented on the map.

Calculation of PT

For each radio tracking session, the proportion of diet obtained in freshly drilled cereal fields (PT) was calculated as the proportion of total ‘potentially foraging’ time during a tracking session the individual wood mouse spent in freshly drilled cereal fields in relation to the time spent in all visited habitat types. Thus, the ‘time potentially foraging’ is the sum of the time periods in freshly drilled cereal fields covered by behavioural categories when foraging could not be excluded. The overall mean PT (= PT factor), standard error of the mean PT and 90th percentile were then calculated from the individual-based PT values. For individuals radio tracked twice, an individual mean PT was calculated based on both radio tracking sessions before calculation of the PT factor based on individual PT values. PT factors were calculated for:

- “Potential consumers”: since all radio tracked wood mice were captured directly adjacent to the cereal fields, they all had access to the study fields and could therefore be determined as potential consumers for the PT calculations. This corresponds to the term “consumers” according to EFSA (2009). Therefore, all successfully radio tracked individuals with all sessions were considered to calculate a PT estimate regardless of the use of the freshly drilled cereal fields during radio tracking.
- “Confirmed consumers”: additionally PT was estimated only from individuals located potentially foraging (i.e. active) at least once in a freshly drilled cereal field.

Results

In total, 22 radio tracking sessions of 18 individual wood mice were performed after drilling and before emergence of cereal plants in Germany (Central Europe). Four individuals were radio tracked twice. The calculated single PT values ranged from 0 to 0.170, resulting in a mean PT factor of 0.025 (90th percentile of 0.082). Of these, 11 individuals were “confirmed consumers” (with 14 radio tracking sessions conducted on these individuals), resulting in a PT factor for the “confirmed consumers” approach of 0.040 (90th percentile of 0.136). A summary of the PT results is provided in the table below.

Summary of PT results for wood mice in freshly drilled cereal fields

	PT factor potential consumers (22 sessions)	PT factor confirmed consumers (14 sessions)
PT factor (overall mean)	0.025	0.040
SEM	0.003	0.005
90th percentile	0.082	0.136

Conclusion

Wood mice showed a PT factor of 0.025 in freshly drilled cereal fields. The 90th percentile was 0.082. The PT factor for “confirmed consumer” approach was 0.040 (90th percentile 0.136). The low PT factor indicates that freshly drilled cereal fields are no attractive foraging habitat for wood mice. The PT data from 18 different individuals and 22 radio tracking sessions represent a robust data set for the use in wildlife risk assessments according to EFSA (2009)

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

No new studies submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.6.1
Report	The growth inhibition of Prosulfocarb Technical to the algae <i>Scenedesmus subspicatus</i> over a 72 hour exposure period, Sacker D., 2008b, ENV8187/110707
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive Summary

The toxicity of prosulfocarb to the alga *Scenedesmus subspicatus* was determined. Algae were exposed to nominal concentrations of 0.010, 0.032, 0.1, 0.32 and 1.0 mg prosulfocarb/L (mean measured: 0.007, 0.017, 0.035, 0.165 and 0.511 mg prosulfocarb/L), alongside a culture medium control. Based on mean measured concentrations, the 72-hour $E_{rC_{50}}$ was 0.086 mg prosulfocarb/L and the E_yC_{50} was 0.038 mg prosulfocarb/L.

Materials

Test Material	Prosulfocarb technical
Lot/Batch #:	071005
Purity:	97.39%
Description:	Clear liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	October 2009
Treatments	
Test rates:	Culture medium control and nominal concentrations of 0.010, 0.032, 0.1, 0.32 and 1.0 mg prosulfocarb/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentration:	Yes, 0 and 96 h by HPLC/UV analysis
Test organisms	
Species:	<i>Scenedesmus subspicatus</i> Strain No. CCAP 278/4
Source:	Obtained from Culture Collection of Algae and Protozoa (Institute of Freshwater Ecology, Windermere Laboratory)
Test design	
Test vessels:	250 mL conical flasks containing 100 mL of media
Test medium:	Filtered and sterilised deionised water with added nutrients, pH adjusted to 8.0 ± 0.2
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static

Aeration	None reported
Duration:	72 h
Environmental conditions	
Temperature:	Test start: 22°C Test end: 21.5°C
pH:	Test start: 7.7 - 8.1 Test end: 6.8 - 7.0
Lighting:	Continuous illumination at approximately 6000 to 10000 Lux

Study design and methods

Experimental dates: 21 October 2008 to 24 October 2008

A stock solution of 10 mg prosulfocarb/L was prepared and stirred well. Appropriate volumes of the stock solution were added to the test media and were then made up to the mark in volumetric flasks to give the required test concentrations. The control consisted of culture medium only.

The test was started by an inoculation of 10000 algal cells per mL of test medium. Test solutions were continuously shaken at 200 rpm under continuous illumination.

A small volume was taken from each replicate flask after 24, 48 and 72 hours of exposure. The algal cell counts were determined by haemocytometer and microscope, and the shape and size of the algal cells were examined microscopically in these samples.

The pH and temperature were measured at the start and at the end of the test in each test concentration and the control using pooled replicates.

The test concentrations were verified by chemical analysis of prosulfocarb at 0 and 72 hours, using HPLC/UV.

Results and Discussion

At the start of the test, the analytically determined concentrations of prosulfocarb were in the range 39.09 to 94.63 % of the nominal values and at the end of the test, aged solutions were in the range 45.00 to 77.54 % of fresh solutions (see table below). The method detection limit in this study was 0.010 mg prosulfocarb/L. Mean measured concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations (mg prosulfocarb/L)	Fresh solutions		Aged solutions as a percentage of fresh concentrations	Mean measured concentrations (mg prosulfocarb/L)
	Measured concentrations (mg prosulfocarb/L)	Percentage recovery		
Control	0.000	-	-	-
0.010	0.009	94.63	50.42	0.007
0.032	0.024	73.67	45.00	0.017
0.10	0.039	39.09	77.54	0.035
0.32	0.213	66.47	54.93	0.165
1.0	0.599	59.93	70.66	0.511

The algal cell densities were measured at 24, 48 and 72 hours and the growth rate and yield were calculated. The 0 to 48- and 0 to 72-hour EC50 values and their 95 % confidence intervals were estimated using the Maximum Likelihood-Probit Method. The Bonferroni T-test was used to determine the 0 to 72-hour NOECs.

There were no abnormalities, observed microscopically, in the control or test concentrations at 24, 48 and 72 hours.

Mean cell density measurements, percent inhibition data, and the summary of biological effects are shown below:

Toxicity of prosulfocarb to *Scenedesmus subspicatus* – mean cell density at 24, 48 and 72 hours

Mean measured concentrations (mg prosulfocarb/L)	Mean cell density* measurements (x 104 cells/mL)		
	24 h	48 h	72 h
Control	2.5	11.4	62.7
0.007	2.8	9.6	57.9
0.017	2.4	9.2	59.7
0.035	2.2	5.9	26.2
0.165	1.7	3.0	3.7
0.511	1.1	0.8	0.9

*Mean initial cell density: 1 x 104 cells/mL

Toxicity of prosulfocarb to *Scenedesmus subspicatus* – percent inhibition by yield and growth rate at 0 – 48 and 0 – 72 hours

Mean measured concentrations (mg prosulfocarb/L)	Percent inhibition by yield		Percent inhibition by growth rate	
	0 – 48 h	0 – 72 h	0 – 48 h	0 – 72 h
Control	0	0	0	0
0.007	10	9	7	2
0.017	17	9	8	1
0.035*	45	56	29	21
0.165*	75	91	55	69
0.511*	100	100	100	100

*Growth was inhibited at these concentrations, compared to the control

Summary of biological effects for toxicity of prosulfocarb to *Scenedesmus subspicatus* after 48 and 72 hours

Parameter	0 – 48 h (mg prosulfocarb/L)		0 – 48 h (mg prosulfocarb/L)	
	Growth rate (ErC50)	Yield (EyC50)	Growth rate (ErC50)	Yield (EyC50)
EC50	0.083	0.048	0.086	0.038
95% CI	0.030-0.35	0.027-0.092	0.046-0.18	0.020-0.087
NOEC	n.r.	n.r.	0.017	0.017

95% CI: 95% confidence interval

n.r.: not reported

Validity criteria

The validity criteria were met.

Cell density in the control increased by a factor of 63 over 72 hours. The mean coefficient of variation of the daily growth rates in the control cultures was 31.56 % over 72 hours (must be ≤ 35 %). The coefficient of variation of average specific growth rates in the control cultures was 1.30 % over 72 hours (must be ≤ 7 %).

Conclusions

Based on mean measured concentrations, the 72-hour E_rC_{50} was 0.086 mg prosulfocarb/L and the 72-hour E_yC_{50} was 0.038 mg prosulfocarb/L. The NOEC at 72 hours, based on growth rate and yield, was 0.017 mg prosulfocarb/L.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.6.1
Report	Effects of Prosulfocarb sulfoxide on <i>Chlamydomonas reinhardtii</i> in an algal growth inhibition test, Juckeland D., 2012a, 12 10 48 057 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb sulfoxide to the alga *Chlamydomonas reinhardtii* was determined. Algae were exposed to nominal concentrations of 31.3, 62.5, 124.9, 249.9 and 499.8 µg prosulfocarb sulfoxide/L, alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 281.6 µg prosulfocarb sulfoxide/L and the E_yC_{50} was 111.5 µg prosulfocarb sulfoxide/L.

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017
Treatments	
Test rates:	Culture medium control and nominal concentrations of 31.3, 62.5, 124.9, 249.9 and 499.8 µg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentration:	Yes, 0 and 72 h using RP-HPLC with MS and UV detection
Test organisms	
Species:	<i>Chlamydomonas reinhardtii</i> DANGEARD Strain: 11-32b SAG
Source:	Laboratory cultures, originally obtained from MBM ScienceBridge GmbH (Göttingen, Germany)
Test design	
Test vessels:	250 mL Erlenmeyer flasks with air-permeable stoppers, containing 100 mL of medium
Test medium:	OECD algal medium
Replication:	Control: 6 Treated: 3 + 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	5 x 10 ³ cells/mL

Exposure regime:	Static
Aeriation	None reported
Duration:	72 h
Environmental conditions	
Temperature:	22.0 – 23.9°C
pH:	Test start: 8.05 – 8.12 Test end: 8.25 – 9.50
Lighting:	Continuous fluorescent illumination at an average of 113 $\mu\text{E}/\text{m}^2\cdot\text{s}^{-1}$

Study design and methods

Experimental dates: 29 June 2012 to 02 July 2012

A primary stock solution with a nominal concentration of 137.6 mg prosulfocarb sulfoxide/L was prepared by weighing 34.4 mg of the test item and making up to 250 mL with test medium. A secondary stock solution with a nominal concentration of 5.0 mg prosulfocarb sulfoxide/L was prepared using 9.08 mL of the primary stock solution and making up to 250 mL with test medium. Appropriate volumes of the secondary stock solution were diluted to give the test concentration series. The control consisted of culture medium only. The test media were prepared just before the start of the test.

The test was started by inoculation of 5,000 algal cells per mL of test medium. Test solutions were placed on a rotary shaker and were held in a temperature controlled water bath under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48 and 72 hours of exposure. The algal biomass in these samples was determined by microscopic counting using a Neubauer counting chamber. In addition, the shape and size of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in the water bath. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of prosulfocarb sulfoxide at 0 and 72 hours, using reverse phase-high performance liquid chromatography with mass spectrometric and diode-array UV detection.

Results and Discussion

At the start of the test, the analytically determined concentrations of prosulfocarb sulfoxide were in the range 90 to 103 % of the nominal values and at the end of the test were in the range 36 to 86 % (see table below). The limit of quantification in this study was 6.21 μg prosulfocarb sulfoxide/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations of prosulfocarb sulfoxide ($\mu\text{g}/\text{L}$)	% of nominal measured at 0 h*	% of nominal measured at 72 h*
Control	n.a.	n.a.
31.3	99	36
62.5	99	83
124.9	90	81
249.9	102	86
499.8	103	77

*determined by mass spectrometric detection

n.a. = not applicable

The algal cell densities were measured at 24, 48 and 72 hours and the mean growth rate and yield were calculated. The 48- and 72-hour E_rC_{50} and E_yC_{50} values and their 95% confidence intervals were calculated by Probit analysis. (Regression analysis was performed using individual replicate responses, not treatment group means.) A Welch-t-test with Bonferroni-Holm adjustment ($p \leq 0.05$, one-sided smaller) or

Williams t-test ($p \leq 0.05$, one-sided smaller) were used to identify significant differences in the calculated mean growth rate and yield of test item treatments compared to the control.

Growth rate

The growth rate 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the growth rate at 48 and 72 hours for *C. reinhardtii*

Nominal concentrations of prosulfocarb sulfoxide ($\mu\text{g/L}$)	Mean growth rate (1/day) 0-48 h	Percentage inhibition	Mean growth rate (1/day) 0-72 h	Percentage inhibition
Control	1.616	0.0	1.404	0.0
31.3	1.607	0.6	1.417	-1.0
62.5	1.555	3.8	1.289	8.2 ⁺
124.9	1.453	10.1*	1.179	16.0 ⁺
249.9	0.864	46.5*	0.702	50.0 ⁺
499.8	0.384	76.3*	0.428	69.5 ⁺

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

⁺ statistically significant different from control (Welch-t-test, $p \leq 0.05$, one-sided smaller)

Negative values indicate an increase in growth, relative to control

Yield

The yield 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the yield at 48 and 72 hours for *C. reinhardtii*

Nominal concentrations of prosulfocarb sulfoxide ($\mu\text{g/L}$)	Mean yield ($\times 10^4$ cell/mL) 0-48 h	Percentage inhibition	Mean yield ($\times 10^4$ cell/mL) 0-72 h	Percentage inhibition
Control	12.25	0.0	33.25	0.0
31.3	12.08	1.4	34.67	-4.3
62.5	10.75	12.2	23.42	29.6*
124.9	8.67	29.3*	16.67	49.9*
249.9	2.33	81.0*	3.67	89.0*
499.8	0.58	95.2*	1.33	96.0*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

Negative values indicate an increase in growth, relative to control

Summary of biological effects for toxicity of prosulfocarb sulfoxide to *C. reinhardtii* after 48 and 72 hours

Parameter	After 48 h ($\mu\text{g prosulfocarb sulfoxide/L}$)		After 72 h ($\mu\text{g prosulfocarb sulfoxide/L}$)	
	Growth rate	Yield	Growth rate	Yield
EC ₅₀	281.9	159.9	281.6	111.5
95% CI	246.7-324.1	127.3-201.1	225.6-365.3	80.0-155.3
EC ₂₀	151.6	97.3	123.4	57.5
95% CI	116.2-180.1	57.4-123.1	75.0-161.8	25.6-80.2
EC ₁₀	109.6	75.0	80.1	40.7
95% CI	75.5-137.5	35.7-101.0	38.8-115.0	13.0-61.8
NOEC	62.5	62.5	31.3	31.3
LOEC	124.9	124.9	62.5	62.5

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

- The algal biomass in the control increased by a factor of 67.5 over 72 hours (must be least 16).
- The mean coefficient of variation of the daily growth rates in the control cultures was 34.8 % over 72 hours (must be ≤ 35 %).
- The coefficient of variation of average specific growth rates in the control cultures was 1.4 % over 72 hours (must be ≤ 7 %).

Conclusion

Based on nominal concentrations, the 72-hour E_rC_{50} was 281.6 μg prosulfocarb sulfoxide/L and the E_yC_{50} was 111.5 μg prosulfocarb sulfoxide/L. The LOEC at 72 hours, based on growth rate and yield, was 62.5 μg prosulfocarb sulfoxide/L. The corresponding NOEC was 31.3 μg prosulfocarb sulfoxide/L.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.6.1
Report	Effects of Prosulfocarb sulfoxide on <i>Chlorella vulgaris</i> in an algal growth inhibition test, Juckeland D., 2012b, 12 10 48 059 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb sulfoxide to the green alga *Chlorella vulgaris* was determined. Algae were exposed to nominal concentrations of 0.19, 0.34, 0.62, 1.11 and 2.00 mg prosulfocarb sulfoxide/L, alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 1.32 mg prosulfocarb sulfoxide/L and the E_yC_{50} was 0.73 mg prosulfocarb sulfoxide/L.

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017
Treatments	
Test rates:	Culture medium control and nominal concentrations of 0.19, 0.34, 0.62, 1.11 and 2.00 mg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentration:	Yes, 0 and 72 h using RP-HPLC with MS and UV detection
Test organisms	
Species:	<i>Chlorella vulgaris</i> BEIJERINCK Strain: 211-11b SAG
Source:	Laboratory cultures, originally obtained from MBM ScienceBridge GmbH (Göttingen, Germany)

Test design

Test vessels:	250 mL Erlenmeyer flasks with air-permeable stoppers, containing 100 mL of medium
Test medium:	OECD algal medium
Replication:	Control: 6 Treated: 3 + 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	5×10^3 cells/mL
Exposure regime:	Static
Aeriation	None reported
Duration:	72 h
Environmental conditions	
Temperature:	22.0 – 23.9°C
pH:	Test start: 8.01 – 8.05 Test end: 8.29 – 9.09
Lighting:	Continuous fluorescent illumination at an average of $113 \mu\text{E}/\text{m}^2 \cdot \text{s}^{-1}$

Study design and methods

Experimental dates: 29 June 2012 to 02 July 2012

A primary stock solution with a nominal concentration of 42.12 mg prosulfocarb sulfoxide/L was prepared by weighing 21.06 mg of the test item and making up to 500 mL with test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only. The test media were prepared just before the start of the test.

The test was started by inoculation of 5,000 algal cells per mL of test medium. Test solutions were placed on a rotary shaker and were held in a temperature controlled water bath under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48 and 72 hours of exposure. The algal biomass in these samples was determined by microscopic counting using a Neubauer counting chamber. In addition, the shape and size of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in the water bath. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of prosulfocarb sulfoxide at 0 and 72 hours, using reverse phase-high performance liquid chromatography with mass spectrometric and diode-array UV detection.

Results and Discussion

At the start of the test, the analytically determined concentrations of prosulfocarb sulfoxide were in the range 98 to 104% of the nominal values and at the end of the test were in the range 81 to 96% (see table below). The limit of quantification in this study was 38.0 μg prosulfocarb sulfoxide/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	% of nominal measured at 0 h*	% of nominal measured at 72 h*
Control	n.a.	n.a.
0.19	98	94
0.34	104	96
0.62	103	90
1.11	103	81
2.00	101	84

*determined by mass spectrometric detection

n.a. = not applicable

The algal cell densities were measured at 24, 48 and 72 hours and the mean growth rate and yield were calculated. The 48- and 72-hour E_rC_{50} and E_yC_{50} values and their 95% confidence intervals were calculated by Probit analysis. (Regression analysis was performed using individual replicate responses, not treatment group means.) A Welch-t-test with Bonferroni-Holm adjustment ($p \leq 0.05$, one-sided smaller) or Williams t-test ($p \leq 0.05$, one-sided smaller) were used to identify significant differences in the calculated mean growth rate and yield of test item treatments compared to the control.

Growth rate

The growth rate 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the growth rate at 48 and 72 hours for *C. vulgaris*

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean growth rate (1/day) 0-48 h	Percentage inhibition	Mean growth rate (1/day) 0-72 h	Percentage inhibition
Control	1.547	0.0	1.308	0.0
0.19	1.570	-1.5	1.331	-1.8
0.34	1.556	-0.5	1.302	0.5
0.62	1.401	9.4 ⁺	1.175	10.2*
1.11	0.750	51.5 ⁺	0.698	46.6*
2.00	0.288	81.4 ⁺	0.415	68.3*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

⁺ statistically significant different from control (Welch-t-test, $p \leq 0.05$, one-sided smaller)

Negative values indicate an increase in growth, relative to control

Yield

The yield 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the yield at 48 and 72 hours for *C. vulgaris*

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean yield ($\times 10^4$ cell/mL) 0-48 h	Percentage inhibition	Mean yield ($\times 10^4$ cell/mL) 0-72 h	Percentage inhibition
Control	10.58	0.0	24.88	0.0
0.19	11.08	-4.7	26.75	-7.5
0.34	10.83	-2.4	24.42	1.8
0.62	7.75	26.8*	16.50	33.7*
1.11	1.75	83.5*	3.58	85.6*
2.00	0.42	96.1*	1.25	95.0*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

Negative values indicate an increase in growth, relative to control

Summary of biological effects for toxicity of prosulfocarb sulfoxide to *C. vulgaris* after 48 and 72 hours

Parameter	After 48 h (mg prosulfocarb sulfoxide/L)		After 72 h (mg prosulfocarb sulfoxide/L)	
	Growth rate	Yield	Growth rate	Yield
EC_{50}	1.15	0.78	1.32	0.73
95% CI	1.01-1.31	0.73-0.83	1.07-1.70	0.68-0.79
EC_{20}	0.73	0.57	0.72	0.53
95% CI	0.56-0.85	0.52-0.62	0.24-0.73	0.45-0.58
EC_{10}	0.57	0.49	0.53	0.44
95% CI	0.39-0.70	0.43-0.53	0.24-0.73	0.36-0.50

NOEC	0.34	0.34	0.34	0.34
LOEC	0.62	0.62	0.62	0.62

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

- The algal biomass in the control increased by a factor of 50.8 over 72 hours (must be least 16).
- The mean coefficient of variation of the daily growth rates in the control cultures was 34.4 % over 72 hours (must be ≤ 35 %).
- The coefficient of variation of average specific growth rates in the control cultures was 1.9 % over 72 hours (must be ≤ 7 %).

Conclusion

Based on nominal concentrations, the 72-hour E_rC_{50} was 1.32 mg prosulfocarb sulfoxide/L and the E_yC_{50} was 0.73 mg prosulfocarb sulfoxide/L. The LOEC at 72 hours, based on growth rate and yield, was 0.62 mg prosulfocarb sulfoxide/L. The corresponding NOEC was 0.34 mg prosulfocarb sulfoxide/L.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference: KCA 8.2.6.2

Report: Effects of Prosulfocarb sulfoxide on *Anabaena flos-aquae* in an algal growth inhibition test, Juckeland D., 2012c, 12 10 48 058 W

Guideline(s): Yes, OECD 201

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb sulfoxide to the blue-green alga *Anabaena flos-aquae* was determined. Algae were exposed to nominal concentrations of 0.51, 1.62, 5.17, 16.6 and 53.0 mg prosulfocarb sulfoxide/L, alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 42.5 mg prosulfocarb sulfoxide/L and the E_yC_{50} was 19.5 mg prosulfocarb sulfoxide/L.

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017
Treatments	
Test rates:	Culture medium control and nominal concentrations of 0.51, 1.62, 5.17, 16.6 and 53.0 mg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	Potassium dichromate

Analysis of test concentration:	Yes, 0 and 72 h using RP-HPLC with MS and UV detection
Test organisms	
Species:	<i>Anabaena flos-aqua</i> de Brébisson Strain: 30.87 SAG
Source:	Laboratory cultures, originally obtained from MBM ScienceBridge GmbH (Göttingen, Germany)
Test design	
Test vessels:	250 mL Erlenmeyer flasks with air-permeable stoppers, containing 100 mL of medium
Test medium:	Reconstituted water prepared according to SAG
Replication:	Control: 6 Treated: 3 + 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	10 ⁴ cells/mL
Exposure regime:	Static
Aeriation	None reported
Duration:	72 h
Environmental conditions	
Temperature:	22.0 – 23.9°C
pH:	Test start: 7.40 – 7.47 Test end: 7.38 – 8.37
Lighting:	Continuous fluorescent illumination at an average of 52 µE/m ² .s ⁻¹

Study design and methods

Experimental dates: 26 June 2012 to 29 June 2012

A primary stock solution with a nominal concentration of 530 mg prosulfocarb sulfoxide/L was prepared by weighing 265 mg of the test item and making up to 500 mL with test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only. The test media were prepared just before the start of the test.

The test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were placed on a rotary shaker and were held in a temperature controlled water bath under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48 and 72 hours of exposure. The algal biomass in these samples was determined by microscopic counting using a Neubauer counting chamber. In addition, the shape and size of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in the water bath. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of prosulfocarb sulfoxide at 0 and 72 hours, using reverse phase-high performance liquid chromatography with mass spectrometric and diode-array UV detection.

Results and Discussion

At the start of the test, the analytically determined concentrations of prosulfocarb sulfoxide were in the range 99 to 110% of the nominal values and at the end of the test were in the range 91 to 103% (see table below). The limit of quantification in this study was 100.15 µg prosulfocarb sulfoxide/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	% of nominal measured at 0 h*	% of nominal measured at 72 h*
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Control	n.a.	n.a.
0.51	110	100
1.62	104	93
5.17	99	91
16.6	109	103
53.0	108	96

*determined by mass spectrometric detection
n.a. = not applicable

The algal cell densities were measured at 24, 48 and 72 hours and the mean growth rate and yield were calculated. The 48- and 72-hour E_rC_{50} and E_yC_{50} values and their 95% confidence intervals were calculated by Probit analysis. (Regression analysis was performed using individual replicate responses, not treatment group means.) A Williams t-test ($p \leq 0.05$, one-sided smaller) was used to identify significant differences in the calculated mean growth rate and yield of test item treatments compared to the control.

Growth rate

The growth rate 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the growth rate at 48 and 72 hours for *A. flos-aqua*

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean growth rate (1/day) 0-48 h	Percentage inhibition	Mean growth rate (1/day) 0-72 h	Percentage inhibition
Control	1.245	0.0	1.118	0.0
0.51	1.258	-1.0	1.127	-0.8
1.62	1.228	1.4	1.125	-0.6
5.17	1.103	11.4*	1.055	5.7*
16.6	0.938	24.6*	0.978	12.5*
53.0	0.314	74.8*	0.442	60.5*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)
Negative values indicate an increase in growth, relative to control

Yield

The yield 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the yield at 48 and 72 hours for *A. flos-aqua*

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean yield ($\times 10^4$ cell/mL) 0-48 h	Percentage inhibition	Mean yield ($\times 10^4$ cell/mL) 0-72 h	Percentage inhibition
Control	11.13	0.0	27.79	0.0
0.51	11.42	-2.6	28.50	-2.5
1.62	10.75	3.4	28.33	-1.9
5.17	8.08	27.3*	22.67	18.4*
16.6	5.58	49.8*	17.83	35.8*
53.0	0.92	91.8*	2.83	89.8*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)
Negative values indicate an increase in growth, relative to control

Summary of biological effects for toxicity of prosulfocarb sulfoxide to *A. flos-aqua* after 48 and 72 hours

Parameter	After 48 h (mg prosulfocarb sulfoxide/L)		After 72 h (mg prosulfocarb sulfoxide/L)	
	Growth rate	Yield	Growth rate	Yield
EC_{50}	29.1	13.1	42.5	19.5

95% CI	24.4-34.9	10.4-16.7	38.8-46.8	15.9-24.0
EC₂₀	12.6	4.22	20.8	8.17
95% CI	8.95-15.8	2.67-5.74	17.4-23.8	5.47-10.6
EC₁₀	8.17	2.33	14.3	5.18
95% CI	5.05-11.0	1.24-3.49	11.1-17.2	2.97-7.24
NOEC	1.62	1.62	1.62	1.62
LOEC	5.17	5.17	5.17	5.17

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

- The algal biomass in the control increased by a factor of 28.8 over 72 hours (must be least 16).
- The mean coefficient of variation of the daily growth rates in the control cultures was 34.8 % over 72 hours (must be ≤ 35 %).
- The coefficient of variation of average specific growth rates in the control cultures was 3.4 % over 72 hours (must be ≤ 7 %).

Conclusion

Based on nominal concentrations, the 72-hour E_rC_{50} was 42.5 mg prosulfocarb sulfoxide/L and the E_yC_{50} was 19.5 mg prosulfocarb sulfoxide/L. The LOEC at 72 hours, based on growth rate and yield, was 5.17 mg prosulfocarb sulfoxide/L. The corresponding NOEC was 1.62 mg prosulfocarb sulfoxide/L.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.6.2
Report	Effects of Prosulfocarb sulfoxide on <i>Navicula pelliculosa</i> in an algal growth inhibition test, Juckeland D., 2012d, 12 10 48 053 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb sulfoxide to the freshwater diatom *Navicula pelliculosa* was determined. Algae were exposed to nominal concentrations of 0.48, 1.53, 4.88, 15.6 and 50.0 mg prosulfocarb sulfoxide/L, alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 7.97 mg prosulfocarb sulfoxide/L and the E_yC_{50} was 2.04 mg prosulfocarb sulfoxide/L.

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017

Treatments

Test rates:	Culture medium control and nominal concentrations of 0.48, 1.53, 4.88, 15.6 and 50.0 mg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentration:	Yes, 0 and 72 h using RP-HPLC with MS and UV detection

Test organisms

Species:	<i>Navicula pelliculosa</i> HILSE Strain: 1050-3 SAG
Source:	Laboratory cultures, originally obtained from MBM ScienceBridge GmbH (Göttingen, Germany)

Test design

Test vessels:	250 mL Erlenmeyer flasks with air-permeable stoppers, containing 100 mL of medium
Test medium:	Reconstituted water prepared according to SAG
Replication:	Control: 6 Treated: 3 + 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	10^4 cells/mL
Exposure regime:	Static
Aeriation	None reported
Duration:	72 h

Environmental conditions

Temperature:	21.8 – 23.5°C
pH:	Test start: 7.45 – 7.66 Test end: 7.80 – 8.98
Lighting:	Continuous fluorescent illumination at an average of $74 \mu\text{E}/\text{m}^2 \cdot \text{s}^{-1}$

Study design and methods

Experimental dates: 03 July 2012 to 06 July 2012

A primary stock solution with a nominal concentration of 504 mg prosulfocarb sulfoxide/L was prepared by weighing 50.4 mg of the test item and making up to 100 mL with test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only. The test media were prepared just before the start of the test.

The test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were placed on a rotary shaker and were held in a temperature controlled water bath under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48 and 72 hours of exposure. The algal biomass in these samples was determined by microscopic counting using a Neubauer counting chamber. In addition, the shape and size of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in the water bath. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of prosulfocarb sulfoxide at 0 and 72 hours, using reverse phase-high performance liquid chromatography with mass spectrometric and diode-array UV detection.

Results and Discussion

At the start of the test, the analytically determined concentrations of prosulfocarb sulfoxide were in the range 102 to 111% of the nominal values and at the end of the test were in the range 0 to 105% (see table below). The limit of quantification in this study was $100.2 \mu\text{g}$ prosulfocarb sulfoxide/L. The limit of

quantification was used for the calculation of the geometrical mean measured concentration for the nominal test concentration of 1.53 mg prosulfocarb sulfoxide/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	% of nominal measured at 0 h*	% of nominal measured at 72 h*
Control	n.a.	n.a.
0.48	102	82
1.53	107	0**
4.88	108	92
15.6	109	98
50.0	111	105

*determined by mass spectrometric detection

n.a. = not applicable

**at or below the limit of quantification (LOQ = 100.2 µg/L)

The algal cell densities were measured at 24, 48 and 72 hours and the mean growth rate and yield were calculated. The 48- and 72-hour E_rC_{50} and E_yC_{50} values and their 95% confidence intervals were calculated by Probit analysis. (Regression analysis was performed using individual replicate responses, not treatment group means.) A Welch-t-test with Bonferroni-Holm adjustment ($p \leq 0.05$, one-sided smaller) or Williams t-test ($p \leq 0.05$, one-sided smaller) was used to identify significant differences in the calculated mean growth rate and yield of test item treatments compared to the control.

Growth rate

The growth rate 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the growth rate at 48 and 72 hours for *N. pelliculosa*

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean growth rate (1/day) 0-48 h	Percentage inhibition	Mean growth rate (1/day) 0-72 h	Percentage inhibition
Control	1.487	0.0	1.265	0.0
0.48	1.487	0.0	1.263	0.2
1.53	1.361	8.4 ⁺	1.067	15.7*
4.88	1.097	26.3 ⁺	0.813	35.7*
15.6	0.322	78.4 ⁺	0.408	67.7*
50.0	0.026	98.2 ⁺	0.132	89.6*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

⁺statistically significant different from control (Welch-t-test, $p \leq 0.05$, one-sided smaller)

Negative values indicate an increase in growth, relative to control

Yield

The yield 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the yield at 48 and 72 hours for *N. pelliculosa*

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean yield ($\times 10^4$ cell/mL) 0-48 h	Percentage inhibition	Mean yield ($\times 10^4$ cell/mL) 0-72 h	Percentage inhibition
Control	18.58	0.0	43.58	0.0
0.48	18.58	0.0	43.33	0.6
1.53	14.25	23.3*	23.58	45.9*
4.88	8.00	57.0*	10.50	75.9*

15.6	0.92	95.1*	2.42	94.5*
50.0	0.08	99.6*	0.50	98.9*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

Negative values indicate an increase in growth, relative to control

Summary of biological effects for toxicity of prosulfocarb sulfoxide to *N. pelliculosa* after 48 and 72 hours

Parameter	After 48 h (mg prosulfocarb sulfoxide/L)		After 72 h (mg prosulfocarb sulfoxide/L)	
	Growth rate	Yield	Growth rate	Yield
EC ₅₀	8.08	3.70	7.97	2.04
95% CI	6.48-10.1	2.84-4.82	6.66-9.55	1.18-3.50
EC ₂₀	3.84	1.52	2.30	0.83
95% CI	2.49-4.99	0.89-2.09	1.66-2.94	0.20-1.36
EC ₁₀	2.60	0.96	1.20	0.52
95% CI	1.43-3.63	0.46-1.43	0.77-1.65	0.07-0.96
NOEC	0.48	0.48	0.48	0.48
LOEC	1.53	1.53	1.53	1.53

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

- The algal biomass in the control increased by a factor of 44.6 over 72 hours (must be least 16).
- The mean coefficient of variation of the daily growth rates in the control cultures was 33.0 % over 72 hours (must be ≤ 35 %).
- The coefficient of variation of average specific growth rates in the control cultures was 1.4 % over 72 hours (must be ≤ 7 %).

Conclusion

Based on nominal concentrations, the 72-hour E_rC₅₀ was 7.97 mg prosulfocarb sulfoxide/L and the E_yC₅₀ was 2.04 mg prosulfocarb sulfoxide/L. The LOEC at 72 hours, based on growth rate and yield, was 1.53 mg prosulfocarb sulfoxide/L. The corresponding NOEC was 0.48 mg prosulfocarb sulfoxide/L.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.6.2
Report	Effects of Prosulfocarb sulfoxide on <i>Skeletonema costatum</i> in an algal growth inhibition test, Juckeland D., 2012e, 12 10 48 060 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb sulfoxide to the marine diatom *Skeletonema costatum* was determined. Algae were exposed to nominal concentrations of 21.5, 49.3, 113.4, 261.0 and 600.2 µg prosulfocarb sulfoxide/L, alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC₅₀ was 134.8 µg prosulfocarb sulfoxide/L and the E_yC₅₀ was 53.8 µg prosulfocarb sulfoxide/L.

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017
Treatments	
Test rates:	Culture medium control and nominal concentrations of 21.5, 49.3, 113.4, 261.0 and 600.2 µg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentration:	Yes, 0 and 72 h using RP-HPLC with MS and UV detection
Test organisms	
Species:	<i>Skeletonema costatum</i> CLEVE Strain: 19.99 SAG
Source:	Laboratory cultures, originally obtained from MBM ScienceBridge GmbH (Göttingen, Germany)
Test design	
Test vessels:	250 mL Erlenmeyer flasks with air-permeable stoppers, containing 100 mL of medium
Test medium:	Reconstituted water prepared according to SAG
Replication:	Control: 6 Treated: 3 + 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	10 ⁴ cells/mL
Exposure regime:	Static
Aeration	None reported
Duration:	72 h
Environmental conditions	
Temperature:	21.8 – 23.5°C
pH:	Test start: 7.48 – 7.65 Test end: 7.90 – 9.04
Lighting:	Continuous fluorescent illumination at an average of 74 µE/m ² .s ⁻¹

Study design and methods

Experimental dates: 03 July 2012 to 06 July 2012

A primary stock solution with a nominal concentration of 123.6 mg prosulfocarb sulfoxide/L was prepared by weighing 30.9 mg of the test item and making up to 250 mL with test medium. A secondary stock solution with a nominal concentration of 6.0 mg prosulfocarb sulfoxide/L was prepared by using 12.14 mL of the primary stock solution and making up to 250 mL with test medium. Appropriate volumes of the secondary stock solution were diluted to give the test concentration series. The control consisted of culture medium only. The test media were prepared just before the start of the test.

The test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were placed on a rotary shaker and were held in a temperature controlled water bath under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48 and 72 hours of exposure. The algal biomass in these samples was determined by microscopic counting using a Neubauer counting chamber. In addition, the shape and size of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in the water bath. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of prosulfocarb sulfoxide at 0 and 72 hours, using reverse phase-high performance liquid chromatography with mass spectrometric and diode-array UV detection.

Results and Discussion

At the start of the test, the analytically determined concentrations of prosulfocarb sulfoxide were in the range 110 to 113% of the nominal values and at the end of the test were in the range 91 to 98% (see table below). The limit of quantification in this study was 10.76 µg prosulfocarb sulfoxide/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations of prosulfocarb sulfoxide (µg/L)	% of nominal measured at 0 h*	% of nominal measured at 72 h*
Control	n.a.	n.a.
21.49	113	97
49.34	111	98
113.44	110	98
260.97	112	91
600.20	111	94

*determined by mass spectrometric detection

n.a. = not applicable

The algal cell densities were measured at 24, 48 and 72 hours and the mean growth rate and yield were calculated. The 48- and 72-hour E_rC_{50} and E_yC_{50} values and their 95% confidence intervals were calculated by Probit analysis. (Regression analysis was performed using individual replicate responses, not treatment group means.) A Welch-t-test with Bonferroni-Holm adjustment ($p \leq 0.05$, one-sided smaller) or Williams t-test ($p \leq 0.05$, one-sided smaller) was used to identify significant differences in the calculated mean growth rate and yield of test item treatments compared to the control.

Growth rate

The growth rate 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the growth rate at 48 and 72 hours for *S. costatum*

Nominal concentrations of prosulfocarb sulfoxide (µg/L)	Mean growth rate (1/day) 0-48 h	Percentage inhibition	Mean growth rate (1/day) 0-72 h	Percentage inhibition
Control	1.415	0.0	1.181	0.0
21.49	1.426	-0.8 ²	1.178	0.3
49.34	1.121	20.8*	0.922	22.0*
113.44	0.680	52.0*	0.736	37.7*
260.97	0.093	93.4*	0.254	78.5*
600.20	-0.048 ¹	100.0*	0.115	90.3*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

¹ Negative values in mean growth rate indicate no increase in growth

² Negative values in percentage inhibition indicate an increase in growth, relative to control

Yield

The yield 0 to 48 hours and 0 to 72 hours were calculated for each replicate culture and the means are shown below.

Mean values at each concentration of prosulfocarb sulfoxide for the yield at 48 and 72 hours for *S. costatum*

Nominal concentrations of prosulfocarb sulfoxide (µg/L)	Mean yield (x 10 ⁴ cell/mL) 0-48 h	Percentage inhibition	Mean yield (x 10 ⁴ cell/mL) 0-72 h	Percentage inhibition
Control	15.96	0.0	33.58	0.0
21.49	16.33	-2.3 ²	33.25	1.0
49.34	8.42	47.3*	14.92	55.6*
113.44	2.92	81.7*	8.17	75.7*
260.97	0.25	98.4*	1.17	96.5*
600.20	-0.08 ¹	100.0*	0.42	98.8*

*statistically significant different from control (Williams t-test, $p \leq 0.05$, one-sided smaller)

¹ Negative values in mean growth rate indicate no increase in growth

² Negative values in percentage inhibition indicate an increase in growth, relative to control

Summary of biological effects for toxicity of prosulfocarb sulfoxide to *S. costatum* after 48 and 72 hours

Parameter	After 48 h (µg prosulfocarb sulfoxide/L)		After 72 h (µg prosulfocarb sulfoxide/L)	
	Growth rate	Yield	Growth rate	Yield
EC ₅₀	101.2	55.9	134.8	53.8
95% CI	90.4-113.3	51.3-61.0	119.0-152.7	45.8-63.1
EC ₂₀	53.6	32.1	56.6	28.0
95% CI	43.6-62.2	27.5-36.1	45.3-67.1	20.3-34.2
EC ₁₀	38.4	24.0	36.0	19.9
95% CI	29.0-46.7	19.4-28.0	26.5-45.0	12.8-25.8
NOEC	21.5	21.5	21.5	21.5
LOEC	49.3	49.3	49.3	49.3

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

- The algal biomass in the control increased by a factor of 34.6 over 72 hours (must be least 16).
- The mean coefficient of variation of the daily growth rates in the control cultures was 34.5 % over 72 hours (must be ≤ 35 %).
- The coefficient of variation of average specific growth rates in the control cultures was 0.6 % over 72 hours (must be ≤ 7 %).

Conclusion

Based on nominal concentrations, the 72-hour E_rC₅₀ was 134.8 µg prosulfocarb sulfoxide/L and the E_yC₅₀ was 53.8 µg prosulfocarb sulfoxide/L. The LOEC at 72 hours, based on growth rate and yield, was 49.3 µg prosulfocarb sulfoxide/L. The corresponding NOEC was 21.5 µg prosulfocarb sulfoxide/L.

Comments of zRMS: The study has been already assessed and accepted in dRAR, December 2022.

Reference: KCA 8.2.7

Report Effects of prosulfocarb sulfoxide on *Myriophyllum spicatum* in a growth inhibition test under semi-static conditions, Juckeland D., 2013b, 13 10 48 017 W

Guideline(s): Yes, ASTM designation E 1913-04

Deviations: No

GLP: Yes
 Acceptability: Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb sulfoxide to the aquatic plant *Myriophyllum spicatum* was determined in a 14-day semi-static test. The *Myriophyllum* were exposed to nominal test item concentrations of 4.12, 6.80, 11.2, 18.5, 30.6, 50.4, 83.2 and 137.3 µg prosulfocarb sulfoxide/L alongside a culture medium control.

Based on nominal test item concentrations, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) for main shoot length were 40.9 and 29.8 µg Prosulfocarb sulfoxide/L, respectively. For total shoot length, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) were 42.6 and 26.0 µg of prosulfocarb sulfoxide/L, respectively. For biomass (dry weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 36.4 µg prosulfocarb sulfoxide/L. For biomass (fresh weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 21.3 µg prosulfocarb sulfoxide/L. For number of whorls, the 14-day EC₅₀ for yield (E_yC₅₀) was 24.5 µg of prosulfocarb sulfoxide/L.

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017
Treatments	
Test rates:	Dilution water control and nominal test item concentrations of 4.12, 6.80, 11.2, 18.5, 30.6, 50.4, 83.2 and 137.3 µg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	None
Analysis of test concentration:	Yes, analysis of prosulfocarb sulfoxide on days 0, 7 and 14, in fresh and replaced solutions, using HPLC with MS detection
Test organisms	
Species:	<i>Myriophyllum spicatum</i> L.
Source:	Cultures prepared under test conditions (originally obtained from Federal Environment Agency; Division IV: Chemical and Biological Safety, Germany)
Test design	
Test vessels:	100 mL glass tubes with air-permeable stoppers filled with 50 mL of test medium
Test medium:	Modified Andrews' medium with 3 % sucrose
Replication:	Ten vessels for the control and five vessels for each test concentration (+ 1 additional vessel for analysis and retained specimen per concentration and control)
Number of plants:	One plant per replicate
Exposure regime:	Semi-static
Duration:	14 days
Environmental conditions	
Temperature:	23.1 – 24.9°C
pH:	5.57 - 5.89 new solutions, 5.81 - 9.04 aged solutions
Lighting:	16 : 8 hours fluorescent light : dark at an average of approximately 10000 lux

Study design and methods

Experimental dates: 04 April 2013 to 18 April 2013

A primary stock solution with a nominal test item concentration of 25.0 mg prosulfocarb sulfoxide/L was prepared by weighing 25.0 mg of the test item and making up to 1000 mL with test medium. A secondary stock solution with a nominal test item concentration of 0.5 mg prosulfocarb sulfoxide/L was prepared using 10.0 mL of the primary stock solution and making up to 500 mL with test medium, and a tertiary stock solution with a nominal test item concentration of 0.05 mg prosulfocarb sulfoxide/L was prepared using 50.0 mL of the secondary stock solution and making up to 1000 mL with test medium. Appropriate volumes of the secondary or tertiary stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

50 mL of the test solutions were transferred into 100 mL glass tubes and inoculated with *Myriophyllum* plants. Cultures were then transferred to an environment chamber where they were maintained under the conditions indicated above. On day 7 fresh solutions were prepared and test solutions were renewed.

Assessments of main shoot length and number of whorls of the test organism were made on days 0, 7 and 14. Plants were harvested for measurement of fresh and dry weight after 14 days; the initial fresh and dry weight was determined using a sample of 10 untreated test organisms identical to that used to inoculate the test on day 0.

Temperature was measured continuously, light intensity was recorded once at test start and pH was recorded on day 0, 7 and 14, in fresh and aged solutions.

The test concentrations were verified by chemical analysis of prosulfocarb sulfoxide at days 0, 7 and 14 in fresh and aged solutions, using high performance liquid chromatography with mass spectrometry detection.

Results and discussions

In the fresh solutions, the concentrations of the test item were found to be in the range 93 to 112 % of the nominal values, and in the aged solutions they were in the range 0 to 84 % (see table below). The limit of quantification in this study was 2.0 µg prosulfocarb sulfoxide/L. Nominal test item concentrations were used for the reporting of results.

Analytical results

Nominal test item concentrations (µg prosulfocarb sulfoxide/L)	% of nominal measured at day 0 (fresh solutions)	% of nominal measured at day 7 (aged solutions)	% of nominal measured at day 7 (fresh solutions)	% of nominal measured at day 14 (aged solutions)
Control	-	-	-	-
4.12	98	77	106	0*
6.80	95	67	108	31
11.23	106	69	109	11
18.52	112	67	107	37
30.56	100	70	105	46
50.43	101	81	99	49
83.21	96	84	97	58
137.30	93	81	95	48

*The measured concentration was less than the defined limit of quantification (2.0 µg/L)

Data for main shoot length, total shoot length, fresh weight and dry weight were used to calculate growth rate and yield for the control and each exposure concentration. Probit analysis using linear maximum likelihood regression was used to calculate the EC₅₀ values. (The nominal 4.12 µg test item/L treatment group was omitted from the EC₅₀ value determination because the measured concentration was less than the defined limit of quantification.) For the Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) values, was used a Williams t-test, Welch-t-test, or U-test, as appropriate, to determine values significantly different to the control.

Mean growth rate based on main shoot length and total shoot length is presented below along with the growth and yield inhibition values:

Effect of prosulfocarb sulfoxide on growth rate and yield of *Myriophyllum spicatum* for main shoot length and total shoot length

Nominal test item concentrations (µg prosulfocarb sulfoxide/L)	Mean final main shoot length	Mean final shoot length	% inhibition			
			Average specific growth rate (%I _r)		Yield (% I _y)	
			Main shoot length	Total shoot length	Main shoot length	Total shoot length
Control	7.0	9.1	-	-	-	-
4.12	7.0	8.6	-12.3	-4.4	-7.9	4.4
6.80	6.2	9.5	0.8	-15.2	12.4	-11.3
11.23	7.1	9.2	-7.9	-5.7	-6.7	-3.8
18.52	6.5	6.6	5.7	24.1*	10.8	38.8
30.56	4.2	4.9	62.8*	58.3*	71.2*	70.7*
50.43	3.6	4.4	60.8*	52.3*	74.7*	70.8*
83.21	3.7	4.2	77.3*	70.4*	84.1*	81.2*
137.30	3.3	3.4	66.5*	72.0*	79.4*	85.4*

* = significantly different to untreated control (Williams t-test; U-test $p \leq 0.05$, one-sided)

(-) = increase in growth relative to the control

Mean final number of whorls, mean biomass fresh weight, and mean biomass dry weight are presented below along with the respective inhibition values:

Effect of prosulfocarb sulfoxide on yield of *Myriophyllum spicatum* for biomass (fresh and dry weight) and number of whorls

Nominal test item concentrations (µg prosulfocarb sulfoxide/L)	Mean final number of whorls	Mean biomass (fresh weight) (g)	Mean bio-mass (dry weight) (g)	% inhibition		
				Number of whorls	Biomass (fresh weight)	Biomass (dry weight)
Control	19.1	293.8	43.4	-	-	-
4.12	18.5	285.6	46.3	-3.2	3.4	-9.2
6.80	16.4	272.2	42.8	15.9	8.8	2.1
11.23	18.4	345.4	51.8	7.9	-21.0	-28.8
18.52	16.0	209.7	38.4	23.8	34.3	15.9
30.56	9.6	82.5	25.8	73.0*	86.2*	56.4*
50.43	7.6	65.3	20.7	87.3*	93.2*	72.9*
83.21	7.0	65.6	20.7	92.1*	93.1*	72.9*
137.30	6.6	57.4	19.1	96.8*	96.4*	77.8*

* = significantly different to untreated control (Welch t-test, $p \leq 0.05$, one-sided)

(-) = increase in growth relative to the control

Summary of biological effects for toxicity of prosulfocarb sulfoxide to *Myriophyllum spicatum* after 14 days

Parameter	After 14 days (µg prosulfocarb sulfoxide/L)						
	Average specific growth inhibition		Yield inhibition				
	Main shoot length	Total shoot length	Biomass (fresh weight)	Biomass (dry weight)	Main shoot length	Total shoot length	Number of whorls
EC ₅₀ ¹	40.9	42.6	21.3	36.4	29.8	26.0	24.5
95%CI	32.2-52.4	34.9-52.6	18.6-24.7	21.9-61.4	21.9-61.4	22.2-30.4	18.7-32.1
NOEC ¹	18.5	11.2	18.5	18.5	18.5	18.5	18.5
LOEC ¹	30.6	18.5	30.6	30.6	30.6	30.6	30.6

95% CI: 95% confidence interval

¹ Based on nominal test item concentrations

Validity criteria

The main shoot length in the control tripled in length by day 14 (must at least double). 100% of the control replicates were sterile on day 14 (must be $\geq 50\%$). Therefore, all validity criteria were met.

Conclusions

Based on nominal test item concentrations, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) for main shoot length were 40.9 and 29.8 µg prosulfocarb sulfoxide/L, respectively. For total shoot length, the 14-day EC₅₀ growth rate (E_rC₅₀) and yield (E_yC₅₀) were 42.6 and 26.0 µg prosulfocarb sulfoxide/L, respectively. For biomass (dry weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 36.4 µg prosulfocarb sulfoxide/L. For biomass (fresh weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 21.3 µg prosulfocarb sulfoxide/L. For number of whorls, the 14-day EC₅₀ for yield (E_yC₅₀) was 24.5 µg of prosulfocarb sulfoxide/L. The 14-day NOEC for growth rate for total shoot length was determined to be 11.2 µg of prosulfocarb sulfoxide/L, and the corresponding LOEC was determined to be 18.5 µg of prosulfocarb sulfoxide/L. For all other parameters, the 14-day NOEC was determined to be 18.5 µg prosulfocarb sulfoxide/L and the 14-day LOEC was determined to be 30.6 mg prosulfocarb sulfoxide/L.

Comments of zRMS:	The study was conducted in line to OECD guideline 202 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.2.1
Report	GLOB1913H: acute toxicity to <i>Daphnia magna</i> in a static 48-hour immobilisation test, Siche O., Wydra V., 2021a, 155401220
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	Yes, the test item instead of the reference item was used to prepare the chromatographic stock and standard solutions. This has no impact on the study since the identity of the analyte was confirmed by the high specificity of the mass transitions.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine the influence of the test item GLOB1913H on the mobility of *Daphnia magna*. For this purpose, young daphnids (< 24 hours old) were exposed in a static test to various concentrations under defined conditions for 48 hours. The recorded effects were the mobility of the daphnids after 24 and 48 hours.

The 48-hour NOEC was determined to be 0.254 mg test item/L. The 48-hour LOEC was determined to be 0.569 mg test item/L and the 48-hour EC₅₀ value was calculated to be 0.421 mg test item/L.

Materials and Methods

Test Item:	GLOB1913H; batch no.: 200701/01; content of Prosulfocarb: 900 g/L (nominal) 886.1 g/L (analytical, equivalent to 98.46% of the declared content), according to certificate of analysis
Test Species:	<i>Daphnia magna</i> , clone 5; 4.50 to 21.75 hours old. Source: The daphnids introduced in the test were taken from ibacon's in-house laboratory culture.
Test Design:	This study encompassed 8 treatment groups (7 dose rates of the test item and a control) each containing 20 individuals. The mobility of the daphnids was determined in a static 48-hour test by visual observation after 24 and 48 hours.

Endpoints:	Number of immobile organisms after 24 and 48 hours
Test Concentrations:	3.0, 1.4, 0.6, 0.3, 0.1, 0.06 and 0.03 mg test item/L (spacing factor 2.2) and a control, corresponding to mean measured concentrations of 2.70, 1.23, 0.569, 0.254, 0.0955, 0.0494 and 0.0243 mg test item/L, and a control.
Test Conditions:	Water temperature: 20.1 to 20.7 °C; pH value: 7.9 to 8.1; dissolved oxygen concentration: 9.2 to 10.3 mg/L; photoperiod: 16 h light - 8 h dark; light intensity: 407 to 974 lux; and thus were within the ranges requested by guideline OECD 202.
Statistical Analysis:	The 24-hour and 48-hour EC ₅₀ , EC ₂₀ and EC ₁₀ and the 95 % confidence limits were calculated by Weibull analysis. The NOEC and LOEC after 24 and 48 hours were calculated by Step-down Cochran Armitage test procedure. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and Discussion

Experimental dates (biological phase): 06 January 2021 - 08 January 2021

Experimental dates (analytical phase): 20 January 2021 – 21 January 2021

The control immobilisation rate was 0% and the dissolved oxygen concentration at test end was ≥ 9.3 mg O₂/L; and thus, the validity criteria were met.

After 48 hours of exposure no immobilisation of the test animals was observed in the control and up to and including the test item concentration of 0.0494 mg test item/L. At the concentrations of 0.0955 and 0.254 mg test item/L, one daphnia was immobile, at the test item concentration of 0.569 mg test item/L 17 daphnids were immobile and at the concentrations of 1.23 and 2.70 mg test item/L all daphnids were immobile. After 48 hours 3 daphnia were moving slower compared to the control at the concentration 0.569 mg test item/L.

Influence of GLOB1913H on the Mobility of *Daphnia magna*

Mean Measured Concentration [mg test item/L]	% of immobilised daphnids after	
	24 hours	48 hours
Control	0	0
0.0243	0	0
0.0494	0	0
0.0955	0	5
0.254	0	5
0.569	10	85
1.23	100	100
2.70	100	100
EC ₅₀ [mg test item/L]:	0.775	0.421
95 % CI [mg test item/L]:	0.537 - 1.12	0.354 - 0.501
EC ₂₀ [mg test item/L]:	0.644	0.292
95 % CI [mg test item/L]:	0.508 - 0.818	0.220 - 0.387
EC ₁₀ [mg test item/L]:	0.570	0.229
95 % CI [mg test item/L]:	0.456 - 0.713	0.157 - 0.334
NOEC [mg test item/L]:	0.254	0.254
LOEC [mg test item/L]:	0.569	0.569

Values refer to mean measured test concentrations

CI: Confidence interval

The quantification of the test item GLOB1913H in the test samples was performed using liquid chromatography with MS/MS detection. At the start of the test 86% of the nominal test concentrations were found (average of all test concentrations). After 48 hours test duration, 90% of the nominal value was

determined (average of all test concentrations). During the test the daphnids were exposed to a mean of 88% of nominal.

Conclusion

The toxic effect of the test item GLOB1913H to *Daphnia magna* was assessed in a static concentration-response test. The 48-hour NOEC was determined to be 0.254 mg test item/L. The 48-hour LOEC was determined to be 0.569 mg test item/L and the 48-hour EC₅₀ value was calculated to be 0.421 mg test item/L.

Since the concentrations of some samples were slightly below 80% of the nominal test concentrations, all biological endpoints were based on arithmetic mean measured test concentrations.

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.2.1
Report	GLOB1913H: toxicity to <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test, Siche O., Wydra V., 2021b, 155401210
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this test was to determine the inhibitory effect of the test item GLOB1913H on the growth of the freshwater green algae *Pseudokirchneriella subcapitata*. For this purpose, exponentially growing cultures of this unicellular green algal species were exposed to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations.

The 72-hour E_yC₅₀ was calculated to be 0.087 mg test item/L and the 72-hour E_rC₅₀ value was calculated to be 0.342 mg test item/L. The 72-hour NOEC for yield and growth rate was determined to be 0.032 mg test item/L and the associated 72-hour LOEC was 0.1 mg test item/L.

Materials and Methods

Test Item:	GLOB1913H; Batch No.: 200701/01; content of Prosulfocarb: 900 g/L (nominal), 886.1 g/L (analytical, equivalent to 98.46% of the declared content), according to certificate of analysis.
Test Species:	<i>Pseudokirchneriella subcapitata</i> , Strain No. 61.81 SAG formerly known as <i>Selenastrum capricornutum</i> , and recently renamed as <i>Raphidocelis subcapitata</i> (KORSHIKOV). Cultivated in the laboratories of ibacon; original source: "Sammlung von Algenkulturen, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen", 37073 Göttingen, Germany.
Test Design:	This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with three replicates per test concentration and six replicates for the control. At test start 50 mL of the test media were inoculated with nominal 5000 algal cells per mL test medium and defined volumes of the algal suspensions were sampled after 24, 48 and 72 hours for determination of cell densities by spectrophotometric

	measurement.
Endpoints:	Yield and growth rate of the algae
Test Concentrations:	1.0, 0.316, 0.100, 0.032 and 0.010 mg test item/L (spacing factor 3.16), and a control.
Test Conditions:	Water temperature: 21.8 to 22.6 °C; pH value in the control at test start: 8.0, pH value in the control at test end: 9.2; pH values in the test item treatments at test start: 8.1 to 8.2, pH values in the test item treatments at test end: 8.4 to 9.2; continuous illumination; mean light intensity: 5145 lux (4470 to 5670 lux).
Statistical Analysis	Based on the calculated cell densities, the 72 hour E_rC_{50} and the 72 hour E_yC_{50} (see Definitions), the corresponding EC_{20} and EC_{10} values and where possible their 95 %-confidence limits were calculated by Probit analysis. For the determination of the 72 hour LOEC and the 72 hour NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams t-test (growth rate) and Bonferoni Welsh t-test (yield). The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and Discussion

Experimental dates (biological phase): 19 January 2021 – 22 January 2021

Experimental dates (analytical phase): 29 January 2021- 30 January 2021

There was a 215.5-fold increase of cell density within 72 hours, a CV of sectional (daily) growth rate of control of 15.9 % and a CV of average growth of control replicates of 2.8 %; and thus, the validity criteria were met.

The 72-hour E_yC_{50} was calculated to be 0.087 mg test item/L and the E_rC_{50} 0.342 mg test item/L. The 72-hour E_yC_{10} was calculated to be 0.042 mg test item/L and the E_rC_{10} 0.055 mg test item/L. The 72-hour NOE_yC was determined to be 0.032 mg test item/L and the associated 72-hour LOE_yC of 0.1 mg test item/L. The 72-hour NOE_rC was determined to be 0.032 mg test item/L and the associated 72-hour LOE_rC is 0.1 mg test item/L.

The microscopic examination of the shape of the algal cells after 72 hours of test duration did not show any difference between the algae that had been growing up to a nominal test concentration of 1.0 mg test item/L and the algal cells in the control. Thus, the shape of the algal cells was not obviously affected up to this test concentration, the highest concentration tested.

Influence of GLOB1913H on the Growth of *Pseudokirchneriella subcapitata*

Parameter	Yield [mg test item/L]	Growth rate [mg test item/L]
72-hour EC_{50}	0.087	0.342
95 % conf. interval	0.080 - 0.095	0.291 - 0.403
72-hour EC_{20}	0.054	0.103
95 % conf. interval	0.044 - 0.065	0.080 - 0.133
72-hour EC_{10}	0.042	0.055
95 % conf. interval	0.032 - 0.054	0.039 - 0.078
72-hour NOEC	0.032	0.032
72-hour LOEC	0.1	0.1

Values refer to nominal test concentrations

The quantification of the active ingredient Prosulfocarb of the test item GLOB1913H in the test samples was performed using liquid chromatography with MS/MS detection. At the start of the test 96 % of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 97 % of the nominal value was determined (average of all test concentrations). During the test the algae were exposed to a mean of 96 % of nominal.

Conclusion

The influence of GLOB1913H on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static concentration-response test. The 72-hour E_yC_{50} was calculated to be 0.087 mg test item/L and the 72-hour E_rC_{50} value was calculated to be 0.342 mg test item/L. The 72-hour NOEC for yield and growth rate was determined to be 0.032 mg test item/L and the associated 72-hour LOEC was 0.1 mg test item/L.

The initial concentrations and the maintenance of the exposure concentrations during the test were verified in the analytical part. All reported results refer to nominal values since the concentrations of the test item were within $\pm 20\%$ of the nominal concentrations during the test

Comments of zRMS:	The study was conducted in line to OECD guideline 221 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.2.1
Report	GLOB1913H: Toxicity to the aquatic plant <i>Lemna gibba</i> in a static growth inhibition test, Siche O., Wydra V., 2021c, 155401240
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine the inhibitory effect of the test item GLOB1913H on the growth of the freshwater aquatic plant *Lemna gibba*.

For this purpose, cultures of *Lemna gibba* were exposed in a static test to various concentrations under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 7 days.

The 7-day E_yC_{50} was calculated to be 0.376 and 0.769 mg test item/L for frond number and dry weight, respectively. The 7-day E_rC_{50} was calculated to be 0.944 and > 3.95 mg test item/L for frond number and dry weight, respectively.

The 7-day NOE_yC and the LOE_yC were determined to be 0.0304 and 0.119 mg test item/L for frond number and 0.119 and 0.385 mg test item/L for dry weight, respectively. The 7-day NOE_rC and the LOE_rC were determined to be 0.119 and 0.385 mg test item/L for frond number and 0.119 and 0.385 mg test item/L for dry weight, respectively

Materials and Methods

Test Item:	GLOB1913H; batch no.: 200701/01; content of a.i.: Prosulfocarb: 900 g/L (nominal) 886.1 g/L (analytical, equivalent to 98.46% of the declared content), according to certificate of analysis.
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Test Species:	<i>Lemna gibba</i> G 3
Test Design:	<p>This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with three replicates per test concentration and control.</p> <p>At test start 12 fronds were introduced in each replicate and incubated for 7 days under static conditions. The frond numbers were determined on day 2, 5 and 7. The dry weight of each replicate was determined at test termination.</p>
Endpoints:	Yield and growth rate based on frond number and dry weight.
Test Concentrations:	<p>4.5, 1.4, 0.44, 0.14 and 0.043 mg test item/L (spacing factor 3.2) corresponding to following arithmetic mean measured concentrations of the test item:</p> <p>3.95, 1.30, 0.385, 0.119 and 0.0304 mg test item/L, and a control.</p>
Test Conditions:	<p>Water temperature: 22.8 to 23.5 °C;</p> <p>pH values in the freshly prepared control medium at test start: 7.5</p> <p>pH values in the aged control medium at test end: 8.9</p> <p>pH values at test start in the test item treatments: 7.5</p> <p>pH values at test end in the test item treatments: 8.6 to 8.9</p> <p>continuous illumination; mean light intensity: 6587 lux (6510 to 6660 lux).</p>
Statistical Analysis:	<p>The ErC50 and the EyC50, the corresponding EC20 and EC10 values and where possible their 95 %-confidence limits were calculated by Probit analysis.</p> <p>For the determination of the 7-day LOEC and NOEC values for yield and growth rate significant differences at the test concentrations compared to the control values were tested by the Williams t-test (frond number and dry weight).</p> <p>The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.</p>

Results and Discussion

Experimental dates (biological part): 27 January 2021 – 03 February 2021
Experimental dates (analytical part): 04 February 2021 – 24 February 2021

Validity criteria:

Doubling Time of fronds in the control was 1.7 days, and therefore the validity criterion was met.

Biological results:

The shape of fronds and colonies after the test period of 7 days was not different to those in the control up to and including the test concentration of 0.119 mg test item/L.

At the higher test item concentrations the fronds showed deviations from the control replicates after 7 days; *i.e.* gibbous growth (0.385 up to 3.95 mg test item/L), chlorosis (0.385 mg test item/L) and slightly pronounced necrosis (1.30 and 3.95 mg test item/L).

Summary of Biological Results

Parameter	Yield (frond number) [mg test item/L]	Growth rate (frond number) [mg test item/L]	Yield (dry weight) [mg test item/L]	Growth rate (dry weight) [mg test item/L]
EC ₅₀ (7-day)	0.376	0.944	0.769	> 3.95
95 % conf. limits	0.348 - 0.407	0.819 - 1.09	0.570 - 1.05	n.d.
EC ₂₀ (7-day)	0.171	0.304	0.156	0.681
95 % conf. limits	0.149 - 0.191	0.236 - 0.371	0.085 - 0.233	0.464 - 0.909

EC ₁₀ (7-day)	0.113	0.168	0.068	0.204
95 % conf. limits	0.094 - 0.131	0.119 - 0.218	< 0.0304 - 0.116	0.102 - 0.320
7-day NOEC	0.0304	0.119	0.119	0.119
7-day LOEC	0.119	0.385	0.385	0.385

n.d.: not determinable,

Values refer to arithmetic mean measured test concentrations

Analytical results:

The quantification of the active ingredient prosulfocarb of the test item GLOB1913H in the test samples was performed using liquid chromatography with MS/MS detection.

At the start of the test 84 % of the nominal test concentrations were found (average of all test concentrations). After 7 days test duration, 85 % of the nominal value was determined (average of all test concentrations). During the test the *Lemna* were exposed to a mean of 85 % of nominal.

Conclusion

The influence of GLOB1913H on the growth of the freshwater plant *Lemna gibba* was assessed in a static concentration-response test.

The 7-day E_yC₅₀ was calculated to be 0.376 and 0.769 mg test item/L for frond number and dry weight, respectively. The 7-day E_rC₅₀ was calculated to be 0.944 and > 3.95 mg test item/L for frond number and dry weight, respectively.

The 7-day NOE_yC and the LOE_yC were determined to be 0.0304 and 0.119 mg test item/L for frond number and 0.119 and 0.385 mg test item/L for dry weight, respectively. The 7-day NOE_rC and the LOE_rC were determined to be 0.119 and 0.385 mg test item/L for frond number and 0.119 and 0.385 mg test item/L for dry weight, respectively.

The initial concentrations and the maintenance of the exposure concentrations during the test were verified in the analytical part. All reported results refer to arithmetic mean concentrations, since the mean measured concentration of the lowest test concentration of nominal 0.043 mg test item/L was slightly below 80% of the nominal concentration.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference: KCP 10.2.1

Report Effects of Prosulfocarb 800 EC on *Myriophyllum spicatum* in a growth inhibition test under semi-static test conditions, Juckeland D., 2013a, 13 10 48 018 W

Guideline(s): Yes, ASTM Designation E 1913-04

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

The toxicity of prosulfocarb to the aquatic plant *Myriophyllum spicatum* was determined in a 14-day semi-static test. The *Myriophyllum* were exposed to nominal test item concentrations of 0.06, 0.13, 0.29, 0.64, 1.41, 3.10, 6.82 and 15.0 mg prosulfocarb/L alongside a culture medium control.

Based on nominal test item concentrations, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) for main shoot length were 2.00 and 1.34 mg prosulfocarb/L, respectively. For total shoot length, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) were 2.70 and 1.75 mg prosulfocarb/L, respectively. For biomass (fresh weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 0.74 mg prosulfocarb/L. For biomass (dry

weight), the 14-day EC_{50} for yield (E_yC_{50}) was 11.8 mg prosulfocarb/L. For number of whorls, the 14-day EC_{50} for yield (E_yC_{50}) was 1.13 mg prosulfocarb/L.

Materials and Methods

Test Material	Prosulfocarb 800 EC
Lot/Batch #:	1910121005
Actual content of a.i.:	Prosulfocarb: 795 g/L
Description:	Clear yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	September 2014
Density	1.03 g/mL
Treatments	
Test rates:	Dilution water control and nominal test item concentrations of 0.06, 0.13, 0.29, 0.64, 1.41, 3.10, 6.81 and 15.0 mg prosulfocarb/L
Solvent:	None
Positive control:	None
Analysis of test concentration:	Yes, analysis of prosulfocarb on days 0, 7 and 14, in fresh and replaced solutions, using HPLC with MS detection
Test organisms	
Species:	<i>Myriophyllum spicatum</i> L.
Source:	Cultures prepared under test conditions (originally obtained from Federal Environment Agency; Division IV: Chemical and Biological Safety, Germany)
Test design	
Test vessels:	100 mL glass tubes with air-permeable stoppers filled with 50 mL of test medium
Test medium:	Modified Andrews' medium with 3 % sucrose
Replication:	Ten vessels for the control and five vessels for each test concentration (+ 1 additional vessel for analysis and retained specimen per concentration and control)
Number of plants:	One plant per replicate
Exposure regime:	Semi-static
Duration:	14 days
Environmental conditions	
Temperature:	22.9 – 24.5°C
pH:	5.72 - 5.87 new solutions, 5.77 – 8.36 aged solutions
Lighting:	16 : 8 hours fluorescent light : dark at an average of approximately 10000 lux

Study Design and Methods

Experimental dates: 26 March 2013 to 10 April 2013

A primary stock solution with a nominal test item concentration of 300.0 mg prosulfocarb/L was prepared by weighing 75.0 mg of the test item and making up to 250 mL with test medium. A secondary stock solution with a nominal test item concentration of 15.0 mg prosulfocarb/L was prepared using 50.0 mL of the primary stock solution and making up to 1000 mL with test medium. Appropriate volumes of the secondary stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

50 mL of the test solutions were transferred into 100 mL glass tubes and inoculated with *Myriophyllum* plants. Cultures were then transferred to an environment chamber where they were maintained under the conditions indicated above. On Day 7 fresh solutions were prepared and test solutions were renewed.

Assessments of main shoot length and number of whorls of the test organism were made on days 0, 7 and 14. Plants were harvested for measurement of fresh and dry weight after 14 days; the initial fresh and dry weight was determined using a sample of 10 untreated test organisms identical to that used to inoculate the test on day 0.

Temperature was measured continuously, light intensity was recorded once at test start and pH was recorded on day 0, 7 and 14, in fresh and aged solutions.

The test concentrations were verified by chemical analysis of prosulfocarb at days 0, 7 and 14, in fresh and aged solutions, using high performance liquid chromatography with mass spectrometry detection.

Results and Discussion

In the fresh solutions, the concentrations of the test item were found to be in the range 21 to 43 % of the nominal values, and in the aged solutions they were in the range 15 to 31 % (see table below). The limit of quantification in this study was 0.022 mg prosulfocarb/L. Nominal test item concentrations were used for the calculation and reporting of results.

Analytical results

Nominal test item concentrations (mg prosulfocarb /L)	% of nominal measured at day 0 (fresh solutions)	% of nominal measured at day 7 (aged solutions)	% of nominal measured at day 7 (fresh solutions)	% of nominal measured at day 14 (aged solutions)
Control	-	-	-	-
0.06	21*	17*	28*	22*
0.13	22	18*	34	22
0.29	22	19	41	26
0.64	26	21	43	30
1.41	21	17	33	31
3.10	21	19	33	27
6.82	21	15	33	22
15.0	22	16	30	26

*The measured concentration was less than the defined limit of quantification (0.022 µg/L)

Data for main shoot length, total shoot length, fresh weight and dry weight were used to calculate growth rate and yield for the control and each exposure concentration. Probit analysis using linear maximum likelihood regression was used to calculate the EC₅₀ values. (The nominal 0.06 and 0.13 mg test item/L treatment group were omitted from the EC₅₀ value determination because the measured concentration was less than the defined limit of quantification.) For the Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) values, was used a Williams t-test, Welch-t-test, or U-test, as appropriate, to determine values significantly different to the control.

Mean growth rate based on main shoot length and total shoot length is presented below along with the growth and yield inhibition values:

Effect of prosulfocarb on growth rate and yield of *Myriophyllum spicatum* for main shoot length and total shoot length

Nominal test item concentrations (mg prosulfocarb/L)	Mean final main shoot length	Mean final shoot length	% inhibition			
			Average specific growth rate (%I _r)		Yield (% I _y)	
			Main shoot length	Total shoot length	Main shoot length	Total shoot length
Control	7.2	8.0	-	-	-	-
0.06	8.3	9.6	-4.5	-6.5	-18.1	-26.1
0.13	9.5	10.8	-22.8	-22.7	-48.2	-50.4
0.29	9.6	10.8	-18.0	-17.8	-45.2	-47.0
0.64	5.9	6.2	30.4*	32.9*	34.8*	39.6*
1.41	4.6	5.9	45.0*	31.6*	57.0*	39.5*
3.10	3.7	4.1	66.4*	59.9*	76.5*	71.6*
6.82	3.9	4.3	74.6*	66.2*	80.9*	74.7*
15.0	3.4	3.4	78.5*	78.7*	85.8*	86.7*

* = significantly different to untreated control (Williams t-test; Welch t-test, or U-test $p \leq 0.05$, one-sided)

(-) = increase in growth relative to the control

Mean final number of whorls, mean biomass fresh weight, and mean biomass dry weight are presented below along with the respective inhibition values:

Effect of prosulfocarb on yield of *Myriophyllum spicatum* for biomass (fresh and dry weight) and number of whorls

Nominal test item concentrations (mg prosulfocarb/L)	Mean final number of whorls	Mean biomass (fresh weight) (g)	Mean biomass (dry weight) (g)	% inhibition		
				Number of whorls	Biomass (fresh weight)	Biomass (dry weight)
Control	18.3	274.2	41.4	-	-	-
0.06	19.2	331.4	48.9	-9.2	-25.8	-24.6
0.13	19.4	335.3	53.1	-9.2	-27.5	-38.4
0.29	20.4	378.8	59.4	-17.6	-47.1	-59.0
0.64	14.4	133.8	32.5	36.1*	63.2*	28.9*
1.41	11.2	111.5	29.5	61.3*	73.3*	38.8
3.10	8.4	93.1	30.9	81.5*	84.6*	34.2
6.82	8.6	87.4	27.9	86.6*	84.1*	44.0*
15.0	7.4	90.6	26.5	91.6*	82.7*	48.8*

* = significantly different to untreated control (Welch t-test or Welch t-test, $p \leq 0.05$, one-sided)

(-) = increase in growth relative to the control

Summary of biological effects for toxicity of prosulfocarb to *Myriophyllum spicatum* after 14 days

Parameter	After 14 days (mg prosulfocarb /L)						
	Average specific growth inhibition		Yield inhibition				
	Main shoot length	Total shoot length	Biomass (fresh weight)	Biomass (dry weight)	Main shoot length	Total shoot length	Number of whorls
EC ₅₀ ¹	0.381	0.505	0.149	2.05	0.263	0.336	0.225
95%CI	0.206-0.695	0.280-0.963	0.133-0.1941	0.88-41.1	0.139-0.467	0.165-0.655	0.146-0.336
NOEC ¹	0.058	0.058	0.058	0.058	0.058	0.058	0.058
LOEC ¹	0.143	0.143	0.143	0.143	0.143	0.143	0.143

95% CI: 95% confidence interval

¹ Based on nominal test item concentrations

Validity Criteria

The main shoot length in the control tripled in length by day 14 (must at least double). 100 % of the control replicates were sterile on day 14 (must be ≥ 50 %). Therefore, all validity criteria were met.

Conclusions

Based on nominal test item concentrations, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) for main shoot length were 2.00 and 1.34 mg prosulfocarb/L, respectively. For total shoot length, the 14-day EC₅₀ for growth rate (E_rC₅₀) and yield (E_yC₅₀) were 2.70 and 1.75 mg prosulfocarb/L, respectively. For biomass (fresh weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 0.74 mg prosulfocarb/L. For biomass (dry weight), the 14-day EC₅₀ for yield (E_yC₅₀) was 11.8 mg prosulfocarb/L. For number of whorls, the 14-day EC₅₀ for yield (E_yC₅₀) was 1.13 mg prosulfocarb/L. For all parameters, the 14-day NOEC was determined to be 0.29 mg prosulfocarb/L and the 14-day LOEC was determined to be 0.64 mg prosulfocarb/L.

Comments of zRMS:	The study was conducted in line to OECD guideline 239 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference: KCP 10.2.1

Report GLOB1913H: Toxicity to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test with a prior rooting phase, Siche O., Wydra V., 2021d, 155401215

Guideline(s): Yes, OECD 239 (2014)

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

The purpose of this test was to determine the inhibitory effect of the test item GLOB1913H on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum*. Plants of *Myriophyllum spicatum* were exposed in a static test to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 14 days.

The 14-day NOE_yC and the LOE_yC were determined to be 0.037 and 0.103 mg test item/L for total shoot length, 0.103 and 0.310 mg test item/L for fresh weight and 0.103 and 0.310 mg test item/L for dry weight, respectively. The 14-day NOE_rC and the LOE_rC were determined to be 0.037 and 0.103 mg test item/L for total shoot length, 0.103 and 0.310 mg test item/L for fresh weight and 0.310 and 0.900 mg test item/L for dry weight, respectively.

The 14-day E_yC₅₀ was calculated to be 0.437, 0.496 and 1.05 mg test item/L for total shoot length, fresh weight and dry weight, respectively. The 14-day E_rC₅₀ was calculated to be 1.00, 1.02 and 1.99 mg test item/L for shoot length, fresh weight and dry weight, respectively.

Materials and Methods

Test Item: GLOB1913H; Batch No.: 200701/01; Content of Prosulfocarb.: 886.1 g/L (analytical), according to certificate of analysis.

Test Species: *Myriophyllum spicatum*

Test Design: This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with five replicates per test concentration and ten replicates for the control.

After a pre-rooting phase of 7 days, 1 plant per replicate was incubated for 14 days under static conditions. The shoot length was determined at test start and day 14. Sublethal parameters were assessed at test start, once during the test (e.g. day 7) and at test end. At test end fresh and dry weight of each replicate was determined. The samples collected at start and after 14 days were analysed.

Endpoints: Inhibition of growth expressed in terms of yield and growth rate, based on total shoot length, fresh and dry weight

Test Concentrations: Nominally 5.00, 1.58, 0.501, 0.158 and 0.050 mg test item/L corresponding to time weighted average measured concentrations of 2.95, 0.90, 0.31, 0.103 and 0.037 mg test item/L, and a control.

Test Conditions: Water temperature: 19.4 - 22.2 °C;

light regime: 16 h light : 8 h dark; mean light intensity: 8716 lux (8050- 9460 lux);

pH values at pre-rooting phase: 7.9,

pH values of the control:

test start: 7.9 , on day 7: 9.6 -10.0, at the end of the test: 10.0 - 10.2 *

pH values of the test item treatments:

test start: 8 -8, on day 7: 8.8 - 9.8, at the end of the test: 8.6 - 10.1

* According to the OECD guideline 239 the increase of pH of >1.5 does not invalidate the study.

The pH increases because the HCO₃ from the medium is metabolised by growing plants. They need the CO₂ for their cell growth and leave OH⁻, which in consequence increases the pH of the test medium. This is a natural reaction called biogenic decalcification.

oxygen concentrations at test start: 8.7 - 10.8 mg/L, on day 7: 11.2 - 18.1 mg/L, at the end of the test: 8.6 - 12.9 mg/L.

Statistical Analysis:

The EC_{50/20/10} (the concentrations of the test item corresponding to 50, 20 and 10 % inhibition of growth rate (total shoot length, fresh weight and dry weight) and yield (total shoot length, fresh weight and dry weight) compared to the control), and their 95 %-confidence limits were calculated by 3-parametric normal CDF.

For the determination of the 14-day LOEC and the 14-day NOEC, the calculated growth rates and yields based on total shoot length, fresh weight and dry weight at each test concentration were tested for significant differences compared to the control values by Williams t-test (yield) and Bonferroni-Welch t-test (growth rate).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and Discussion

Experimental dates (biological part): 12 January 2021 – 05 February 2021

Experimental dates (analytical part): 09 February 2021 – 24 February 2021

Validity criteria:

Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film. The total shoot length increased by a factor of 4.5 after 14 days of exposure. The fresh weight increased by a factor of 6.9 after 14 days of exposure. The coefficient of variation of yield fresh weight was 16.9 %. Thus, all validity criteria were met.

Biological results:

Observed effects followed a concentration-response-relationship. Phytotoxic symptoms were observed at 0.31, 0.9 and 2.95 mg test item/L and number and extent of the symptoms increased with increasing test concentration. Symptoms observed were distorted leaves, shortened shoot tips, chlorosis, lack of buoyancy, shortened roots and fewer roots.

Summary of Biological Results

Parameter	Yield (total shoot length) [mg test item/L]	Growth rate (total shoot length) [mg test item/L]	Yield (fresh weight) [mg test item/L]	Growth rate (fresh weight) [mg test item/L]	Yield (dry weight) [mg test item/L]	Growth rate (dry weight) [mg test item/L]
EC ₅₀ (14-day)	0.437	1.00	0.496	1.02	1.05	1.99
95 % conf. limits	0.335 - 0.570	0.860 - 1.16	0.363 - 0.679	0.810 - 1.29	0.690 - 1.59	1.37 - 2.88
EC ₂₀ (14-day)	0.133	0.327	0.174	0.305	0.246	0.440
95 % conf. limits	0.091 - 0.198	0.260 - 0.413	0.110 - 0.279	0.214 - 0.438	0.128 - 0.481	0.234 - 0.835
EC ₁₀ (14-day)	0.071	0.182	0.100	0.162	0.115	0.200
95 % conf. limits	0.043 - 0.120	0.133 - 0.250	0.054 - 0.187	0.099 - 0.265	0.047 - 0.290	0.083 - 0.486
14-day NOEC	0.037	0.037	0.103	0.103	0.103	0.310
14-day LOEC	0.103	0.103	0.310	0.310	0.310	0.900

Values refer to time weighted average measured test concentrations

Analytical results:

The quantification of the active ingredient prosulfocarb of the test item GLOB1913H in the test samples was performed using liquid chromatography with MS/MS detection. The concentrations of the test item were determined in the overlying test water of the nominal test concentrations of 0.05, 0.158, 0.501, 1.58,

5.0 mg test item/L, the abiotic control (5.0 mg test item/L), the abiotic sediment control (5.0 mg test item/L) and in the control.

The concentrations of the test item were additionally determined in the sediment and pore water of the test concentration of nominal 5.0 mg test item/L, the abiotic sediment control (5.0 mg test item/L) and the control from test start and test end.

At the start of the test 95% of the nominal test concentrations were found in the overlying test media (average of test concentrations of nominal 0.05 to 5.0 mg test item/L). Thus the test item was dosed correctly. After 14 days test duration the recoveries in the aged overlying test media were reduced to 57% (average of test concentrations of nominal 0.05 to 5.0 mg test item/L).

Therefore, the biological endpoints were based on time-weighted average concentrations.

In the aged overlying water samples of the abiotic control (Samples treated like the test samples but without sediment and without plants) the measured concentrations of the test item were close to 100%. The concentration in the overlying water samples of the aged abiotic sediment control (Samples treated like the test samples with sediment but without plants) were reduced to 53%.

Therefore, it can be concluded that the reduced concentrations of the test item in the aged overlying water samples was caused by the presence of sediment.

Conclusion

The influence of GLOB1913H on the growth of the dicotyledonous freshwater plant *Myriophyllum spicatum* was assessed in a static concentration-response test.

The 14-day NOE_yC and the LOE_yC were determined to be 0.037 and 0.103 mg test item/L for total shoot length, 0.103 and 0.310 mg test item/L for fresh weight and 0.103 and 0.310 mg test item/L for dry weight, respectively. The 14-day NOE_rC and the LOE_rC were determined to be 0.037 and 0.103 mg test item/L for total shoot length, 0.103 and 0.310 mg test item/L for fresh weight and 0.310 and 0.900 mg test item/L for dry weight, respectively.

The 14-day E_yC₅₀ was calculated to be 0.437, 0.496 and 1.05 mg test item/L for total shoot length, fresh weight and dry weight, respectively. The 14-day E_rC₅₀ was calculated to be 1.00, 1.02 and 1.99 mg test item/L for shoot length, fresh weight and dry weight, respectively.

The correct application of the test item and the maintenance of the exposure concentrations during the test were verified in the analytical part. All reported results refer to time weighted average concentrations, since the test item concentrations were not within $\pm 20\%$ of the nominal concentrations during the test.

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Comments of zRMS:	The re-analysis has been already assessed and accepted in dRAR, December 2022.
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Reference: KCA 8.2.8

Report Re-analysis of biological data of a mesocosm experiment performed with prosulfocarb, Deneer J., 2015, ICI574_10226

Guideline(s): No official guidelines followed

Deviations: No

GLP: No

Acceptability: Yes/No/Supplementary

Executive summary

The aim was to evaluate the statistical power of the data set from a microcosm study on prosulfocarb previously given in a confidential report: Van Wijngaarden, R.P.A. (2006). Prosulfocarb EC (800) formulation (A8545C): Microcosm experiment to determine population and community level effects on plankton communities and periphyton (GLP compliant study). The van Wijngaarden prosulfocarb mesocosm data set contains 21 potentially sensitive taxa in category 1. Hence the prosulfocarb study meets the EFSA (2013) criterion that at least 8 different taxa of the potentially sensitive taxonomic groups (in this case phytoplankton and periphyton) need to be available to allow the derivation of a Regulatory Acceptable Concentration based on the Ecological Threshold Option (ETO-RAC). At 3 µg/L no clear dose-response effects were demonstrated (effect class 1). At the concentration of 15 µg/L a slight and short-term effect was observed in only one taxon (*Achnanthes* spp.) in periphyton. This taxon was represented in the MDD Category 1 in both, the phytoplankton and the periphyton communities. As phytoplanktonic species, a treatment-related effect on this taxon could not be observed. In the periphyton, the observed abundance decline occurred in one isolated sampling date (on day 14). Note however, that the mean abundance was higher in the 76 µg/L and 380 µg/L treatment groups as compared to the 15 µg/L treatment. According to the decision scheme of Brock et al. (2015), this abundance decline corresponds to an effect class 2. At the concentration of 76 µg/L and the highest test concentration of 380 µg/L, observed effects were more pronounced and longer lasting with recovery not appropriately evaluated due to high %MDD abu values in the recovery period. The observed treatment-related population abundance increases or decreases in these two treatment levels corresponded to an effect class 3A-4B.

Methods

The minimum detectable differences (MDD) were calculated for phytoplankton, periphyton and zooplankton. All phytoplankton and periphyton taxa are a priori considered to be potentially sensitive groups. Zooplankton was expected to be affected as well, not because of direct effects as a result of high sensitivity of zooplankton taxa towards prosulfocarb, but because of indirect effects, i.e. their main source of food being affected. The data given for macrophytes (surface coverage and biomass at the end of the sampling period) were not used in the re-analysis. Surface coverage as given is expected to be relatively imprecise. Differences of less than 10% in coverage can probably not be estimated reliably, and calculation of MDD's for such data gives an overly optimistic view of the reliability of the coverage data. In the original report van Van Wijngaarden (2006) no response to macrophytes was identified, and for that reason the macrophyte data is not included in the MDD re-analysis.

Statistical analysis

The original analysis of algae and invertebrate data sets was performed using multivariate techniques (Principal Response Curve methodology; PRC) and univariate techniques (Williams test). Since the previous multivariate analysis is still valid the PRC analysis was not repeated. However, the Minimal Detectable Difference (MDD) was presented as a supplement to the NOECs calculated by means of the univariate Williams test. The Community Analysis computer program was used for this (Hommen et al., 1994). NOEC (No Observed Effect Concentration) estimations at taxon level ($p \leq 0.05$) were carried out using the Williams test (ANOVA; Williams, 1972). The test assumes that the mean response of the variable is a monotonic function of the treatment, thus expecting increasing effects with increasing dose. The analyses were performed with the Community Analysis (CA) computer program v3.4.08 (Hommen et al., 1994), resulting in an overview of NOECs for each sampling day for the data analyzed. Where statistically significant differences between treatments and controls were observed and these were considered to be treatment-related, the responses for treatment-related declines were categorized into Effect Classes. Abundances, calculated values for NOECs, and MDDs for phytoplankton, periphyton and zooplankton are given in Annexes 1 – 3 of the report. Data for macrophytes, including NOEC as well as MDD and effect classes are also given.

Results and Discussion

All phytoplankton and periphyton taxa are considered to be potentially sensitive groups. In some cases, erratic responses were observed in the evaluated phytoplankton and periphyton taxa. The taxa that did not

show a clear and consistent concentration-response relationship on the sampling date of the calculated NOEC were not used for the effect class derivation. There is overlap in the potentially sensitive taxa in the phytoplankton and periphyton samples, since all three sensitive taxa in the periphyton data set were also present in the phytoplankton data set. However, the phytoplankton data set already contains 21 potentially sensitive taxa in category 1. The data set therefore meets the EFSA (2013) criterion that at least 8 different taxa of the potentially sensitive taxonomic groups (in this case phytoplankton and periphyton) need to be available to allow the derivation of a Regulatory Acceptable Concentration based on the Ecological Threshold Option (ETO – RAC).

Conclusions

The prosulfocarb data set contains 21 potentially sensitive taxa in category 1. Hence the prosulfocarb study meets the EFSA (2013) criterion that at least 8 different taxa of the potentially sensitive taxonomic groups (in this case phytoplankton and periphyton) need to be available to allow the derivation of a Regulatory Acceptable Concentration based on the Ecological Threshold Option (ETO-RAC). At the concentration of 3 µg/L no clear dose-response effects were demonstrated (effect class 1). At the concentration of 15 µg/L a slight and short-term effect was observed in only one taxon (*Achnanthes* spp.) in periphyton. This taxon was represented in the MDD Category 1 in both, the phytoplankton and the periphyton communities. As phytoplanktonic species, a treatment-related effect on this taxon could not be observed. In the periphyton, the observed abundance decline occurred in one isolated sampling date (on day 14). Note however, that the mean abundance was higher in the 76 µg/L and 380 µg/L treatment groups as compared to the 15 µg/L treatment. According to the decision scheme in Figure 3 of Brock et al. (2015), this abundance decline corresponds to an effect class 2. At the concentration of 76 µg/L and the highest test concentration of 380 µg/L, observed effects were more pronounced and longer lasting with recovery not appropriately evaluated due to high %MDD abu values in the recovery period. The observed treatment-related population abundance increases or decreases in these two treatment levels corresponded to an effect class 3A-4B.

Comments of zRMS:	The study has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.8
Report	SYN521384 – The effect on phytoplankton and periphyton in freshwater mesocosms, Taylor S., 2013, CEA.984; SYN521384_10033
Guideline(s):	OECD Guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms) (2006), SETAC Guidance document on testing procedures for pesticides in freshwater microcosms (1991), SETAC Community-level aquatic systems studies – Interpretation criteria (2002)
Deviations:	No
GLP:	Yes, with the exception of water and sediment characterisation, and meteorological data
Acceptability:	Yes/No/Supplementary

Executive Summary

The effects of SYN521384 on phytoplankton, periphyton and macrophyte communities were determined in outdoor mesocosms. Mesocosms were exposed to two treatment applications, seven days apart, of nominal concentrations of 3, 10, 30 and 50 µg SYN521384/L and a water control. Based on nominal concentrations, the NOEC_{population} and NOEC_{coverall} for phytoplankton and macrophytes was 30 µg SYN521384/L, and for periphyton and zooplankton it was 50 µg SYN521384/L. The NOEC_{community}

was 50 µg SYN521384/L, with the exception of phytoplankton for which the NOECcommunity was 30 µg SYN521384/L.

Materials

Test Material:	SYN521384 R331405 Prosulfocarb sulfoxide
Parent:	Prosulfocarb
Lot/Batch #:	MLA-581/4
Purity:	96 % w/w (estimated error: ± 2 %)
Description:	Colourless oil
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	31 August 2013
Density:	Not reported
Test concentrations:	Deionised water control and nominal concentrations of 3, 10, 30 and 50 µg SYN521384/L
Control:	Untreated mesocosms
Test water:	Mature pond water collected from a nearby mesocosm reservoir facility
Analysis of test concentrations:	Yes, at 0, 1, 3, 7, 8, 10, 14, 21, 28, 35, 42, 49 and 56 days after treatment by LC-MS/MS detector

Test animals

Species: plankton	Natural populations of phytoplankton, periphyton, macrophytes and zoo-
Source:	A reservoir comprising a partially sunken lagoon (12 x 12 m wide and 0.8 m deep) with areas of sediment and mature populations of macroinvertebrates, zooplankton, aquatic plants and algae

Test design

Test vessel:	Fibreglass tanks (1.8 x 0.9 x 0.8 m) located in the ground to a depth of approximately 0.6 m with approximately 0.2 m remaining above ground. Each mesocosm contained approximately 10 cm of sediment and 50 cm of overlying water.
Exposure regime:	Static
Aeration:	None
Replication:	4

Environmental conditions

Water temperature:	11.37 to 24.76 °C
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pH: 7.1 to 10.38

Dissolved oxygen: 7.76 to 16.31 mg/L

Total hardness of dilution water: 64.10 to 207.19 mg/L as CaCO₃

Lighting: Natural conditions

Length of test: 117 days

Study Design and Methods

Experimental dates: 26 April 2012 to 31 October 2012

The study was conducted in ponds exposed to natural conditions at Boxworth, UK. Mesocosms were established between May and December 2010 and contained a layer of clay loam and mature lake sediment plus overlying water and populations of phytoplankton, periphyton, zooplankton and macroinvertebrates. A mesocosm reservoir, established in February 2010, was used to supply the water and most of the organisms for the study.

A concentrated stock solution of 100 mg SYN521384/L was prepared by adding 0.5 g of SYN521384 to approximately 1.5 L of deionised water in a 2 L volumetric flask, followed by ultrasound treatment and vigorous shaking. The contents of the flask were then made up to 2 L with deionised water and added to a 10 L aspirator (calibrated to 5 L). The flask was then refilled with 2 L of deionised water and added to the aspirator in order to rinse any test item remaining in the flask. The aspirator was then made up to 5 L with an additional aliquot of deionised water, covered with a black bag and stirred, after which the required volumes to prepare the dosing solutions were removed. The stock solution was stirred for the duration of the dosing period. Aliquots of this stock solution were added to volumetric flasks and made up to 1 L with deionised water, followed by inversion, giving dosing solutions at nominal concentrations of 3, 10, 30 and 50 µg SYN521384/L. The treatment solutions were poured into the appropriate mesocosm in a figure of eight pattern and each flask refilled with deionised water to rinse any remaining test item into the same mesocosm. The water was then mixed in a figure of eight pattern for one minute. A second application was made seven days after the initial application. The control mesocosms consisted of deionised water only.

Test item concentrations were verified by analysing water and/or sediment samples for SYN521384 on Days 0, 1, 3, 7, 8, 10, 14, 21, 28, 35, 42, 49 and 56 days after initial application, and also by analysing dose and stock solutions. Analyses were performed using LC-MS/MS detector. Water temperature, pH, turbidity, conductivity and dissolved oxygen concentration were measured in situ at a depth of approximately 25 cm on Days 1, 3, 8, 11, 14, 21, 28, 36, 42, 56, 63, 77, 92, 105 and 116 days after initial application. Total phosphorus, oxidised nitrogen, alkalinity, hardness and suspended solids were recorded on Days 14, 28, 56, 77 and 117 after initial application. Air temperature, precipitation and sunshine were recorded daily, and were provided from three UK Meteorological Office weather stations in the same geographical region as the test facility

Phytoplankton, periphyton, macrophytes and zooplankton were all sampled on Days 42, 56, 63, 77, 92, 105 and 117 after initial application. Additional sampling was conducted on Days 1, 3, 8, 11, 14, 21, 28 and 36 for phytoplankton, Days 14 and 28 for periphyton, Days 3, 11, 14 and 28 for macrophytes, and Days 21 and 36 for zooplankton. Macrophyte distribution was visually assessed and plant health was qualitatively assessed based on appearance, coverage, structure, stem number and stem length. Total biomass of the macrophytes was determined upon study completion. Depth-integrated water samples were collected from several spots in each mesocosm and analysed for chlorophyll-a, and diversity and abundance of zooplankton and algae. Periphyton were colonised in periphytometers for typically two weeks and the diversity and abundance of periphyton was determined.

Statistical evaluation of the data for phytoplankton, periphyton and zooplankton communities were performed using multivariate (Principal Response Curve method) and univariate (ANOVA) analyses on the log-transformed data. Univariate analysis was used for macrophyte data. Comparisons were made between each test item treatment group and the control using a two-sided Dunnett's t-test and/or a two-sided Mann-Whitney U test, at the 5 % probability level.

Results and Discussion

For the initial treatment (Day 0), the measured concentrations in the mesocosms were in the range 69 to 87 % of the nominal values and for the second treatment (Day 7) were in the range 69 to 86 % (see table below). The limit of quantification in this study was 0.05 µg SYN521384/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Test item: SYN521384								
Treatment group (µg/L)	Day 0				Day 7			
	Mesocosm		Dosing solutions		Mesocosm		Dosing solutions	
	Mean measured conc. (µg/L)	% of nominal conc.	Nominal conc. Mg/L	% of nominal (range)	Mean measured conc. (µg/L) ^a	% of nominal conc.	Nominal conc. Mg/L	% of nominal (range)
3	2.61	87	2.43	79-87	2.45	82	2.43	74-89
10	8.43	84	8.10	85-96	6.88	69	8.10	80-91
30	21.4	71	24.3	96-110	25.8	86	24.3	79-92
50	34.7	69	40.5	88-120	34.2	69	40.5	86-92

^a adjusted for residues remaining after initial treatment

Conc. = concentration

Where possible, a NOEC (No Observed Effect Concentration) and a NOEAEC (No Observed Ecological Adverse Effect Concentration) were determined for each community. Effects were classified according to the effects classes published by de Jong *et al* (2008), as shown in the table below:

Criteria for categorising effects of SYN521384 on phytoplankton, periphyton macrophytes and zooplankton

Effect class	Description	Criteria
1	Effects could not be demonstrated (NOECmicro/microcosm)	No (statistically significant) effects observed as a result of treatment
		Observed differences between treatment and control show no clear causal relationship
2	Slight and transient effects	Effects reported as 'slight' or 'transient', or other similar description
		Short-term and/or quantitatively restricted response of one or a few sensitive endpoints, and only observed at individual samplings
3A	Pronounced effects; recovery within 8 weeks after the first application or total period of effects	Clear response of sensitive endpoints, but full recovery within 8 weeks after the first application, or total period of effects < 8 weeks
		Effects reported as 'temporary effects on several sensitive species', 'temporary effects on less sensitive species/endpoints' or other similar descriptions
		Effects observed at some subsequent sampling instances
3B	Pronounced effects; recovery within 8 weeks after the last application (dilution)	Clear effects of sensitive endpoints, but full recovery within 8 weeks following the last application (dilution). In the case of repeated treatments (dilutions), a total duration of the effects of > 8 weeks is possible
		Effects reported as 'temporary effects on several sensitive species', 'temporary effects on less sensitive species/endpoints' or other similar descriptions

		Effects observed at some subsequent sampling instances
4	Pronounced effects; study too short to demonstrate recovery within 8 weeks after the last application	Clear effects observed as in Effect class 3, but the study is too short to demonstrate complete recovery within 8 weeks after the (last) application
5A	Pronounced effects; total period of effects >8 weeks and no recovery within 8 weeks after the last application; full recovery within the test period	Clear response of sensitive endpoints and recovery time is longer than 8 weeks after the last application
		Full recovery is reported before the end of the study
		Effects reported as 'long-term effects followed by recovery on several sensitive and less sensitive species/endpoints or other similar descriptions
		On consecutive time points
5B	Pronounced effects; total period of effects >8 weeks and no recovery within 8 weeks after the last application; and no full recovery within the test period	Clear response of sensitive endpoints and recovery time is longer than 8 weeks after the last application
		Full recovery is not reported before the end of the study
		Effects reported as 'long-term effects followed by recovery on several sensitive and less sensitive species/endpoints or other similar descriptions
		On consecutive time points

In total, 56 phytoplankton taxa were identified, belonging to 20 groups (mainly orders of algae). The overall abundance of phytoplankton was dominated by Chlorococcales, Cryptomonadales, Pyrenomonadales and Volvocales. For periphyton, 51 taxa were identified belonging to 17 orders of algae. The overall abundance of periphyton was dominated by Chlorococcales, Oscillatoriales, Pennate diatoms and Volvocales. The macrophyte community comprised of eight introduced species, with five additional taxa that were also observed to have colonised the mesocosms during the test. For zooplankton, 28 taxa were observed, comprising mostly of arthropoda, rotifera and tardigrada.

Clear and consistent treatment related effects were not seen for individual phytoplankton or periphyton taxa, but were seen for the total number of phytoplankton on Days 14 and 21 within the 50 µg/L treatment group; recovery of the total number of phytoplankton was seen at the affected level on Day 28. A clear and consistent dose response was observed for *Glyceria maxima* mean stem lengths and *Hippuris vulgaris* total stem numbers due to the statistically significant direct effects occurring in mesocosms treated at 50 µg/L; recovery at this level was observed for *Glyceria maxima* by Day 77 and *Hippuris vulgaris* by Day 92.

No effects on any other parameter including zooplankton, primary productivity and environmental parameters including diurnal dissolved oxygen were observed.

Due to the effects observed on total number of phytoplankton, *Glyceria maxima* and *Hippuris vulgaris* at the highest treatment level, the class 1 NOEC was estimated to be 30 µg/L. The class NOEAEC (Class 5A) was estimated to be 50 µg/L, due to recovery of all affected parameters at this level by the end of the test.

Summary results where statistically significant derived consistent NOECs could be calculated are presented in the tables below:

Effects of SYN521384 on phytoplankton, periphyton macrophytes and zooplankton

Community	NOEC (µg SYN521384/L)			NOEAEC (µg SYN521384/L)		
	NOEC _{population}			NOEC _{population}		
Phytoplankton	30	30	30	3A*	50	3A*
Periphyton	50	50	50	50	50	50
Macrophytes	30	50	50	50	50	50
Zooplankton	50	50	50	50	50	50

*Please see table defining the effect categories

Effects of SYN521384 on specific macrophytes

Parameter	NOEC (µg SYN521384/L)
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<i>Glyceria maxima</i> , NOEC _{stem length}	30
<i>Hippuris vulgaris</i> , NOEC _{total stem numbers}	30

No clear treatment-related effects on measurements of physical or chemical parameters were observed at any concentration.

Conclusions

Based on nominal concentrations, the NOEC_{population} and NOEC_{overall} for phytoplankton and macrophytes was 30 µg SYN521384/L, and for periphyton and zooplankton it was 50 µg SYN521384/L. The NOEC_{community} was 50 µg SYN521384/L, with the exception of phytoplankton for which the NOEC_{community} was 30 µg SYN521384/L.

Comments of zRMS:	The statistical analysis has been already assessed and accepted in dRAR, December 2022.
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Reference:	KCA 8.2.8
Report	Prosulfocarb sulfoxide – Statistical (MDD) analysis of existing data from a mesocosm study with prosulfocarb sulfoxide (SYN521384), Taylor S. & Dark R., 2014, CEA.1294; SYN521384_10076
Guideline(s):	No official guideline followed
Deviations:	No
GLP:	No
Acceptability:	Yes/No/Supplementary

Executive Summary

The aim was to evaluate the statistical power of the data set from a microcosm study on prosulfocarb sulfoxide previously given in a confidential report: Taylor, S. (2013) SYN521384 - The Effect on Phytoplankton and Periphyton in Freshwater Mesocosms (GLP-compliant study).

The study is suitable for the derivation of robust NOECs for *Glyceria maxima* and *Sparganium erectum* and it is also suitable for the derivation a robust NOEAEC for *Hippuris vulgaris*. In addition, it was robustly demonstrated that no effects occurred on the areal coverage of *Veronica beccabunga* therefore, the reported endpoints for this parameter are also suitable and relevant to the risk assessment for macrophytes. These observations are supported by the plant health scores in the original study which although were not suitable for MDD analysis, they suggested no effects on these taxa.

For algae, safety has been clearly demonstrated for 5 algal orders, 4 algal Genera, the total number of organisms and the total numbers taxa (both phyto and periphyton) and the total phytoplankton chlorophyll giving a total of 14 parameters with Category 1 MDD values. As robust data suitable for ETO-RAC derivation are available which include recommended tier-1 groups (green algae and diatoms), it is considered that the available data are sufficient to meet the minimum requirement of the aquatic guidance document and the endpoint from the mesocosm study (Taylor, 2013) for algae is appropriate for the regulatory risk assessment. As a result, the recommended NOEC of 30 µg a.s/L and NOEAEC (Class 3A) of 50 µg a.s./L from the original study are supported here.

Methods

The minimum detectable differences (MDD) were calculated for phytoplankton, periphyton and aquatic macrophyte endpoints. The calculated MDD values were assigned to Classes and were classified as follows: MDD >100% = Class 0 (no effects can be determined), MDD 90-100% = Class I (only strong ef-

fects can be determined), MDD 70-90% = Class II (strong to medium effects can be determined), MDD 50-70% = Class III (medium effects can be determined), MDD 10-50% = Class IV (low effects can be determined), (EFSA Aquatic Guidance Document, 2013).

The calculated MDD values/classes are then characterized using the principles outlined by Brock et al (2014) in which three categories of taxa on the basis of their MDD values are recommended.

Statistical Analysis

Where possible MDD values have been obtained for each item selected for analysis at each sampling occasion. The MDD indicates the lowest significant difference between control and treatment which can be detected by a statistical test. The %MDD represents the relative minimum detectable difference between the control and treatment.

In order to compute the MDD the variability of the replicates is estimated and as a result, the MDD becomes larger with increased variability. For this analysis, the best estimate of error comes from an analysis of variance applied to the data from all treatments. If the estimate of error and t-value used in the computation of the MDD and NOEC are in agreement then the MDD and NOEC will be consistent. Therefore, if the MDD was calculated using data from just the control and the NOEC concentration the resulting MDD could contradict the NOEC. As a result, the MDD values from this analysis have been computed using the pooled treatment data.

Results and Discussion

These data include missing values due to some mesocosms being replaced during the establishment phase of the original study. For macrophytes percentage health leaf area, all values were missing for *Glyceria maxima* on study day 28. For total chlorophyll, values were missing for mesocosms M71 and M73 (Control), M70 (10 µg/L) and M72 (50 µg/L), on study days -35 and -21. For periphyton, values were missing for mesocosms M71 and M73 (Control), M70 (10 µg/L) and M72 (50 µg/L), on study day -21. For macrophytes final wet and dry weights many values were missing, for details please consult the original study report (Taylor, 2013). The missing values had no effect on the integrity of the original study or this analysis.

For macrophytes, Category 1 MDD values were calculated for the areal coverage of four plant taxa (*Glyceria maxima*, *Hippuris vulgaris*, *Sparganium erectum* and *Veronica beccabunga*) and the total macrophyte coverage. In addition, Category 1 MDD values were able to be calculated for the wet and dry weights of *Glyceria maxima* leaves, wet weights for *Glyceria maxima* roots and the dry weight of the total macrophyte biomass. As it was clearly demonstrated that typically medium to low effects could be determined for multiple parameters on *Glyceria maxima* and *Sparganium erectum* which included those measurements known to be sensitive for NOEC determination (terminal wet and dry weight biomass estimates), it is recommended that the reported endpoints for these taxa are relevant for the derivation of the ETO-RAC (Ecotoxicological Threshold-Regulatory Acceptable Concentration).

Data for which Category 1 MDD values could be determined were also obtained for multiple parameters (areal coverage, number of stems and mean stem height) for *Hippuris vulgaris* and for the areal coverage of *Veronica beccabunga*. As a result, it is considered that these macrophyte parameters have been successfully evaluated and it is recommended that the reliably reported endpoints (areal coverage, number of stems and mean stem height) for *Hippuris vulgaris* and areal coverage of *Veronica beccabunga* are relevant to the risk assessment. For phytoplankton, Category 1 MDD values were calculated for total chlorophyll, the phytoplankton orders of Chlorococcales, Pyrenomonadales, Volvocales, the total number of organisms and the total numbers taxa. In addition, Category 1 MDD values were able to be calculated for three Genera (*Chlamydomonas* sp., *Chlorella* sp. and *Rhodomonas* sp.) and the data suggested no effects had occurred on their abundance during the test.

As it was clearly demonstrated that typically medium to low effects could be determined for these parameters, and that no statistically significant effects were observed for any treatment, it is recommended that the original study is suitable for demonstrating no effects on total chlorophyll, the phytonplankton orders of Chlorococcales, Pyrenomonadales, Volvocales, the total number of organisms and the total numbers taxa in addition to the phytoplankton Genera of Chlamydomonas sp., Chlorella sp. and Rhodomonas sp at concentrations of up to 50 µg/L.

For periphyton, Category 1 MDD values were calculated for total chlorophyll, the periphyton orders of Chlorococcales, Oscillatoriales, Pennate Diatoms, Volvocales, the total number of organisms and the total numbers taxa. In addition, reliable MDD values were able to be calculated for three Genera (Chlamydomonas sp., Chlorella sp. and Lyngbya sp.) and although their abundance were not statistically evaluated in the original report, subsequent statistical analysis confirmed that no effects had occurred on their abundance during the test at concentrations of up to nominally 50 µg/L.

As it was clearly demonstrated that typically strong to medium effects could be determined for these parameters, and that no statistically significant effects were observed in any treatment, it is recommended that the original study is suitable for demonstrating no effects on the periphyton orders of Chlorococcales, Oscillatoriales, Pennate Diatoms, Volvocales, the total number of organisms and the total numbers taxa in addition to the periphyton Genera of Chlamydomonas sp., Chlorella sp. and Lyngbya sp at concentrations of up to nominally 50 µg/L.

Considering the phytoplankton and periphyton data together, no effects were robustly demonstrated on Chlorococcales (phyto and periphyton), Oscillatoriales (periphyton), Pennate diatoms (periphyton) Pyrenomonadales (phytoplankton), Volvocales (phytoplankton and periphyton), the total number of organisms and the total numbers taxa (phyto and periphyton), Chlamydomonas sp. (phyto and periphyton), Chlorella sp. (phyto and periphyton), Rhodomonas sp. (phytoplankton) and Lyngbya sp. (periphyton).

The Aquatic Guidance Document (EFSA, 2013) states that for substances with a herbicidal mode of action, tier-1 testing should be conducted on one green alga and on a second species from a different taxonomic group, such as a diatom. In the present study, safety has been clearly demonstrated for 5 algal orders, 4 algal genera, the total number of organisms and the total numbers taxa (both phyto and periphyton) and the total phytoplankton chlorophyll, giving a total of 14 parameters with Category 1 MDD values. Consequently, as robust data suitable for ETO-RAC derivation are available which include endpoints for green algae and diatoms, it is considered that the available data are sufficient to meet the minimum requirement of the aquatic guidance document and the algal endpoint from the mesocosm study (Taylor, 2013) is appropriate for the regulatory risk assessment.

Conclusions

The original study is suitable for the derivation of robust NOECs for *Glyceria maxima* and *Sparganium erectum* and it is also suitable for the derivation a robust NOEAEC for *Hippuris vulgaris*. In addition, it was robustly demonstrated that no effects occurred on the areal coverage of *Veronica beccabunga* therefore, the reported endpoints for this parameter are also suitable and relevant to the risk assessment for macrophytes. These observations are supported by the plant health scores in the original study which although were not suitable for MDD analysis, they suggested no effects on these taxa.

For algae, safety has been clearly demonstrated for 5 algal orders, 4 algal Genera, the total number of organisms and the total numbers taxa (both phyto and periphyton) and the total phytoplankton chlorophyll giving a total of 14 parameters with Category 1 MDD values. As robust data suitable for ETO-RAC derivation are available which include recommended tier-1 groups (green algae and diatoms), it is considered that the available data are sufficient to meet the minimum requirement of the aquatic guidance document and the endpoint from the mesocosm study (Taylor, 2013) for algae is appropriate for the regulatory risk assessment. As a result, the recommended NOEC of 30 µg a.s./L and NOEAEC (Class 3A) of 50 µg a.s./L from the original study are supported here.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD 213 and 214 The validity criteria were met. The following endpoints were derived Oral, acute, 48 h LD50 = 464.1 µg formulation/bee Contact, acute, 72 h LD50 = 149.5 µg formulation/bee
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Reference: KCP 10.3.1.1

Report GLOB1913H: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory, Sekine T., 2020, 155401035

Guideline(s): Yes, OECD 213 (1998) and OECD 214 (1998)

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

The purpose of this study was to determine the acute toxicity of GLOB1913H to the honeybee *Apis mellifera* L. in a laboratory test after oral and contact exposure.

The selected test design corresponds to the recommendations of the OECD Guidelines 213 and 214.

The contact NOED values (24 h + 48 h + 72 h) were all 98.3 µg a.i./bee. The contact LD₅₀ values (24 h + 48 h + 72 h) were 196.5, 129.7 and 129.7 µg a.i./bee, respectively. The oral LD₅₀ values (48 h + 72 h) were 422.0 and 402.6 µg a.i./bee, respectively. The oral NOED values (24 h + 48 h + 72 h) were all 219.1 µg a.i./bee.

Materials and Methods

Test item: GLOB1913H; Batch No.: 200701/01

Content of active substance (a.s.): nominal analysed

Prosulfocarb: 900 g/L 886.1 g/L

Test species: Honeybee – *Apis mellifera* L.

Female worker bees of a healthy and queen-right colony; bred by Ibacon, collected in the morning of use

Test design: Acute contact and oral LD₅₀ test; duration 72 h; 3 replicates, each consisting of 10 bees per cage per treatment. Assessment of mortality after 4, 24, 48 and 72 hours because of increasing mortality between 24 and 48 hours; reference item: dimethoate

Test concentrations	Contact test:	786.1, 393.1, 196.5, 98.3 and 49.1 µg a.i./bee*
	Oral test (target):	393.1, 196.5, 98.3, 49.1 and 24.6 µg a.i./bee*
	Oral test (consumed):	442.9, 219.1, 106.8, 53.6 and 26.8 µg a.i./bee*
<p>*in the following e.g. 800 µg prosulfocarb/bee, will be referred to as 800 µg a.i./bee. The dose level of the test item was adjusted to reflect the percentage a.i. (nominal value with a density of 1.0375 g/mL)</p>		

Statistics: Statistical program used: ToxRat Professional 3.2.1
Calculation of LD₅₀ values:
Test item: Trimmed Spearman-Kärber procedure (Hamilton *et al.*, 1979)
Reference item: Binomial distribution (Stephan, 1977)
Determination of NOED values:
 Test item: Fisher's Exact Binomial (pairwise comparison, one-sided greater, $\alpha = 0.05$)

Results and Discussion

Contact test

During the first 4 hours and 24 hours behavioral impairments such as dis-coordinated movements (= af-fected), moribund and apathy were observed in the 786.1, 393.1 and 196.5 μg a.i./bee treatment groups. After 72 hours 4 bees were affected in the 98.3 μg a.i./bee treatment group.

Oral test

In the oral test during the 4, 24 and 48 hours bees with dis-coordinated movements (affected) and/or moribund bees were observed in the two highest dose groups (442.9 and 219.1 μg a.i./bee). After 72 hours no behavioral abnormalities were found.

LD₅₀-values of the contact and oral toxicity test

LD ₅₀ values	Contact toxicity test	Oral toxicity test
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	24 h	48 h	72 h	24 h	48 h	72 h
LD₅₀ [µg a.i./bee]	196.5	129.7	129.7	> 442.9	422.0	402.6
NOED [µg a.i./bee]	98.3	98.3	98.3	219.1	219.1	219.1

The contact and oral LD₅₀ (24 h) values of the reference item were calculated to be 0.16 µg a.s./bee and 0.15 µg a.s./bee, respectively. All validity criteria have been met.

Conclusions

The toxicity of GLOB1913H was tested in both an acute contact and an acute oral toxicity test on honeybees. The contact NOED values (24 h + 48 h + 72 h) were all 98.3 µg a.i./bee. The contact LD₅₀ values (24 h + 48 h + 72 h) were 196.5, 129.7 and 129.7 µg a.i./bee, respectively. The oral LD₅₀ values (48 h + 72 h) were 422.0 and 402.6 µg a.i./bee, respectively. The oral NOED values (24 h + 48 h + 72 h) were all 219.1 µg a.i./bee.

Comments of zRMS:	<p>The study was accepted.</p> <p>The study was conducted in accordance with OECD guidances 246 and 247.</p> <p>The validity criteria were met:</p> <ul style="list-style-type: none"> control mortality in both the oral and contact test was ≤ 10 %; mortality of the reference item (dimethoate) was ≥ 50 %
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Reference:	KCP 10.3.1.1.1
Report	GLOB1913H: Acute contact and oral toxicity to bumblebees (<i>Bombus terrestris</i> L.) in the laboratory, Chwiesko D., 2021, 155401105
Guideline(s):	Yes, OECD 246 and OECD 247 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The toxicity of GLOB1913H was tested in an acute contact and oral toxicity test on bumblebees.

As there was no mortality above 50.0% in any of the test item groups in the contact test, the contact LD₅₀ (72 h) was estimated to be > 387.2 µg a.s./bumblebee. The contact NOED (72 h) was calculated to be ≥ 387.2 µg a.s./bumblebee.

As there was no mortality above 50.0% in any of the test item groups in the oral test, the oral LD₅₀ (72 h) was estimated to be > 285.3 µg a.s./bumblebee. The oral NOED (72 h) was calculated to be 176.2 µg a.s./bumblebee.

Materials and Methods

Test Item:	<p>GLOB1913H, Batch No.: 200701/01, content: Prosulfocarb: 886.1 g/L (analytical), density: 1.0375 g/mL (at 20 °C) (according to certificate of analysis).</p> <p>The concentrations were calculated taking into account the analytical content of the active ingredient in the test item (886.1 g/L) and the density 1.0375 g/mL.</p> <p>The dose levels according to the study plan were 400, 200, 100, 50 and 25 µg a.s./bumblebee for the contact and oral test. After the GLP certificate of analysis was provided by the sponsor the dose rates were recalculated.</p>
Test Species:	<p>Bumblebee (<i>Bombus terrestris</i> L.); female worker bumblebees; obtained from a commercial bumblebee breeding company (Koppert Deutschland GmbH, Zeppelinstr. 32, D-47638 Straelen).</p>
Test Design:	<p><u>Acute Contact Dose Response Test:</u></p> <p>Duration: 72 h;</p> <p>replicates: 30 per each dose of the test item treatment group (five doses), 30 for the water control and 30 for the reference item, each consisting of 1 bumblebee per cage per treatment;</p> <p>assessment of mortality and behavioural abnormalities:</p> <p>after 4 (± 0.5 h); 24 (± 2 h), 48 (± 2 h) and 72 (± 2 h) hours;</p> <p>reference item: dimethoate 414 g/L (analytical).</p> <p>Analytical verification of the concentrations of the active ingredient Prosulfocarb in the highest and lowest concentrated contact application solutions.</p> <p><u>Acute Oral Dose Response Test:</u></p> <p>Duration: 72 h;</p> <p>replicates: 50 per each dose of the test item treatment group (five doses), 50 for the water control and 45 for the reference item, each consisting of 1 bumblebee per cage per treatment (individual bumblebees which did not take up at least 80 % of the mean food uptake per treatment group were excluded from the evaluation ;</p> <p>assessment of mortality and behavioural abnormalities:</p> <p>after 4 (± 0.5 h); 24 (± 2 h), 48 (± 2 h) and 72 (± 2 h) hours;</p> <p>reference item: dimethoate 414 g/L (analytical).</p> <p>Analytical verification of the concentrations of the active ingredient Prosulfocarb in the highest and lowest concentrated oral feeding solutions.</p>

Test Item Dose Levels: Contact Dose Response Test (nominal):
387.2, 193.6, 96.8, 48.4 and 24.2 µg a.s./bumblebee
Oral Dose Response Test (nominal):
387.2, 193.6, 96.8, 48.4 and 24.2 µg a.s./bumblebee
Oral Dose Response Test (mean consumption):
285.3, 176.2, 99.4, 50.4 and 25.6 µg a.s./bumblebee

The dose levels according to the study plan were 400, 200, 100, 50 and 25 µg a.s./bumblebee for the contact test and oral test. After the GLP certificate of analysis was provided by the sponsor the dose rates were recalculated taking into account the actual content of the a.s. (886.1 g/L) and the density (1.0375 g/mL) (analytical).

Analytical Results of the Contact Test:	387.2 µg a.s./bumblebee	118	%
	24.2 µg a.s./bumblebee	89	%
Analytical Results of the Oral Test:	387.2 µg a.s./bumblebee	79	%
	24.2 µg a.s./bumblebee	92	%

Test Conditions:

<u>Contact Test:</u>	Acclimatisation:	Temperature: 24.8 – 24.9°C Relative Humidity: 46.1 – 56.0%
	Exposure:	Temperature: 24.8 - 25.1°C Relative Humidity: 43.2 – 61.8%
<u>Oral Test:</u>	Acclimatisation:	Temperature: 25.2 – 25.3°C Relative Humidity: 57.6 – 63.1%
	Application :	Temperature: 22.5 – 24.9°C Relative Humidity: 45.9 – 62.7%
	Exposure:	Temperature: 25.0 – 25.4°C Relative Humidity: 45.4 – 63.2%
Photoperiod:	Photoperiod:	24 h darkness (except handling procedures, including treatment and observations).
Study Validity:	This study met the OECD 246 (2017) and OECD 247 (2017) validity criteria as the control mortality in both the oral and contact test was ≤ 10 % and the mortality of the reference item (dimethoate) was ≥ 50 % at test end.	

Results and Discussion

Experimental dates: 23 September 2020 – 27 September 2020

Biological results:

Contact Test:

In the contact test a droplet of 2 µL containing the targeted dose levels of 387.2, 193.6, 96.8, 48.4 and 24.2 µg a.s./bumblebee was applied on the dorsal thorax of each exposed bumblebee. At the end of the contact toxicity test (72 hours after application) the 387.2, 193.6, 96.8, 48.4 and 24.2 µg a.s./bumblebee resulted in 33.3, 6.7, 6.7, 3.3 and 0.0 % mortality. 10.0 % mortality occurred in the water control treatment group (tap water containing 0.1 % v/v Triton X-100). During the 24 hours assessment, one moribund bumblebee was observed in the 48.4 µg a.s./bumblebee test item treatment group. 48 hours after

application behavioural abnormalities (e.g. affected and/or moribund) were observed in the 387.2 and 96.8 µg a.s./bumblebee test item treatment groups. During the 72 hours assessment one affected bumblebee was observed in the 96.8 µg a.s./bumblebee test item treatment group. No test item induced behavioural effects were observed at any time in the 193.6 and 24.2 µg a.s./bumblebee test item treatment groups.

The contact target dose level of the reference item of 10 µg dimethoate/bumblebee was applied on the dorsal thorax of each exposed bumblebee. The mortality in the reference item treatment group was 100.0 % (24 hours after application). The contact test is considered valid as the control mortality (tap water containing 0.1 % v/v Triton X-100) was ≤ 10 % and the reference item mortality (dimethoate) was ≥ 50 %.

Oral Test:

In the oral test the targeted dose levels of 387.2, 193.6, 96.8, 48.4 and 24.2 µg a.s./bumblebee would have been achieved if an exact amount of 40 mg treated feeding solution were consumed by each exposed bumblebee. This was not the case and the food uptake per bumblebee in the different treatment groups varied between 4 and 50 mg. The 80 % of the mean food uptake was calculated considering all 50 replicates for the test item and water control treatment groups and all 45 replicates for the reference item treatment group. Bumblebees which did not consume at least 80% of the mean food uptake per treatment group were excluded from the derivation of the end points, as well as from the calculation of the actual mean oral doses in the test and reference item treatment groups. This was done to avoid potentially overestimating the final endpoints.

Thus, the actual mean consumed oral doses of the test item were 285.3, 176.2, 99.4, 50.4 and 25.6 µg a.s./bumblebee. For the 285.3, 176.2, 99.4, 50.4 and 25.6 µg a.s./bumblebee test item treatment groups 17, 17, 27, 33 and 36 bumblebees were considered for the evaluation (≥ 80 % of the mean food uptake). For the water control treatment groups (50 % w/v sucrose solution) 47 bumblebees were considered for the evaluation.

At test end (72 hours after application) the actual mean consumed oral doses of 285.3, 176.2, 99.4, 50.4 and 25.6 µg a.s./bumblebee resulted in 35.3, 17.6, 11.1, 0.0 and 2.8 % mortality. 2.1 % mortality occurred in the water control treatment group (50 % w/v sucrose solution). No test item related behavioural effects were observed at any time in the oral test.

The reference item targeted dose level of 4 µg dimethoate/bumblebee would have been achieved if exactly 40 mg treated feeding solution were consumed by each bumblebee. Considering bumblebees with a food uptake of at least 80 % of the mean food uptake, the mean consumption corresponded to an actual mean oral dose of 4.3 µg dimethoate/bumblebee. For the reference item treatment group 33 bumblebees were considered for the evaluation. The mortality in the reference item treatment group was 100.0 % 72 hours after application. The oral test is considered valid as the control (50 % w/v sucrose solution) mortality was ≤ 10 % and the reference item (dimethoate) mortality was ≥ 50 %.

Toxicity to bumblebees; laboratory tests

Test Item	GLOB1913H	
Test Species	<i>Bombus terrestris</i> L.	
Exposure	Contact (tap water containing 0.1 % v/v Triton X-100)	Oral (50 % w/v sucrose solution) (based on recorded consumption considering bumblebees with food uptake of at least 80 % of the mean uptake per treatment group ²)
Target (nominal) dose rates ¹ [µg a.s./bumblebee]	387.2, 193.6, 96.8, 48.4 and 24.2	387.2, 193.6, 96.8, 48.4 and 24.2

Actual dose rates [µg a.s./bumblebee]	387.2, 193.6, 96.8, 48.4 and 24.2			285.3, 176.2, 99.4, 50.4 and 25.6		
Test Duration:	24 h	48 h	72 h	24 h	48 h	72 h
LD ₅₀ [µg a.s./bumblebee] ^{3,4}	> 387.2	> 387.2	> 387.2	> 285.3	> 285.3	> 285.3
NOED [µg a.s./bumblebee] ^{3,5}	≥ 387.2	≥ 387.2	≥ 387.2	≥ 285.3	176.2	176.2

¹ The dose levels according to the study plan were 400, 200, 100, 50 and 25 µg a.s./bumblebee for the contact test and oral test. After the GLP certificate of analysis was provided by the sponsor the dose rates were recalculated taking into account the actual content of the a.s. (886.1 g/L) and the density (1.0375 g/mL) (analytical).

² For the 285.3, 176.2, 99.4, 50.4 and 25.6 µg a.s./bumblebee test item treatment group 17, 17, 27, 33 and 36 bumblebees were considered for the evaluation (≥ 80 % of the mean food uptake).

³ Results obtained from test item treated groups were compared to those obtained from the water control treatment group.

⁴ As the test item treatment groups in the contact and oral test did not show mortality above 50.0 %, no statistical evaluation on the LD₅₀ was carried out. The contact and oral LD₅₀ values were considered as higher as the highest dose rate.

⁵ The contact and oral NOED was estimated using Fisher's Exact Test after Bonferroni-Holm (pairwise comparison, one-sided greater, α = 0.05)

Analytical results:

The analytical recovery rates of the active substance Prosulfocarb were as follows:

Concentration/bumblebee	Nominal concentration of the active substance in the Solution	Recovery of the nominal value of the active substance in the Solution
<u>Contact Test:</u> Highest concentrated application solution (387.2 µg a.s./bumblebee*)	193.6 g a.s./L	118 %
<u>Contact Test:</u> Lowest concentrated application solution (24.2 µg a.s./bumblebee*)	12.1 g a.s./L	89 %
<u>Oral Test:</u> Highest concentrated feeding solution (387.2 µg a.s./bumblebee*)	9.68 g a.s./kg	92 %
<u>Oral Test:</u> Lowest concentrated feeding solution (24.2 µg a.s./bumblebee*)	0.65 g a.s./kg	79 %

Conclusion

The toxicity of GLOB1913H was tested in an acute contact and oral toxicity test on bumblebees.

As there was no mortality above 50.0% in any of the test item groups in the contact test, the contact LD₅₀ (72 h) was estimated to be > 387.2 µg a.s./bumblebee. The contact NOED (72 h) was calculated to be ≥ 387.2 µg a.s./bumblebee.

As there was no mortality above 50.0% in any of the test item groups in the oral test, the oral LD₅₀ (72 h) was estimated to be > 285.3 µg a.s./bumblebee. The oral NOED (72 h) was calculated to be 176.2 µg a.s./bumblebee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted in accordance with OECD 245. All validity criteria for the study were met. After 10 days of continuous exposure, mortality in the control was 10 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %. The mean recovery rates in all test item concentrations of Prosulfocarb of Days 1-9 were within ± 20 % of the nominal concentrations. Study is acceptable.
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Reference:	KCP 10.3.1.2
Report	GLOB1913H: Chronic oral toxicity test on the honey bees (<i>Apis mellifera</i> L.) in the laboratory, Berg C., 2021, 155401136
Guideline(s):	Yes, OECD 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The chronic oral toxicity of GLOB1913H on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

The mean recovery rates in all test item concentrations of Prosulfocarb of Days 1-9 were within ± 20 % of the nominal concentrations.

The LDD₅₀ was estimated to be >178.8 µg a.i./bee/day, respectively. This corresponds to a LC₅₀ of >9831 mg a.i./kg food, respectively. The NOEDD was determined to be 111.1 µg a.i./bee/day and the NOEC was 4916 mg a.i./kg food.

Materials and Methods

Test Item:	GLOB1913H, Batch No.: 200701/01 content: Prosulfocarb 900 g/L (nominal), 886.1 g/L (analytical), according to certificate of analysis.
Test Species:	Honey bee (<i>Apis mellifera</i> L.); freshly emerged young female worker bees; obtained from a healthy and queen-right colony, bred by ibacon. After hatch, the bees were collected and thereafter acclimatized under test conditions for one day.
Age of the Honey Bees:	Two days old worker bees.
Test Design:	10 days chronic oral feeding test in the laboratory (dose response test).

	<p>Young honey bees were provided with 5 concentrations of the test item treated sugar solutions <i>ad libitum</i> over a period of 10 days.</p> <p>An untreated control and a reference item (Danadim; 400 g/L dimethoate) were included in this study.</p> <p>3 replicates per treatment, each consisting of 10 bees per test cage.</p>
Endpoints:	<p>Daily assessment of mortality and behavioral abnormalities up to day 10.</p> <p>Endpoints: LC₅₀, LDD₅₀, NOEC, NOEDD.</p>
Test Concentrations:	<p>Test item: 9831, 4916, 2458, 1229 and 614 ppm (mg a.i./kg feeding solution)</p> <p>Reference item: 1 ppm dimethoate (1 mg dimethoate/kg feeding solution)</p>
Target Dose Level:	<p>Test item: 197, 98.3, 49.2, 24.6 and 12.3 µg a.i./bee per day*</p> <p>Reference item: 0.02 µg a.i./bee per day*</p> <p>* taking into account a mean uptake of feeding solution of 20 mg/bee/day; the exact dose per bee per day was calculated after determination of the definitive food uptake of the bees at test end (see “Actual Mean Dose Level”).</p>
Actual Mean Dose Level:	<p>Test item: 178.8, 111.1, 72.7, 35.2, and 16.9 µg a.i./bee/day**</p> <p>Reference item: 0.015 µg a.i./bee/day**</p> <p>** based on daily actual intake taking into consideration loss by evaporation</p>
Evaporation:	<p>In order to adjust for possible evaporation of test solutions from the feeders, evaporation figure was subtracted from the calculated uptake to give the real uptake accounting the loss by evaporation.</p>
Test Conditions (Exposure):	<p>Temperature: 32 - 33°C; relative humidity: 53 - 69% mean relative humidity: 67%; photoperiod: 24 h darkness.</p>

Results and Discussion

Experimental dates (biological phase): 25 August 2020 – 04 September 2020

The test item was administered daily to the bees in sucrose solution at the following concentrations: 9831, 4916, 2458, 1229 and 614 mg a.i./kg feeding solution. These concentrations resulted in a daily mean dose of 178.8, 111.1, 72.7, 35.2, and 16.9 µg a.i./bee after 10 days.

Significantly elevated mortality occurred only in the highest test item treated dose level with 30.0 % at test end (10 days following the start of chronic exposure, Cochran Armitage Test, one-sided greater, $\alpha=0.05$).

There was 10.0 % mortality in the control (50 % w/v sucrose solution).

The reference item (dimethoate) at a concentration of 1 ppm (1 mg dimethoate/kg feeding solution) corresponding to 0.015 µg a.i./bee/day caused 100 % mortality at day 5.

Two affected bees were observed in the highest test item treatment group on day 6. Furthermore two moribund bees occurred on day 10 in the second highest treatment (111.1 µg a.i./bee/day).

10 days Chronic Oral Toxicity of GLOB1913H to young honey bees; laboratory test

Test Organism		<i>Apis mellifera</i> L.	
Exposure		Oral 10 days chronic exposure	
Treatment Group	Concentration [mg a.i./kg]	Dose Level ¹ [µg a.i./bee/day]	Mortality at day 10 ² [% Mean]
GLOB1913H	9831	178.9	30.0 (*)
	4916	111.1	3.3 (n.s.)

	2458	72.7	6.7 (n.s.)
	1229	35.2	0.0 (n.s.)
	614	16.9	6.7 (n.s.)
Water control	0.0	0.0	10.0
Reference Item	1.0	0.015	100.0
Endpoint at test termination (day 10)			
LC₅₀	LDD₅₀	NOEC	NOEDD
>9831 mg a.i./kg	>178.9 µg a.i./bee	4916 mg a.i./kg	111.1 µg a.i./bee

1) mean dose per bee per day; dose measured based on consumed feeding solution adjusted for evaporation

2) Mortality at study termination 10 days after start of first feeding

Statistics:

LC₅₀/LDD₅₀: was estimated as the mortality in the highest test item dose did not exceed 50.0 %.

NOEC/NOEDD: was determined using Step-down Cochran-Armitage Test (one-sided greater, $\alpha = 0.05$).

n.s. = no statistically significant difference compared to the control, * = statistically significant difference compared to the control

All validity criteria for the study were met. After 10 days of continuous exposure, mortality in the control was 10 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %.

The analytical recovery rates of the active ingredient prosulfocarb in the feeding solutions were as follows:

	Feeding Solution 614 mg a.i./kg	Feeding Solution 1229 mg a.i./kg	Feeding Solution 2458 mg a.i./kg	Feeding Solution 4916 mg a.i./kg	Feeding Solution 9831 mg a.i./kg
DAA0	111 %	99 %	103 %	102 %	101 %
DAA1	88 %	105 %	97 %	100 %	99 %
DAA2	108 %	104 %	102 %	103 %	100 %
DAA3	92 %	90 %	80 %	77 %	100 %
DAA4	80 %	84 %	68 %	67 %	78 %
DAA5	68 %	60 %	67 %	71 %	133 %
DAA6	107 %	82 %	71 %	63 %	71 %
DAA7	102 %	65 %	58 %	52 %	108 %
DAA8	117 %	103 %	103 %	104 %	94 %
DAA9	86 %	61 %	85 %	85 %	62 %
Mean recovery rate	96 %	85 %	83 %	82 %	95 %

DAA = Days after 1st Application (1st Application = DAA0)

Conclusion

The chronic oral toxicity of GLOB1913H on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

The mean recovery rates in all test item concentrations of Prosulfocarb of Days 1-9 were within ± 20 % of the nominal concentrations.

The LDD₅₀ was estimated to be >178.8 µg a.i./bee/day, respectively. This corresponds to a LC₅₀ of >9831 mg a.i./kg food, respectively. The NOEDD was determined to be 111.1 µg a.i./bee/day and the NOEC was 4916 mg a.i./kg food.

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

Comments of zRMS:	The study was conducted in accordance with OECD 239. The validity criteria with regards to control larval mortality on D8, control adult emergence on D22 and toxicity of the reference item were met. Study is acceptable.
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Reference:	KCP 10.3.1.3
Report	Effects of GLOB1913H on honey bees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure, Colli M., 2020, BT273/20
Guideline(s):	Yes, OECD 239 (2016)
Deviations:	Yes, during the test the temperature was out of the range for more than two hours. The deviations were minimal and did not affect the test results, as is also demonstrated because all the validity criteria were met.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The effects of the test item GLOB1913H on the larval development and subsequent adult emergence of honeybees (*Apis mellifera* L.), were tested in a GLP compliant laboratory study.

Regarding the effects on larvae on D8 (developmental period), the test item GLOB1913H caused statistically significant mortality starting from the dose of 100.00 µg prod./larva. Therefore, the NOED for larvae on D8 was determined to be 50.00 µg prod./larva/developmental period (corresponding to 42.70 µg a.s./larva/developmental period) equivalent to 324.68 mg prod./kg diet (corresponding to 277.30 mg a.s./kg diet).

Regarding the effects on adult emergence on D22, the test item GLOB1913H caused statistically significant reduction in emergence rate only at the maximum tested dose of 200.00 µg test item/larva.

The NOED and the NOEC for adult emergence rate was evaluated to be 100.00 µg prod./larva (corresponding to 85.41 µg a.s./larva) and 649.356 mg prod./kg diet (corresponding to 554.59 mg a.s./kg diet) respectively.

Materials and Methods

Test Item:	GLOB1913H, Batch No.: 200701/01 content: Prosulfocarb 900 g/L (nominal), 886.1 g/L (analytical), density 1.0375 g/mL (at 20°C), according to certificate of analysis.
Test Species:	Honey bee (<i>Apis mellifera</i> L.); 3 days old larvae; obtained from a healthy and queen-right colony, bred by BioTecnologie BT S.r.l.
Diet:	Diet A (D1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose Diet B (D3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose Diet C (D4-D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose

Test Design:	The test item was dissolved in ultrapure water in order to get the highest stock solution. The other stock solutions were obtained by sequential dilution. The stock solutions were mixed into the diet in a range of five increasing concentrations and administered daily to the larvae at a constant concentration, from day 3 to 6 of the test. Three replicates of 12 larvae each were prepared for each experimental group. The reference item Dime-thoate was dissolved in ultrapure water and simultaneously tested at a single concentration (equivalent to a cumulative dose of 7.39 µg a.s./larva).
Endpoints:	Assessments on mortality and any developmental/behavioral abnormality were performed from D4 to D8 and on D15 and on D22. The pupal mortality and the adults' emergence rate on D22 were also assessed.
Test Concentrations:	Test item: 12.5, 25, 50, 100, 200 µg product/larva equivalent to 81.17, 162.34, 324.68, 649.35 and 1298.7 mg product/kg diet (10.68, 21.35, 42.70, 85.41 and 170.81 µg a.s./larva equivalent to 96.32, 138.65, 277.30, 554.59 and 1109.18 mg a.s./kg diet). Reference item: 7.39 µg a.i./larva equivalent to 48 mg a.s./kg diet.
Test Conditions:	Temperature: D1 to D8: 32.3-34.7°C; D8 to D15: 34.3-34.5°C; D15 to D22: 33.9-35.3°C Relative humidity: D1 to D8: 77.7-98.5%; D8 to D15: 77.0-84.6%; D15 to D22: 65.5-74.4% Photoperiod: 24 h darkness (except during observations)
Statistics	To evaluate the statistical significance of the data and the NOED/NOEC, the Step-down Cochran-Armitage test, for the D8 and D22 data were performed. To evaluate the LD/LCx and ED/ECx values a Weibull analysis using linear max. likelihood regression was performed. The software Tox-RatPro Version 3.3.0 was used for the statistics.

Results and Discussion

Experimental dates (including analytical phase): 09 September 2020 – 28 September 2020

The following tables show the mean mortalities on D8 and D22, the pupal mortality and the effects on adult emergence on D22. Until D8 the test item caused statistically significant mortality of the treated larvae starting from the dose of 100.00 µg prod./larva. The adult emergence on D22 was affected by the test item administration to the larvae at the highest dose.

Mortality (M) and Corrected Mortality (CM) of larvae (on D8)

Treatment	Dose [µg prod./larva]	Concentration [mg prod./kg diet]	Larvae mortality on D8		
			M - Mean [%]	CM - Mean [%]	Sign.
Control	0.00	0.00	8.3	n.a.	n.a.
Test item (T1)	12.50	81.17	0.0	0.0	-
Test item (T2)	25.00	162.34	5.6	0.0	-
Test item (T3)	50.00	324.68	2.8	0.0	-
Test item (T4)	100.00	649.35	22.2	15.2	+
Test item (T5)	200.00	1298.70	100.0	100.0	+

n.a. = not applicable CM = negative values are set as 0.00

+ : significant; - : non-significant (Step-down Cochran-Armitage test; $\alpha = 0.05$, one-sided greater).

Pupal Mortality

Treatment	Dose [µg prod./larva]	Concentration [mg prod./kg diet]	Pupal mortality from D8 to D15*	Pupal mortality from D8 to D22**
			Mean [%]	Mean [%]
Control	0.00	0.00	0.0	15.2
Test item (T1)	12.50	81.17	5.6	8.3

Test item (T2)	25.00	162.34	2.9	11.8
Test item (T3)	50.00	324.68	2.9	14.3
Test item (T4)	100.00	649.35	3.6	10.7
Test item (T5)	200.00	1298.70	0.0	0.0

*calculated in percentage comparing the number of dead pupae from D8 to D15 to the number of alive pupae on D8

**calculated in percentage comparing the number of dead pupae from D8 to D22 to the number of alive pupae on D8

Total mortality, corrected mortality (CM) and adult emergence from D3 to D22 and emergence on D22

Treatment	Dose [µg prod./larva]	Concentration [mg prod./kg diet]	Mortality (larvae + pupae) on D22			Adult emergence on D22*	
			M - Mean [%]	CM - Mean [%]	Sign.	Mean [%]	Sign.
Control	0.00	0.00	22.2	n.a.	n.a.	77.8	n.a.
Test item (T1)	12.50	81.17	8.3	0.0	-	91.7	-
Test item (T2)	25.00	162.34	16.7	0.0	-	83.3	-
Test item (T3)	50.00	324.68	16.7	0.0	-	83.3	-
Test item (T4)	100.00	649.35	30.6	10.7	-	69.4	-
Test item (T5)	200.00	1298.70	100.0	100.0	+	0.0	+

n.a. = not applicable CM = negative values are set as 0.00 *n° adult emerged/n° initial larvae x 100

+ : significant; - : non-significant (Step-down Cochran-Armitage test - $\alpha = 0.05$, one-sided greater).

Reference item - mean mortality

Treatment	Dose [µg a.s./larva]	Concentration [mg a.s./kg diet]	Mortality on D8 Mean [%]
Reference item	7.39	48.00	100.00

All validity criteria were met:

- in the control plates the cumulative larval mortality from D3 to D8 was 8.3%;
- in the control plates the adult emergence rate on D22 was 77.8%;
- in the reference item group treated with Dimethoate larval mortality at D8 was 100%.

The analytical results demonstrate that the Prosulfocarb content in the stock solutions prepared on D3 at the highest and lowest concentration was in the range of $\pm 20\%$ of nominal concentrations.

Results of the samples analysis – D3

Sample code	Nominal Concentration [g/L]	Measured concentration [g/L]	Recovery [%]	Mean Recovery [%]	SD	RSD [%]
S1D3 001	0.854	0.8851	103.64	103.09	0.78	0.76
S1D3 002		0.8756	102.53			
S5D3 001	13.665	14.0914	103.12	103.15	0.04	0.04
S5D3 002		14.0997	103.18			

SD: standard deviation - RSD%: relative standard deviation

Conclusion

Regarding the effects on larvae on D8 (developmental period), the test item GLOB1913H caused statistically significant mortality starting from the dose of 100.00 µg prod./larva. Therefore, the NOED for larvae on D8 was determined to be 50.00 µg prod./larva/developmental period (corresponding to 42.70 µg a.s./larva/developmental period) equivalent to 324.68 mg prod./kg diet (corresponding to 277.30 mg a.s./kg diet).

Regarding the effects on adult emergence on D22, the test item GLOB1913H caused statistically significant reduction in emergence rate only at the maximum tested dose of 200.00 µg test item/larva.

The NOED and the NOEC for adult emergence rate was evaluated to be 100.00 µg prod./larva (corresponding to 85.41 µg a.s./larva) and 649.356 mg prod./kg diet (corresponding to 554.59 mg a.s./kg diet) respectively.

The mortality and emergence data allowed the extrapolation of the LD/EC₁₀, LD/EC₂₀ and LD/EC₅₀.

Summary results for all endpoints in terms of formulated product

Critical dose	Larvae Mortality D8 [µg prod./larva]	Adult Emergence D22 [µg prod./larva]
LD/ED₁₀	93.58 (c.l. 95%: 68.95 – 106.93)	99.24 (c.l. 95%: 85.16 – 115.64)
LD/ED₂₀	105.40 (c.l. 95%: 89.81 – 129.80)	111.37 (c.l. 95%: 96.45 – 128.58)
LD/ED₅₀	126.15 (c.l. 95%: 109.84 – 212.02)	132.56 (c.l. 95%: 110.69 – 158.75)
NOED	50.00	100.00
Critical concentration	Larvae Mortality D8 [mg prod./kg diet]	Adult Emergence D22 [mg prod./kg diet]
LC/EC₁₀	607.66 (c.l. 95%: 447.74 – 694.32)	644.38 (c.l. 95%: 552.97 – 750.90)
LC/EC₂₀	684.45 (c.l. 95%: 583.14 – 842.74)	723.16 (c.l. 95%: 626.32 – 834.96)
LC/EC₅₀	819.22 (c.l. 95%: 713.30 – 1375.85)	860.77 (c.l. 95%: 718.78 – 1030.81)
NOEC	324.68	649.35

LD/ED - LC/ECx evaluated by Weibull analysis
c.l.: confidence limits

Summary results for all endpoints in terms of Prosulfocarb

Critical dose	Larvae Mortality D8 [µg a.s./larva]	Adult Emergence D22 [µg a.s./larva]
LD/ED₁₀	79.92 (c.l. 95%: 58.89 – 91.33)	84.75 (c.l. 95%: 72.73 – 98.76)
LD/ED₂₀	90.02 (c.l. 95%: 76.70 – 110.86)	95.12 (c.l. 95%: 82.38 – 109.82)
LD/ED₅₀	107.74 (c.l. 95%: 93.81 – 181.08)	113.22 (c.l. 95%: 94.54 – 135.58)
NOED	42.70	85.41
Critical concentration	Larvae Mortality D8 [mg a.s./kg diet]	Adult Emergence D22 [mg a.s./kg diet]
LC/EC₁₀	518.98 (c.l. 95%: 382.40 – 593.00)	550.35 (c.l. 95%: 472.28 – 641.32)
LC/EC₂₀	584.57 (c.l. 95%: 498.04 – 719.76)	617.63 (c.l. 95%: 534.92 – 713.11)
LC/EC₅₀	699.67 (c.l. 95%: 609.21 – 1175.07)	735.16 (c.l. 95%: 613.89 – 880.38)
NOEC	277.30	554.59

LD/ED - LC/ECx evaluated by Weibull analysis.
c.l.: confidence limits

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No new studies submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No new studies submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No new studies submitted.

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Using artificial substrates

No new studies submitted.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory tests

Comments of zRMS:	The study was accepted. Validity criteria were met. The following endpoints for <i>Aphidius rhopalosiphi</i> were derived: LR50 = 1.501 L/ha ER50 > 0.6921 L/ha NOERmortality = 0.6921 L/ha NOERreproduction ≥ 0.6921 L/ha These endpoints were used for risk assessment.
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Reference: KCP 10.3.2.2

Report GLOB1913H: Effects on the parasitoid *Aphidius rhopalosiphi* extended laboratory study, Leopold J., 2020, 155401002

Guideline(s): Yes, IOBC (Mead-Briggs *et al.*, 2009)

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1913H on the parasitic wasp *Aphidius rhopalosiphi*. Under extended laboratory conditions the LR₅₀ of GLOB1913H is 1501.0 mL product/ha in 400 L water/ha (95% confidence limits: 1238.3 – 1819.3 mL product/ha). For mortality effects the LOER (lowest observed effect rate) is 1730.3 mL product/ha and the NOER (no observed effect rate) is 692.1 mL product/ha.

At 4325.8 and 10814.5 mL product/ha, the settling rate was 24.8 and 16.6 % during the initial 3 hours and below the threshold value of 30%. This might be an indication for an initial repellent effect for these two test item application rates. Afterwards, the threshold value was clearly exceeded in all test item treatments at further two observations with exception of the highest application rate of 10814.5 mL product/ha after 48 hours. The low settling rate (0 %) observed for this rate was caused by the high mortality at this time

point and not by a repellent effect. Therefore it can be concluded that sufficient exposure was given at all test item application rates during the entire study.

The reproductive capacity of *A. rhopalosiphi* was tested at 277.2 and 692.1 mL product/ha. There was no effect on reproduction up to and including 692.1 mL product/ha compared to the control. ER₅₀ is estimated to be greater than 692.1 mL product/ha. For reproduction effects the NOER is equal or greater than 692.1 mL product/ha and the LOER is greater than 692.1 mL product/ha.

Materials and Methods

Test item:	GLOB1913H, batch No.: 200701/01 Prosulfocarb: 886.1 g/L (nominal 900 g/L)
Test species:	Parasitoid (<i>Aphidius rhopalosiphi</i>), adults not older than 48 hours; source: Katz Biotech AG, Baruth, Germany
Test design:	This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 6 replicates each containing 5 female parasitoids. The parasitoids were exposed to dried residues on treated plant surfaces (barley plants). Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups with < 50 % corrected mortality survived females were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, <i>Rhopalosiphum padi</i> . The adult parasitoids were removed after 24 hours and the aphid-infested plants left for further 11 days before the numbers of aphid mummies that had developed were assessed.
Endpoints:	Mortality of exposed parasitoids; additionally, reproductive capacity for female survivors was assessed.
Validity criteria:	Control mortality should not exceed 10%. Reference item mortality should be > 50% corrected mortality. Mean control reproduction rate should be ≥ 5 mummies per female. There should be no more than 2 parasitoids producing zero values.
Reference item:	DANADIM PROGRESS (nominal: 400 g/L dimethoate)
Test rates:	Control, 277.2, 692.1, 1730.3, 4325.8 and 10814.5 mL product/ha and reference item. The reference item was applied at an application rate of 10.0 mL DANADIM PROGRESS/ha. All treatments were applied in 400 L deionised water/ha. The spraying dilutions were sprayed onto barley plants <i>via</i> laboratory spraying equipment, which were then air dried.
Test conditions:	Temperature: 19 - 22°C; relative humidity: 69 - 78% (acclimatisation and exposure period), 74 - 77% (post-exposure period, within the test units); photoperiod: 16 h light : 8 h dark; light intensity: 560 - 810 lux (acclimatisation and exposure period), 1130 - 1370 lux (parasitisation period), 5870 - 6800 lux (post-parasitisation period).
Statistics:	Mortality data: Step-down Cochran-Armitage Test, Fisher Exact Binomial Test (both test: one-sided greater, $\alpha = 0.05$); LR ₅₀ : Trimmed Spearman-Kärber Procedure Reproduction data: Dunnett's t-test (one-sided smaller, $\alpha = 0.05$) Settling data: Bonferroni-Holm Welch t-Test; Student t-test (both tests: one-sided smaller, $\alpha = 0.05$)

Results and discussion

Experimental dates: 08 September 2020 – 22 September 2020

The mortality of *Aphidius rhopalosiphi* was statistically significantly increased compared to the control at 1730.3, 4325.8 and 10814.5 mL product/ha (Step-down Cochran-Armitage Test, $\alpha = 0.05$; one-sided greater). At 277.2 and 692.1 mL product/ha, mortality was not statistically significantly increased compared to the control.

Behavioural abnormalities (affected and/or moribund parasitoids) were observed at all test item application rates after 2, 24 and 48 hours with exception of the lowest test item rate of 277.2 mL product/ha where no behavioural abnormalities were noted at any observation.

During the initial 3 hours the settling rate of the parasitoids on the plants was between 16.6 and 72.0 % in the test treatments and was statistically significantly reduced in all test item rates compared to the control (Bonferroni-Holm Welch t-test, one-sided smaller, $\alpha = 0.05$). At the control and reference item treatment, it was 88.7 and 65.8 %, respectively. At 4325.8 and 10814.5 mL product/ha, the settling rate was 24.8 and 16.6 % and below the threshold value of 30%. As this is an indication for a repellent effect at a further two time points, after 24 and 48 hours of the study, additional observations were performed.

After 24 hours the settling rate was clearly increased in all test item treatments and ranged from 52.7 to 80.0 %. There was no statistically significant difference at any test item application rate compared to the control (Bonferroni-Holm Welch t-test, one-sided smaller, $\alpha = 0.05$).

Mortality and parasitisation efficiency of *Aphidius rhopalosiphi*

	Rate ¹⁾ [mL/ha]	Mortality ²⁾ [%]	Mortality corr. ³⁾ [%]	Reproduction ⁴⁾ [mummies/female]	Effect on reproduction ⁵⁾ [%]
Control	-	3.3	--	57.8	--
GLOB1913H	277.2	0.0 n.s.	-3.4	56.8 n.s.	1.9
GLOB1913H	692.1	6.7 n.s.	3.4	56.3 n.s.	2.6
GLOB1913H	1730.3	73.3 *	72.4	--	--
GLOB1913H	4325.8	93.3 *	93.1	--	--
GLOB1913H	10814.5	96.7 *	96.6	--	--
Endpoints ⁶⁾					
LR ₅₀ (95 % CL): 1501.0 mL product/ha (1238.3 – 1819.3 mL product/ha)					
ER ₅₀ > 692.1 mL product/ha					

1) Application rate in 400 L deionised water/ha

2) Mortality: after 48 hours of exposure to spray residues on plant surfaces

(Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$; n.s. = not significant, * = significant)

3) Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate lower mortality compared to the control

4) Reproduction: mean number of parasitised aphids/female

(Dunnett's t-test, one-sided smaller, $\alpha = 0.05$; n.s. = not significant)

5) Calculated on the exact raw data

6) LR₅₀ was calculated with Trimmed Spearman-Kärber Procedure; CL = confidence limits

After 48 hours, the settling rate was between 40.0 and 82.5 % in all test item treatments with exception of the highest application rate. At 10814.5 mL product/ha, it was 0% which was caused by the high mortality of 96.7 % at this time point. At 1730.3, 4325.8 and 10814.5 mL product/ha, the settling rate was statistically significantly reduced compared to the control (Bonferroni-Holm Welch t-test, one-sided smaller, $\alpha = 0.05$).

Reproduction of *A. rhopalosiphi* was assessed at the control and at 277.2 and 692.1 mL product/ha. At both test item application rates, no statistically significant difference compared to the control was observed (Dunnett's t-test, one-sided smaller, $\alpha = 0.05$).

The reference item applied at a rate of 10.0 mL DANADIM PROGRESS/ha produced a statistically significant corrected mortality of 100.0 % after 48 hours.

Conclusions

Under extended laboratory conditions the LR₅₀ of GLOB1913H is 1501.0 mL product/ha in 400 L water/ha (95% confidence limits: 1238.3 – 1819.3 mL product/ha). For mortality effects the LOER (lowest observed effect rate) is 1730.3 mL product/ha and the NOER (no observed effect rate) is 692.1 mL product/ha.

At 4325.8 and 10814.5 mL product/ha, the settling rate was 24.8 and 16.6 % during the initial 3 hours and below the threshold value of 30%. This might be an indication for an initial repellent effect for these two test item application rates. Afterwards, the threshold value was clearly exceeded in all test item treatments at further two observations with exception of the highest application rate of 10814.5 mL product/ha after 48 hours. The low settling rate (0 %) observed for this rate was caused by the high mortality at this time point and not by a repellent effect. Therefore it can be concluded that sufficient exposure was given at all test item application rates during the entire study.

The reproductive capacity of *A. rhopalosiphi* was tested at 277.2 and 692.1 mL product/ha. There was no effect on reproduction up to and including 692.1 mL product/ha compared to the control. ER₅₀ is estimated to be greater than 692.1 mL product/ha. For reproduction effects the NOER is equal or greater than 692.1 mL product/ha and the LOER is greater than 692.1 mL product/ha.

Comments of zRMS:	<p>The study was accepted. The validity criteria were met:</p> <p>LR₅₀ = 0.6013 L/ha ER₅₀ > 0.6921 L/ha NOER_{mortality} < 0.2772 L/ha NOER_{reproduction} ≥ 0.6921 L/ha</p> <p>This endpoint was used for risk assessment.</p>
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Reference: KCP 10.3.2.2

Report GLOB1913H: Effects on the predatory mite *Typhlodromus Pyri* (Acari: Phytoseiidae), extended laboratory study -dose response test-, Leopold J., 2020, 155401062

Guideline(s): Yes, Blümel *et al.* 2000 and Oomen, 1988

Deviations: Yes, at 692.1 mL product/ha the reproduction assessment was performed although the corrected mortality (M_{corr}) was 57.7 %. The corrected mortality was very close to 50 %. Therefore it was decided to run the reproduction at this rate too in order to have additional information on reproduction at 692.1 mL product/ha.

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1913H on the predatory mite *Typhlodromus pyri* SCHEUTEN.

Under extended laboratory conditions the LR₅₀ of GLOB1913H is 601.3 mL product/ha in 200 L water/ha (95% confidence limits: 512.6 – 692.7 mL product/ha). The NOER (no observed effect rate) for mortality

effects was < 277.2 mL product/ha and the LOER (lowest observed effect rate) for mortality effects was 277.2 mL product/ha in 200 L water/ha.

The reproductive capacity of *Typhlodromus pyri* was tested at 277.2 and 692.1 mL product/ha. There was no effect on reproduction up to and including 692.1 mL product/ha compared to the control. ER₅₀ is estimated to be greater than 692.1 mL product/ha in 200 L water/ha. For reproduction effects the NOER is equal or greater than 692.1 mL product/ha and the LOER is greater than 692.1 mL product/ha in 200 L water/ha.

Materials and Methods

Test item:	GLOB1913H, batch No.: 200701/01 Prosulfocarb: 886.1 g/L (nominal 900 g/L)
Test species:	Predatory mite (<i>Typhlodromus pyri</i>), protonymphs less than 24 hours old; source: Katz Biotech AG, Baruth, Germany
Test design:	This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 6 replicates each containing 10 mites. The mites were exposed to dried residues on treated leaf surfaces (bean leaves). Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where the corrected mortality was ≤ 57.7 % were sexed and the number of eggs per females was recorded on 3 assessment days within one week.
Endpoints:	Mortality after 7 days of exposure; additionally, reproduction capacity for all variants with less than 50% corrected mortality.
Reference item:	DANADIM PROGRESS (nominal: 400 g dimethoate/L)
Validity criteria:	Control mortality should not exceed 20 % at day 7 after exposure. Reference item mortality should result in at least 50 % corrected mortality at day 7 after exposure. Control reproduction: number of eggs per female should be ≥ 4 eggs for the second week.
Test rates:	Control, 277.2, 692.1, 1730.3, 4325.8 and 10814.5 mL product/ha and reference item. The reference item was applied at an application rate of 40 mL DANADIM PROGRESS/ha. All treatments were applied in 200 deionised L water/ha. The spraying dilutions were sprayed onto leaves <i>via</i> laboratory spraying equipment, which were then air dried.
Test conditions:	Temperature: 24 - 26°C; relative humidity: 66 - 74%; photoperiod: 16 h light: 8 h dark; light intensity: 250 - 440 lux.
Statistics:	Mortality data: Step-down Cochran-Armitage Test, Fisher Exact Binomial Test (both test: one-sided greater, $\alpha = 0.05$); LR ₅₀ : Weibull Analysis. Reproduction data: Bonferroni-Holm Welch t-Test (one-sided smaller, $\alpha = 0.05$).

Results and Discussion

Experimental dates: 04 September 2020 – 18 September 2020

The mortality of *Typhlodromus pyri* was statistically significantly increased compared to the control at all test item application rates (Step-down Cochran-Armitage Test, $\alpha = 0.05$; one-sided greater).

Reproduction of *T. pyri* was assessed at the control and at 277.2 and 692.1 mL product/ha. At both test item application rates, no statistically significant difference compared to the control was observed (Bonferroni-Holm Welch t-Test, one-sided smaller, $\alpha = 0.05$).

Mortality and reproduction of *Typhlodromus pyri*

	Rate ¹⁾ [mL/ha]	Mortality ²⁾ [%]	Mortality corr. ³⁾ [%]	Reproduction ⁴⁾ [eggs/female]	Effect on reproduction ⁵⁾ [%]
Control	--	13.3	--	7.0	--
GLOB1913H	277.2	26.7 *	15.4	6.3 n.s.	9.8
GLOB1913H	692.1	63.3 *	57.7	4.4 n.s.	37.7
GLOB1913H	1730.3	100.0 *	100.0	--	--
GLOB1913H	4325.8	100.0 *	100.0	--	--
GLOB1913H	10814.5	100.0 *	100.0	--	--
Endpoints ⁶⁾					
LR ₅₀ (95 % CL): 601.3 mL product/ha (512.6 – 692.7 mL product/ha) ER ₅₀ > 692.1 mL product/ha					

1) Application rate in 200 L deionised water/ha

2) Mortality: after 7 days of exposure to spray residues on leaf surfaces

(Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$; * = significant)

3) Corrected mortality according to Abbott and improvements by Schneider-Orelli

4) Reproduction: mean number of eggs/female,

(Bonferroni-Holm Welch t-Test, one-sided smaller, $\alpha = 0.05$; n.s. = not significant, * = significant)

5) Calculated on the exact raw data

6) LR₅₀ was calculated with Weibull Analysis; CL = confidence limits. ER₅₀ could not be calculated as no effects on reproduction above 50 % were noted.

The reference item applied at a rate of 40 mL DANADIM PROGRESS/ha produced a statistically significant mortality of 100.0 % (corrected mortality 100.0 %) after 7 days.

Conclusions

Under extended laboratory conditions the LR₅₀ of GLOB1913H is 601.3 mL product/ha in 200 L water/ha (95% confidence limits: 512.6 – 692.7 mL product/ha). The NOER (no observed effect rate) for mortality effects was < 277.2 mL product/ha and the LOER (lowest observed effect rate) for mortality effects was 277.2 mL product/ha in 200 L water/ha.

The reproductive capacity of *Typhlodromus pyri* was tested at 277.2 and 692.1 mL product/ha. There was no effect on reproduction up to and including 692.1 mL product/ha compared to the control. ER₅₀ is estimated to be greater than 692.1 mL product/ha in 200 L water/ha. For reproduction effects the NOER is equal or greater than 692.1 mL product/ha and the LOER is greater than 692.1 mL product/ha in 200 L water/ha.

Comments of zRMS:	<p>The study was accepted.</p> <p>The validity criteria were met.</p> <p>The following endpoints for <i>Aleochara bilineata</i> were derived: ER₅₀ > 10.814 L/ha NOERreproduction = 1.730 L/ha</p> <p>These endpoints were used for risk assessment.</p>
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Reference: KCP 10.3.2.2

Report GLOB1913H: Effects on the reproduction of rove beetles *Aleochara bilineata* Gyll. -extended laboratory study- -dose response test-, Berg C., 2021, 155401071

Guideline(s): Yes, Grimm *et al.* 2000

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1913H on the rove beetle *Aleochara bilineata*.

In this extended laboratory study the effects of GLOB1913H on the reproduction capacity of the rove beetle *Aleochara bilineata* at rates of 277, 692, 1730, 4326 and 10814 mL product/ha in 400 L water/ha were determined.

The ER₅₀ (50 % effect rate) for the reproduction was estimated to be > 10814 mL GLOB1913H /ha.

The ER₂₀ (20 % effect rate) for the reproduction was determined to be 5820 mL GLOB1913H /ha.

The ER₁₀ (10 % effect rate) for the reproduction was determined to be 3405 mL GLOB1913H /ha.

The NOER (no observed effect rate) for the reproduction was determined to be 1730 mL GLOB1913H /ha.

Materials and Methods

Test item: GLOB1913H, batch No.: 200701/01
Prosulfocarb: 886.1 g/L (nominal 900 g/L)

Test species: Staphylinid beetle (*Aleochara bilineata*), adults (1 to 5 days old); source: De groene Vlieg, Duivenwaardsedijk 1, NL-3244 LG – Nieuwe Tonge.

Test design: Dose response design with endpoint effect on reproduction of *Aleochara bilineata*. The test item at 5 concentrations, control and reference item were sprayed via laboratory spray applicator on the soil surface. Exposure of the beetles was reached via treated natural soil LUFA 2.1. The results were compared to a deionised water treated control and a reference item group. The beetles were introduced into the test units immediately after treatment. Each replicate contained 10 female and 10 male beetles; 4 replicates per treatment. The beetles were exposed to control, test and reference item for 28 days. On days 7, 14 and 21 approx. 500 pupae of *Delia antiqua* were buried into the soil of each replicate to be parasitized by the larvae of the beetles. On day 28 the adults were separated from the soil and the soil with the pupae was allowed to dry for seven days. On day 35 the pupae were sieved out of the natural soil and transferred into an emergence container. The emergence of the F1-generation of beetles was observed from day 37 - 82.

Endpoint: Reproductive capacity (average number of hatched beetles of the F₁ generation)

Reference item: DANADIM PROGRESS (dimethoate, nominal: 400 g/L)

Validity criteria: Mean number of emerged beetles in the control group: > 400 beetles per replicate.
Effect on reproduction in the reference item compared to the control: at least 50%.

Test rates:	Test item: 277, 692, 1730, 4326 and 10814 mL product/ha. The maximum field rate per season (recalculated 4326 mL product/ha) was selected as the second highest concentration. The reference item (Danadim Progress) was applied at an application rate of 2000 mL product/ha. The control group was sprayed with 400 L water/ha.
Test conditions:	Temperature: 18°C - 22°C; relative humidity: 66 - 85%; light intensity: 700 – 900 lux; photoperiod: 16 h light: 8 h dark; food: frozen midge larvae (commercial food for aquarium fish; <i>Chironomus sp.</i>).
Statistics:	Test Item: Williams multiple sequential t-test procedure, probit analysis (Finney 1971); Reference Item: Student t-test for homogeneous variances, both tests one-sided smaller, $\alpha = 0.05$.

Results and Discussion

Experimental dates: 21 October 2020 – 11 January 2021

The reduction of the reproduction capacity of the rove beetle *Aleochara bilineata* after exposure to GLOB1913H at rates of 277, 692, 1730, 4326 and 10814 mL GLOB1913H/ha compared to the control group (=100 %) was -0.3, -1.7, 0.5, 15.9 and 36.4 %, respectively. Negative values represent an increased, positive values a decreased reproduction compared to the control.
The reference item (2000 mL product/ha) led to a significantly reduced reproduction by 98.8 %.

Effects on reproduction of staphylinid beetles (*Aleochara bilineata*) exposed to GLOB1913H in an extended laboratory trial

Treatment	Application Rate ¹⁾	Reproduction Efficiency [mean number of emerged beetles ± Standard Deviation]		Effect on Reproduction ²⁾ [%]
Control	-	866	± 20	-
GLOB1913H	277 mL product/ha	868	± 42	-0.3 (n.s.)
	692 mL product/ha	881	± 8	-1.7 (n.s.)
	1730 mL product/ha	862	± 28	0.5 (n.s.)
	4326 mL product/ha	728	± 65	15.9 (*)
	10814 mL product/ha	551	± 109	36.4 (*)
Reference Item	2000 mL product/ha	11	± 6	98.8 (*)
NOER	1730 mL product/ha	ER ₅₀	> 10814 mL product /ha	
ER ₂₀	5820 mL product /ha	ER ₁₀	3405 mL product /ha	

1) The test item and reference item were sprayed with an application rate of 400 L water/ha, the control was sprayed with deionised water

2) The effect on reproduction was calculated with exact raw data according to the following formula: $(1 - R_t/R_c) \cdot 100\%$ (negative values represent an increased, positive values a decreased reproduction compared to the control)

Statistics:

* = statistically significantly difference compared to the control; n.s. = not statistically significant difference compared to the control; test item: NOER: Williams multiple sequential test, one-sided smaller, $\alpha = 0.05$; ER_x values: probit analysis, reference item: Student t-test, pairwise comparison, one-sided smaller, $\alpha = 0.05$

All validity criteria were met.

Conclusions

In this extended laboratory study the effects of GLOB1913H on the reproduction capacity of the rove beetle *Aleochara bilineata* at rates of 277, 692, 1730, 4326 and 10814 mL product/ha in 400 L water/ha were determined.

The ER₅₀ (50 % effect rate) for the reproduction was estimated to be > 10814 mL GLOB1913H /ha.
The ER₂₀ (20 % effect rate) for the reproduction was determined to be 5820 mL GLOB1913H /ha.
The ER₁₀ (10 % effect rate) for the reproduction was determined to be 3405 mL GLOB1913H /ha.
The NOER (no observed effect rate) for the reproduction was determined to be 1730 mL GLOB1913H /ha.

Comments of zRMS:	The study was accepted. The validity criteria were met. The following endpoints for <i>Poecilus cupreus</i> L. were derived: LR ₅₀ > 10.814 L/ha NOERmortality ≥ 10.814 L/ha These endpoints were used for risk assessment.
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Reference: KCP 10.3.2.2
Report GLOB1913H: Effects on the carabid beetle *Poecilus cupreus* L. -extended laboratory study- -dose response study-, Berg C., 2021, 155401007
Guideline(s): Yes, BBA guideline part VI, 23-2.1.8, 1991 and Heimbach *et al.* 2000
Deviations: No
GLP: Yes
Acceptability: Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1913H on the carabid beetle *Poecilus cupreus* L.

In this extended laboratory study, no effects on either mortality, food consumption or behaviour of the ground dwelling predator *Poecilus cupreus* were observed after exposure of the beetles to the presence of 277, 692, 1730, 4326 and 10814 mL GLOB1913H/ha. The LR₅₀ was estimated to be > 10814 mL GLOB1913H/ha. The NOER (no observed effect rate) was determined to be ≥ 10814 mL GLOB1913H /ha.

Materials and Methods

Test item: GLOB1913H, batch No.: 200701/01
Prosulfocarb: 886.1 g/L (nominal 900 g/L)

Test species: Carabid beetle (*Poecilus cupreus* L.), age: about 5-6 weeks old; source: Bio-Test Labor GmbH, Sagerheide, Germany.

Test design: The beetles were exposed to GLOB1913H in five different concentrations in a dose-response design on natural soil (LUFA 2.1). The test item was applied directly onto the soil surface via a laboratory spray applicator at a water amount of 400 L/ha. The control group was sprayed with 400 L deionised water and dime-thoate (Danadim Progress, nominal 400g/L) was used as reference item.
Each treatment consisted of 5 test units each containing 6 individuals (3 males and 3 females)/test unit. Assessment of mortality and behaviour were conducted *ca.* 2 hours, 1, 2, 4, 7, 10 and 14 days after application. Food consumption was assessed on days 2, 4, 7, 10 and 14 after application.

Endpoints:	Mortality, behaviour and feeding rate of exposed beetles.
Validity criteria:	Control mortality: should not exceed 6.7% at day 14 Reference item mortality: should result in at least 65% ± 35% at day 14
Test rates:	Test item: 277, 692, 1730, 4326 and 10814 mL product/ha. The maximum field rate per season (recalculated 4326 mL product/ha) was selected as the second highest concentration. The reference item was applied at an application rate of 2000 mL Danadim Progress/ha.
Test conditions:	Temperature: 19°C - 22°C; relative humidity: 69% - 74%; photoperiod: 16 h light : 8 h dark; light intensity: 570 – 730 lux; food: fly pupae (<i>Delia</i> spec.).
Statistics:	NOER: multiple sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided greater, $\alpha = 0.05$); LR ₅₀ : As no mortality occurred in any of the test item treatment groups the LR ₅₀ of the test item was be considered as > the highest tested rate (10814 mL product/ha).

Results and Discussion

Experimental dates: 03 November 2020 – 17 November 2020

No mortality occurred after exposure of the beetles to the presence of 277, 692, 1730, 4326 and 10814 mL GLOB1913H/ha at the end of the experiment (14 days after application). No mortality occurred in the untreated control group. In the reference item group 100.0 % mortality was observed on day 2 of the experiment.

No test item related behavioral abnormalities occurred at any time for the duration of the experiment.

The mean food consumption (mean number of eaten pupae per beetle over the entire observation period) was 5.0 (corresponding to 100 %) for all test item treatment groups. No differences in food consumption were found between any of the test item treated groups and the control group with 5.0 pupae per beetle. The mean food consumption in the reference item group with 0.4 pupae per beetle was lower compared to the control group.

Effects of GLOB1913H on ground beetles (*Poecilus cupreus*) in an extended laboratory trial

	Rate ¹⁾ [mL]	Mortality ²⁾ [%]	Pupae consumed per beetle ³⁾	Food consumption [%]
GLOB1913H	277	0	5.0	100
	692	0	5.0	100
	1730	0	5.0	100
	4326	0	5.0	100
	10814	0	5.0	100
NOER: ≥ 10814 mL GLOB1913H/ha		LR ₅₀ : > 10814 mL GLOB1913H/ha		
Control	0.0	0.0	5.0	100 %
Reference Item	2000 mL	100	0.4	9 %

¹⁾ Test item and reference item were applied as spraying application in 400 L water/ha onto the soil surface

²⁾ Mortality: mean of 5 replicates with 6 beetles/replicates, 30 beetles on total

³⁾ rounded values

Statistics:

NOER: multiple sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided greater, $\alpha = 0.05$); LR₅₀: As no mortality occurred in any of the test item treatment groups the LR₅₀ of the test item was be considered as > the highest tested rate (10814 mL product/ha).

All validity criteria were met.

Conclusions

In this extended laboratory study, no effects on either mortality, food consumption or behaviour of the ground dwelling predator *Poecilus cupreus* were observed after exposure of the beetles to the presence of 277, 692, 1730, 4326 and 10814 mL GLOB1913H/ha. The LR₅₀ was estimated to be > 10814 mL GLOB1913H/ha. The NOER (no observed effect rate) was determined to be \geq 10814 mL GLOB1913H/ha.

Comments of zRMS:	The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.3.2.2
Report	Effects of GLOB1913H on the parasitic wasp <i>Aphidius rhopalosiphii</i> DeStephani-Perez under extended laboratory conditions (under semi-field conditions aged residues on potted barley plants), Röhlig U., 2022a, 22 48 NAR 0003
Guideline(s):	Yes, IOBC (MEAD-BRIGGS <i>et al.</i> 2009), modified for an aged residue test
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

An extended laboratory study with aged residues on plant surfaces was carried out to determine the effects and the duration of the residual activity of the test item GLOB1913H on the parasitic wasp *Aphidius rhopalosiphii* (Hymenoptera: Braconidae). For determination of mortality and reproduction, adult wasps were exposed to fresh dried or under semi-field conditions aged residues of GLOB1913H on potted barley plants at several exposure times. Effects on mortality were assessed by the number of surviving, affected, moribund and dead wasps, and effects on reproduction were assessed by the number of parasitised aphids (mummies) produced per female.

The study encompassed one test item rate, one reference item rate and a water treated control, each with 6 replicates for each bioassay. GLOB1913H was sprayed on potted barley plants (*Hordeum vulgare*), variety “Xanadu”, at an application rate of 4.4 L product/ha with a water volume corresponding to 400 L/ha. Additional test plants were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 401.7 g Dimethoate/L) as the reference item. The application was carried out under semi-field (outdoor) conditions using a spray equipment for small plot applications (plot-sprayer). In the bioassay started on DAT14 and DAT28, untreated potted barley plants were treated in the laboratory with DANADIM PROGRESS with a water volume corresponding to 400 L/ha as the reference item. Endpoints of the study were the mortality and, additionally, effects on reproduction.

The 1st bioassay commenced within 1 hour after treatment of the plants, as soon as the spray residues had dried, i.e. 0 days after treatment (DAT0). A 2nd bioassay was started 14 days after treatment (DAT14) and a 3rd bioassay was started 28 days after treatment (DAT28). All bioassays were set up with 6 replicates (consisting of 5 females) per treatment. Exposure of the wasps lasted until 48 hours after start of each bioassay. Mortality assessments were carried out 2, 24 and 48 hours after exposure of the wasps and additionally behavioural impacts were assessed during the initial 3 hours after exposure. In addition, for the control and the test item rates, the reproduction, i.e. number of mummies per female, was determined (1 assessment, 14 days after start of each bioassay).

Effects < 50 % on survival and reproduction of *Aphidius rhopalosiphi* were observed in two consecutive bioassays, when the wasps were exposed to 14-day-old residues (bioassay started on DAT14) and to 28-day-old residues (bioassay started on DAT28) of GLOB1913H applied at a rate of 4.4 L product/ha in 400 L water/ha.

Materials and Methods

Test item:	GLOB1913H, batch No.: 200701/01 analysed content of a.i.: Prosulfocarb: 886.1 g/L (nominal 900 g/L) density: 1.0375 g/mL
Test species:	Parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEPHANI-PEREZ), adults (< 48 hours old) source (in the stage of mummies): “Katz Biotech AG”, An der Birkenpfehlheide 10, 15837 Baruth, Germany, three days before start of each bioassay.
Test design:	<p>Adult wasps were exposed via fresh dried or semi-field aged residues of the test item on potted barley plants. Initial bioassay started on the day of application (DAT0), once residues had dried. The plants intended for the bioassay initiated 14 days after application (DAT14) until bioassay initiated 28 days after application (DAT28) were maintained outside under rain protection roof permeable to UV-light. The test comprised</p> <p>3 treatment groups (1 test item rate, water treated control, reference item).</p> <p>Treatments were applied to potted barley plants (<i>Hordeum vulgare</i>) using a spray equipment for small plot applications (plot-sprayer).</p> <p>Extended laboratory bioassays were initiated within 1 h after treatment application, i.e. 0 days after treatment (DAT0), 14 days (DAT14) and 28 days after treatment (DAT28), set up with 6 replicates for test item, control and reference item treatments, consisting of 5 female wasps each. Exposure of the adults was achieved via air-dried residues on treated potted barley plants.</p> <p>Mortality assessments were carried out 2, 24 and 48 hours after exposure of the wasps. In the bioassays started on DAT14 and DAT28, after 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (<i>Rhopalosiphum padi</i>). Assessment of reproduction capacity i.e. number of mummies per female was made for the control and test item rates (1 assessment, 14 days after start of each exposure).</p>
Endpoints:	Mortality: number of surviving, affected, moribund and dead wasps Reproductive capacity: number of mummies per surviving female.
Reference item:	DANADIM PROGRESS (401.7 g Dimethoate/L, nominal: 400 g/L) The reference item was applied at a rate of 30 mL product/ha in 400 L/ha of water on DAT0 (outdoor conditions) and at a rate of 10 mL product/ha in 400 L/ha of water on DAT14 and DAT28 (laboratory conditions).
Test rates:	Control (deionised water) Test item (GLOB1913H): 4.4 L product/ha in 400 L/ha of water
Test conditions:	<u>Controlled-environment test room:</u>

Temperature: 21 - 22 °C (1st bioassay, DAT0)
21 - 22 °C (2nd bioassay, DAT14)
20 – 22 °C (3rd bioassay, DAT28)

Relative humidity: 64 - 72 % (1st bioassay, DAT0)
64 - 73 % (2nd bioassay, DAT14)
65 - 73 % (3rd bioassay, DAT28)

Light-dark-cycle: 16 hours light, 8 hours dark

Light intensity: (1st bioassay, DAT0):
1120 lx (exposure phase)

Light intensity: (2nd bioassay, DAT14):
1080 lx (exposure phase)
2470 lx (parasitisation phase)
6530 lx (reproduction phase)

Light intensity: (3rd bioassay, DAT28):
1120 lx (exposure phase)
2530 lx (parasitisation phase)
6590 lx (reproduction phase)

Semi-field (outdoor) conditions (non-GLP):

(valid for the full time of ageing)

Temperature (mean/day): 17.5 °C – 28.9 °C

Temperature (min/max): 9.2 °C – 38.2 °C

Relative humidity (mean/day): 43 % - 89 %

Rainfall: 39.7 mm (not relevant, since the treated plants were placed rain-protected under a roof)

Food: 10 % w/w aqueous fructose solution

Statistics: Chi² 2x2 Table Test ($\alpha = 0.05$) for mortality (test item and reference item)
STUDENT-t-test ($\alpha = 0.05$) for reproductive capacity and for repellence (test item)

Results and Discussion

DAT0:

In the bioassay started on DAT0, in the water-treated control a mortality of 6.7 % was observed. In the test item treatment mortality was 73.3 % at 4.4 L product/ha. This resulted in a corrected mortality rate of 71.4 %. Statistically significant effects on mortality were determined at the test item rate compared to the control (Chi² 2x2 Table test, $\alpha = 0.05$). In the bioassay initiated on DAT0, the toxic reference item caused a mortality of 100 % of the exposed wasps, resulting in a corrected mortality of 100 % (Chi² 2x2 Table test, $\alpha = 0.05$).

In the bioassay started on DAT0 no reproduction test was performed, due to 73.3 % mortality in the test item treatment group.

DAT14:

In the bioassay started on DAT14, in the water-treated control a mortality of 3.3 % was observed. In the test item treatment mortality was 3.3 % at 4.4 L product/ha. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were determined at the test item rate compared to the control (Chi² 2x2 Table test, $\alpha = 0.05$). In the bioassay initiated on DAT14, the toxic reference item caused a mortality of 100 % of the exposed wasps, resulting in a corrected mortality of 100 % (Chi² 2x2 Table test, $\alpha = 0.05$).

In the bioassay started on DAT14 a reproduction rate of 16.6 mummies/female at the test item rate of 4.4 L product/ha were obtained. In the control 17.6 mummies/female were observed. Thus an effect on reproduction of 5.7 %, was calculated for the test item treated group compared to the control. No statistically significant effect on reproduction was observed at the 4.4 L product/ha test item rate (STUDENT-t-test, $\alpha = 0.05$).

DAT28:

In the bioassay started on DAT28, in the water-treated control a mortality of 3.3 % was observed. In the test item treatment mortality was 3.3 % at 4.4 L product/ha. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were determined at the test item rate compared to the control (χ^2 2x2 Table test, $\alpha = 0.05$). In the bioassay initiated on DAT28, the toxic reference item caused a mortality of 100 % of the exposed wasps, resulting in a corrected mortality of 100 % (χ^2 2x2 Table test, $\alpha = 0.05$).

In the bioassay started on DAT28 a reproduction rate of 16.9 mummies/female at the test item rate of 4.4 L product/ha were obtained. In the control 17.9 mummies/female were observed. Thus an effect on reproduction of 5.6 %, was calculated for the test item treated group compared to the control. No statistically significant effect on reproduction was observed at the 4.4 L product/ha test item rate (STUDENT-t-test, $\alpha = 0.05$).

The results are summarised below.

Effects on the parasitic wasp *Aphidius rhopalosiphi* exposed to fresh dry or under semi-field conditions aged residues of GLOB1913H in an extended laboratory test

Treatment	Rate ¹	Mortality ² [%]	Corrected mortality ³ [%]	Reproduction [mean number of mummies/female] ⁴	Effect on Reproduction ⁵ [%]
Bioassay initiated on DAT0 ⁶					
Control	-	6.7	-	n.d.	-
GLOB1913H	4.4 L product/ha	73.3*	71.4	n.d.	-
Reference item DANADIM PROGRESS	30 mL product/ha	100*	100	n.d.	-
Bioassay initiated on DAT14 ⁶					
Control	-	3.3	-	17.6	-
GLOB1913H	4.4 L product/ha	3.3 (n.s.)	0	16.6 (n.s.)	5.7
Reference item DANADIM PROGRESS	10 mL product/ha	100*	100	n.d.	-
Bioassay initiated on DAT28 ⁶					
Control	-	3.3	-	17.9	-
GLOB1913H	4.4 L product/ha	3.3 (n.s.)	0	16.9 (n.s.)	5.6
Reference item DANADIM PROGRESS	10 mL product/ha	100*	100	n.d.	-

¹ Application rate in 400 L water/ha.

- ² Mortality after 48 hours of exposure to the test item on treated barley plants. The results for mortality in individual treatments were compared to that in the control using the χ^2 2x2 Table Test ($\alpha = 0.05$) for mortality (test item and reference item)
- ³ Corrected mortality according to ABBOTT (1925).
- ⁴ Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results were compared to the control by the STUDENT-t-test ($\alpha = 0.05$).
- ⁵ Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease relative to the control.
- ⁶ DAT = Days After Treatment (equivalent to days over which residues aged before bioassay was initiated)
- n.s. not statistically significant different compared to the control
- * statistically significant different compared to the control
- n.d. not determined

In the bioassays started on DAT0, DAT14 and DAT28, no unusual observations were noted in the control and the test item treated group at any observation point during the test. There were no statistically significant differences in the behaviour (wasps settled on the plants, as a criterion for repellence) in all treated groups in comparison to control (STUDENT-t-test, $\alpha = 0.05$).

All validity criteria were met.

- mortality in the control group: $\leq 10\%$ (48 hours)
(observed: 6.7 %, 1st bioassay DAT0,
3.3 %, 2nd bioassay, DAT14)
3.3 %, 3rd bioassay, DAT28)
- reproduction in the control group: ≥ 5 mummies per female
(observed: 17.6 mummies/female, 2nd bioassay DAT14,
17.9 mummies/female, 3rd bioassay, DAT28)
- no more than 2 control replicates (with surviving wasps) with zero values
(observed: 1 replicate, 2nd bioassay DAT14,
2 replicates, 3rd bioassay, DAT28)
- corrected mortality in the reference item group: $> 50\%$ (48 hours)
(observed: 100 %, 1st bioassay DAT0)
(observed: 100 %, 2nd bioassay DAT14)
(observed: 100 %, 3rd bioassay DAT28)

Conclusions

To assess the duration and extent of possible effects of GLOB1913H on survival and reproduction of the parasitic wasp, *Aphidius rhopalosiphi*, at an application rate of 4.4 L product/ha, a control (treated with water) and a reference item (DANADIM PROGRESS) were applied to potted barley plants (*Hordeum vulgare*, var. “Xanadu”) under outdoor conditions. After defined time periods, adults of *A. rhopalosiphi* were exposed to the residues on the potted barley plants in a series of extended laboratory tests. Effects $< 50\%$ on survival and reproduction of *Aphidius rhopalosiphi* were observed in two consecutive bioassays, when the wasps were exposed to 14-day-old residues (bioassay started on DAT14) and to 28-day-old residues (bioassay started on DAT28) of GLOB1913H applied at a rate of 4.4 L product/ha in 400 L water/ha.

Comments of zRMS:	The study is considered to be reliable and suitable for the risk assessment,
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Reference: KCP 10.3.2.2

Report	Effects of GLOB1913H on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in an extended laboratory test (under semi-field conditions aged-residues on potted bean plants), Röhlig U., 2022b, 22 48 NTR 0002
Guideline(s):	Yes, IOBC (BLÜMEL <i>et al.</i> , 2000), modified for an aged residue test
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

An extended laboratory study with aged residues on plant surfaces was carried out to determine the effects and the duration of the residual activity of the test item GLOB1913H on the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari: Phytoseiidae). For determination of mortality and reproduction, protonymphs of the predatory mites were exposed in the laboratory to fresh dried or under semi-field conditions aged residues of GLOB1913H, on detached bean leaves at three exposure times. Effects on mortality were assessed by the number of surviving, dead and escaped predatory mites. Effects on reproduction were assessed by the number of eggs laid per female and number of juveniles per evaluation period.

The study encompassed 3 treatment groups (1 test item rate, control, reference item), each with 5 replicates. 20 protonymphs per replicate were exposed to fresh dried or under semi-field conditions aged residues of the test item sprayed on potted bean plants (*Phaseolus vulgaris*), variety “Jutta”, at an application rate of 4.4 L product/ha with a water volume corresponding to 400 L/ha. Additional test plants were treated with deionised water for the water control in the same way as the test item groups. A reference item group with DANADIM PROGRESS (active substance 401.7 g Dimethoate/L) was treated on DAT0 under semi-field (outdoor) conditions. On DAT14 and on DAT28, the reference item was freshly applied on excised untreated bean leaves under laboratory conditions. Endpoints of the study were the mortality and additionally effects on reproduction.

The 1st bioassay commenced within 1 hour after treatment of the plants, as soon as the spray residues had dried, i.e. 0 days after treatment (DAT0). A 2nd bioassay was started 14 days after treatment (DAT14) and a 3rd bioassay was started 28 days after treatment (DAT28).

Exposure of the mites lasted until 14 days after the start of each bioassay. Mortality assessments were carried out 3 and 7 days after exposure of the mites and, additionally, after 7, 9, 11 and 14 days, the number of males and females were counted. In addition, the reproduction, i.e. number of eggs per female, was determined (3 assessments on 9, 11 and 14 days after start of bioassay) for the control and the test item treatment.

Effects < 50 % on survival and reproduction of *Typhlodromus pyri* were observed in two consecutive bioassays, when the mites were exposed to 14-day-old residues (bioassay started on DAT14) and to 28-day-old residues (bioassay started on DAT28) of GLOB1913H applied at a rate of 4.4 L product/ha in 400 L water/ha.

Materials and Methods

Test item:	GLOB1913H, batch No.: 200701/01 analysed content of a.i.: Prosulfocarb: 886.1 g/L (nominal 900 g/L) density: 1.0375 g/mL
Test species:	Predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (protonymphs < 24 hours old) source: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Test design:	<p>Protonymphs were exposed via freshly dried or aged residues of the test item on bean leaves. The test comprised 3 treatment groups on DAT0, DAT14 and DAT28 (1 test item rate, water treated control, reference item) set up with 5 replicates (consisting of 20 protonymphs per replicate).</p> <p>Treatments were applied to potted bean plants using a spray equipment for commercial applications (plot-sprayer). For each bioassay, the replicate leaves were gently cut to leaf discs, which were placed with the treated side upward on moistened cotton wool in Petri dishes.</p> <p>The ageing of spray residues on potted bean plants took place under semi-field (outdoor) conditions with rain protection (under a UV-permeable roof) from the application until the start of the respective bioassay.</p> <p>Exposure lasted until 14 days after start of each bioassay started on DAT0, DAT14 and DAT28. Mortality assessments were carried out 3 and 7 days after exposure of the mites. Additionally after 7, 9, 11 and 14 days the number of females and males was counted. In addition, the reproduction, <i>i.e.</i> number of eggs per female, was determined (3 assessments on 9, 11 and 14 days after start of bioassay DAT 14 and DAT28).</p> <p>Extended laboratory bioassays were initiated 0, 14 and 28 days after the application (DAT0, DAT14 and DAT28)</p>																						
Endpoints:	<p>Mortality: number of surviving, dead, escaped mites (trapped or not found) after start of each bioassay over 7 days</p> <p>Reproduction: number of eggs laid and number of juveniles per evaluation period per female from day 7-14</p>																						
Test rates:	<p>Control (deionised water): 400 L/ha</p> <p>Test item (GLOB1913H): 4.4 L product/ha in 400 L/ha of deionised water</p> <p>Reference item (DANADIM PROGRESS, 401.7 g Dimethoate/L):</p> <p>200 mL product/ha (nominally equivalent to 80 g a.i./ha) in 400 L/ha of deionised water, DAT0) (outdoor conditions)</p> <p>30 mL product/ha (nominally equivalent to 12 g a.i./ha) in 200 L/ha of deionised water, DAT14 and DAT28) (laboratory conditions)</p>																						
Test conditions:	<p><u>Controlled-environment test room:</u></p> <table><tr><td>Temperature:</td><td>24 °C – 27 °C (1st bioassay DAT 0)</td></tr><tr><td></td><td>24 °C – 27 °C (2nd bioassay DAT 14)</td></tr><tr><td></td><td>24 °C – 26 °C (3rd bioassay DAT 28)</td></tr><tr><td>Relative humidity:</td><td>68 % – 86 % (1st bioassay DAT0)</td></tr><tr><td></td><td>71 % – 87 % (2nd bioassay DAT14)</td></tr><tr><td></td><td>75 % – 89 % (3rd bioassay DAT28)</td></tr><tr><td>Light-dark-cycle:</td><td>16 hours light, 8 hours dark</td></tr><tr><td>Light intensity:</td><td>2090 lx (1st bioassay DAT0)</td></tr><tr><td></td><td>2070 lx (2nd bioassay DAT 14)</td></tr><tr><td></td><td>2020 lx (3rd bioassay DAT 28)</td></tr><tr><td>Food:</td><td>pollen: pine (<i>Pinus nigra</i>) and birch (<i>Betula pendula</i>) 1:1</td></tr></table> <p><u>Outdoor weather conditions (non-GLP):</u> (valid for the full time of ageing)</p>	Temperature:	24 °C – 27 °C (1 st bioassay DAT 0)		24 °C – 27 °C (2 nd bioassay DAT 14)		24 °C – 26 °C (3 rd bioassay DAT 28)	Relative humidity:	68 % – 86 % (1 st bioassay DAT0)		71 % – 87 % (2 nd bioassay DAT14)		75 % – 89 % (3 rd bioassay DAT28)	Light-dark-cycle:	16 hours light, 8 hours dark	Light intensity:	2090 lx (1 st bioassay DAT0)		2070 lx (2 nd bioassay DAT 14)		2020 lx (3 rd bioassay DAT 28)	Food:	pollen: pine (<i>Pinus nigra</i>) and birch (<i>Betula pendula</i>) 1:1
Temperature:	24 °C – 27 °C (1 st bioassay DAT 0)																						
	24 °C – 27 °C (2 nd bioassay DAT 14)																						
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Light-dark-cycle:	16 hours light, 8 hours dark																						
Light intensity:	2090 lx (1 st bioassay DAT0)																						
	2070 lx (2 nd bioassay DAT 14)																						
	2020 lx (3 rd bioassay DAT 28)																						
Food:	pollen: pine (<i>Pinus nigra</i>) and birch (<i>Betula pendula</i>) 1:1																						

Temperature (mean/day): 17.5 °C – 28.9 °C
Temperature (min/max): 9.2 °C – 38.2 °C
Relative humidity (mean/day): 43 % - 89 %

Rainfall: 39.7 mm (not relevant, since the treated plants were placed rain-protected under a roof)

Statistics: Chi² 2x2 Table test ($\alpha = 0.05$) for mortality (test item)
Chi² 2x2 Table test ($\alpha = 0.05$) for mortality (reference item)
STUDENT-t-test ($\alpha = 0.05$) for reproduction (DAT14, DAT28)
ToxRat Professional 3.3.0 (RATTE 2018)

Results and Discussion

DAT0:

In the bioassay started on DAT0, in the water-treated control a mortality of 0 % was observed after 7 days. In the test item treatment, mortality was 93.0 % at 4.4 L product/ha. This resulted in a corrected mortality rate of 93.0 %. Statistically significant effects on mortality were determined at the test item rate of 4.4 L product/ha compared to the control (Chi² 2x2 Table test, $\alpha = 0.05$). In the bioassay initiated on DAT0, the toxic reference item caused a mortality of 100 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 100 % (Chi² 2x2 Table test, $\alpha = 0.05$).

In the bioassay started on DAT0 no reproduction test was performed, due to 93.0 % mortality in the test item treatment group.

DAT14:

In the bioassay started on DAT14, in the water-treated control a mortality of 1.0 % was observed after 7 days. In the test item treatment, mortality was 4.0 % at 4.4 L product/ha. This resulted in a corrected mortality rate of 3.0 %. No statistically significant effects on mortality were determined at the test item rate of 4.4 L product/ha compared to the control (Chi² 2x2 Table test, $\alpha = 0.05$). In the bioassay initiated on DAT14, the toxic reference item caused a mortality of 78.0 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 77.8 % (Chi² 2x2 Table test, $\alpha = 0.05$).

In the bioassay started on DAT14, the reproduction rate in the 4.4 L product/ha test item treated group resulted in 6.50 eggs/female, compared to 6.42 eggs/female in the control. Thus, an effect on reproduction of -1.2 % was calculated for the test item treated group compared to the control. No statistically significant effects on reproduction were observed at the 4.4 L product/ha test item treatment group (STUDENT-t-test, $\alpha = 0.05$).

DAT28:

In the bioassay started on DAT28, in the water-treated control a mortality of 1.0 % was observed after 7 days. In the test item treatment, mortality was 2.0 % at 4.4 L product/ha. This resulted in a corrected mortality rate of 1.0 %. No statistically significant effects on mortality were determined at the test item rate of 4.4 L product/ha compared to the control (Chi² 2x2 Table test, $\alpha = 0.05$). In the bioassay initiated on DAT28, the toxic reference item caused a mortality of 75.0 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 74.7 % (Chi² 2x2 Table test, $\alpha = 0.05$).

In the bioassay started on DAT28, the reproduction rate in the 4.4 L product/ha test item treated group resulted in 6.11 eggs/female, compared to 6.46 eggs/female in the control. Thus, an effect on reproduction of 5.4 % was calculated for the test item treated group compared to the control. No statistically significant effects on reproduction were observed at the 4.4 L product/ha test item treatment group (STUDENT-t-test, $\alpha = 0.05$).

The results are summarised below.

Effects on the predatory mite (*Typhlodromus pyri*) exposed to fresh and aged residues of GLOB1913H in an extended laboratory trial

Treatment	Rate ¹	Mortality ²	Corrected	Reproduction ⁴	Effects on Repro-
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		[%]	mortality ³ [%]	[mean number of eggs/female]	duction ⁵ [%]
Bioassay initiated DAT0 ⁶					
Control	-	0	-	n.d.	-
Test item	4.4 L product/ha	93.0*	93.0	n.d.	-
Reference item	200 mL product/ha	100*	100	-	-
Bioassay initiated DAT14 ⁶					
Control	-	1.0	-	6.42	-
Test item	4.4 L product/ha	4.0 (n.s.)	3.00	6.50 (n.s.)	-1.2
Reference item	30 mL product/ha	78.0*	77.8	-	-
Bioassay initiated DAT28 ⁶					
Control	-	1.0	-	6.46	-
Test item	4.4 L product/ha	2.0 (n.s.)	1.0	6.11 (n.s.)	5.4
Reference item	30 mL product/ha	75.0*	74.7	-	-

¹ Application rate in 400 L water/ha (control, test item, reference item DAT0)

Application rate in 200 L water/ha (reference item, DAT14 and DAT28)

² Mortality: percentage of individuals (after 7 days of each exposure)

³ Corrected mortality according to ABBOTT (1925)

⁴ Reproduction: mean number of eggs per female.

⁵ Change in mean numbers of eggs per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control.

⁶ DAT = Days After Treatment (equivalent to days over which residues aged before bioassay was initiated)

n.s. not statistically significantly different compared to the corresponding control

* statistically significantly different compared to the corresponding control

n.d. not determined, due to > 50 % corrected mortality in the test item treatment group

No unusual observations regarding behavior were noted in the control and the test item treatment groups at any observation point during the test.

All validity criteria were met.

- mortality in the control group: ≤ 20 % (dead and escaped mites) on day 7
(observed: 0 % bioassay DAT0)
(observed: 1.0 % bioassay DAT14)
(observed: 1.0 % bioassay DAT28)
- corrected mortality in the reference group: 50 – 100 % on day 7
(observed: 100 % bioassay DAT0)
(observed: 77.8 % bioassay DAT14)
(observed: 74.7 % bioassay DAT28)
- reproduction in the control group: ≥ 4 eggs per female
(observed: 6.42 eggs per female in bioassay DAT14)
(observed: 6.46 eggs per female in bioassay DAT28)

Conclusions

To assess the duration and extent of possible effects of GLOB1913H on survival and reproduction of the predatory mite *Typhlodromus pyri*, at an application rate of 4.4 L product/ha, A control (treated with water) and a reference item (DANADIM PROGRESS) were applied on potted bean plants (*Phaseolus vulgaris*, var. “Jutta”) under outdoor conditions. After defined time periods, protonymphs of the predatory mite *Typhlodromus pyri* were exposed to the residues on the bean leaf discs in a series of extended laboratory tests.

Effects < 50 % on survival and reproduction of *Typhlodromus pyri* were observed in two consecutive bioassays, when the mites were exposed to 14-day-old residues (bioassay started on DAT14) and to 28-day-old residues (bioassay started on DAT28) of GLOB1913H applied at a rate of 4.4 L product/ha in 400 L water/ha.

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted in line to OECD guideline 222 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.4.1.1
Report	GLOB1913H: Effects on reproduction and growth of earthworms <i>Eisenia andrei</i> in artificial soil, Straube D., 2020, 155401022
Guideline(s):	Yes, OECD 222 (2016), ISO 11268-2 (2012)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine potential effects of the test item on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil in a laboratory test. The test was performed according to the recommendations of the OECD Guideline 222 (2016).

In an earthworm reproduction and growth study with GLOB1913H the No Observed Effect Concentration (NOEC) for mortality of the earthworm *Eisenia andrei* was determined to be 97.0 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for mortality was determined to be 150 mg test item/kg soil. The LC₅₀ was determined to be 199.4 mg test item/kg soil. The NOEC for weight changes was determined to be the concentration of 150 mg test item/kg soil. The LOEC for weight changes was determined to be 233 mg test item/kg soil. The NOEC for reproduction was determined to be the test concentration of 62.6 mg test item/kg soil. The LOEC for reproduction was determined to be 97.0 mg test item/kg soil. The EC values for earthworms based on reproduction and nominal concentrations calculated by Weibull analysis are not reliable due to overly wide confidence intervals caused by the missing clear test item related dose response.

Materials and Methods

Test item:	GLOB1913H, batch 200701/01, prosulfocarb: 900 g/L (nominal)
Test species:	Earthworm (<i>Eisenia andrei</i>), adult earthworms (with clitellum and weight range 301 to 597 mg), approximately 10 months old, source: from an in-house culture

Test design:	56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Validity criteria:	Control mortality: should not exceed 10% over initial 4-week test period. Reproduction of control: should be ≥ 30 earthworms per replicate container. Coefficient of variation of reproduction in control: should not exceed 30%.
Test conditions:	Artificial soil according to OECD 222; initial pH 6.5, pH at experimental end 6.5; water content 26.2% to 27.0% (54.6% to 56.2% of maximum water holding capacity, WHC) at experimental start and 26.5% to 28.8% (55.2% to 60.1% of the maximum WHC) at experimental end; temperature: within the range of 18°C to 22°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Test concentrations:	Control, 10.8, 16.8, 26.0, 40.4, 62.6, 97.0, 150 and 233 mg GLOB1913H/kg soil. All concentrations are indicated per kg soil dry weight.
Dates of work:	Experimental start date: August 20, 2020 Experimental completion date: October 16, 2020
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Williams t-test (body weight changes), Welch t-test after Bonferroni-Holm (reproduction), Weibull Analysis (LC ₅₀ and EC values).

Results and Discussion

All study validity criteria were met.

A mortality of 5.0% was found at the test concentration of 97.0 mg test item/kg soil, which was not statistically significantly different compared to the control, where 0% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater). At the test concentration of 150 mg test item/kg soil and above, mortality was statistically significantly increased compared to the control.

The body weight changes of the earthworms after 4 weeks exposure to GLOB1913H were not statistically significantly different compared to the control up to and including the test concentration of 150 mg test item/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 233 mg test item/kg soil body weight changes were statistically significantly increased compared to the control.

The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 62.6 mg test item/kg soil (Welch t-test after Bonferroni-Holm, $\alpha = 0.05$, one-sided smaller). At the test concentration of 97.0 mg test item/kg soil and 233 mg test item/kg soil, the reproduction was statistically significantly increased compared to the control. The test concentration of 150 mg test item/kg soil reproduction was not statistically significantly different compared to the control. No behavioural abnormalities were observed in any of the treatment groups.

The feeding activity in all the treated groups was comparable to the control up to and including the test concentration of 150 mg test item/kg soil. The feeding activity in the highest test concentration of 233 mg test item/kg soil was reduced.

Effect of GLOB1913H on earthworms (*Eisenia andrei*) in a 56-day reproduction study

GLOB1913H [mg test item/kg soil]	Control	10.8	16.8	26.0	40.4	62.6	97.0	150	233
Mortality (day 28) [%]	0	0	0	0	0	0	5	13	78
Statistical Significance ¹⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
Body weight change (day 28) [%]	16.5	15.6	11.9	17.5	11.5	12.9	15.5	18.2	-4.2
Statistical Significance ²⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Mean No. of juveniles (day 56)	77	74	88	61	66	67	49	54	2
Statistical Significance ³⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*
Reproduction in [%] of control (day 56)	-	96	114	79	85	86	64	69	2
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	8.8
Endpoints [mg test item/kg soil]									
NOEC (day 28 mortality)	97.0								
LOEC (day 28 mortality)	150								
LC ₅₀ ⁴⁾	199.4								
NOEC (day 28 weight changes)	150								
LOEC (day 28 weight changes)	233								
NOEC (day 56 reproduction)	62.6								
LOEC (day 56 reproduction)	97.0								
EC Values (reproduction) ⁴⁾	EC ₁₀			EC ₂₀			EC ₅₀		
	49.4			75.3			142.6		
95% confidence limits	0.36 to 86.2			3.49 to 113.4			73.27 to 249.9		

The results represent rounded values calculated from the exact raw data.

- = not applicable

n.s. = not significantly different compared to the control

* = significantly different compared to the control

¹⁾ Fisher's Exact Test, $\alpha = 0.05$, one-sided greater

²⁾ Williams t-test, $\alpha = 0.05$, one-sided smaller

³⁾ Welch t-test after Bonferroni-Holm, $\alpha = 0.05$, one-sided smaller

⁴⁾ Weibull Analysis

In the most recent test with the reference item Carbendazim (performed under ibacon Study No. 105685022 from May to July 2019), there were statistically significant effects on reproduction at a concentration of 0.695 mg a.i./kg soil and above, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg a.i./kg soil). The EC₅₀ for reproduction was calculated as 0.92 mg a.i./kg soil.

Conclusions

In an earthworm reproduction and growth study with GLOB1913H the No Observed Effect Concentration (NOEC) for mortality of the earthworm *Eisenia andrei* was determined to be 97.0 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for mortality was determined to be 150 mg test item/kg soil. The LC₅₀ was determined to be 199.4 mg test item/kg soil. The NOEC for weight changes was determined to be the concentration of 150 mg test item/kg soil. The LOEC for weight changes was determined to be 233 mg test item/kg soil. The NOEC for reproduction was determined to be the test concentration of 62.6 mg test item/kg soil. The LOEC for reproduction was determined to be 97.0 mg test item/kg soil. The EC values for earthworms based on reproduction and nominal concentrations calculated by Weibull analysis are not reliable due to overly wide confidence intervals caused by the missing clear test item related dose response.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Comments of zRMS:	The study has been already assessed in dRAR, December 2022.
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Reference:	KCP 10.4.1.2
Report	Effects of Prosulfoarb 800 g/L EC on earthworms under field conditions, Schulz L., Biochem Agrar, 14 10 48 008 F
Guideline(s):	Yes, ISO 11268-3 (1999), Kula <i>et al.</i> , 2006 - Technical recommendations to ISO 11268-3
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	/

Executive Summary

The objective of this field study was to investigate potential effects and the potential recovery of field populations of earthworms after the application of the test item Prosulfocarb 800 g/L EC. Therefore, a field experiment lasting about one year was performed and the effects of the test item on different earthworm species, biomass and abundance were compared to an untreated control and to a reference item.

The trial was placed on arable land near Machern in Saxony/Germany. The test item Prosulfocarb 800 g/L EC (prosulfocarb 800 g/L (nominal)) was applied once at a rate of 5 L/ha corresponding to 4 kg prosulfocarb/ha. Nutdazim 50 FLOW (carbendazim 500 g/L (nominal)) was applied once to the plots as reference item at a rate of 20 L/ha corresponding to 10 kg carbendazim/ha. Tap water was applied once as a control.

Twelve plots, each 10 m x 10 m, were arranged in a 3 x 4 formation, each plot surrounded by a 2 m wide path between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site. Defined areas were sampled to assess earthworm populations before application and three times after application, i.e. about 1, 6 and 12 months after test item application.

No measurable residues (< LOD) of prosulfocarb were determined in any of the soil samples of the control plots taken after test item application. After the application of Prosulfocarb 800 g/L EC a mean residue value of 121 % of the application rate was found in soil samples of the test item treatment group. The mean recovery was in the recommended range of 50 - 150%.

Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole. The mean earthworm abundance in the control plots was 200.5 ind./m² at pre-sampling, 93.0 ind./m² at 1st sampling, 304.0 ind./m² at 2nd sampling and 269.5 ind./m² at 3rd sampling. Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Allolobophora chlorotica* (12.2% of total earthworms), *Aporrectodea caliginosa* (45.0% of total earthworms) and *Aporrectodea rosea* (3.7% of total earthworms) as well as the anecic species *Aporrectodea longa* (3.3% of total earthworms) and *Lumbricus terrestris* (28.6% of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris* representing different ecological groups indicated the suitability of the field site.

The toxic reference item reduced total earthworm abundance and biomass by 22.0 % and 50.3 % at 1st sampling, respectively. *Lumbricus terrestris* was the most sensitive species and was reduced in total abundance and biomass by 65.3% and 66.6% on this sampling date, respectively. The statistically significant reduction in total earthworm biomass of 50.3% at 1st sampling (about 1 month after test item application) confirmed the validity of the test system.

Surface monitoring on days 1 - 3 after test item application showed that there was no acute primary effect on earthworms by Prosulfocarb 800 g/L EC. No alive, moribund or dead earthworms were found on the soil surface neither in the test item nor in the control monitoring areas.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rate of 5 L Prosulfocarb 800 g/L EC about 1, 6 and 12 months after test item application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species and ecological groups could be observed for the tested application rate of 5 L/ha about 1, 6 and 12 months after test item application. Only for the total biomass of the earthworm species *Lumbricus terrestris* a statistically significant reduction of 27.7 % could be observed about 12 months after test item application. However, since no effects on total biomass of *Lumbricus terrestris* could be observed about 1 and 6 months after test item application and a reduction in biomass of *Lumbricus terrestris* of 27.7 % is within the range of the natural variability of earthworm populations, the statistically significant reduction in total biomass of *Lumbricus terrestris* can be considered as ecologically not relevant.

Materials and methods

Test material	Prosulfocarb 800 g/L EC
Active ingredient	Prosulfocarb, 806 g/L (analysed), 800 g/L (nominal)
Control	Tap water
Toxic reference	Nutdazim 50 FLOW (carbendazim 500 g/L, nominal)

Test site and maintenance:

The study was located near Machern in Saxony, Germany. Cultural practices performed on the test field during 2011 until 2013 followed the usual agricultural practice. The only cultivated crop within this time span was *Phacelia tanacetifolia*. Maintenance of the field during the present study was according to general agricultural practice. The application was performed on bare soil. About one month after test item application, the test field was seeded with the fodder crop “Landsberger Gemenge” (clover grass mixture) which stayed on the field until the end of the study. The test field was mulched once in autumn 2014 (see table above). No further plant protection products others than the test item and the reference item were applied on the test field. Furthermore, no mineral or organic fertilisers were applied to the test field.

Application replicates:

Application was conducted on 11 April 2014, a day with low wind and no rain forecast, 2 weeks after the presampling. The application was performed with a plot sprayer (PL 1, agrotop GmbH, Obertraubling) with Lechler DG TEEJET 80015 VS nozzles.

For the control only tap water without test item was used. Each treatment and control consisted of four replicates. For the reference an application rate of 20 L/ha was used. For each application the test item or reference item was dissolved in a water volume equivalent to 600 L/ha. The test item was applied at 5 L/ha.

Rainfall was recorded on day 1 after test item application (1.0 mm). The test field was irrigated with 10.0 mm tap water on day 3 after application.

Earthworm sampling:

The surface of all plots was carefully searched for moribund or dead earthworms on three following days after application.

Defined areas were sampled to assess earthworm populations before and three times after application. Sampling was conducted on 31 March 2014 (2 weeks before application), 14 May 2014 (1 month after application), 29 October 2014 (6 months after application) and 30 March 2015 (12 months after application). Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion. Sampling was performed by a combination of hand-sorting and formalin extraction in the excavated hole.

Adult earthworms were identified to the species level and juveniles were identified to species level if possible, otherwise to the genus level. Total abundance, total biomass, total adult and total juvenile abundance and biomass, total adult and total juvenile abundance and biomass of endogeic and anecic, total adult and total juvenile abundance and biomass of single species were determined.

Analytical verification:

For the verification of the actual exposure concentrations, soil samples were collected after application. On each plot 10 sub-specimens (soil cores) were taken in an “X” shape sampling scheme across the plot, which were pooled to one specimen per plot.

Meteorological conditions:

Data on air and soil temperature, precipitation, relative humidity and wind speed were collected on site.

Statistical evaluation:

For the pre-treatment sampling, data were analysed with a two-factorial analysis of variance (ANOVA, 5 % significance level) with treatment as fixed factor and block as random factor.

For the post-treatment sampling, data were analysed by a one-sided Dunnett-t-test with test item treatment group < control at 5% significance level.

Normality and homogeneity of variances were tested with Shapiro-Wilk W- test and Levenes test.

Analyses were conducted with the software STATISTICA 7.1 (Statsoft, Tulsa, USA).

Results and discussion

Residue analysis:

No measurable residues (< LOD) of prosulfocarb were determined in any of the soil samples of the control plots taken after test item application. After the application of Prosulfocarb 800 g/L EC a mean residue value of 121 % of the application rate was found in soil samples of the test item treatment group. The mean recovery was in the recommended range of 50 - 150 %.

Biological system:

Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Allolobophora chlorotica* (12.2% of total earthworms), *Aporrectodea caliginosa* (45.0% of total earthworms) and *Aporrectodea rosea* (3.7% of total earthworms) as well as the anecic species *Aporrectodea longa* (3.3% of total earthworms) and *Lumbricus terrestris* (28.6% of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris* representing different ecological groups indicated the suitability of the field site.

The toxic reference item reduced total earthworm abundance and biomass by 22.0 % and 50.3 % at 1st sampling, respectively. *Lumbricus terrestris* was the most sensitive species and was reduced in total abundance and biomass by 65.3% and 66.6% on this sampling date, respectively. The statistically significant reduction in total earthworm biomass of 50.3% at 1st sampling (about 1 month after test item application) confirmed the validity of the test system.

Surface monitoring on days 1 - 3 after test item application showed that there was no acute primary effect on earthworms by Prosulfocarb 800 g/L EC. No alive, moribund or dead earthworms were found on the soil surface neither in the test item nor in the control monitoring areas.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rate of 5 L Prosulfocarb 800 g/L EC about 1, 6 and 12 months after test item application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species and ecological groups could be observed for the tested application rate of 5 L/ha about 1, 6 and 12 months after test item application. Only for the total biomass of the earthworm species *Lumbricus terrestris* a statistically significant reduction of 27.7% could be observed about 12 months after test item application. However, since no effects on total biomass of *Lumbricus terrestris* could be observed about 1 and 6 months after test item application and a reduction in biomass of *Lumbricus terrestris* of 27.7% is within the range of the natural variability of earthworm populations, the statistically significant reduction in total biomass of *Lumbricus terrestris* can be considered as ecologically not relevant.

Results are summarised in the table below.

Group	Treatment	Abundance (individuals/m ²)				Biomass (g/m ²)			
		Sampling				Sampling			
		0 ^a	1 ^b	2 ^c	3 ^d	0 ^a	1 ^b	2 ^c	3 ^d

Total earth-worms	Control	200.5	93.0	304.0	269.5	147.21	90.71	209.34	220.81
	Prosulfocarb 800 g/L EC (5 L/ha)	198.0 (98.8%)	93.5 (100.5%)	293.0 (96.4%)	223.5 (82.9%)	159.01 (108.0%)	75.90 (83.7%)	187.91 (89.8%)	181.17 (82.1%)
	Reference	193.0 (96.3%)	72.5 (78.0%)	303.5 (99.8%)	215.0 (79.8%)	191.10 (129.8%)	45.05 (49.7%)	187.85 (83.1%)	182.44 (82.6%)
Total adults	Control	87.0	44.0	120.5	92.0	101.02	70.31	136.63	137.35
	Prosulfocarb 800 g/L EC (5 L/ha)	88.5 (101.7%)	43.5 (98.9%)	112.5 (93.4%)	90.5 (98.4%)	112.87 (111.7%)	65.61 (80.5%)	119.76 (87.7%)	121.91 (88.8%)
	Reference	83.0 (95.4%)	28.5 (64.8%)	130.0 (107.9%)	112.0 (121.7%)	143.6 (142.1%)	29.28 (41.6%)	125.73 (92.0%)	140.87 (102.6%)
Total juveniles	Control	104.0	45.5	172.0	167.0	43.55	19.46	69.17	79.84
	Prosulfocarb 800 g/L EC (5 L/ha)	99.5 (95.7%)	40.0 (87.9%)	172.0 (100.0%)	124.0 (74.3%)	43.16 (103%)	14.98 (90%)	65.93 (118%)	56.57 (88%)
	Reference	83.0 (90.9%)	28.5 (87.9%)	130.0 (95.4%)	112.0 (58.1%)	39.94 (91.7%)	14.89 (76.6%)	45.65 (66.0%)	40.05 (50.2%)
<i>A. caliginosa</i>	Control	87.5	28.0	166.0	136.0	48.75	11.63	81.73	61.33
	Prosulfocarb 800 g/L EC (5 L/ha)	97.5 (111.4%)	29.5 (105.4%)	168.5 (101.5%)	122.5 (90.1%)	54.34 (111.5%)	11.78 (101.3%)	80.47 (98.5%)	64.34 (104.9%)
	Reference	81.5 (93.1%)	44.0 (157.1%)	202.0 (121.7%)	152.5 (112.1%)	41.0 (84.1%)	18.25 (156.9%)	100.37 (122.8%)	89.74 (146.3%)
<i>A. chlorotica</i>	Control	30.5	23.5	44.0	41.5	9.46	6.60	12.21	9.50
	Prosulfocarb 800 g/L EC (5 L/ha)	26.5 (86.9%)	21.5 (91.5%)	48.0 (109.1%)	25.5 (61.5%)	7.86 (83.2%)	6.48 (98.1%)	14.13 (115.7%)	6.70 (70.6%)
	Reference	15.5 (50.8%)	7.0 (29.8%)	37.0 (84.1%)	15.0 (36.1%)	5.40 (57.1%)	1.80 (27.3%)	11.43 (93.7%)	4.11 (43.2%)
<i>A. rosea</i>	Control	7.5	1.5	13.0	6.0	0.95	0.13	2.35	1.02
	Prosulfocarb 800 g/L EC (5 L/ha)	6.5 (86.7%)	2.5 (166.7%)	10.5 (80.8%)	4.0 (66.7%)	1.46 (153.3%)	0.46 (351.9%)	1.84 (78.2%)	0.67 (65.8%)
	Reference	8.0 (106.7%)	3.0 (200.0%)	13.0 (100.0%)	5.0 (83.3%)	1.73 (181.8%)	0.33 (250.0%)	2.22 (94.5%)	1.35 (132.9%)
<i>A. longa</i>	Control	7.5	0.0	27.5	21.0	11.20	0.00	29.71	21.59
	Prosulfocarb 800 g/L EC (5 L/ha)	5.0 (66.7%)	0.5 (0.0%)	20.5 (74.6%)	15.0 (71.4%)	5.79 (51.7%)	0.78 (0.0%)	23.15 (77.9%)	17.17 (79.5%)
	Reference	7.0 (93.3%)	0.5 (0.0%)	21.5 (78.2%)	15.0 (71.4%)	5.77 (51.5%)	0.10 (0.0%)	34.0 (114.4%)	29.65 (137.3%)
<i>L. terrestris</i>	Control	55.0	36.0	41.5	54.0	73.25	71.75	80.62	124.74
	Prosulfocarb 800 g/L EC (5 L/ha)	48.5 (88.2%)	30.5 (84.7%)	36.0 (86.8%)	47.5 (88.0%)	86.03 (117.4%)	54.22 (75.6%)	66.56 (82.6%)	90.16 (72.3%)
	Reference	65.5 (119.1%)	12.5 (34.7%)	18.0 (43.4%)	20.0 (37.0%)	131.66 (179.7%)	23.94 (33.4%)	23.07 (28.6%)	55.91 (44.8%)

In brackets: % from control. Statistics: test item vs control and reference vs. control: one-sided Dunnett-t-test. Bold: significant different from control.

Statistically not analysed (due to low abundances)

^a two weeks before application

^b about 1 month after application

^c about 6 months after application

^d about 12 months after application

Validity criteria:

All validity criteria were met.

The mean abundance of earthworms of the test field at trial start was 197.0 ind./m² thus fulfilling the guideline recommendation of 60 ind./m² for arable soils).

At least one representative of endogeic and anecic earthworms was present at the field site in a sufficient number (>10 % of total earthworms or 10-15 ind./m²), with abundances of 88.8 ind./m² for *Aporrectodea caliginosa* (endogeic) and 56.3 ind./m² for *Lumbricus terrestris* (anecic; pre-sampling values).

Conclusions

It can be concluded that the application of Prosulfocarb 800 g/L EC tested at an application rate of 5 L/ha had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after test item application.

MDD analysis

Minimum Detectable Differences (MDDs) were calculated *a posteriori* for the results of the earthworm field study by Schulz (2015), as appropriate for the statistical method used in the original analysis (Dunnett's t-test), considering the actual test design (replication, selected type-I error level alpha) and the sample variation.

As there is no guidance available yet to classify the calculated MDDs for terrestrial field studies, the MDD classes proposed in the Aquatic Guidance Document (EFSA Journal 2013;11(7):3290) were used.

Results for the MDD calculations (one-sided Dunnett's test, $p < 0.05$), given in % relative to control values, for earthworm abundance and earthworm biomass are summarized in the table below.

% MDD	Abundance				Biomass			
	sampling date				sampling date			
Taxon	0 (pre)	1 m	6 m	12 m	0 (pre)	1 m	6 m	12 m
• Total	29	48	49	42	35	57	37	39
• Total adults	19	51	28	33	49	65	42	42
• Total juvenile	45	69	71	50	53	78	71	59
• Endogeic total	30	65	52	38	36	73	42	46
• Endogeic adults	20	56	26	33	33	55	28	58
• Endogeic juvenile	51	99	80	47	71	144	75	54
• <i>Aporrectodea caliginosa</i> total	49	121	63	53	48	118	52	56
• <i>Aporrectodea caliginosa</i> adults	51	99	48	55	50	100	46	78
• <i>Aporrectodea caliginosa</i> juvenile	60	144	86	60	76	181	78	59
• <i>Allolobophora chlorotica</i> total	61	92	78	50	61	99	81	57
• <i>Allolobophora chlorotica</i> adults	58	103	80	65	58	105	81	67
• <i>Allolobophora chlorotica</i> juvenile	158	89	96	42	218	106	125	39
• <i>Aporrectodea rosea</i> total	74	242	92	92	79	402	85	103
• <i>Aporrectodea rosea</i> adults	147	-	94	261	249	-	120	220
• <i>Aporrectodea rosea</i> juvenile	83	219	101	74	125	246	96	123
• Anecic total	37	43	48	57	65	60	48	51
• Anecic adults	93	83	61	67	96	75	71	62
• Anecic juvenile	48	65	62	64	62	74	87	81
• <i>Aporrectodea longa</i> total	85	-	82	93	72	-	84	165
• <i>Aporrectodea longa</i> adults	88	-	90	149	82	-	89	266
• <i>Aporrectodea longa</i> juvenile	132	-	90	87	117	-	104	106
• <i>Lumbricus terrestris</i> total	33	42	37	49	71	60	53	38
• <i>Lumbricus terrestris</i> adults	93	83	61	67	96	75	71	62
• <i>Lumbricus terrestris</i> juvenile	48	65	62	64	62	74	87	81
• Taxon is statistically evaluated in the study MDD class 0: >100 % MDD class I: 90-100 % MDD class II: 70-90 % MDD class III: 50-70 % MDD class IV: <50 %								

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was conducted in line to OECD guideline 232 and according to the principles of GLP. Validity criteria were met.
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	The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.4.2.1
Report	GLOB1913H: Effects on reproduction of the Collembola <i>Folsomia candida</i> in artificial soil, Straube D., 2020, 155401016
Guideline(s):	Yes, OECD 232 (2016), ISO 11267 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine potential effects of the test item on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks, the number of offspring (juveniles) and surviving parental collembolans were counted. The test was performed according to the OECD Guideline 232 (2016). The NOEC of GLOB1913H for mortality of *Folsomia candida* was determined to be 40.8 mg test item/kg soil. The LOEC for mortality was determined to be 63.2 mg test item/kg soil. The LC₅₀ was determined to be 66.9 mg test item/kg soil. The NOEC of GLOB1913H for reproduction of *Folsomia candida* was determined to be 26.3 mg test item/kg soil. The LOEC for reproduction was determined to be 40.8 mg test item/kg soil. The EC₁₀ was determined to be 35.5 mg test item/kg soil, EC₂₀ was determined to be 38.9 mg test item/kg soil and the EC₅₀ was determined to be 46.4 mg test item/kg soil.

Materials and Methods

Test Item:	GLOB1913H; batch no.: 200701/01; content of a.i.: Prosulfocarb: nominal 900 g/L, authenticated 886.1 g/L
Test Species:	Collembola <i>Folsomia candida</i> , 9 - 11 days old, from cultures held at the laboratory.
Test Design:	28-d exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was placed into glass vessels before the Collembola were introduced on top of the soil; 8 concentrations and one control; 4 replicates/concentration with 10 Collembola each (8 replicates for the control). Feeding of Collembola with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 d.
Endpoints:	Mortality of adult Collembola, behavioural effects, number of juveniles.
Reference Item:	Boric acid (the effects of the reference item were investigated in a separate GLP study.)
Test Concentrations:	Control, 4.56, 7.07, 11.0, 17.0, 26.3, 40.8, 63.2 and 98.0 mg GLOB1913H/kg soil ⁶ .
Test Conditions:	Artificial soil according to OECD 232; pH at experimental start 5.7 to 5.9, pH at experimental end 5.7 to 5.8; water content at experimental start 19.5% to 20.0% (51.3% to 52.6% of the maximum water holding capacity); at experimental end 17.1% to 18.9% (44.9% to 49.7% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark, light intensity within the range of 400 to 800 lux.
Statistics:	Standard procedures, Step-down Cochran-Armitage Test and Probit Analysis

⁶ All concentrations are indicated per kg soil dry weight.

(mortality), Williams t-test and Probit Analysis (reproduction).

Results and Discussion

Experimental dates: 16 October 2020 – 16 November 2020

All validity criteria for the study were met.

A mortality of up to 10% was observed in the test item treated groups up to and including 40.8 mg test item/kg soil, which was not statistically significantly different compared to the control, where 6.3% of the Collembola died (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). At the test item concentration of 63.2 mg test item/kg soil and above, mortality was statistically significantly increased compared to the control.

Reproduction of the Collembolan exposed to GLOB1913H was not statistically significantly different compared to the control up to and including the test concentration of 26.3 mg/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test item concentration of 40.8 mg test item/kg soil and above, reproduction was statistically significantly reduced compared to the control.

No behavioural abnormalities were observed in any of the treatment groups.

In a separate study, the reference item Boric acid showed statistically significant effects on reproduction at concentrations of ≥ 48.8 mg/kg soil. The EC_{50} for reproduction was calculated to be 104.6 mg/kg soil.

Effect of GLOB1913H on Collembola (*Folsomia candida*) in a 28-day reproduction study

GLOB1913H [mg test item/kg soil]	Control	4.56	7.07	11.0	17.0	26.3	40.8	63.2	98.0
Mortality (day 28) [%]	6.3	0.0	7.5	10.0	5.0	0.0	5.0	40.0	97.5
Significance ¹⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
No. of juveniles (day 28)	1360	1301	1243	1417	1220	1186	1004	84	0
Reproduction in [%] of control (day 28)	-	96	91	104	90	87	74	6	0
Statistical significance ²⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
Endpoints [mg test item/kg soil]									
NOEC (mortality)	40.8								
LOEC (mortality)	63.2								
LC ₅₀ (mortality) ³⁾	66.9								
NOEC (reproduction)	26.3								
LOEC (reproduction)	40.8								
EC Values (reproduction) ³⁾	EC ₁₀			EC ₂₀			EC ₅₀		
	35.5			38.9			46.4		
95% confidence limits	21.4 to 40.1			28.3 to 43.3			41.5 to 58.9		

n.s. = not significantly different compared to the control

* = significantly different compared to the control

¹⁾ Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater

²⁾ Williams t-test, $\alpha = 0.05$, one-sided smaller

³⁾ Probit Analysis

- not applicable

Conclusions

The NOEC of GLOB1913H for mortality of *Folsomia candida* was determined to be 40.8 mg test item/kg soil. The LOEC for mortality was determined to be 63.2 mg test item/kg soil. The LC₅₀ was determined to be 66.9 mg test item/kg soil.

The NOEC of GLOB1913H for reproduction of *Folsomia candida* was determined to be 26.3 mg test item/kg soil. The LOEC for reproduction was determined to be 40.8 mg test item/kg soil. The EC₁₀ was determined to be 35.5 mg test item/kg soil, EC₂₀ was determined to be 38.9 mg test item/kg soil and the EC₅₀ was determined to be 46.4 mg test item/kg soil.

Comments of zRMS:	The study was conducted in line to OECD guideline 226 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.4.2.1
Report	GLOB1913H: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil, Straube D., 2021, 155401089
Guideline(s):	Yes, OECD 226 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine potential effects of the test item on mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative species of soil micro-arthropods during a test period of 14 days. The test was performed according to the OECD Guideline 226 (2016). GLOB1913H caused no statistically significant effects on mortality of *Hypoaspis aculeifer* up to and including the test concentration of 236 mg test item/kg soil. Therefore, the NOEC for mortality was determined to be 236 mg test item/kg soil. The LOEC for mortality was determined to be 365 mg test item/kg soil. The LC₅₀ value was estimated to be >365 mg test item/kg soil. The NOEC of GLOB1913H for reproduction of *Hypoaspis aculeifer* was determined to be 98.0 mg test item/kg soil. The LOEC for reproduction was determined to be 152 mg test item/kg soil. The EC₁₀ for *Hypoaspis aculeifer* in artificial soil was determined to be 169.4 mg test item/kg soil, EC₂₀ was determined to be 202.7 mg test item/kg soil and the EC₅₀ was determined to be 285.7 mg test item/kg soil.

Materials and Methods

Test Item:	GLOB1913H; batch no.: 200701/01; content of a.i.: Prosulfocarb: nominal 900 g/L, authenticated 886.1 g/L
Test Species:	Predatory mite <i>Hypoaspis aculeifer</i> , adult females, approximately 7 days after reaching the adult stage (28 days after placing adult females in clean rearing vessels and the start of the egg laying period in the synchronisation), cultured by ibacon.
Test Design:	14 days exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil, which was filled into glass vessels before the predatory mites were introduced on top of the soil; 8 concentrations and one control were tested; 4 replicates per test item concentration and 8 replicates for the control, with 10 female predatory mites in each replicate. Feeding of the mites with cheese mites (<i>Tyrophagus putrescentiae</i>) <i>ad libitum</i> at test start and on day 2, 4, 7, 9 and 11. Assessment of adult mortality and reproduction performed after 14 days.
Endpoints:	Adult mortality, number of juveniles.

Reference Item:	Dimethoate (the effects of the reference item were investigated in a separate GLP study.)
Test Concentrations:	Control, 17.0, 26.3, 40.8, 63.2, 98.0, 152, 236 and 365 mg GLOB1913H/kg soil ⁷ .
Test Conditions:	Artificial soil based on OECD 226; initial pH 5.8 to 5.9, pH at experimental end 5.9 to 6.2; water content at experimental start 19.5% to 20.0% (51.4% to 52.7% of the maximum water holding capacity); at experimental end 18.6% to 19.7% (48.9% to 51.9% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).
Statistics:	Standard procedures, Chi ² Test (mortality), Williams t-test (reproduction), Probit Analysis (EC values).

Results and Discussion

Experimental dates: 04 January 2021 – 20 January 2021

All validity criteria for the study were met.

A mortality of up to 20% was observed in the test item treated groups up to and including 236 mg test item/kg soil, which was not statistically significantly different compared to the control, where 6% of the adult mites died (Chi² Test, $\alpha = 0.05$, one-sided greater). At the test concentration of 365 mg test item/kg soil mortality was 23%, which was statistically significantly increased compared to the control.

Reproduction of the predatory mites exposed to GLOB1913H was not statistically significantly different compared to the control up to and including the test concentration of 98.0 mg test item/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 152 mg test item/kg soil and above, reproduction was statistically significantly reduced compared to the control.

No behavioural abnormalities were observed in any of the treatment groups.

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 4.69 mg dimethoate/kg soil and above. The EC₅₀ for reproduction was 3.18 mg dimethoate/kg soil.

Summary of the Effects of GLOB1913H on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study

GLOB1913H [mg test item/kg soil]	Control	17.0	26.3	40.8	63.2	98.0	152	236	365
Mortality (day 14) [%]	6	10	13	15	10	20	13	15	23
Statistical significance ¹⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
No. of juveniles (day 14)	189	201	186	198	192	189	162	140	46
Reproduction in [%] of control (day 14)	-	106	98	104	101	100	86	74	24
Statistical significance ²⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*

⁷ All concentrations are indicated per kg soil dry weight.

GLOB1913H [mg test item/kg soil]	Control	17.0	26.3	40.8	63.2	98.0	152	236	365
Endpoints [mg test item/kg soil]									
NOEC (mortality)	236								
LOEC (mortality)	365								
LC ₅₀ (mortality) ³⁾	>365								
NOEC (reproduction)	98.0								
LOEC (reproduction)	152								
EC Values (reproduction) ⁴⁾	EC ₁₀			EC ₂₀			EC ₅₀		
	169.4			202.7			285.7		
95% confidence limits	129.0 to 196.2			167.3 to 226.6			261.1 to 314.6		

n.s. = not significantly different compared to the control

* = significantly different compared to the control

¹⁾ Chi² Test, $\alpha = 0.05$, one-sided greater

²⁾ Williams t-test, $\alpha = 0.05$, one-sided smaller

³⁾ estimated value

⁴⁾ Probit Analysis

- not applicable

Conclusion

GLOB1913H caused no statistically significant effects on mortality of *Hypoaspis aculeifer* up to and including the test concentration of 236 mg test item/kg soil. Therefore, the NOEC for mortality was determined to be 236 mg test item/kg soil. The LOEC for mortality was determined to be 365 mg test item/kg soil. The LC₅₀ value was estimated to be >365 mg test item/kg soil. The NOEC of GLOB1913H for reproduction of *Hypoaspis aculeifer* was determined to be 98.0 mg test item/kg soil. The LOEC for reproduction was determined to be 152 mg test item/kg soil. The EC₁₀ for *Hypoaspis aculeifer* in artificial soil was determined to be 169.4 mg test item/kg soil, EC₂₀ was determined to be 202.7 mg test item/kg soil and the EC₅₀ was determined to be 285.7 mg test item/kg soil.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Comments of zRMS:	<p>The study was conducted in line to ISO 11268-3 and according to the principles of GLP. There are no strict validity criteria concerning soil mesofauna mentioned in the test guideline ISO 11268-3. Nevertheless, the following criteria were fulfilled:</p> <ul style="list-style-type: none"> - At least at one post-treatment sampling date, the reduction of at least one major taxonomic group or total Collembola by the application of the reference item should be $\geq 50\%$ in comparison to the control. The following collembolan taxa are defined as major taxonomic groups: Isotomoidea, Entomobryoidea, Poduromorpha, Symphypleona. - Recovery of the test item in soil samples between 50% - 150%. <p>The study is considered to be reliable and suitable for the risk assessment.-</p>
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Reference: KCP 10.4.2.2

Report Prosulfocarb: GLP-compliant Collembola field study in Germany, Henkes G., 2022, 2140003

Guideline(s): Yes, de Jong et al., 2010, ISO 11268-3 (2014), ISO/FDIS 23611-2 (2006), Kula et al., 2006, Candolfi et al., 2000, de Jong et al., 2006

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

This study aims to identify the possible impact of prosulfocarb on a natural site specific Collembola community after application in an agricultural scenario.

Materials and methods

Test item

Product name:	GLOB1913H
Type of formulation:	Emulsifiable concentrate (EC)
Active substance content:	Nominal: 900 g/L Analysed: 886.1 g/L
Batch ID.:	200701/01
Date of production:	07.07.2020
Expiry date of batch:	07.07.2022
Form:	Liquid
Density (at 20°C):	1.0375 g/ml
Colour:	Golden yellow
Storage:	Ambient temperature
Proposed use:	Herbicide

Reference item

Danadim Progress (400 g/L, EC)

Test organism

Collembola

Location and condition of the study field

The study will be conducted on an agricultural field in Germany. The application of the test item will be conducted before drilling before emergence (bare soil).

The study field will be chosen to support large populations of soil micro-arthropods (Collembola).

The active substances of the test item and the reference item have not been applied on the study field for at least three years prior to the application.

Study plots

On the study field at least twelve study plots each with a size of 10 m x 10 m (100 m²) will be marked.

The twelve study plots will be evenly distributed among the following three treatment groups:

- C: control/tap water
- T: test item with an nominal application rate of 4.4 L GLOB1913H/ha
- R: reference item with an application rate of 8,000 g dimethoate/ha

Samples will be taken from a 6 m x 6 m core area in the central part of each study plot. At each sampling, two virtual squares will be sampled (three soil cores per square).

Application

The application will be carried out in compliance with GLP and will be conducted in the following order to avoid contamination: control (C), test item (T) and reference item (R). The test item will be applied in a spray volume of 300 L/ha (T 3960 g a.s./ha). The reference item (R) will be applied with a nominal rate

of 8,000 g a.s./ha in a spray volume of 300 L/ha. The control plots (C) will be treated with tap water with the same spray volume than the test and the reference item plots (300 L/ha). The application of the three different treatments will be conducted with a wheel driving sprayer-system (Schachtner PSG-FE 5.3 B) with compressed air and equipped with drift reducing nozzles.

Samplings

In total five micro-arthropod samplings will be conducted.

Time schedule for soil core (Collembola) sampling

Sampling event	Timing of sampling
1 st	14-21 days before application
2 nd	Approx. 1 month after application
Backup	Approx. 2-3 months after application
3 rd	Approx. 6 months after application
4 th	Approx. 12 months after application

Sampling, heat-gradient extraction and determination of Collembola will be conducted in accordance with ISO 23611-2 (2006). At each sampling event, on each study plot six cores (10 cm depth, at least 5 cm in diameter) will be taken by means of a split corer in a sampling area of 1.5 m x 1.5 m.

Verification of initial exposure concentrations

For the verification of initial exposure concentrations, soil samples will be collected within 72 hours after application, i.e. about one day after rainfall/irrigation have reached or exceeded 10 mm. The collection of soil samples will be carried out with a suitable hand sampler. Sampling depth will be approximately 10 cm, samples will include the turf. In the four test item study plot and in the four control study plots, five insertions randomly distributed over the study plots will be performed (approximately 1.5 kg soil). The soil cores taken in each study plot will be combined to one sample per study plot in the field.

For the verification of the application rates glass Petri-dishes (120 x 20) filled with LUFA standard soil No. 2 (80 g dry weight/Petri-dish) will be distributed as spray targets during the application. The Petri-dishes will be placed directly on the soil surface. The Petri-dishes will be opened shortly before onset of the application and closed with a glass lid and a suitable tape immediately after the application of the respective study plot. For each study plot the five Petri-dish samples will be pooled and mixed together to create a representative sample of the respective study plot.

Maintenance of the study field

All study plots will be prepared according to Good Agricultural Practice. Approximately one week before the application the vegetation from the study plot area will be incorporated into the soil by harrowing to ensure bare soil conditions during application. Approximately 4 weeks after application (and after the 2nd collembolan sampling), a cover crop will be sown.

A data logger will be set-up in the study field to collect data of the soil moisture

For soil characterization, a mixed sample collected over the study plot area will be taken before the application.

During 72 hours after the end of application an amount of at least 10 mm natural and/or artificial precipitation is required. If there is no rainfall or the amount of rain is distinctly below 10 mm (recording by rain gauge on the study field), the study plots will be artificially irrigated. To facilitate the soil core samplings after dry weather conditions (e. g. hard soil), irrigation could be conducted also before each sampling, if necessary.

Temperature data (daily minimum, maximum and mean temperature, precipitation) will be obtained from a RIFCON weather station situated close to the study field or an official weather station.

Extraction and determination

The extraction of soil samples will be carried out in high-gradient extractors (Macfadyen, 1961). The extraction will run for at least seven days. During this time the temperature will increase from approximately $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (measured at the upper layer of the sample). After termination of the extraction the extracted micro-arthropods will be washed with tap water and transferred to ethanol (70%). The total number of Collembola from the pre-application sampling will be roughly estimated to ensure a homogeneous distribution of the study plots to the four different treatments. All Collembola from each sample will be counted and determined to species level by use of e.g. a microscope. If the determination to species level is not possible (e.g. juveniles, damaged individuals) individuals will be determined to the next possible higher taxonomic unit (e.g. genus, family).

Data evaluation and statistics

The collected biological data will be subjected to appropriate statistical analyses. As an initial step data will be tested for normality (e.g. Shapiro Wilks' test) and homogeneity of variances (e.g. Levene test). If normality and/or homogeneity of variances are not given, data may be transformed (e.g. log, square-root) to achieve or at least to improve these criteria. The data will be analysed as described in the recommendations of Kula et al (2006): comparisons between treatment and control by ANOVA followed e.g. by Dunnett's post hoc test; comparisons between reference and control e.g. by Mann Whitney U test. Furthermore, Principle Response Curves (PRC) might be used to analyze the response of the collembolan community to the test item concentration and to the reference item if needed.

Analytics

The objective of the analytical phase is to determine the magnitude of residues of prosulfocarb in soil to verify the initial exposure concentration applications with GLOB1913H. Furthermore, a validation set in terms of matrix effects, linearity, recovery, repeatability (precision) and selectivity/specificity will be carried out.

Results and discussion

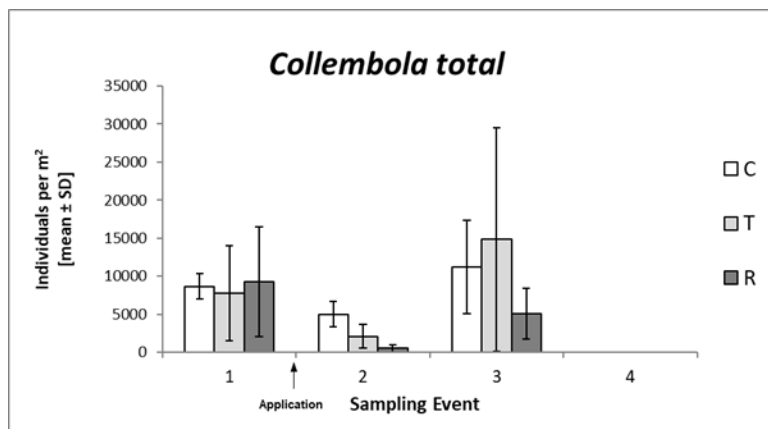
Validity criteria

There are no strict validity criteria concerning soil mesofauna mentioned in the test guideline ISO 11268-3. Nevertheless, the following criteria were fulfilled:

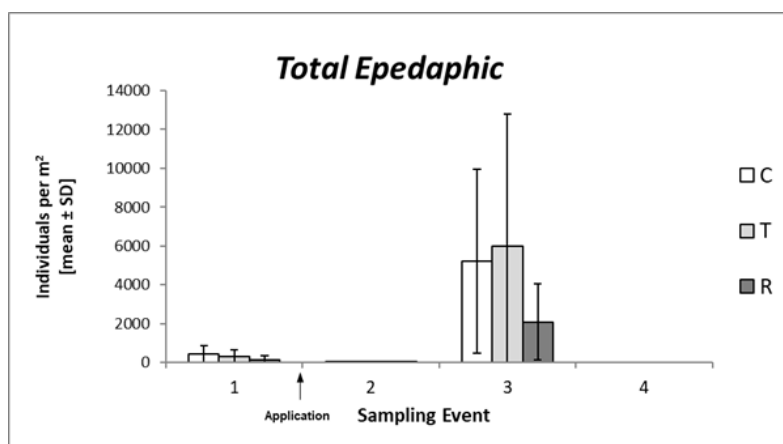
- At least at one post-treatment sampling date, the reduction of at least one major taxonomic group or total Collembola by the application of the reference item should be $\geq 50\%$ in comparison to the control. The following collembolan taxa are defined as major taxonomic groups: Isotomoidea, Entomobryoidea, Poduromorpha, Symphyleona.
- Recovery of the test item in soil samples between 50% - 150%.

A slight reduction of the total number of Collembola caused by the test item was found at the 2nd sampling. However, a recovery was already observed at the 3th sampling. For the 4th sampling, no effects of the test item are expected.

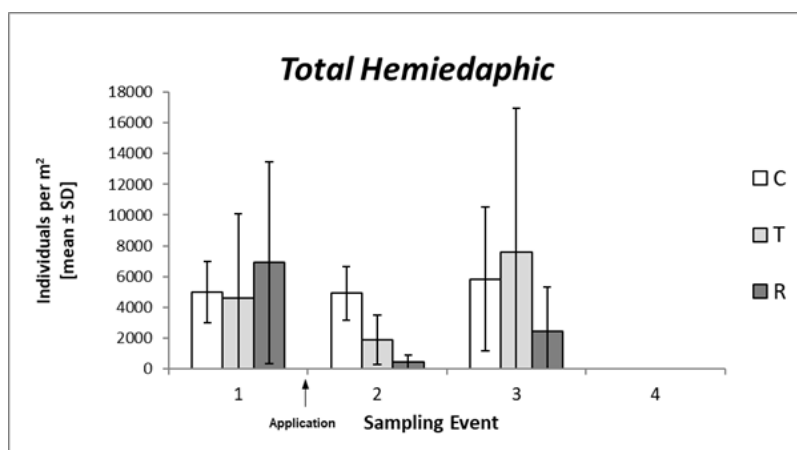
A significant effect of the reference item on the total number of Collembola was observed at the 2nd sampling demonstrating that the system is sensitive.



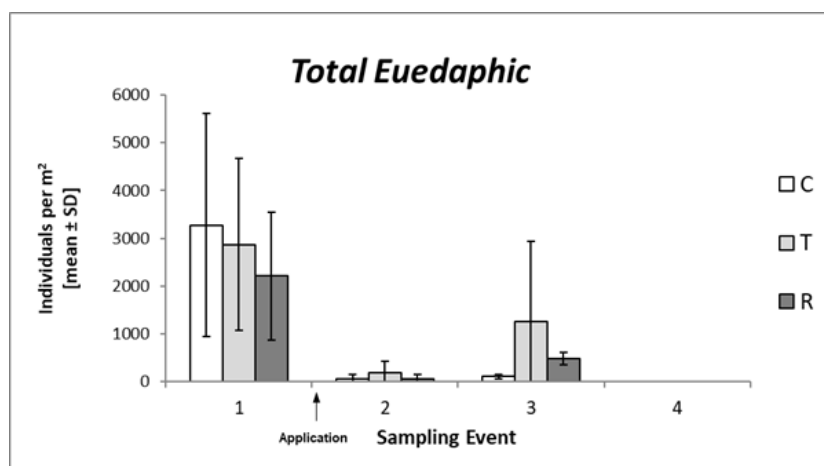
Epedaphic collembola species are living on the soil surface and need some vegetation or litter. This might explain, why species from this group were only found in low numbers at the presampling and the 2nd sampling (bare soil) and show a high abundance at the 3rd sampling event.



The hemiedaphic Collembola showed a similar pattern than the total collembolan: a clear reference effect, and also a transient test item effect at the 2nd sampling.



Euedaphic Collembola species living deeper in the soil. Here a reduction after the presampling was observed. This might be caused by the mechanical treatment before the application to adjust bare soil conditions and the drilling of the cover crop.



Conclusion

It can be concluded that the application of GLOB1913H tested at an application rate of 4.4 L/ha had no adverse effects on Collembola about one year after test item application.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted in line208 to OECD guideline 216 and according to the principles of GLP. Validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.3.2.2
Report	GLOB1913H: Effects on the activity of soil microflora in the laboratory (nitrogen transformation), Hammesfahr U., 2020, 155401080
Guideline(s):	Yes, OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation (mineralization) in a laboratory test over a period of 28 days of exposure. The test was performed in accordance with the OECD Guideline 216 (2000) by measuring the nitrogen turnover.

The test item had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rate) of soil microorganisms when applied at 6.0 mg and 59.8 mg test item/kg soil dry weight treatment.

Materials and Methods

Test Item:	GLOB1913H, Batch No. 200701/01
Test System:	Biologically active agricultural soil: Loamy sand
Test Design:	Determination of nitrogen-transformation in soil enriched with lucerne meal. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment. NH ₄ ⁺ , NO ₂ ⁻ and NO ₃ ⁻ -nitrogen formed in

	the nitrification process was determined by continuous flow analysis. Sampling scheme: 0, 7, 14 and 28 days after treatment
Test Rates:	Control 6.0 mg GLOB1913H/kg soil dry weight 59.8 mg GLOB1913H/kg soil dry weight
Endpoints:	Effects on NO ₃ -nitrogen production after 28 days exposure (soil nitrogen transformation).
Reference Item:	Effects of sodium chloride were determined at a rate of 16 g/kg dry soil in a separate study (ibacon study code: 116524080) within one year before start of the experimental phase of this study
Test Conditions:	Moisture: 44% to 46% of maximum water holding capacity (WHC _{max}). Temperature: 20°C ± 2°C, in the dark.
Statistics:	Calculation of mean values per treatment, standard deviation and coefficient of variation. Normality and homogeneity of variances were tested using the R/S-Test ($\alpha = 0.01$) and Levene's test ($\alpha = 0.01$), respectively and pair-wise comparisons of treated and control values according to Student t-test ($\alpha = 0.05$) were conducted.

Results and Discussion

Experimental dates: 03 August 2020 – 10 September 2020

All validity criteria were met. The variation between the replicate control samples did not exceed the validity criterion of 15% throughout the study.

No adverse effects of the test item on nitrate content in soil were observed at day 28. At day 28 differences to the control were 6.76% and 23.11% in the 6.0 mg and 59.8 mg test item/kg soil dry weight treatment, respectively.

The mineral nitrogen contents in soil were within the trigger range of $\pm 25\%$ set by EPPO and SETAC guidelines at day 28. At day 28 differences to the control were 6.51% and 22.41% in the 6.0 mg and 59.8 mg test item/kg soil dry weight treatment, respectively.

The incremental soil nitrate formation rates did not exceed the trigger range of $\pm 25\%$ set by OECD guideline 216 at the 14 - 28 day determination. Differences to the control were -3.18% and 2.00% in the 6.0 mg and 59.8 mg test item/kg soil dry weight treatment, respectively.

Effects of the test item on Nitrogen Transformation in a Loamy Sand Soil

Nitrogen Transformation - NO ₃ – Nitrogen (mg / kg soil dry weight) Mean Values						
	Control		6.0 mg GLOB1913H/kg soil dw		59.8 mg GLOB1913H/kg soil dw	
Sampling	Nitrate-N Content	Replicate Variation ¹	Nitrate-N Content	Deviation ²	Nitrate-N Content	Deviation ²
Day 0	14.850	0.66	14.899	0.33	14.913	0.42
Day 7	15.231	1.34	17.324*	13.74	19.892*	30.60
Day 14	25.122	1.03	28.015*	11.52	33.450*	33.15
Day 28	37.028	3.50	39.531*	6.76	45.586*	23.11
Nitrogen Transformation - Mineral Nitrogen ³ (mg / kg soil dry weight) Mean Values						
	Control		6.0 mg GLOB1913H/kg soil dw		59.8 mg GLOB1913H/kg soil dw	
Sampling	Mineral-N Content	Replicate Variation ¹	Mineral -N Content	Deviation ²	Mineral -N Content	Deviation ²
Day 0	22.558	1.77	23.176	2.74	23.262	3.12
Day 7	17.016	0.86	19.151*	12.55	21.849*	28.40
Day 14	26.268	0.91	29.202*	11.17	34.651*	31.91
Day 28	38.003	3.37	40.477*	6.51	46.521*	22.41
Nitrogen Transformation - NO ₃ – Nitrogen Formation Rate (mg / kg soil dry weight per day) ⁴						
	Control		6.0 mg GLOB1913H/kg soil dw		59.8 mg GLOB1913H/kg soil dw	
Interval ⁴	Nitrate-N Formation		Nitrate-N Formation	Deviation ²	Nitrate-N Formation	Deviation ²
Day 0 - 7	0.054		0.346*	540.74	0.711*	1216.67
Day 7 - 14	1.413		1.527*	8.07	1.937*	37.08
Day 14 - 28	0.850		0.823	-3.18	0.867	2.00

¹ = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value x 100)

² = % deviation to control

³ = mineral nitrogen = sum of nitrite- nitrate- and ammonium-nitrogen

⁴ = related to successive intervals between samplings

positive values = stimulatory effect; negative values = inhibitory effect

dw = dry weight

* statistically significantly different from control (Student t-test; $\alpha = 0.05$)

In a separate study the reference item sodium chloride had a retarding effect of more than $\pm 25\%$ compared to the control at days 28 and 98 after application. The results of the study proved sensitivity of the test system and provided assurance that the laboratory test conditions are adequate.

Conclusions

The test item had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rate) of soil microorganisms when applied at 6.0 mg and 59.8 mg test item/kg soil dry weight treatment.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

No new studies submitted.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study was conducted in line to OECD guideline 208 and according to the principles of GLP. All the validity criterion are met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference:	KCP 10.6
Report	GLOB1913H: Effects on terrestrial (non-target) plant: seedling emergence and seedling growth test, Bützler R., 2021a, 155401086
Guideline(s):	Yes, OECD 208 (2006)
Deviations:	For fresh weight, emergence, mortality, phytotoxicity and growth stages only 9 pots (18 seeds) were evaluated for the control group for <i>Solanum lycopersicum</i> instead of 10 pots with 2 seeds each because the soil of one pot silted up during the application procedure.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine a dose dependence of the test item on the seedling emergence and seedling growth of six non-target plant species representing five plant families. Parameters measured were plant fresh weight, phytotoxicity, emergence and mortality.

The most sensitive species in terms of fresh weight was *Lolium perenne* with an ER₅₀ value of 72.7 mL test item/ha (calculated on all replicates), followed by *Brassica napus*, *Solanum lycopersicum* and *Echinochloa crus-galli* with ER₅₀ values of 1127, 1325 and 960 mL test item/ha, respectively. *Daucus carota* showed a NOER of ≥ 1467 mL test item/ha and therefore an ER₅₀ value of > 1467 mL test item/ha and *Phaseolus vulgaris* showed a NOER of ≥ 4400 mL test item/ha and therefore an ER₅₀ value of > 4400 mL test item/ha.

Materials and Methods

Test Item:	GLOB1913H; batch no.: 200701/01, content: Prosulfocarb: 900 g/L (nominal), 886.1 g/L (analytical), according to certificate of analysis.
Test Species and Rates:	Six species, four dicotyledonous and two monocotyledonous species were tested, representing five plant families: <i>Brassica napus</i> , <i>Phaseolus vulgaris</i> , <i>Daucus carota</i> , <i>Solanum lycopersicum</i> , <i>Echinochloa crus-galli</i> and <i>Lolium perenne</i> . Based on a non GLP range finding test following rates beside a control with deionised water were tested:

Plant species and tested rates						
Species	Rate [mL test item/ha]					
	18.1	54.3	163	489	1467	4400
<i>Brassica napus</i>		x	x	x	x	x
<i>Phaseolus vulgaris</i>		x	x	x	x	x
<i>Daucus carota</i>	x	x	x	x	x	
<i>Solanum lycopersicum</i>		x	x	x	x	x
<i>Echinochloa crus-galli</i>	x	x	x	x	x	
<i>Lolium perenne</i>	x	x	x	x	x	

Test Design:	On the day after sowing different rates of the test item were sprayed in 200 L/ha of deionised water onto the soil. At least 20 seeds were tested per rate and species. The exposure time was 14 or 21 days after 50% emergence in the control depending on the growth of the seedlings. The concentration of the active ingredient in the stock solutions was verified analytically.
Endpoints:	ER ₁₀ , ER ₂₀ , ER ₅₀ and NOER and LOER based on plant fresh weight; ER ₁₀ , ER ₂₀ , ER ₅₀ based on phytotoxicity; Observation of emergence, mortality and phytotoxicity.
Test Conditions:	The study was performed in a growth chamber. For all plant species except <i>Daucus carota</i> the exposure conditions were as follows: Mean temperature was 19.4 °C (14.9 °C to 23.5 °C). Mean humidity was 68% (46% to 82%). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 233 µE/m ² /s (200 to 320 µE/m ² /s). For <i>Daucus carota</i> the exposure conditions were as follows: Mean temperature was 20.3 °C (15.0 °C to 24.7 °C). Mean humidity was 66% (53% to 78%). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 238 µE/m ² /s (210 to 280 µE/m ² /s).
Statistical Analysis:	Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). If the data were normally distributed and homogeneous and showed a monotonic dose response the Williams t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control. If the data were normally distributed and not homogeneous the Bonferroni-Welch t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used. If the data were not normally distributed and homogeneous the Bonferroni-Holm U-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used. In order to determine the ER ₁₀ , ER ₂₀ and ER ₅₀ values, a regression analysis was performed (Probit-analysis). In the case that no significant dose response relation on the mean values for each treatment group was found ($p(F) > 0.05$) the regression analysis was performed using all replicates for fitting. For the mortality and emergence data Fisher's Exact Binomial Test (with Bonferroni Correction, multiple comparison, one-sided greater, $\alpha = 0.05$) was used or in case that a linear trend was shown the Step-down Cochran-Armitage Test (one-sided greater, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH. In order to determine the ER ₁₀ , ER ₂₀ and ER ₅₀ values on phytotoxicity data, a regression analysis was performed (Probit-analysis or Weibull analysis). The software used to perform this statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Experimental dates (biological phase): 13 January 2020 – 6 April 2020

Experimental dates (analytical phase): 25 January 2021 – 7 April 2020

All validity criteria were met:

Emergence Rate of the Control Seeds:	89% -100%
Mean Survival of Emerged Control Seedlings:	100%
Growth and Morphology of the Control Seedlings:	The control seedlings exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species.

The most sensitive species in terms of fresh weight was *Lolium perenne* with an ER₅₀ value of 72.7 mL test item/ha (calculated on all replicates), followed by *Brassica napus*, *Solanum lycopersicum* and *Echinochloa crus-galli* with ER₅₀ values of 1127, 1325 and 960 mL test item/ha, respectively. *Daucus carota* showed a NOER of ≥ 1467 mL test item/ha and therefore an ER₅₀ value of > 1467 mL test item/ha and *Phaseolus vulgaris* showed a NOER of ≥ 4400 mL test item/ha and therefore an ER₅₀ value of > 4400 mL test item/ha.

The emergence rate was statistically significantly reduced for *Echinochloa crus-galli* at 1467 mL test item/ha (50%).

No statistically significant mortality was observed for any plant species tested.

Phytotoxic effects observed were deformation (all species), necrosis (all species except *Daucus carota*) and chlorosis (*Solanum lycopersicum* and *Lolium perenne*).

Summary of effect rates (based on fresh weight)

	NOER [mL test item/ha]	LOER [mL test item/ha]	Statistical Analysis	ER ₁₀ [mL test item/ha]	ER ₂₀ [mL test item/ha]	ER ₅₀ [mL test item/ha]	Statistical Analysis
<i>Brassica napus</i>	(489) 163 ^s	(1467) 489 ^s	¹	50.8 lower 95%-cl upper 95%-cl $r^2 = 0.814$	147 n.d. n.d.	1127 237 54582	⁴
<i>Phaseolus vulgaris</i>	≥ 4400	> 4400	²	n.d.	n.d.	> 4400 ^s	
<i>Daucus carota</i>	≥ 1467	> 1467	¹	n.d.	n.d.	> 1467 ^s	
<i>Solanum lycopersicum</i>	489	1467	¹	258 lower 95%-cl upper 95%-cl $r^2 = 0.957$	452 209 680	1325 934 1910	⁴
<i>Echinochloa crus-galli</i>	489	1467	³	281 lower 95%-cl upper 95%-cl $r^2 = 0.950$	428 213 607	960 698 1319	⁴
<i>Lolium perenne</i>	18.1	54.3	³	9.09* lower 95%-cl upper 95%-cl $r^2 = 0.649$	18.8* n.d. n.d.	75.0* n.d. n.d.	⁴
				7.82 [#] lower 95%-cl upper 95%-cl $r^2 = 0.506$	16.8 [#] 3.07 33.6	72.7 [#] 37.7 129	⁴

results represent rounded values based on exact data

n.d. not determined due to mathematical reasons

^s expert judgement

^s extrapolated to be higher than highest test rate due to the absence of effects ≥ 50%

* The ER_x-values are with reservation ($p(F) = 0.100 > \alpha = 0.05$)

[#] the ER_x-values are calculated on each replicate per rate

¹ multiple comparison Bonferroni-Welch t-test, $\alpha = 0.05$

² multiple comparison Bonferroni-Holm U-test, $\alpha = 0.05$

³ multiple comparison Williams t-test, $\alpha = 0.05$

⁴ Probit Analysis, cl = confidence limits

The values for the phytotoxicity data used for the ER_x calculation are a rating system and are not measured. The most sensitive species in terms of phytotoxicity was *Echinochloa crus-galli* with an ER₅₀ value of 639 mL test item/ha followed by *Lolium perenne* with an ER₅₀ value of 1562 mL test item/ha. They were followed by *Brassica napus* and *Solanum lycopersicum* with ER₅₀ values of 2116 and 2525 mL test item/ha, respectively. For *Daucus carota* the ER₅₀ value is estimated to be > 1467 mL test item/ha and for *Phaseolus vulgaris* the ER₅₀ value is estimated to be > 4400 mL test item/ha, the highest rates tested.

Summary of effect rates (based on phytotoxicity)					
		ER ₁₀	ER ₂₀	ER ₅₀	Statistical Analysis
		[mL test item/ha]			
<i>Brassica napus</i>		448	832	2116	¹
	lower 95%-cl	24.1	119	969	
	upper 95%-cl	976	1543	4237	
<i>Phaseolus vulgaris</i>		1253	> 4400 [#]	> 4400 [#]	²
	lower 95%-cl	n.d.	n.d.		
	upper 95%-cl	n.d.	n.d.		
<i>Daucus carota</i>		n.d.	n.d.	> 1467 [#]	
<i>Solanum lycopersicum</i>		576	957	2525	²
	lower 95%-cl	90.8	288	1379	
	upper 95%-cl	1092	1750	8221	
<i>Echinochloa crus-galli</i>		262	374	639	¹
	lower 95%-cl	0.11	2.31	132	
	upper 95%-cl	507	685	1950	
<i>Lolium perenne</i>		206	413	1562	²
	lower 95%-cl	n.d.	n.d.	n.d.	
	upper 95%-cl	n.d.	n.d.	n.d.	

results represent rounded values based on exact data

n.d. not determined due to mathematical reasons

[#] extrapolated to be higher than highest test rate due to the absence of effects $\geq 50\%$

¹ Weibull Analysis, cl = confidence limits

² Probit Analysis, cl = confidence limits

The analytical recovery rate of the active ingredient Prosulfocarb in the stock solution for application 1 (22.4 g test item/L) was 107%, and for application 2 and 3 (7.61 g test item/L) 101% and 103%, respectively of the nominal value.

Conclusion

GLOB1913H was tested for effects on seedling emergence and seedling growth of six plant species out of five different plant families.

The most sensitive species in terms of fresh weight was *Lolium perenne* with an ER₅₀ value of 72.7 mL test item/ha (calculated on all replicates), followed by *Brassica napus*, *Solanum lycopersicum* and *Echinochloa crus-galli* with ER₅₀ values of 1127, 1325 and 960 mL test item/ha, respectively. *Daucus carota* showed a NOER of ≥ 1467 mL test item/ha and therefore an ER₅₀ value of > 1467 mL test item/ha and *Phaseolus vulgaris* showed a NOER of ≥ 4400 mL test item/ha and therefore an ER₅₀ value of > 4400 mL test item/ha.

The emergence rate was statistically significantly reduced for *Echinochloa crus-galli* at 1467 mL test item/ha (50%). No statistically significant mortality was observed for any plant species tested. Phytotoxic effects observed were deformation (all species), necrosis (all species except *Daucus carota*) and chlorosis (*Solanum lycopersicum* and *Lolium perenne*).

Comments of zRMS:	The study was conducted in line to OECD guideline 227 and according to the principles of GLP. All the validity criterion are met. The study is considered to be reliable and suitable for the risk assessment.-
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Reference: KCP 10.6

Report GLOB1913H: Effects on terrestrial (non-target) plants: vegetative vigour test, Bützler R., 2021b, 155401087

Guideline(s): Yes, OECD 227 (2006)

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

The purpose of this study was to determine a dose dependence of the test item on the vegetative vigour of six non-target plant species representing five plant families. Parameters measured were plant fresh weight, phytotoxicity and mortality.

The most sensitive species in terms of fresh weight was *Lolium perenne* with an ER₅₀ value of 3749 mL test item/ha. For all other plant species the ER₅₀ value is estimated to be > 4400 mL test item/ha, the highest rate tested. No mortality was observed for any species tested. Phytotoxic effects observed were chlorosis (all species except *Phaseolus vulgaris*), necrosis (all species) and deformation (all species). Beside chlorosis *Lolium perenne* showed additional discoloration.

Materials and Methods

Test Item: GLOB1913H; batch no.: 200701/01, content: Prosulfocarb: 900 g/L (nominal), 886.1 g/L (analytical), according to certificate of analysis.

Test Species and Rates: Six species, four dicotyledonous and two monocotyledonous species were tested, representing five plant families: *Brassica napus*, *Phaseolus vulgaris*, *Daucus carota*, *Amaranthus retroflexus*, *Avena sativa* and *Lolium perenne*. Based on a non GLP range finding test following rates beside a control with deionised water were tested:

Plant species and tested rates

Species	Rate [mL test item/ha ⁸]				
	157	362	832	1913	4400
<i>Brassica napus</i>	x	x	x	x	x
<i>Phaseolus vulgaris</i>	x	x	x	x	x
<i>Daucus carota</i>	x	x	x	x	x
<i>Avena sativa</i>	x	x	x	x	x
<i>Lolium perenne</i>	x	x	x	x	x

Species	Rate [mL test item/ha ¹]				
	54.3	163	489	1467	4400
<i>Amaranthus retroflexus</i>	x	x	x	x	x

Test Design: The plants were grown until they had reached the 2 to 4 true leaf stage prior to dosing. Test rates were calculated for a water amount of 200 L/ha and were administered onto the plants using laboratory spraying equipment. At least 20 plants were tested per rate and species. The concentration of the active ingredient in the stock solution was verified analytically. The exposure time was 21 days.

Endpoints: ER₁₀, ER₂₀, ER₅₀ and NOER and LOER based on plant fresh weight. Observation of mortality.

Test Conditions: The study was performed in a growth chamber. Pre-application conditions were as follows:
Mean temperature was 20.6 °C (15.0 °C to 24.2 °C). Mean humidity was 65% (52% to 89%). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 265 µE/m²/s (200 to 430 µE/m²/s).
Exposure conditions were as follows:
Mean temperature was 20.7°C (15.0°C to 24.1°C). Mean humidity was 66% (54% to 83%). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 293 µE/m²/s (200 to 410 µE/m²/s).

⁸ The calculation of the amount of test item was done with the relative density value of 1.02 according to the MSDS

Statistical Analysis: Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). If the data were normally distributed and homogeneous the Dunnett's t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) or if the data showed a monotonic dose response the Williams t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control. In order to determine the ER₁₀, ER₂₀ and ER₅₀ values, a regression analysis was performed (Probit-analysis). In the case that no significant dose response relation on the mean values for each treatment group was found ($p(F) > 0.05$) the regression analysis was performed using all replicates for fitting. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

Experimental dates (biological phase): 02 December 2020 – 23 December 2020

Experimental dates (analytical phase): 25 January 2021 – 25 January 2020

All validity criteria were met:

- Seedling emergence in the untreated control pots is at least 70%, except for *Amaranthus retroflexus*.
- Untreated control seedlings must not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation) and plants must exhibit only normal variation in growth and morphology for that particular species.
- The mean survival of emerged untreated control seedlings is at least 90% for the duration of the study.

The most sensitive species in terms of fresh weight was *Lolium perenne* with an ER₅₀ value of 3749 mL test item/ha. For all other plant species the ER₅₀ value is estimated to be > 4400 mL test item/ha, the highest rate tested. Looking at the ER₂₀ values for these plant species the most sensitive species was *Amaranthus retroflexus* with an ER₂₀ value of 982 mL test item/ha (calculated per replicate), followed by *Avena sativa* with an ER₂₀ value of 3073 mL test item/ha (calculated per replicate) and *Phaseolus vulgaris* with an ER₂₀ value of 3901 mL test item/ha. The least sensitive species were *Brassica napus* and *Daucus carota* for which the fresh weight was not statistically significantly reduced up to and including 4400 mL test item/ha. Thus these species showed a NOER of ≥ 4400 mL test item/ha.

No mortality was observed for any species tested.

Phytotoxic effects observed were chlorosis (all species except *Phaseolus vulgaris*), necrosis (all species) and deformation (all species). Beside chlorosis *Lolium perenne* showed additional discoloration.

Summary of effect rates (based on fresh weight)

	NOER [mL test item/ha]	LOER [mL test item/ha]	Statistical Analysis	ER ₁₀ [mL test item/ha]	ER ₂₀ [mL test item/ha]	ER ₅₀ [mL test item/ha]	Statistical Analysis
<i>Brassica napus</i>	≥ 4400	> 4400	¹	n.d.	n.d.	n.d.	
<i>Phaseolus vulgaris</i>	832	1913	¹	1813 lower 95%-cl 1132 upper 95%-cl 2335 $r^2 = 0.953$	3901 3120 5330	> 4400 [#]	³
<i>Daucus carota</i>	≥ 4400	> 4400	²	n.d.	n.d.	n.d.	
<i>Amaranthus retroflexus</i>	489	1467	¹	613* lower 95%-cl n.d. upper 95%-cl n.d. $r^2 = 0.677$	1465* n.d. n.d.	> 4400 [#]	³
				347 [§] lower 95%-cl 69.9 upper 95%-cl 674 $r^2 = 0.369$	982 [§] 428 1559	> 4400 [#]	³
<i>Avena sativa</i>	1913	4400	¹	1112* lower 95%-cl n.d. upper 95%-cl n.d. $r^2 = 0.624$	2606* n.d. n.d.	> 4400 [#]	³
				2361 [§] lower 95%-cl 1685 upper 95%-cl 2784 $r^2 = 0.584$	3073 [§] 2523 3413	> 4400 [#]	³
<i>Lolium perenne</i>	832	1913	¹	961 lower 95%-cl 268 upper 95%-cl 1495 $r^2 = 0.923$	1534 692 2121	3749 2811 6253	³

results represent rounded values based on exact data

n.d. not determined due to mathematical reasons

¹ multiple comparison Williams t-test, $\alpha = 0.05$

² multiple comparison Dunnett's t-test, $\alpha = 0.05$

³ Probit Analysis, cl = confidence limits

[#] extrapolated to be higher than highest test rate due to the absence of effects $\geq 50\%$

* The ER_x-values are with reservation ($p(F) > \alpha = 0.05$; $p(F) = 0.087$ for *Amaranthus retroflexus* and 0.112 for *Avena sativa*).

[§] The ER_x-values are calculated on each replicate per rate.

The analytical recovery rate of the active ingredient Prosulfocarb in the stock solution was 102% of the nominal value. In the control solutions no active ingredient was detected.

Conclusion

GLOB1913H was tested for effects on the vegetative vigour using six plant species out of five different plant families.

The most sensitive species in terms of fresh weight was *Lolium perenne* with an ER₅₀ value of 3749 mL test item/ha. For all other plant species the ER₅₀ value is estimated to be > 4400 mL test item/ha, the highest rate tested. No mortality was observed for any species tested. Phytotoxic effects observed were chlorosis (all species except *Phaseolus vulgaris*), necrosis (all species) and deformation (all species). Beside chlorosis *Lolium perenne* showed additional discoloration.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No new studies submitted.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

Not required.

A 2.8 KCP 10.8 Monitoring data

No new studies submitted.