

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB1912H

Product name: **Jura Max**

Chemical active substances:

Prosulfocarb, 667 g/L

Diflufenican, 14 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Update July 2022

Applicant: Globachem NV

Submission date: November 2021

Evaluation date: August 2022

MS Finalisation date: ---

Version history

When	What
November 2021	Initial dossier submission by applicant for new product authorization.
July 2022	Dossier update by applicant after request for additional information
August 2022	zRMS finalized dRR evaluation

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9 Ecotoxicology (KCP 10)

Review Comments:

This document describes the acceptable use conditions required for registration of GLOB1912H, a suspension concentrate containing 667 g/L prosulfocarb and 14 g/L diflufenican for use as a herbicide in winter cereals, potato and sunflower.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

It should be highlighted that no tests were performed on GLOB1912H in the interest of animal welfare. The evaluation was performed based on tests with similar formulation GLOB1817H, which has the prosulfocarb and diflufenican at the same amount. All co-formulants are the same and are not relevant. The difference is from an additional active substance and a safener in very small amount. Thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey.

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 destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ syner-gist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic	Bees	Non-target	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, CZ, DE, BE, HU	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI), Winter durum wheat (TRZDW), Spelt (TRZSP)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre-emergence (BBCH 0-09)	a) 1 b) 1	/	a) 3.2 b) 3.2	a) Prosulfocarb: 2.134 Diflufenican: 0.0448 b) Prosulfocarb: 2.134 Diflufenican: 0.0448	160-300	/	/							
2	PL, CZ, DE, BE, HU	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI), Winter durum wheat (TRZDW), Spelt (TRZSP)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre-emergence (BBCH 0-09)	a) 1 b) 1	/	a) 3.0 b) 3.0	a) Prosulfocarb: 2.001 Diflufenican: 0.042 b) Prosulfocarb: 2.001 Diflufenican: 0.042	160-300	/	/							
3	PL, CZ, DE, BE, HU	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI), Winter durum wheat (TRZDW), Spelt (TRZSP)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	BBCH10-13	a) 1 b) 1	/	a) 3.2 b) 3.2	a) Prosulfocarb: 2.134 Diflufenican: 0.0448 b) Prosulfocarb: 2.134 Diflufenican: 0.0448	160-300	/	/							
4	PL, CZ, DE, BE, HU	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	BBCH10-13	a) 1 b) 1	/	a) 3.0 b) 3.0	a) Prosulfocarb: 2.001 Diflufenican: 0.042 b) Prosulfocarb: 2.001	160-300	/	/							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		(SECCW), Triticale (TTLWI), Winter durum wheat (TRZDW), Spelt (TRZSP)								Diflufenican: 0.042										
5	PL, CZ, DE, BE, HU	Potato (SOLTU)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre- emergence (BBCH 0- 09)	a) 1 b) 1	/	a) 3.2 b) 3.2	a)Prosulfocarb: 2.134 Diflufenican: 0.0448 b)Prosulfocarb: 2.134 Diflufenican: 0.0448	160-300	/	/							
6	PL, CZ, DE, BE, HU	Potato (SOLTU)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre- emergence (BBCH 0- 09)	a) 1 b) 1	/	a) 3.0 b) 3.0	a)Prosulfocarb: 2.001 Diflufenican: 0.042 b)Prosulfocarb: 2.001 Diflufenican: 0.042	160-300	/	/							
7	PL, CZ, DE, HU	Sunflower (HELAN)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre- emergence (BBCH 0- 09)	a) 1 b) 1	/	a) 3.2 b) 3.2	a) Prosulfocarb: 2.134 Diflufenican: 0.0448 b)Prosulfocarb: 2.134 Diflufenican: 0.0448	160-300	/	/							
8	PL, CZ, DE, HU	Sunflower (HELAN)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	Pre- emergence (BBCH 0- 09)	a) 1 b) 1	/	a) 3.0 b) 3.0	a) Prosulfocarb: 2.001 Diflufenican: 0.042 b)Prosulfocarb: 2.001 Diflufenican: 0.042	160-300	/	/							

* 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:

- (1) Numeration necessary to allow references
- (2) Use official codes/nomenclatures of EU
- (3) 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)
- (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
- (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (*e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- (6) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
- (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (8) The maximum number of application possible under practical conditions of use must be provided
- (9) Minimum interval (in days) between applications of the same product.
- (10) For specific uses other specifications might be possible, *e.g.*: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
- (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- (12) If water volume range depends on application equipments (*e.g.* ULVA or LVA) it should be mentioned under “application: method/kind”.
- (13) PHI - minimum pre-harvest interval
- (14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

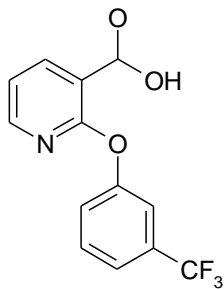
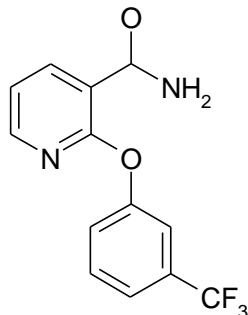
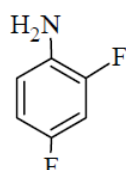
9.1.1.1

9.1.1.2 Review Comments:

The prosulfocarb sulfoxide is a metabolite of prosulfocarb forming in soil but not in water or sediment. According to information included in the DAR B9 2005, no degradation products were detected in either hydrolysis or photolysis studies conducted in water. No metabolite of prosulfocarb reached significant levels in the water/sediment study (<0.8%) at any time. Thus, for surface water the risk assessment for prosulfocarb sulfoxide is not required.

In the DAR for prosulfocarb sulfoxide the PEC_{soil} were not calculated. Taking to consideration that it is soil metabolite, and a high toxicity of prosulfocarb to soil organism, the prosulfocarb sulfoxide was included in the risk assessment of GLOB1912H.

Table 9.1-5 Metabolites of diflufenican

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE B107137		283	Soil: 16.8% Water: 32.6% Sediment: 13.3%	Yes, soil and aquatic organisms
AE 0542291		282	Soil: 26.3%	Yes, soil and aquatic organisms
AE C522392		129	Soil: 10.7% Water: 6.1% Sediment: 1%	No

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

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

GLOB1912H 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 GLOB1912H taking into account the mitigation measures where necessary.

Based on the mixture toxicity assessment, it can be concluded that the mitigation measures based on the risk assessment of the individual active substances will be sufficient to protect aquatic organisms.

The D2 ditch and D6 ditch scenarios are not relevant for Central Zone, thus were not taken to consideration in overall conclusions.

GLOB1912H applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures (10 m no spray buffer zone including a 10 m vegetated buffer strip).

9.1.1.5

9.1.1.6 Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing.

For active substances and relevant metabolites PEC_{sw} calculations were performed with FOCUS STEPS 1-2 (active substances and metabolites) and FOCUS STEP 3 - 4 (prosulfocarb and diflufenican). Additionally for diflufenican FOCUS profiles of scenarios with a maximum PEC_{sw} above 0.1 µg/L (but below 0.42 µg/L) were analysed using EPAT v1.2. Acceptability of this approach should be consider at MSs level. For Poland EPAT is not accepted.

For both active substances the R scenarios require the widest zones to confirm the safe use of GLOB1912H:

- prosulfocarb (RAC of 7.5 µg a.s./L) - 10 m no spray buffer zone including a 10 m vegetated buffer strip
- diflufenican (RAC of 0.045 µg a.s./L) - 20 m no spray buffer zone including a 20 m vegetated buffer strip; only for R4 scenario, use in sunflower, PEC/RAC is 1.0449
- diflufenican (RAC of 0.1 µg a.s./L) - 10 m no spray buffer zone including a 10 m vegetated buffer strip

Based on the mixture toxicity assessment, it can be concluded that the mitigation measures based on the risk assessment of the individual active substances will be sufficient to protect aquatic organisms.

The D2 ditch and D6 ditch scenarios are not relevant for Central Zone, thus were not taken to consideration in overall conclusions.

GLOB1912H applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures (10 m no spray buffer zone including a 10 m vegetated buffer strip).

9.1.1.7 Effects on bees (KCP 10.3.1)

A low risk to bees is expected from the application of GLOB1912H.

9.1.1.8 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field risks of GLOB1912H for arthropods other than bees are acceptable for the intended use.

According to the ESCORT 2 when the acceptable in-field risk cannot be confirmed based on extended laboratory studies, than age residue test for the most sensitive species is require. This study has not been presented.

In zRMS opinion, based on WoE approach, the acceptable in-field risk can be concluded. As it is not standard assessment, the acceptability of this statement should be taken at MSs level.
For Poland risk is acceptable.

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

GLOB1912H poses low risk to earthworms and other non-target soil organisms when applied according to the proposed use rate.

For dose rate of 3.2 L/ha, the TER values for earthworms and collembola due to exposure to prosulfocarb and formulation were below the trigger of 5. An acceptable risk for earthworms can be concluded based on field study with prosulfocarb 800 EC. For collembola exposed to prosulfocarb (endpoint derived from product study - GLOB1817H), earthworms and collembola exposed to formulation, the risk is unresolve based on standard assessment. In zRMS opinion, the acceptable risk for soil organisms can be concluded based on WoE approach.

As it is not standard assessment, the acceptability of this statement should be taken at MSs level.
For Poland risk is acceptable.

There is no unacceptable risk on soil microbial activity for GLOB1912H.

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

GLOB1912H 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, ~~a buffer zone of 3 m in combination with 75% drift reducing techniques~~, a buffer zone of 5 m in combination with 50% drift reducing techniques or a buffer zone of 10 m without drift reduction.

9.1.1.11 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 9.1-2: Critical use pattern of GLOB1912H 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	1, 2	Application timing	Pre-emergence
2	3, 4		Post-emergence
3	5, 6		Pre-emergence
4	7, 8		Pre-emergence

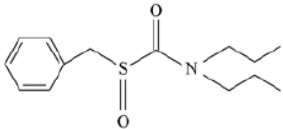
Table 9.1-3: Critical use pattern of GLOB1912H grouped according to dose rate

Grouping according to dose rate			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
5	1, 3, 5, 7	Dose rate	3.2 L/ha
6	2, 4, 6, 8		3.0 L/ha

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of GLOB1912H is indicated in the table.

Table 9.1-4 Metabolites of prosulfocarb

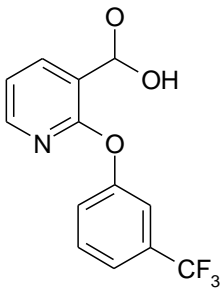
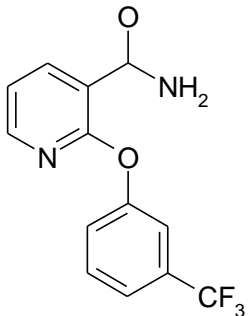
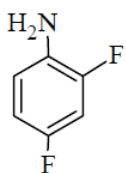
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Prosulfocarb sulfoxide		267.4	Soil: 6.8%	Yes, soil and aquatic organisms

Review Comments:

The prosulfocarb sulfoxide is a metabolite of prosulfocarb forming in soil but not in water or sediment. According to information included in the DAR B9 2005, no degradation products were detected in either hydrolysis or photolysis studies conducted in water. No metabolite of prosulfocarb reached significant levels in the water/sediment study (<0.8%) at any time. Thus, for surface water the risk assessment for prosulfocarb sulfoxide is not required.

In the DAR for prosulfocarb sulfoxide the PEC_{soil} were not calculated. Taking to consideration that it is soil metabolite, and a high toxicity of prosulfocarb to soil organism, the prosulfocarb sulfoxide was included in the risk assessment of GLOB1912H.

Table 9.1-5 Metabolites of diflufenican

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE B107137		283	Soil: 16.8% Water: 32.6% Sediment: 13.3%	Yes, soil and aquatic organisms
AE 0542291		282	Soil: 26.3%	Yes, soil and aquatic organisms
AE C522392		129	Soil: 10.7% Water: 6.1% Sediment: 1%	No

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prosulfocarb, diflufenican and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on birds of GLOB1912H were not evaluated as part of the EU assessment of prosulfocarb or diflufenican.

However, the provision of further data on the GLOB1912H is not considered essential, because the risk for birds from GLOB1912H 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
Bobwhite quail, <i>Colinus virginianus</i>	Diflufenican	Oral 1 d Acute	LD ₅₀ > 2150 mg/kg bw	EFSA, 2007
Bobwhite quail, <i>Colinus virginianus</i>	Diflufenican	Dietary Reproductive toxicity	NOEL = 91.84 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 acute risk assessment of diflufenican will be performed using the LD₅₀ of the acute toxicity study. All reproductive risk assessments will be performed using the NOEL from the reproduction studies 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 1 also covers the risk for birds from all other intended uses in groups 3 and 4 (see 9.1.2). The risk assessment was conducted at the highest application rate (use group 5) covering the intended uses in use group 6 (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 GLOB1912H in winter cereals, potato and sunflower

Intended use		Bare soil				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 2.134				
Acute toxicity (mg/kg bw)		1505.6				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Bare soil	Small granivorous bird	25.3	1	54.0	27.9	
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	12.9	117	
Active substance/product		Diflufenican				
Application rate (kg/ha)		1 × 0.0448				
Acute toxicity (mg/kg bw)		2150				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Bare soil	Small granivorous bird	25.3	1	1.13	1897	
Reprod. toxicity (mg/kg bw/d)		91.84				
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	0.271	339	

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 GLOB1912H in winter cereals

Intended use		Winter cereals				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 2.134				
Acute toxicity (mg/kg bw)		1505.6				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Cereals	Small omnivorous bird	158.8	1	338.9	4.44	
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	30.5	1	65.1	23.13	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24.0	1	51.2	29.40	
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	73.3	1.79	
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	18.3	7.15	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	12.3	10.63	
Active substance/product		Diflufenican				
Application rate (kg/ha)		1 × 0.0448				
Acute toxicity (mg/kg bw)		2150				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Cereals	Small omnivorous bird	158.8	1	7.1	302	
Reprod. toxicity (mg/kg bw/d)		91.84				
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	1.54	59.7	
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	0.385	239	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.259	354.9	

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.

Since GLOB1912H contains 2 active ingredients, a combined risk assessment was performed. According to Appendix B of the Guidance Document on the Risk Assessment for birds and mammals, the basic concept of the risk assessment is that animals are exposed to residues of the active substances in the environment. Thus the assessment of GLOB1912H is not an assessment of the formulation as such, but an assessment of the effects of an exposure to a mixture of active substances in the environment, resulting from the use of the formulation. Toxicity studies for birds with formulated products are typically not available.

For the assessment of acute effects, a surrogate LD₅₀ is calculated. A model often used to estimate the toxicity mixtures is the assumption of dose/concentration additivity of toxicity (Finney approach of concentration additivity of toxicity (Finney, D.J., 1948 and 1971).

The following formula is used to derive a surrogate LD₅₀ for the mixture of active substances with known toxicity assuming additivity:

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

$X(a.s._i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

$LD_{50}(a.s._i)$ = acute toxicity value for active substance [i]

For GLOB1912H, the LD₅₀ (mix) amounts to 1514.68 mg/kg bw (=1/[(0.98/1505.6)+(0.02/2150)]).

Using the same approach, also a NOEL (mix) was calculated which amounts to 129.9 mg/kg bw/d (=1/[(0.98/131)+(0.02/91.84)]).

Intended use		Bare soil				
Active substance/product		Mixture of active substances				
Application rate (kg/ha)						
Acute toxicity (mg/kg bw)		1 × 2.1788				
TER criterion		LD50 (mix) = 1514.68				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Bare soil	Small granivorous bird	25.3	1	29.2	51.9	
Reprod. toxicity (mg/kg bw/d)		129.9				
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	13.16	9.87	
Reprod. toxicity (mg/kg bw/d)		-*				
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	-	-	-	87	

Intended use		Winter cereals				
Active substance/product		Mixture of active substances				
Application rate (kg/ha)						
Acute toxicity (mg/kg bw)		LD ₅₀ (mix) = 1514.68				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Cereals	Small omnivorous bird	158.8	1	346	4.38	
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	30.5	1	66.5	22.8	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24.0	1	52.3	29.0	
Reprod. toxicity (mg/kg bw/d)		129.9				
TER criterion						
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	74.8	1.74	
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	18.2	6.94	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	12.6	10.32	
Reprod. toxicity (mg/kg bw/d)		-*				
TER criterion						
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	-	-	-	1.74	
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	-	-	-	6.94	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	-	-	-	10.32	

*In addition, a combined risk assessment for sublethal effects was performed as well using the following equation and assuming a direct proportionality of the TER to the NOEL:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

TER_(a.s.) = calculated TER for the active substance i

9.2.2.2 Higher-tier risk assessment

Not required.

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 GLOB1812H 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) =	2134		
Acute toxicity (mg/kg bw) =	1505.6	quotient =	1.42
Reprod. toxicity (mg/kg bw/d) =	131	quotient =	16.3

With a $K(f)_{oc}$ of 3091 L/kg, diflufenican belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	44.8		
Acute toxicity (mg/kg bw) =	2150	quotient =	0.021
Reprod. toxicity (mg/kg bw/d) =	91.84	quotient =	0.49

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.

The log P_{ow} of diflufenican amounts to 4.2 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 9.2-4: 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, potatoes and sunflower

Parameter	Prosulfocarb	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	1.7118	dRR B8 Table 8.7-3
log P_{ow} / P_{ow}	4.48/30199	
K _{oc}	1799	Geomean (n = 6)

Parameter	Prosulfocarb	comments
foc	0.02	Default
BCF _{worm}	10.10	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	17.28	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	18.14	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	131	
TER _{lt}	7.22	

TER values shown in bold fall below the relevant trigger.

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

Parameter	Diflufenican	comments
PEC _{soil;accu} (twa = 21 d) (mg/kg soil)	0.1493	dRR B8 Table 8.7-6
log P _{ow} / P _{ow}	4.2/15849	
Koc	3091	Geomean (n = 10)
foc	0.02	Default
BCF _{worm}	3.09	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.461	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.484	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	91.84	
TER _{lt}	190	

TER values shown in bold fall below the relevant trigger.

Since GLOB1912H contains 2 active ingredients, a risk assessment for the mixture of active substances was performed using the ~~the~~ NOEL (mix) of 129.9 mg/kg bw/d.

Table 9.2-6: Assessment of the risk for earthworm-eating birds due to exposure to the mixture via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	18.624	Sum of DDD in Table 9.2-4 to 9.2-5
NOEL (mg/kg bw/d)	129.9	NOEL (mix)
TER _{lt}	6.97	

TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for the mixture was performed as well using the following equation:

$$TER_{(mix)} = \left(\sum_i \frac{1}{TER_{(a.s._i)}} \right)^{-1}$$

where:

$TER_{(a.s._i)}$ = calculated TER for the active substance i

$TER_{mix} = 6.73$.

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, potatoes and sunflower

Parameter	Prosulfocarb	comments
PEC_{sw} (tw = 21 d) (mg/L)	0.009015	dRR B8 Table 8.9-5 (FOCUS Step 3; D1 ditch)
BCF_{fish}	700	
BMF	-	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	6.3105	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.0034	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	131	
TER_{lt}	130.6	

TER values shown in bold fall below the relevant trigger.

Table 9.2-8: Assessment of the risk for fish-eating birds due to exposure to diflufenican via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Diflufenican	comments
PEC_{sw} (tw = 21 d) (mg/L)	0.0001619	dRR B8 Table 8.9-28 (FOCUS Step 3; D1 ditch)
BCF_{fish}	1596	
BMF	-	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.2584	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0411	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	91.84	
TER_{lt}	2235	

TER values shown in bold fall below the relevant trigger.

Since GLOB1912H contains 2 active ingredients, a risk assessment for the mixture of active substances was performed using the NOEL (mix) of 129.9 mg/kg bw/d.

Table 9.2-9: Assessment of the risk for fish-eating birds due to exposure to the mixture via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	1.0445	Sum of DDD in Table 9.2-7 to 9.2-8
NOEL (mg/kg bw/d)	129.9	NOEL (mix)
TER _{It}	124.4	

TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for the mixture was performed as well using the following equation:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

TER_(a.s.i) = calculated TER for the active substance i

$$TER_{mix} = 123.4$$

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 when applying GLOB1912H according to the intended uses.

Review Comments:

The acute and chronic risks of GLOB1912H to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with active ingredients, mixture of active substances and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that GLOB1912H does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low (both active substances).

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

Effects on mammals of GLOB1912H were not evaluated as part of the EU assessment of prosulfocarb and diflufenican.

However, the provision of further data on the formulation GLOB1912H is not considered essential, because the risk for mammals from GLOB1912H 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
Rat	Diflufenican	Oral 1 d Acute	LD₅₀ > 5000 mg/kg bw	EFSA, 2007
Rat	Diflufenican	Dietary Reproductive toxicity	NOAEL = 35.5 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).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for birds from all other intended uses in groups 3 and 4 (see 9.1.2). The risk assessment was conducted at the highest application rate (use group 5) covering the intended uses in use group 6 (see 9.1.2).

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the pre-emergence use of GLOB1912H in winter cereals, potato and sunflower

Intended use		Bare soil				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 2.134				
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	30.7	59.2
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	51.2 7.46	6.7
Active substance/product		Diflufenican				
Application rate (kg/ha)		1 × 0.0448				
Acute toxicity (mg/kg bw)		> 5000				
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	0.645	7751
Reprod. toxicity (mg/kg bw/d)		35.5				
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	0.157	227

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 post-emergence use of GLOB1912H in winter cereals

Intended use		Winter cereals				
Active substance/product		Prosulfocarb				
Application rate (kg/ha)		1 × 2.134				
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						
Cereals	Small herbivorous mammal	118.4	1	237 252.7	7.68 7.20	
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	7.6	1	15.2 16.22	120 112.2	

Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	42.1	1	84.2	89.84	21.6	20.3
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1	34.4	36.70	52.9	49.6
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 _{it}	
Growth stage							
Cereals	Small herbivorous mammal	48.3	0.53	51.2	54.63	0.98	0.91
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	4.2	0.53	4.45	4.75	11.2	10.53
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	22.3	0.53	23.6	25.22	2.12	1.98
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	0.53	8.27	8.82	6.05	5.67
Active substance/product		Diflufenican					
Application rate (kg/ha)		1 × 0.0448					
Acute toxicity (mg/kg bw)		> 5000					
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	5.30		942	
Reprod. toxicity (mg/kg bw/d)		35.5					
TER criterion		5					
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)		TER _{it}	
Growth stage							
Cereals	Small herbivorous mammal	48.3	0.53	1.15		31.0	
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	4.2	0.53	0.100		356	
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	22.3	0.53	0.529		67.1	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	0.53	0.185		192	

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.

Since GLOB1912H contains 2 active ingredients, a combined risk assessment was performed. According to Appendix B of the Guidance Document on the Risk Assessment for birds and mammals, the basic concept of the risk assessment is that animals are exposed to residues of the active substances in the environment. Thus the assessment of GLOB1912H is not an assessment of the formulation as such, but an assessment of the effects of an exposure to a mixture of active substances in the environment, resulting from the use of the formulation.

For the assessment of acute effects, a surrogate LD₅₀ is calculated. A model often used to estimate the toxicity mixtures is the assumption of dose/concentration additivity of toxicity (Finney approach of concentration additivity of toxicity (Finney, D.J., 1948 and 1971).

The following formula is used to derive a surrogate LD₅₀ for the mixture of active substances with known toxicity assuming additivity:

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

$X(a.s._i)$ = fraction of active substance $[i]$ in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

$LD_{50}(a.s._i)$ = acute toxicity value for active substance $[i]$

For GLOB1912H, the $LD_{50}(\text{mix})$ amounts to 1843.4 mg/kg bw ($=1/[(0.98/1820)+(0.02/5000)]$).

Using the same approach, also a NOEL (mix) was calculated which amounts to 49.6 mg/kg bw/d ($=1/[(0.98/50)+(0.02/35.5)]$).

Intended use		Bare soil				
Active substance/product		Mixture of active substances				
Application rate (kg/ha)						
Acute toxicity (mg/kg bw)		LD50 (mix) = 1843.4				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species		SV90	MAF90	DDD90 (mg/kg bw/d)	TERa
Bare soil	Small granivorous mammal		14.4	1	31.4	58.8
Reprod. toxicity (mg/kg bw/d)		49.6				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species		SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Bare soil	Small granivorous mammal		6.6	0.53	7.62	6.5
Reprod. toxicity (mg/kg bw/d)		-*				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species		SVm	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Bare soil	Small granivorous mammal		-	-	-	6.5
Intended use		Winter cereals				
Active substance/product		Mixture of active substances				
Application rate (kg/ha)		1 × 2.1788				
Acute toxicity (mg/kg bw)		LD50 (mix) = 1843.4				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species		SV90	MAF90	DDD90 (mg/kg bw/d)	TERa
Cereals	Small herbivorous mammal		118.4	1	258	7.1
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”		7.6	1	16.6	111
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”		42.1	1	91.7	20.1
Cereals, BBCH 10-29	Small omnivorous mammal		17.2	1	37.5	49.2

	"mouse"				
Reprod. toxicity (mg/kg bw/d)	49.6				
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 herbivorous mammal	48.3	0.53	55.8	0.89
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	4.9	10.23
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.53	25.8	1.93
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	9.0	5.51
Reprod. toxicity (mg/kg bw/d)	-*				
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 herbivorous mammal	-	-	-	0.95 0.88
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	-	-	-	10.9 10.22
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	-	-	-	2.06 1.92
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	-	-	-	5.87 5.51

*In addition, a combined risk assessment for sublethal effects was performed as well using the following equation and assuming a direct proportionality of the TER to the NOEL:

$$TER_{(mix)} = \left(\sum_i \frac{1}{TER_{(a.s._i)}} \right)^{-1}$$

where:

TER_(a.s.) = calculated TER for the active substance i

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 for the post-emergence use in winter cereals. Therefore, a higher-tier risk assessment is provided here.

Table 9.3-4: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of GLOB1912H 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 × 2.134				
Reprod. toxicity (mg/kg bw/d)		50				
TER criterion		5				
Crop scenario	Generic focal species	SV _m	MAF _m × TWA*	DDD _m (mg/kg bw/d)	TER _{It}	
Growth stage						
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	22.3	0.151	7.19	6.96	
Intended use		Winter cereals				
Active substance/product		Mixture of active substances				
Application rate (kg/ha)		Prosulfocarb: 1 x 2.134, diflufenican: 1 x 0.0448				
Reprod. toxicity (mg/kg bw/d)		49.6				
TER criterion		5				
Crop scenario	Indicator/generic focal species	Active substance	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{It}
Growth stage						
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	Prosulfocarb	22.3	0.151	7.19	-
		Diflufenican		0.53	0.529	-
		Sum	-	-	7.71	6.43
Intended use		Winter cereals				
Active substance/product		Mixture of active substances				
Application rate (kg/ha)		1 × 2.1788				
Reprod. toxicity (mg/kg bw/d)		- **				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{It}	
Growth stage						
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	-	-	-	6.31	

**In addition, a combined risk assessment for sublethal effects was performed as well using the following equation and assuming a direct proportionality of the TER to the NOEL:

$$TER_{(mix)} = \left(\sum_i \frac{1}{TER_{(a.s._i)}} \right)^{-1}$$

where:

TER_(a.s.i) = calculated TER for the active substance i

*Refined parameters:

For the reproductive risk assessment of the lagomorph, the exposure was refined using the DT_{50} of prosulfocarb on young cereal plants since the lagomorph feeds on 100% crop leaves. The DT_{50} 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. These DT_{50} values amounted to 1.43, 1.75, 1.92, 1.93 and 2.2 days. This latter (highest) value was used for the refinement of the exposure to prosulfocarb, which leads to a TWA of 0.151.

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	

At the request of zRMS Poland, a kinetic analysis of the above residue decline data was provided.

Residue data were fitted using CAKE v3.3 to determine first order half-lives, with consideration of the guidance of FOCUS kinetics (2006, 2014). 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;

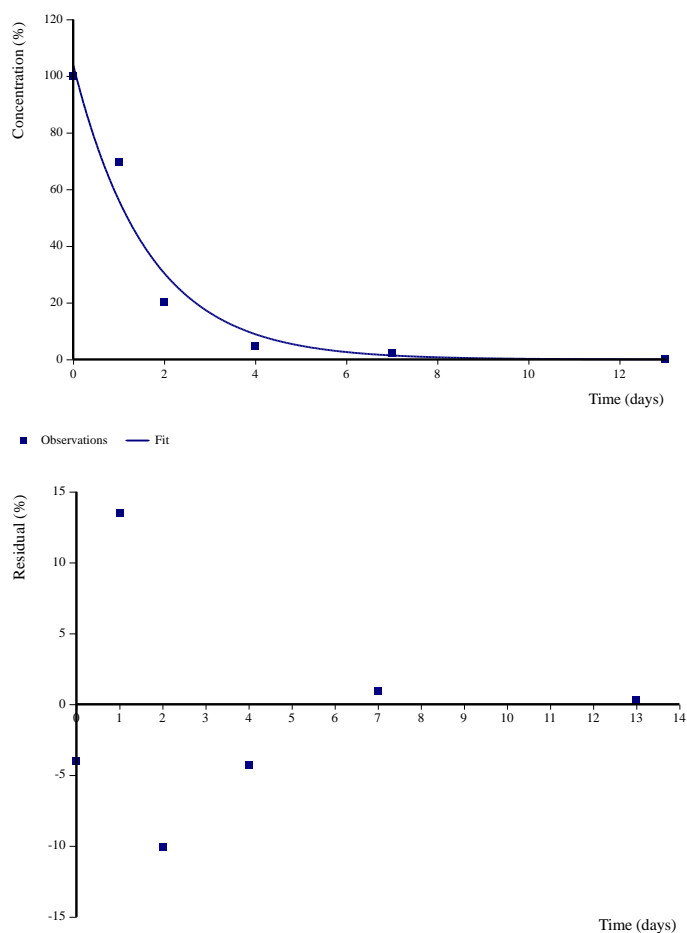
Good = the fitted curve closely follows all the data points, residuals are randomly distributed.

- Fit to the data points (χ^2 error%):

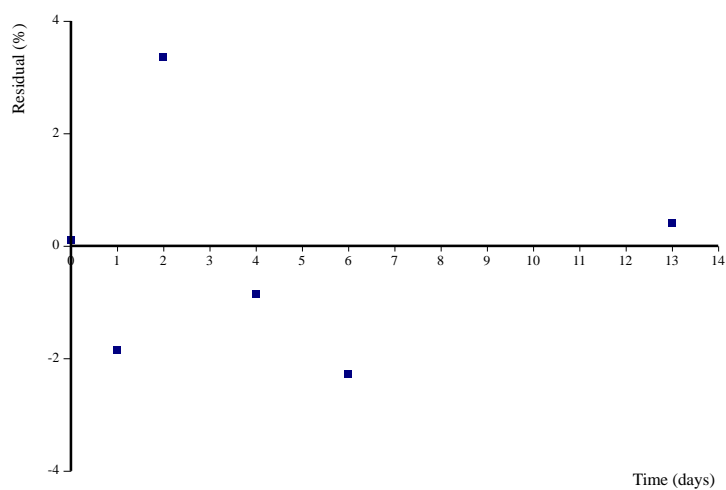
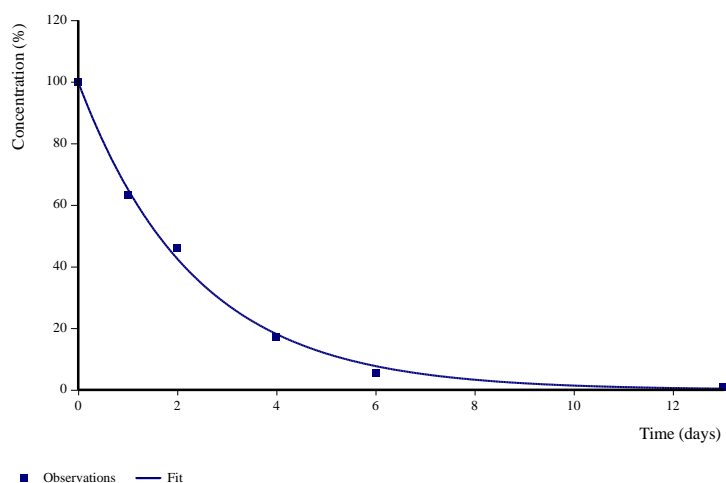
It is recommended that a χ^2 error% of 15% or less indicates acceptable fits, although for data that may include intrinsically variable data, higher values can be tolerated if the visual fit is acceptable or good. Where two or more models are acceptable fits to the data, the χ^2 error% parameter has 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.

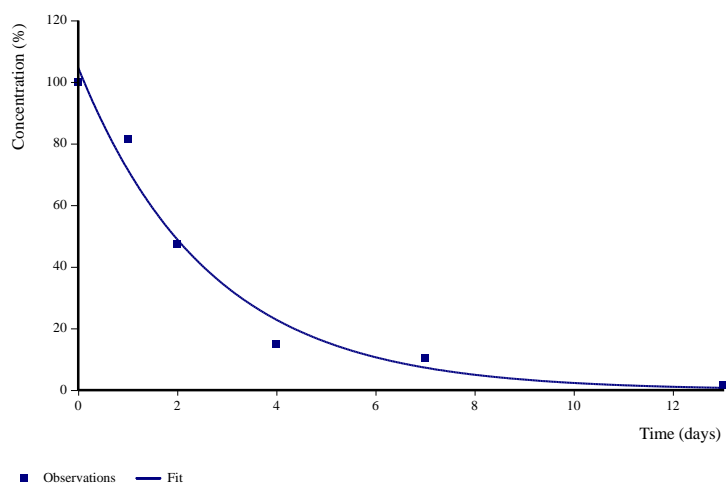
Graphs for trial A9051 AN1

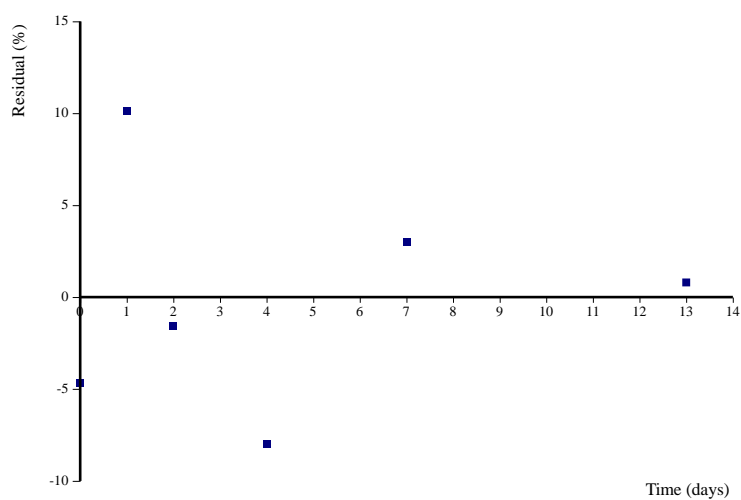


Graphs for trial A9051 GE1

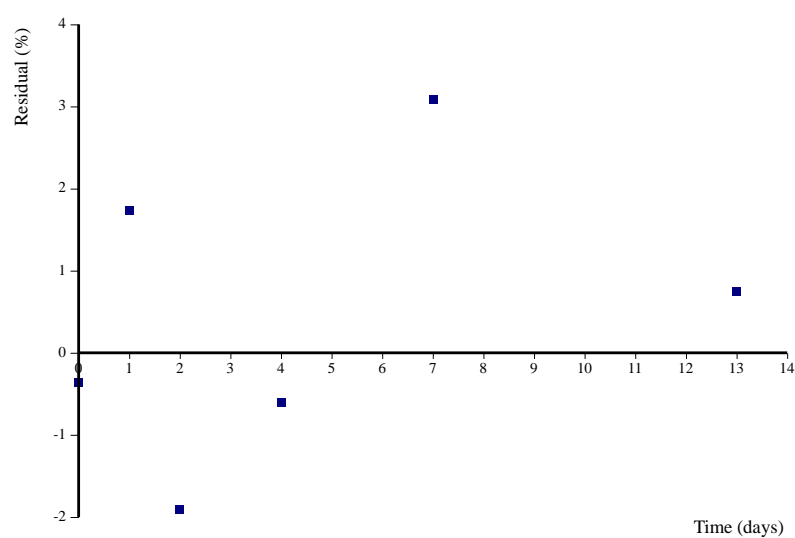
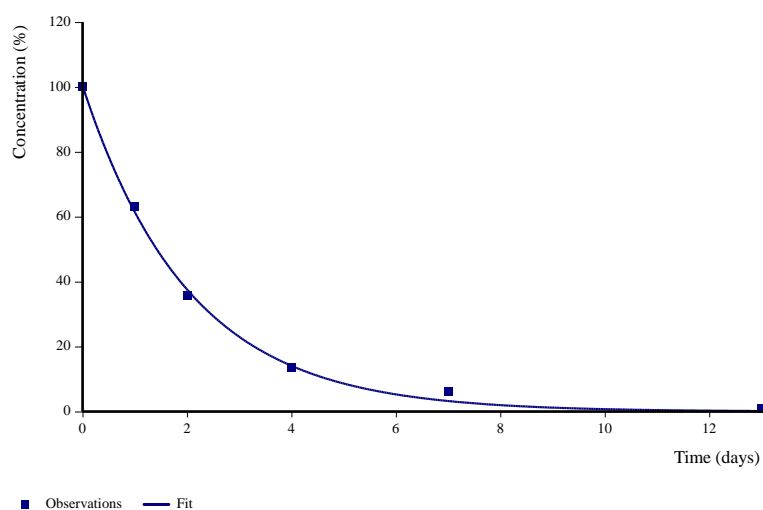


Graphs for trial B1234 AN1

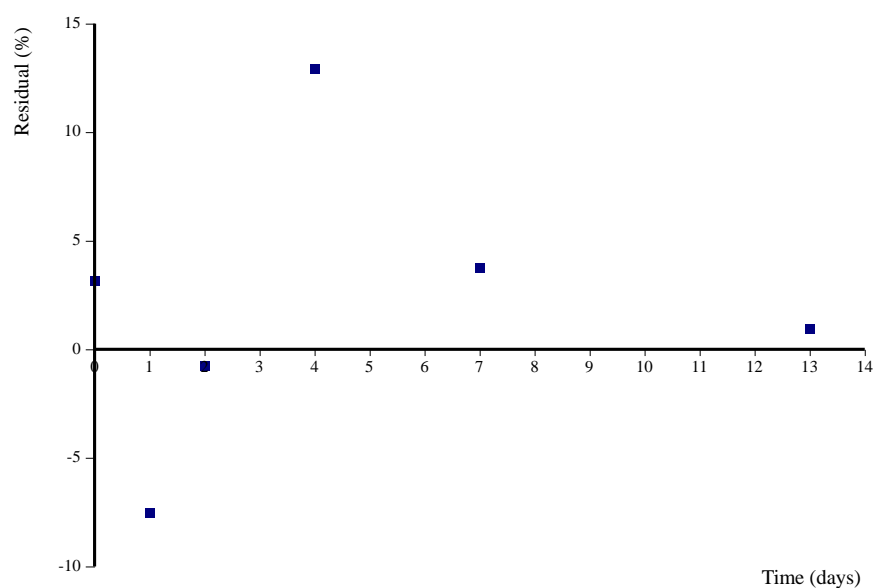
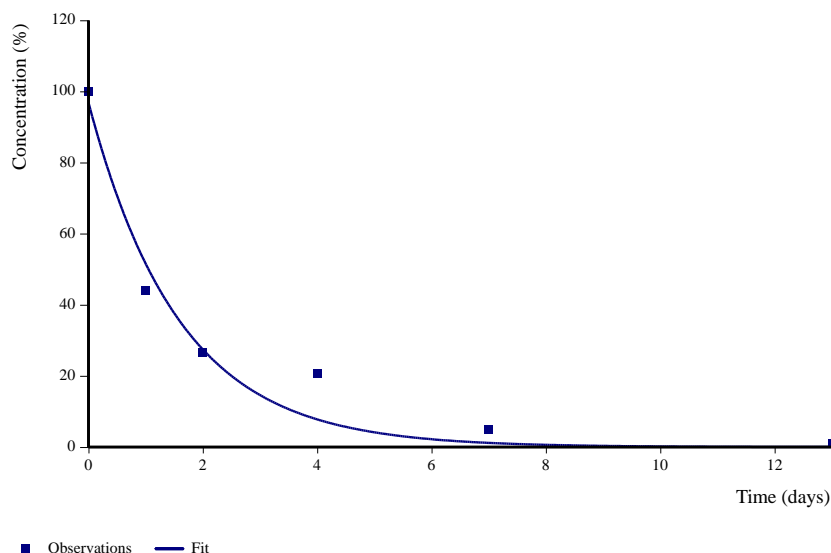




Graphs for trial B1234 BM1



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	3.68	SFO	
B1234 BP1	1.10	3.65	15.6	SFO	

The highest value of 1.82 days can be used to perform the risk assessment. However, as this DT₅₀ is shorter than the one obtained in the reports of Jonchère (2010) and Perny (2012) using an exponential decay equation in Excel, the higher-tier risk assessment already provided here above was considered more conservative and thus not updated.

Review Comments:

Normally a DT₅₀ of 10 days is assumed in the birds and mammals risk assessment as a default value. For prosulfocarb however, a lower DT₅₀ could be expected based on five plant residue trials (winter wheat, BBCH 12-13) that were conducted in Europe (see dRR Part B7 for a description of these studies).

The residue trials were performed in one Central Zone country (Germany) as well as in one Southern Zone country (Northern France). As the environmental conditions during the tests duration were comparable in the countries, thus the results from France, in zRMS opinion, can be included in the overall analysis. Due to intensive rain in second day after application in trial A9051 AN1, error % (χ^2) of 17.6 and shortest DT₅₀ the study results should not to be taken to consideration. Nevertheless, the risk assessment was based on highest DT₅₀ rather than the average, thus it hasn't any impact on the overall conclusion.

The samplings were carried out at 0, then 1, 2, 4, 7 and 14 days after the application. The sampling schedule gave 6 data points for each trial, which is sufficient to perform the reliable kinetic analysis.

A kinetic analysis of the dissipation of prosulfocarb in winter wheat were conducted by the Applicant which is presented above.

FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT₅₀ endpoints for prosulfocarb modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by SFO kinetics and acceptable endpoints were derived for four studies.

The calculated DT₅₀ values and statistics for the decline of prosulfocarb in cereals are shown in the table above. The final DT₅₀ recommended for modelling is the highest DT₅₀ value of 1.82 days.

zRMS agree with the Applicant that the higher-tier risk assessment already provided above is more conservative (based on DT₅₀ of 2.2 days) and thus updated is not required.

For the transparency reason below are added location data of all trials.

The trials were performed on soil types and under cultural practices typical for winter wheat production and on typical cultivars of the regional commercial production.

Trial No.	Trial responsible	Crop	Type of trial	European area	Region country
A9051 AN1	Djamel BOUNAAS	Winter wheat	DC	North	Alsace FRANCE
A9051 GE1	Luc PETERSCHMITT	Winter wheat	DC	North	Baden-Württemberg GERMANY

DC: Decline curve

Location



1 Trial A9051 AN1

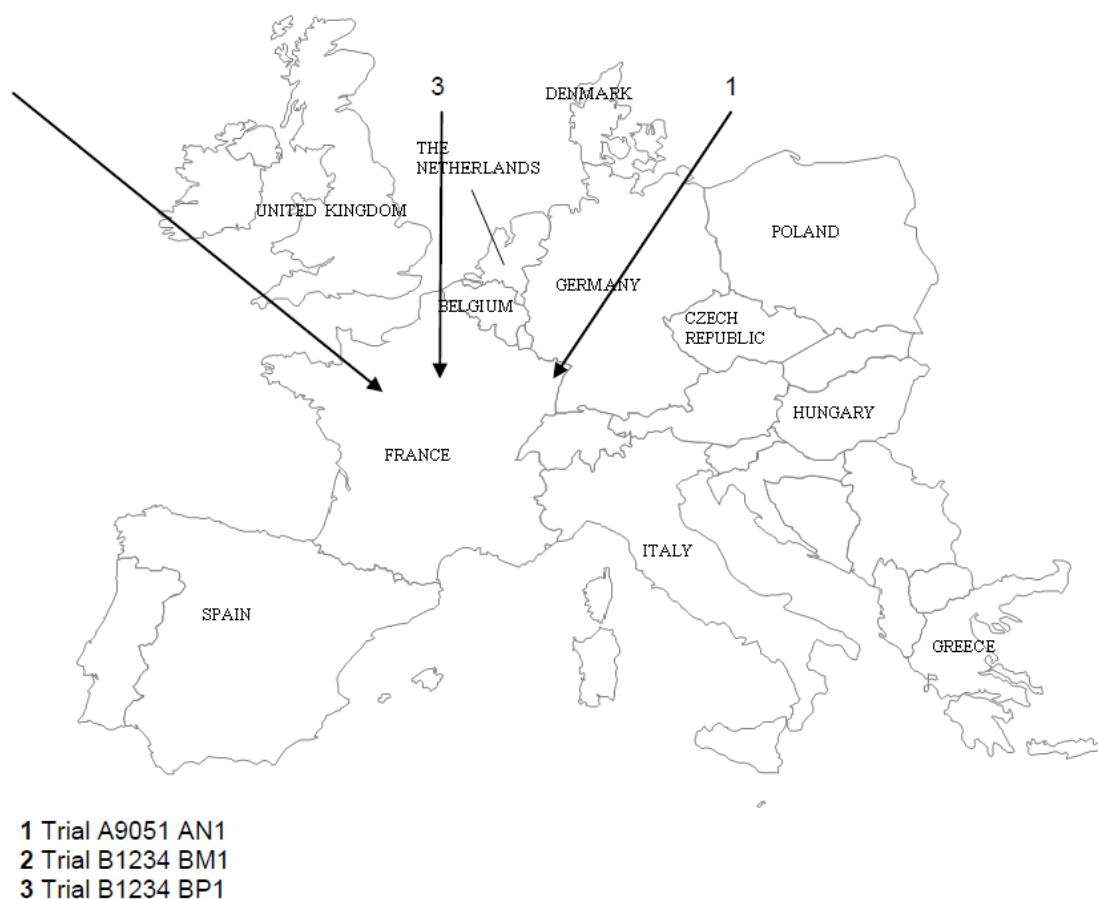
2 Trial A9051 GE1

The trials were performed on soil types and under cultural practices typical for winter wheat production and on typical cultivars of the regional commercial production.

Trial No.	Trial responsible	Crop	Type of trial	European area	Region country
B1234 AN1	Djamel BOUNAAS	Winter wheat	DC	North	Alsace France
B1234 BM1	Annick DORLEANS	Winter wheat	DC	North	Pays de la Loire France
B1234 BP1	Philippe DOUBLIER	Winter wheat	DC	North	Centre France

DC: Decline curve

Location



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) =	2134		
Acute toxicity (mg/kg bw) =	1820	quotient =	1.17
Reprod. toxicity (mg/kg bw/d) =	50	quotient =	42.7

With a $K(f)_{oc}$ of 3091 L/kg, diflufenican belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	44.8		
Acute toxicity (mg/kg bw) =	> 5000	quotient =	< 0.00896
Reprod. toxicity (mg/kg bw/d) =	35.5	quotient =	1.26

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.

The log P_{ow} of diflufenican amounts to 4.2 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 9.3-5: 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, potatoes and sunflower

Parameter	Prosulfocarb	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	1.7118	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}	17.28	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	22.12	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	
TER_{lt}	2.26	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} is under the threshold value, but a bioaccumulation study in earthworms is available for the formulation Prosulfocarb 800 EC, which is considered representative for effects of the active substance. A BCF of 1.39 based on this study is used instead of the calculated value. A refined assessment is provided in the table below.

Table 9.3-6: 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, potatoes and sunflower – refined BCF

Parameter	Prosulfocarb	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	1.7118	dRR B8 Table 8.7-3
BCF_{worm} BAF	4.39 0.77	Sacker D., 2008 Bätscher, 2006 in EFSA, 2007
PEC _{worm}	2.38 1.32	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	2.50 1.69	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	50	
TER _{lt}	20.0 29.6	

TER values shown in bold fall below the relevant trigger.

Table 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to diflufenican via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Diflufenican	comments
PEC _{soil;accu} (twa = 21 d) (mg/kg soil)	0.1493	dRR B8 Table 8.7-6
log P _{ow} / P _{ow}	4.2/15849	
Koc	3091	Geomean (n = 10)
foc	0.02	Default
BCF _{worm}	3.09	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw}) = (0.84 + 0.012 × P _{ow}) / foc × Koc
PEC _{worm}	0.461	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.484	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	35.5	
TER _{lt}	73.3	

TER values shown in bold fall below the relevant trigger.

Since GLOB1912H contains 2 active ingredients, a risk assessment for the mixture of active substances was performed using the NOEL (mix) of 48.7 mg/kg bw/d.

Table 9.3-8: Assessment of the risk for earthworm-eating mammals due to exposure to the mixture via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	2.984	Sum of DDD in Table 9.3-6 to 9.3-7
NOEL (mg/kg bw/d)	49.6	NOEL (mix)
TER _{lt}	16.62	

TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for the mixture was performed as well using the following equation:

$$TER_{(mix)} = \left(\sum_i \frac{1}{TER_{(a.s._i)}} \right)^{-1}$$

where:

$TER_{(a.s._i)}$ = calculated TER for the active substance i

$$TER_{mix} = 15.71 \text{ } 21.09$$

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 9.3-9: 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, potatoes and sunflower

Parameter	Prosulfocarb	comments
PEC _{sw} (twa = 21 d) (mg/L)	0.009015	dRR B8 Table 8.9-5 (FOCUS Step 3; D1 ditch)
BCF _{fish}	700	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	6.3105	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.8961	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	50	
TER _{lt}	55.8	

TER values shown in bold fall below the relevant trigger.

Table 9.3-10: Assessment of the risk for fish-eating mammals due to exposure to diflufenican via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Diflufenican	comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0001619	dRR B8 Table 8.9-28 (FOCUS Step 3; D1 ditch)
BCF _{fish}	1596	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.2584	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.0367	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	35.5	
TER _{lt}	967.5	

TER values shown in bold fall below the relevant trigger.

Since GLOB1912H contains 2 active ingredients, a risk assessment for the mixture of active substances was performed using the NOEL (mix) of 49.6 mg/kg bw/d.

Table 9.3-11: Assessment of the risk for fish-eating mammals due to exposure to the mixture via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals, potatoes and sunflower

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	0.9328	Sum of DDD in Table 9.3-9 to 9.3-10
NOEL (mg/kg bw/d)	49.6	NOEL (mix)
TER _{lt}	53.2	

TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for the mixture was performed as well using the following equation:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

TER_(a.s.i) = calculated TER for the active substance i

$$TER_{mix} = 52.8$$

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 when GLOB1912H is applied according to the intended uses.

Review Comments:

The acute and chronic risks of GLOB1912H to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with active ingredients, mixture of active substances, and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

Almost all TER values exceed the relevant triggers based on screening or first tier risk assessment. Only for prosulfocarb acceptable reproductive risk for lagomorph for the post-emergence use in winter cereals was concluded based on higher tier assessment. The GLOB1912H does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low.

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

Effects on aquatic organisms of GLOB1912H were not evaluated as part of the EU assessment of prosulfocarb and diflufenican. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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, diflufenican 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
Fathead minnow, <i>Pimephales promelas</i>	Prosulfocarb	96 h	LC ₅₀ = 2.4 mg/L	DAR, 2006 (Peter P., 2001)
Fish	Prosulfocarb	Acute	LC ₅₀ = 1420 µg a.s./L	Geomean
Rainbow trout, <i>Oncorhynchus mykiss</i>	Prosulfocarb	96 h (dynamic simulating DT ₅₀ = 1.5 d)	LC ₅₀ = 4.3 mg/L (24h) LC ₅₀ calculated based on DT ₅₀ = 6.2 d in mesocosm study)	EFSA, 2007
Rainbow trout, <i>Oncorhynchus mykiss</i>	Prosulfocarb	21 d, f	NOEC = 0.31 mg/L_{mm}	EFSA, 2007
Common carp, <i>Cyprinus carpio</i>	Diflufenican	96 h	LC ₅₀ > 98.5 µg/L	EFSA, 2007
Rainbow trout, <i>Oncorhynchus mykiss</i>	Diflufenican	35 d	NOEC = 15 µg/L	EFSA, 2007
Rainbow trout, <i>Oncorhynchus mykiss</i>	AE B107137	96 h	LC ₅₀ > 17300 µg/L	EFSA, 2007
Aquatic invertebrate				
Water flea, <i>Daphnia magna</i>	Prosulfocarb	48 h, s	EC ₅₀ = 0.51 mg/L_{mm}	EFSA, 2007
<i>Chaoborus sp.</i>			EC ₅₀ = 790 µg/L	DAR, 2006 (Ashwell,

Species	Substance	Exposure System	Results	Reference
<i>Cleon sp.</i>			EC ₅₀ = 1410 µg/L	2001)
<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
Water flea, <i>Daphnia magna</i>	Diflufenican	48 h	EC ₅₀ > 240 µg/L	EFSA, 2007
Water flea, <i>Daphnia magna</i>	Diflufenican	21 d	NOEC = 52 µg/L	EFSA, 2007
Water flea, <i>Daphnia magna</i>	AE B107137	48 h	EC ₅₀ > 20400 µg/L	EFSA, 2007
Water flea, <i>Daphnia magna</i>	AE 0542291	48 h	EC ₅₀ > 10000 µg/L	EFSA, 2007
Sediment dwelling organisms				
Freshwater midge, <i>Chironomus riparius</i>	Prosulfocarb	25 d	NOEC = 1.25 mg/L	EFSA, 2007
Freshwater midge, <i>Chironomus riparius</i>	Diflufenican	28 d	NOEC = 0.1 mg/L (spiked water)	EFSA, 2007
Freshwater midge, <i>Chironomus riparius</i>	Diflufenican	28 d	NOEC = 2.0 mg/kg (spiked sediment)	EFSA, 2007
Freshwater midge, <i>Chironomus riparius</i>	AE C522392	28 d	NOEC = 1.0 mg/kg	EFSA, 2007
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
<i>Scenedesmus subspicatus</i>	Prosulfocarb	72 h/96 h	72 h E _b C ₅₀ = 112 µg/L 96 h E_rC₅₀ = 113 µg/L	DAR, 2006 (Ellgehausen, 1986)
<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	72 h E _b C ₅₀ = 1540 µg/L 96 h E _r C ₅₀ = 8340 µg/L	DAR, 2006 (Wallace, 2001)
<i>Chlamydomonas reinhardtii</i>	Prosulfocarb	72 h/96 h	72 h E _b C ₅₀ = 3690 µg/L 96 h 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)
Algae	Prosulfocarb	-	EC ₅₀ = 680 1173.5	Geomean

Species	Substance	Exposure System	Results	Reference
			µg/	
<i>Pseudokirchneriella subcapitata</i>	Prosulfocarb sulfoxide	72 h	$E_bC_{50} = 1.28 \mu\text{g/L}$ $E_rC_{50} = 4.33 \mu\text{g/L}$	DAR
<i>Desmodesmus subspicatus</i>	Prosulfocarb sulfoxide		$E_rC_{50} = 85 \mu\text{g/L}$	DAR
<i>Chlamydomonas reinhardtii</i>	Prosulfocarb sulfoxide	72 h	$E_bC_{50} = 97.1 \mu\text{g/L}$ $E_rC_{50} = 253.9 \mu\text{g/L}$	Juckeland D, 2012a
			$E_rC_{50} = 410 \mu\text{g/L}$	DAR
<i>Chlorella vulgaris</i>	Prosulfocarb sulfoxide	72 h	$E_bC_{50} = 730 \mu\text{g/L}$ $E_rC_{50} = 1320 \mu\text{g/L}$	Juckeland D, 2012b
			$E_rC_{50} = 2860 \mu\text{g/L}$	DAR
<i>Anabaena flosaquae</i>	Prosulfocarb sulfoxide	72 h	$E_bC_{50} = 19500 \mu\text{g/L}$ $E_rC_{50} = 42500 \mu\text{g/L}$	Juckeland D, 2012c
			$E_rC_{50} = 43000 \mu\text{g/L}$	DAR
<i>Navicula pelliculosa</i>	Prosulfocarb sulfoxide	72 h	$E_bC_{50} = 1400 \mu\text{g/L}$ $E_rC_{50} = 7650 \mu\text{g/L}$	Juckeland D, 2012d
			$E_rC_{50} = 2700 \mu\text{g/L}$	DAR
<i>Skeletonema costatum</i>	Prosulfocarb sulfoxide	72 h	$E_bC_{50} = 53.8 \mu\text{g/L}$ $E_rC_{50} = 134.8 \mu\text{g/L}$	Juckeland D, 2012e
<i>Scenedesmus subspicatus</i>	Diflufenican	72 h	Without sediment: $E_bC_{50} = 0.25 \mu\text{g/L}$ $E_rC_{50} = 0.45 \mu\text{g/L}$ NOEC = 0.1 µg/L	EFSA, 2007
<i>Scenedesmus subspicatus</i>	Diflufenican	72 h	With sediment: $E_bC_{50} = 2.4 \mu\text{g/L}$ $E_rC_{50} = 4.7 \mu\text{g/L}$ NOEC = 0.76 µg/L	EFSA, 2007
<i>Scenedesmus subspicatus</i>	Diflufenican	72 h	$E_bC_{50} = 0.46 \mu\text{g/L}$ $E_rC_{50} = 1.22 \mu\text{g/L}$ Max conc. From which recovery possible: 4.2 µg/L NOEC = 0.15 µg/L	EFSA, 2007
<i>Scenedesmus subspicatus</i>	AE B107137	72 h	$E_bC_{50} > 20400 \mu\text{g/L}$ $E_rC_{50} > 20400 \mu\text{g/L}$	EFSA, 2007
<i>Scenedesmus subspicatus</i>	AE 0542291	72 h	$E_bC_{50} > 36000 \mu\text{g/L}$ $E_rC_{50} > 66000 \mu\text{g/L}$	EFSA, 2007
<i>Pseudokirchneriella subcapitata</i>	AE 592370	72 h	$E_bC_{50} > 39000 \mu\text{g/L}$ $E_rC_{50} > 58000 \mu\text{g/L}$	EFSA, 2007
<i>Pseudokirchneriella subcapitata</i>	AE C522392	72 h	$E_bC_{50} > 3400 \mu\text{g/L}$ $E_rC_{50} > 16000 \mu\text{g/L}$	EFSA, 2007
Higher plants				
Duckweed, <i>Lemna</i>	Prosulfocarb	14 d	$EC_{50} = 690 \mu\text{g/L}$	EFSA, 2007

Species	Substance	Exposure System	Results	Reference
<i>gibba</i>				
<i>Duckweed, Lemna gibba</i>	Prosulfocarb sulfoxide	7 d	$E_rC_{50} = 13 \mu\text{g/L}$ $E_bC_{50} = 2.8 \mu\text{g/L}$	DAR
<i>Duckweed, Lemna gibba</i>	Diflufenican	14 d	$E_bC_{50} = 56 \mu\text{g/L}$ $EC_{50} \text{ frond density} = 39 \mu\text{g/L}$	EFSA, 2007
Primary producers				
Algae & higher plants	Prosulfocarb sulfoxide	-	HC5 = 4.84 $\mu\text{g/L}$	HC5
Higher-tier studies (micro- or mesocosm studies)				
<i>Microcosm</i>	Prosulfocarb	NOEC = 15 $\mu\text{g a.i./L}$ => ETO-RAC = 7.5 $\mu\text{g/L}$ with a safety factor 2)		EFSA 2007 DAR, 2006 (van Wijngaarden 2006) + Deneer J., Roessink I. & Rico A. (2015)
	Prosulfocarb sulfoxide	NOEC = 30 $\mu\text{g/L}$ => ETO-RAC = 15 $\mu\text{g/L}$ with a safety factor 2		Addendum to DAR, 2013 (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

*EFSA Scientific Report (2007) 11, 1-81 has not been updated or replaced due to new data from Addendum to DAR 2013. Therefore, evaluation will be performed without those information.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB1912H

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	GLOB1817H	48 h, ss	$EC_{50} = 0.954 \text{ mg/L}_{\text{nom}}$	Juckeland D., 2021a
<i>Pseudokirchneriella subcapitata</i>	GLOB1817H	72 h, s	$E_rC_{50} = 0.0597 \text{ mg/L}_{\text{nom}}$ $E_yC_{50} = 0.0310 \text{ mg/L}_{\text{nom}}$	Juckeland D., 2021b
<i>Lemna gibba</i>	GLOB1817H	7 d, ss	$E_rC_{50} = 0.5159 \text{ mg/L}_{\text{nom}}$ $E_yC_{50} = 0.3352 \text{ mg/L}_{\text{nom}}$	Juckeland D., 2021c
<i>Myriophyllum spicatum</i>	GLOB1817H	14 d, ss	$E_rC_{50} = 0.075 \text{ mg/L}_{\text{nom}}$ $E_yC_{50} = 0.040 \text{ mg/L}_{\text{nom}}$	Juckeland D., 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

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.

Review Comments:

According the recommendation of “Working document on Risk Assessment of Plant Protection Products in Central Zone Ecotoxicology” (May 2021), point 3.3.12, following evaluation was performed (Tier 2A): The lowest fish endpoint is LC₅₀ = 840 µg/L and LC_{50geomean} = 1420 µg/L (RAC_{geomean} = 14.2 µg/L)

Step 1: is lowest EP < RAC_{geomean}?

- No (LC₅₀ = 840 µg/L > RAC_{geomean} = 14.2 µg/L)

Step 2: compare RAC_{geomean} and RAC_{lowest}

- RAC_{lowest} is 14.0 µg/L (lowest EP / AF 60)
- RAC_{geomean} is 14.2 µg/L

Thus, the lowest RAC of 14 µg/L will be use in the RA.

“The RAC_{lowest} (i.e. endpoint of the most sensitive species tested divided by an AF of ≥ 60) is considered as a “safety net” to the RAC_{geomean}, especially relevant when the lowest available endpoint of the dataset is in a range close to the RAC_{geomean}. In the current situation, the use of the RAC_{lowest} instead of RAC_{geomean} helps to reduce the shift in the protection level that will be achieved for species situated close to this trigger.”

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
<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).

Review Comments:

A summary of the studies of the toxicity of prosulfocarb or the formulated product Boxer (express as a.s.) to aquatic invertebrates is presented in Table B.9.2.10.b of DAR 11 Vol.3 B9 (April 2005). All those studies are considered to be valid.

According the recommendation of “Working document on Risk Assessment of Plant Protection Products in Central Zone Ecotoxicology” (May 2021), point 3.3.12, following evaluation was performed (Tier 2A): The lowest daphnia endpoint is EC₅₀ = 510 µg/L and EC_{50geomean} = 869.5 µg/L (RAC_{geomean} = 8.695 µg/L)

Step 1: is lowest EP < RAC_{geomean}?

- No (LC₅₀ = 510 µg/L > RAC_{geomean} = 8.695 µg/L)

Step 2: compare RAC_{geomean} and RAC_{lowest}

- RAC_{lowest} is 8.5 µg/L (lowest EP / AF 60)
- RAC_{geomean} is 8.695 µg/L

Thus, the lowest RAC of 8.5 µg/L will be use in the RA.

“The RAC_{lowest} (i.e. endpoint of the most sensitive species tested divided by an AF of ≥ 60) is considered as a “safety net” to the RAC_{geomean}, especially relevant when the lowest available endpoint of the dataset is in a range close to the RAC_{geomean}. In the current situation, the use of the RAC_{lowest} instead of RAC_{geomean} helps to reduce the shift in the protection level that will be achieved for species situated close to this trigger.”

Algae – geomean approach

To address the uncertainty inherent in the estimate of toxicity of prosulfocarb to primary producers, toxicity studies in five additional species of freshwater phytoplankton as well as the *Lemna* value were used. Estimates of toxicity of prosulfocarb to green algae, blue-green algae, and freshwater diatoms and *Lemna* (representing aquatic macrophytes) in single species laboratory tests, as submitted in the Annex I application 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 ₁ C ₅₀	120 µg a.i./L	EFSA, 2007	941

Taxonomic group	Organism	Endpoint	Value	Reference	Geomean (µg a.s./L)
	<i>Scenedesmus subspicatus</i>	96h ErC ₅₀ ¹	113 µg a.i./L	DAR, 2006 (Ellgehausen. 1986)	
	<i>Chlorella vulgaris</i>	96h ErC ₅₀ ¹	8340 µg a.i./L	DAR, 2006 (Wallace. 2001)	
	<i>Chlamydomonas reinhardtii</i>	96h ErC ₅₀ ¹	7720 µg a.i./L	DAR, 2006 (Swarbrick. 2001)	
Blue-green algae	<i>Anabaena flos-aquae</i>	72h ErC ₅₀	7480 µg a.i./L	DAR, 2006 (Wallace. 2001)	7480
Freshwater diatom	<i>Navicula pelliculosa</i>	72h ErC ₅₀	680 µg a.i./L	DAR, 2006 (Smyth. 1998)	680
Monocot macrophyte	<i>Lemna gibba</i>	14d ErC ₅₀	690 µg a.i./L	DAR, 2006 (Smyth. 1999)	690 ²
Lowest geomean EC₅₀					680³
ErC₅₀geomean (n=7)					1173.5

¹ data from either 72 h or 96 h tests is acceptable, according to the EFSA AGD (2013)

² the single species value is taken where there is only one species in that taxonomic group

³ 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” (680 µg/L).

Review Comments:

A summary of the studies of the toxicity of prosulfocarb to algae and aquatic plants is presented in Table B.9.2.10.c of DAR 11 Vol.3 B9 (April 2005). All those studies are considered to be valid.

According the recommendation of “Working document on Risk Assessment of Plant Protection Products in Central Zone Ecotoxicology” (May 2021), point 3.3.12, following evaluation was performed (Tier 2A):
The lowest algae endpoint is ErC₅₀ = 113 µg/L and ErC₅₀geomean = 1173.5 µg/L (RAC_{geomean} = 117.35 µg/L)
Step 1: is lowest EP < RAC_{geomean}?

- Yes (ErC₅₀ = 113 µg/L < RAC_{geomean} = 117.35 µg/L)
- RAC_{lowest} is 18.83 µg/L (lowest EP / AF 6)

Thus, the lowest RAC of 18.83 µg/L will be use in the RA.

“The RAC_{lowest} (i.e. endpoint of the most sensitive species tested divided by an AF_{overall} of ≥ 6) is considered as a “safety net” to the RAC_{geomean}, especially relevant when the lowest available endpoint of the dataset is in a range close to the RAC_{geomean}. In the current situation, the use of the RAC_{lowest} instead of RAC_{geomean} helps to reduce the shift in the protection level that will be achieved for species situated close to this trigger.”

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 µg a.s./L. The lowest NOEC population for zooplankton was 15 µg a.s./L based on effects on the rotifer *Polychaeta remata*. The zooplankton community NOEC was estimated as 76 µg 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 µg 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 µg a.s./L. The overall conclusion of the meeting was that a NOEC of 15 µg 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 µg 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. µg a.s./L			
	3	15	76	380
Phytoplankton				
PRC phytoplankton	1	1	3	5
Desmids	1	1	3↓↑ ¹	5↓ ²
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.

Review Comments:

The decision of use or not the safety factor should be taken at Member State level. Both endpoint will be use in the risk assessment. Nevertheless, in zRMS opinion the AF of 2 should be used. Thus, the endpoint from mesocosms study will driving the aquatic risk assessment.

The value of 7.5 µg a.s./L is very close to acute RAC_{lowest} of 8.5 µg/L for invertebrates and higher than chronic RAC to Daphnia of 4.5 µg/L. Taking to consideration that is only one, quite old mesocosms study with its specific composition of species, ETO-RAC with AF of 2, is the most appropriate endpoint for the risk assessment.

Prosulfocarb sulfoxide

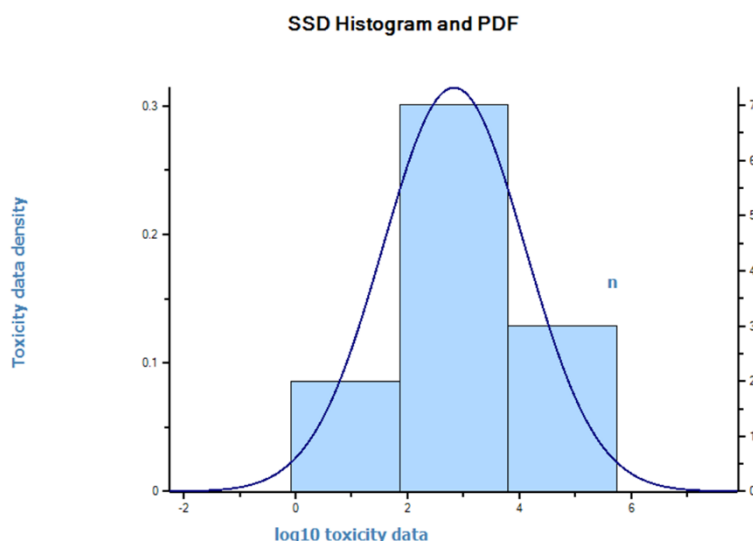
Primary producers — HC_s

A number of studies of the toxicity of the metabolite prosulfocarb sulfoxide to primary producers have been conducted and endpoints are summarised in the table below.

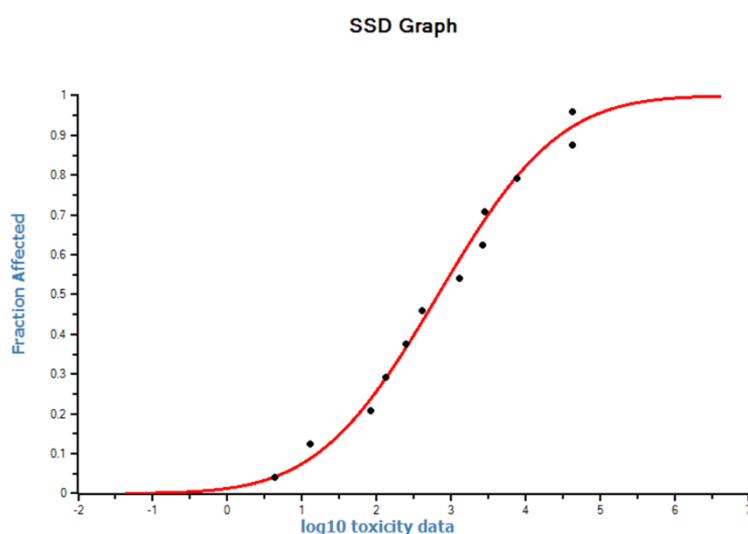
Summary of the toxicity values of prosulfocarb sulfoxide used for the risk assessment to primary producers (algae and aquatic plants)

Taxonomic group	Organism	Endpoint	Value	Reference
Green algae	<i>Pseudokirchneriella subcapitata</i>	E _r C ₅₀	4.3 µg/L	DAR
	<i>Desmodesmus subspicatus</i>	E _r C ₅₀	85 µg/L	DAR
	<i>Chlorella vulgaris</i>	E _r C ₅₀	2860 µg/L	DAR
		E _r C ₅₀	1320 µg/L	Juckeland, 2012b
	<i>Chlamydomonas reinhardtii</i>	E _r C ₅₀	410 µg/L	DAR
		E _r C ₅₀	253.9 µg/L	Juckeland, 2012a
Blue-green algae	<i>Anabaena flos-aquae</i>	E _r C ₅₀	43000 µg/L	DAR
		E _r C ₅₀	42500 µg/L	Juckeland, 2012e
Freshwater diatom	<i>Navicula pelliculosa</i>	E _r C ₅₀	2700 µg/L	DAR
		E _r C ₅₀	7650 µg/L	Juckeland, 2012d
	<i>Skeletonema costatum</i>	E _r C ₅₀	134.8 µg/L	Juckeland, 2012e
Monocot macrophyte	<i>Lemna gibba</i>	E _r C ₅₀	13 µg/L	DAR

Anses calculated a HC_s of 4.84 µg/L from a SSD constructed with the 12 previous endpoints. (see below).



The median HC_5 be 1.61 $\mu\text{g/L}$ 3). Anses finally RAC issued study (see study).



—RAC would (median HC_5 considered the from the cosm Mesocosm

Mesocosm study

A statistical analysis of the cosm study is available (MDD report). Based on these elements an overall NOEC of 30 $\mu\text{g/L}$ can be derived from this study from which an ETO RAC of 15 $\mu\text{g/L}$ can be derived for use in the higher tier aquatic risk assessment for prosulfocarb sulfoxide.

Review Comments:

The prosulfocarb sulfoxide is a metabolite of prosulfocarb forming in soil but not in water or sediment. According to information included in the DAR B9 2005, no degradation products were detected in either hydrolysis or photolysis studies conducted in water. No metabolite of prosulfocarb reached significant levels in the water/sediment study (<0.8%) at any time. Thus, the studies on metabolite are not required. Furthermore, according to EFSA Scientific Report (2007) 111, 1-81, for surface water the risk assessment for prosulfocarb sulfoxide is not required.

9.5.2 Risk assessment

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

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

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

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

Table 9.5-3: 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 GLOB1912H in winter cereals (pre-emergence)

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		RAC _{lowest}	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		RAC _{lowest}	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>		RAC _{lowest}	Higher tier for <i>Daphnia</i> and primary producers	
Endpoint (µg/L)		LC ₅₀	Geomean LC ₅₀	LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	EC ₅₀	NOEC	ErC ₅₀	NOEC	ErC ₅₀	Geomean ErC ₅₀	ErC ₅₀	NOEC _{community}	NOEC _{community}
		840	1420	840	310	510	869.5	510	45	113	1250	690	680	113	15	15
AF		100	100	60	10	100	100	60	10	10	10	10	10	6	2	1
RAC (µg/L)		8.40	14.2	14	31.0	5.10	8.695	8.5	4.5	11.3	125.0	69.0	68.0	18.83	7.50	15
FOCUS Scenario	PEC _{gl-max} (µg/L)															
Step 1																
	228.92	27.252	16.121	16.35	7.385	44.886	26.328	26.93	50.871	20.258	1.831	3.318	3.366	12.16	30.523	15.261
Step 2																
N-Europe	90.77	10.806	6.392	6.48	2.928	17.798	10.439	10.68	20.171	8.033	0.726	1.316	1.335	4.82	12.103	6.051
S-Europe	74.13	8.825	5.220	5.30	2.391	14.535	8.526	8.72	16.473	6.560	0.593	1.074	1.090	3.94	9.884	4.942
Step 3																
D1/ditch	13.66	1.626	0.962	0.98	0.441	2.678	1.571	1.61	3.036	1.209	0.109	0.198	0.201	0.725	1.821	0.911

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D1/stream	11.95	1.423	0.842	0.85	0.385	2.343	1.374	1.41	2.656	1.058	0.096	0.173	0.176	0.635	1.593	0.797
D2/ditch	13.68	1.629	0.963	0.98	0.441	2.682	1.573	1.61	3.040	1.211	0.109	0.198	0.201	0.726	1.824	0.912
D2/stream	12.17	1.449	0.857	0.87	0.393	2.386	1.400	1.43	2.704	1.077	0.097	0.176	0.179	0.646	1.623	0.811
D3/ditch	13.46	1.602	0.948	0.96	0.434	2.639	1.548	1.58	2.991	1.191	0.108	0.195	0.198	0.715	1.795	0.897
D4/pond	0.4659	0.055	0.033	0.033	0.015	0.091	0.054	0.05	0.104	0.041	0.004	0.007	0.007	0.025	0.062	0.031
D4/stream	11.68	1.390	0.823	0.83	0.377	2.290	1.343	1.37	2.596	1.034	0.093	0.169	0.172	0.620	1.557	0.779
D5/pond	0.4668	0.056	0.033	0.033	0.015	0.092	0.054	0.05	0.104	0.041	0.004	0.007	0.007	0.025	0.062	0.031
D5/stream	12.60	1.500	0.887	0.90	0.406	2.471	1.449	1.48	2.800	1.115	0.101	0.183	0.185	0.669	1.680	0.840
D6/ditch	13.62	1.621	0.959	0.97	0.439	2.671	1.566	1.60	3.027	1.205	0.109	0.197	0.200	0.723	1.816	0.908
R1/pond	1.309	0.156	0.092	0.09	0.042	0.257	0.151	0.154	0.291	0.116	0.010	0.019	0.019	0.070	0.175	0.087
R1/stream	10.28	1.224	0.724	0.73	0.332	2.016	1.182	1.21	2.284	0.910	0.082	0.149	0.151	0.546	1.371	0.685
R3/stream	13.92	1.657	0.980	0.99	0.449	2.729	1.601	1.64	3.093	1.232	0.111	0.202	0.205	0.739	1.856	0.928
R4/stream	8.931	1.063	0.629	0.64	0.288	1.751	1.027	1.05	1.985	0.790	0.071	0.129	0.131	0.474	1.191	0.595
Step 4: 5 unsprayed buffer zone																
D1/ditch	3.799	Not required													0.51	
D1/stream	4.37														0.58	
D2/ditch	3.807														0.51	

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb · prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers	Mesocosm	
D2/stream	4.451									0.59	
D3/ditch	3.65									0.49	
D4/stream	4.284									0.57	
D5/stream	4.284									0.57	
D6/ditch	7.401									0.63	
Step 4: 10 unsprayed buffer zone + 10 m vegetative strip											
R1/stream	4.600									0.61	Not required
R3/stream	6.354									8.50	
R4/stream	3.640									0.49	

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 GLOB1912H in winter cereals (post-emergence)

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverte b. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		RAC _{lowest}	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		RAC _{lowest}	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>		RAC _{lowest}	Higher tier for Daphnia and primary producers	
Endpoint (µg/L)		LC ₅₀	Geomean LC ₅₀	LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	EC ₅₀	NOEC	ErC ₅₀	NOEC	ErC ₅₀	Geomean ErC ₅₀	ErC ₅₀	NOEC _{community}	NOEC _{community}
AF		840	1420	840	310	510	869.5	510	45	113	1250	690	680	113	15	15
RAC (µg/L)		100	100	60	10	100	100	60	10	10	10	10	10	6	2	1
FOCUS Scenario	PEC _{gl-max} (µg/L)	8.40	14.2	14	31.0	5.10	8.695	8.5	4.5	11.3	125.0	69.0	68.0	18.83	7.50	15
Step 1																
	228.92	27.252	16.121	16.35	7.385	44.886	26.328	26.93	50.871	20.258	1.831	3.318	3.366	12.157	30.523	15.261
Step 2																
N-Europe	90.77	10.806	6.392	6.48	2.928	17.798	10.439	10.68	20.171	8.033	0.726	1.316	1.335	4.820	12.103	6.051
S-Europe	74.13	8.825	5.220	5.30	2.391	14.535	8.526	8.72	16.473	6.560	0.593	1.074	1.090	3.937	9.884	4.942
Step 3																
D1/ditch	13.66	1.626	0.962	0.98	0.441	2.678	1.571	1.61	3.036	1.209	0.109	0.198	0.201	0.725	1.821	0.911
D1/stream	11.95	1.423	0.842	0.85	0.385	2.343	1.374	1.41	2.656	1.058	0.096	0.173	0.176	0.635	1.593	0.797

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverte b. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D2/ditch	13.58	1.617	0.956	0.97	0.438	2.663	1.562	1.60	3.018	1.202	0.109	0.197	0.200	0.721	1.811	0.905
D2/stream	11.02	1.312	0.776	0.79	0.355	2.161	1.267	1.30	2.449	0.975	0.088	0.160	0.162	0.585	1.469	0.735
D3/ditch	13.46	1.602	0.948	0.96	0.434	2.639	1.548	1.58	2.991	1.191	0.108	0.195	0.198	0.715	1.795	0.897
D4/pond	0.4659	0.055	0.033	0.033	0.015	0.091	0.054	0.05	0.104	0.041	0.004	0.007	0.007	0.025	0.062	0.031
D4/stream	11.68	1.390	0.823	0.83	0.377	2.290	1.343	1.37	2.596	1.034	0.093	0.169	0.172	0.620	1.557	0.779
D5/pond	0.4673	0.056	0.033	0.03	0.015	0.092	0.054	0.05	0.104	0.041	0.004	0.007	0.007	0.025	0.062	0.031
D5/stream	12.60	1.500	0.887	0.90	0.406	2.471	1.449	1.48	2.800	1.115	0.101	0.183	0.185	0.669	1.680	0.840
D6/ditch	13.62	1.621	0.959	0.97	0.439	2.671	1.566	1.60	3.027	1.205	0.109	0.197	0.200	0.723	1.816	0.908
R1/pond	1.302	0.155	0.092	0.09	0.042	0.255	0.150	0.15	0.289	0.115	0.010	0.019	0.019	0.069	0.174	0.087
R1/stream	10.20	1.214	0.718	0.73	0.329	2.000	1.173	1.20	2.267	0.903	0.082	0.148	0.150	0.542	1.360	0.680
R3/stream	12.97	1.544	0.913	0.93	0.418	2.543	1.492	1.53	2.882	1.148	0.104	0.188	0.191	0.689	1.729	0.865
R4/stream	14.95	1.780	1.053	1.07	0.482	2.931	1.719	1.76	3.322	1.323	0.120	0.217	0.220	0.794	1.993	0.997
Step 4: 5 unsprayed buffer zone																
D1/ditch	3.8	Not required												0.51	Not required	
D1/stream	4.37													0.58		
D2/ditch	3.683													0.49		

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverte b. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers	Mesocosm	
D2/stream	4.09									0.55	
D3/ditch	3.648									0.49	
D4/stream	4.284									0.57	
D5/stream	4.608									0.61	
D6/ditch	7.401									0.99	
Step 4: 10 unsprayed buffer zone + 10 m vegetative strip											
R1/stream	4.564	Not required								0.61	Not required
R3/stream	5.840									0.78	
R4/stream	6.748									0.90	

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 GLOB1912H in potato

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		RAC _{lowest}	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		RAC _{low est}	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>		RAC _{lowest}	Higher tier for <i>Daphnia</i> and primary producers	
Endpoint (µg/L)		LC ₅₀	Geomean LC ₅₀	LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	EC ₅₀	NOEC	E _r C ₅₀	NOEC	E _r C ₅₀	Geomean E _r C ₅₀	E _r C ₅₀	NOEC _{community}	NOEC _{community}
		840	1420	840	310	510	869.5	510	45	113	1250	690	680	113	15	15
AF		100	100	60	10	100	100	60	10	10	10	10	10	6	2	1
RAC (µg/L)		8.40	14.2	14	31.0	5.10	8.695	8.5	4.5	11.3	125.0	69.0	68.0	18.83	7.50	15
FOCUS Scenario	PEC _{gl-max} (µg/L)															
Step 1																
	228.92	27.252	16.121	16.35	7.385	44.886	26.328	26.93	50.871	20.258	1.831	3.318	3.366	12.157	30.523	15.261
Step 2																
N-Europe	40.84	4.862	2.876	2.92	1.317	8.008	4.697	4.80	9.076	3.614	0.327	0.592	0.601	2.169	5.445	2.723
S-Europe	74.13	8.825	5.220	5.30	2.391	14.535	8.526	8.72	16.473	6.560	0.593	1.074	1.090	3.937	9.884	4.942
Step 3																
D3/ditch	11.17	1.330	0.787	0.80	0.360	2.190	1.285	1.31	2.482	0.988	0.089	0.162	0.164	0.593	1.489	0.745
D4/pond	0.4510	0.054	0.032	0.03	0.015	0.088	0.052	0.05	0.100	0.040	0.004	0.007	0.007	0.024	0.060	0.030

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D4/stream	9.224	1.098	0.650	0.66	0.298	1.809	1.061	1.09	2.050	0.816	0.074	0.134	0.136	0.490	1.230	0.615
D6/ditch, 1st	11.05	1.315	0.778	0.79	0.356	2.167	1.271	1.30	2.456	0.978	0.088	0.160	0.163	0.587	1.473	0.737
D6/ditch, 2nd	11.24	1.338	0.792	0.80	0.363	2.204	1.293	1.32	2.498	0.995	0.090	0.163	0.165	0.597	1.499	0.749
R1/pond	0.7623	0.091	0.054	0.05	0.025	0.149	0.088	0.09	0.169	0.067	0.006	0.011	0.011	0.040	0.102	0.051
R1/stream	7.721	0.919	0.544	0.55	0.249	1.514	0.888	0.91	1.716	0.683	0.062	0.112	0.114	0.410	1.029	0.515
R2/stream	10.22	1.217	0.720	0.73	0.330	2.004	1.175	1.20	2.271	0.904	0.082	0.148	0.150	0.543	1.363	0.681
R4/stream	10.90	1.298	0.768	0.78	0.352	2.137	1.254	1.28	2.422	0.965	0.087	0.158	0.160	0.579	1.453	0.727
Step 4: 5 unsprayed buffer zone																
D3 ditch	3.662	Not required												0.49	Not required	
D4 stream	3.937													0.52		
D6 ditch, 1st	3.622													0.49		
D6 ditch, 2nd	3.712													0.49		

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb · prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers	Mesocosm	
Step 4: 10 unsprayed buffer zone + 10 m vegetative strip											
R1 stream	2.543	Not required								0.34	Not required
R2 stream	2.345									0.31	
R3 stream	3.671									0.49	

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 GLOB1912H in sunflower

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
Test species		<i>Oncorhynchus mykiss</i>		RAC _{lowest}	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		RAC _{low est}	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>		RAC _{lowest}	Higher tier for <i>Daphnia</i> and primary producers	
Endpoint (µg/L)		LC ₅₀	Geomean LC ₅₀	LC ₅₀	NOEC	EC ₅₀	Geomean EC ₅₀	EC ₅₀	NOEC	ErC ₅₀	NOEC	ErC ₅₀	Geomean ErC ₅₀	ErC ₅₀	NOEC _{community}	NOEC _{community}
AF		840	1420	840	310	510	869.5	510	45	113	1250	690	680	113	15	15
RAC (µg/L)		100	100	60	10	100	100	60	10	10	10	10	10	6	2	1
FOCUS Scenario	PEC _{gl-max} (µg/L)	8.40	14.2	14	31.0	5.10	8.695	8.5	4.5	11.3	125.0	69.0	68.0	18.83	7.50	15
Step 1																
	228.92	27.252	16.121	16.35	7.385	44.886	26.328	26.93	50.871	20.258	1.831	3.318	3.366	12.157	30.523	15.261
Step 2																
N-Europe	40.84	4.862	2.876	2.92	1.317	8.008	4.697	4.80	9.076	3.614	0.327	0.592	0.601	2.169	5.445	2.723
S-Europe	74.13	8.825	5.220	5.30	2.391	14.535	8.526	8.72	16.473	6.560	0.593	1.074	1.090	3.937	9.884	4.942
Step 3																
D3/ditch	11.18	1.3310	0.7873	0.80	0.3606	2.1922	1.2858	1.32	2.4844	0.9894	0.089	0.162	0.164	0.594	1.491	0.745
D4/pond	0.4511	0.0537	0.0318	0.03	0.0146	0.0885	0.0516	0.05	0.1002	0.0399	0.004	0.007	0.007	0.024	0.060	0.030

Group		Fish acute			Fish prolonged	Inverteb. acute			Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers		Mesocosm	
D4/stream	9.571	1.1394	0.6740	0.68	0.3087	1.8767	1.1007	1.13	2.1269	0.8470	0.077	0.139	0.141	0.508	1.276	0.638
D5/pond	0.4512	0.054	0.032	0.03	0.015	0.088	0.052	0.05	0.100	0.040	0.004	0.007	0.007	0.024	0.060	0.030
D5/stream	10.10	1.202	0.711	0.72	0.326	1.980	1.162	1.19	2.244	0.894	0.081	0.146	0.149	0.536	1.347	0.673
R1/pond	0.7971	0.095	0.056	0.06	0.026	0.156	0.092	0.09	0.177	0.071	0.006	0.012	0.012	0.042	0.106	0.053
R1/stream	7.714	0.918	0.543	0.55	0.249	1.513	0.887	0.91	1.714	0.683	0.062	0.112	0.113	0.410	1.029	0.514
R3/stream	10.90	1.298	0.768	0.78	0.352	2.137	1.254	1.28	2.422	0.965	0.087	0.158	0.160	0.579	1.453	0.727
R4/stream	15.72	1.871	1.107	1.12	0.507	3.082	1.808	1.85	3.493	1.391	0.126	0.228	0.231	0.835	2.096	1.048
Step 4: 5 unsprayed buffer zone																
D3 ditch	3.663	Not required												0.49	Not required	
D4 stream	4.091													0.55		
D5 stream	4.316													0.58		
Step 4: 10 unsprayed buffer zone + 10 m vegetative strip																
R1 stream	2.574	Not required												0.34	Not required	
R3 stream	4.643													0.62		

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 sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in winter cereals (pre-emergence)

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)		EC ₅₀	EC ₅₀	HC ₅	NOEC _{community}
AF		13	43	4.84	30
RAC (µg/L)		10	10	3	2
RAC (µg/L)		13	0.43	1.61	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
-	55.12	42.400	128.186	34.236	3.675
Step 2					
N-Europe	10.79	8.300	25.093	6.702	0.719
S-Europe	8.65	6.654	20.116	5.373	0.577
Step 3					
D1/ditch	43.39	33.377	100.907	26.950	2.893
D1/stream	28.45	21.885	66.163	17.671	1.897
D2/ditch	81.08	62.369	188.558	50.360	5.405
D2/stream	51.65	39.731	120.116	32.081	3.443
D3/ditch	<0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	1.827	1.405	4.249	1.135	0.122
D4/stream	3.376	2.597	7.851	2.097	0.225

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D5/pond	5.964	4.588	13.870	3.704	0.398
D5/stream	9.050	6.962	21.047	5.621	0.603
D6/ditch	19.55	15.038	45.465	12.143	1.303
R1/pond	0.2618	0.201	0.609	0.163	0.017
R1/stream	9.351	7.193	21.747	5.808	0.623
R3/stream	7.610	5.854	17.698	4.727	0.507
R4/stream	7.242	5.571	16.842	4.498	0.483

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

Review Comments:

According to EFSA Scientific Report (2007) 111, 1-81, for aquatic organisms the risk assessment for prosulfocarb sulfoxide is not required.

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 GLOB1912H in winter cereals (post-emergence)

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	ErC ₅₀	HC ₅	NOEC _{community}
AF		13	4.3	4.84	30
RAC (µg/L)		10	10	3	2
		1.3	0.43	1.61	15

Group		Aquatic plants	Algae	Primary producers	Mesocosm
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
-	55.12	42.400	128.186	34.236	3.675
Step 2					
N-Europe	10.79	8.300	25.093	6.702	0.719
S-Europe	8.65	6.654	20.116	5.373	0.577
Step 3					
D1/ditch	49.35	37.962	114.767	30.652	3.290
D1/stream	30.94	23.800	71.953	19.217	2.063
D2/ditch	73.44	56.492	170.791	45.615	4.896
D2/stream	46.43	35.715	107.977	28.839	3.095
D3/ditch	<0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	2.816	2.166	6.549	1.749	0.188
D4/stream	5.242	4.032	12.191	3.256	0.349
D5/pond	5.964	4.588	13.870	3.704	0.398
D5/stream	9.050	6.962	21.047	5.621	0.603
D6/ditch	19.42	14.938	45.163	12.062	1.295
R1/pond	0.2601	0.200	0.605	0.162	0.017
R1/stream	9.248	7.114	21.507	5.744	0.617
R3/stream	8.615	6.627	20.035	5.351	0.574
R4/stream	10.20	7.846	23.721	6.335	0.680

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

Review Comments:

According to EFSA Scientific Report (2007) 111, 1-81, for aquatic organisms the risk assessment for prosulfocarb sulfoxide is not required.

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 GLOB1912H 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)		ErC ₅₀ 13	ErC ₅₀ 4.3	HC ₅ 4.84	NOEC _{community} 30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	1.61	15
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
-	55.12	42.400	128.186	34.236	3.675
Step 2					
N-Europe	4.37	3.362	10.163	2.714	0.291
S-Europe	8.65	6.654	20.116	5.373	0.577
Step 3					
D3/ditch	<0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	0.02693	0.021	0.063	0.017	0.002
D4/stream	0.04689	0.036	0.109	0.029	0.003

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D6/ditch, 1st	4.857	3.736	11.295	3.017	0.324
D6/ditch, 2nd	5.796	4.458	13.479	3.600	0.386
R1/pond	0.3752	0.289	0.873	0.233	0.025
R1/stream	7.073	5.441	16.449	4.393	0.472
R2/stream	7.806	6.005	18.153	4.848	0.520
R4/stream	11.74	9.031	27.302	7.292	0.783

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

Review Comments:

According to EFSA Scientific Report (2007) 111, 1-81, for aquatic organisms the risk assessment for prosulfocarb sulfoxide is not required.

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 GLOB1912H in sunflower

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	E _r C ₅₀	HC ₅	NOEC _{community}
AF		13	4.3	4.84	30
RAC (µg/L)		10	10	3	2
		1.3	0.43	1.61	15

Group		Aquatic plants	Algae	Primary producers	Mesocosm
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
-	55.12	42.400	128.186	34.236	3.675
Step 2					
N-Europe	4.37	3.362	10.163	2.714	0.291
S-Europe	8.65	6.654	20.116	5.373	0.577
Step 3					
D3/ditch	<0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	0.04099	0.0315	0.0952	0.0258	0.0027
D4/stream	0.07392	0.0569	0.1719	0.0459	0.0049
D5/pond	0.003939	0.003	0.009	0.002	<0.001
D5/stream	0.005903	0.005	0.014	0.004	<0.001
R1/pond	0.4289	0.330	0.997	0.266	0.029
R1/stream	7.430	5.715	17.279	4.615	0.495
R3/stream	12.40	9.538	28.837	7.702	0.827
R4/stream	15.13	11.638	35.186	9.398	1.009

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

Review Comments:

According to EFSA Scientific Report (2007) 111, 1-81, for aquatic organisms the risk assessment for prosulfocarb sulfoxide is not required.

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for diflufenican for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in winter cereals (pre-emergence)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
Test species		<i>Cyprinus carpio</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>			<i>Chironomus riparius</i>	<i>Lemna gibba</i>	Test species	<i>Chironomus riparius</i>
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	E _r C ₅₀	NOEC	NOEC	E _b C ₅₀	Endpoint (µg/kg)	NOEC
AF		98.5	15	240	52	0.25	0.45	0.1	100	39		2000
RAC (µg/L)		100	10	100	10	10	10	1	10	10	AF	10
		0.985	1.5	2.40	5.2	0.025	0.045	0.1	10	3.9	RAC (µg/kg)	200
FOCUS Scenario	PEC _{gl-max} (µg/L)										PEC _{gl-max} (µg/kg)	
Step 1												
	3.33	3.381	2.220	1.388	0.640	133.200	74.000	33.300	0.333	0.854	92.25	0.461
Step 2												
N-Europe	1.54	1.563	1.027	0.642	0.296	61.600	34.222	15.400	0.154	0.395	46.36	0.232
S-Europe	1.25	1.269	0.833	0.521	0.240	50.000	27.778	12.500	0.125	0.321	37.58	0.188
Step 3												
D1/ditch	0.2879	0.292	0.192	0.120	0.055	11.516	6.398	2.879	0.029	0.074	1.402	0.007
D1/stream	0.2501	0.254	0.167	0.104	0.048	10.004	5.558	2.501	0.025	0.064	0.6336	0.003
D2/ditch	0.3069	0.312	0.205	0.128	0.059	12.276	6.820	3.069	0.031	0.079	1.263	0.006
D2/stream	0.2618	0.266	0.175	0.109	0.050	10.472	5.818	2.618	0.026	0.067	0.8155	0.004
D3/ditch	0.2818	0.286	0.188	0.117	0.054	11.272	6.262	2.818	0.028	0.072	0.1514	0.001
D4/pond	0.009742	0.010	0.006	0.004	0.002	0.390	0.216	0.097	0.001	0.002	0.1277	0.001
D4/stream	0.2444	0.248	0.163	0.102	0.047	9.776	5.431	2.444	0.024	0.063	0.05230	< 0.001

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
D5/pond	0.009792	0.010	0.007	0.004	0.002	0.392	0.218	0.098	0.001	0.003	0.08721	< 0.001
D5/stream	0.2637	0.268	0.176	0.110	0.051	10.548	5.860	2.637	0.026	0.068	0.07345	< 0.001
D6/ditch	0.2850	0.289	0.190	0.119	0.055	11.400	6.333	2.850	0.029	0.073	0.7374	0.004
R1/pond	0.02288	0.023	0.015	0.010	0.004	0.915	0.508	0.229	0.002	0.006	0.3056	0.002
R1/stream	0.1858	0.189	0.124	0.077	0.036	7.432	4.129	1.858	0.019	0.048	0.3328	0.002
R3/stream	0.2580	0.262	0.172	0.108	0.050	10.320	5.733	2.580	0.026	0.066	13.72	0.069
R4/stream	0.1869	0.190	0.125	0.078	0.036	7.476	4.153	1.869	0.019	0.048	0.2446	0.001

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 diflufenican for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in winter cereals (post-emergence)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
Test species		<i>Cyprinus carpio</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>			<i>Chironomus riparius</i>	<i>Lemna gibba</i>	Test species	<i>Chironomus riparius</i>
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	E _r C ₅₀	NOEC	NOEC	E _b C ₅₀	Endpoint (µg/kg)	NOEC
		98.5	15	240	52	0.25	0.45	0.1	100	39		2000
AF		100	10	100	10	10	10	1	10	10	AF	10
RAC (µg/L)		0.985	1.5	2.40	5.2	0.025	0.045	0.1	10	3.9	RAC (µg/kg)	200
FOCUS Scenario	PEC _{gl-max} (µg/L)										PEC _{gl-max} (µg/kg)	
Step 1												
	3.33	3.381	2.220	1.388	0.640	133.200	74.000	33.300	0.333	0.854	92.25	0.461

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
Step 2												
N-Europe	1.54	1.563	1.027	0.642	0.296	61.600	34.222	15.400	0.154	0.395	46.36	0.232
S-Europe	1.25	1.269	0.833	0.521	0.240	50.000	27.778	12.500	0.125	0.321	37.58	0.188
Step 3												
D1/ditch	0.2876	0.292	0.192	0.120	0.055	11.504	6.391	2.876	0.029	0.074	1.378	0.007
D1/stream	0.2501	0.254	0.167	0.104	0.048	10.004	5.558	2.501	0.025	0.064	0.6359	0.003
D2/ditch	0.3130	0.318	0.209	0.130	0.060	12.520	6.956	3.130	0.031	0.080	1.275	0.006
D2/stream	0.2437	0.247	0.162	0.102	0.047	9.748	5.416	2.437	0.024	0.062	0.6295	0.003
D3/ditch	0.2817	0.286	0.188	0.117	0.054	11.268	6.260	2.817	0.028	0.072	0.1451	0.001
D4/pond	0.009742	0.010	0.006	0.004	0.002	0.390	0.216	0.097	0.001	0.002	0.1234	0.001
D4/stream	0.2444	0.248	0.163	0.102	0.047	9.776	5.431	2.444	0.024	0.063	0.05230	< 0.001
D5/pond	0.009782	0.010	0.007	0.004	0.002	0.391	0.217	0.098	0.001	0.003	0.08666	< 0.001
D5/stream	0.2637	0.268	0.176	0.110	0.051	10.548	5.860	2.637	0.026	0.068	0.07344	< 0.001
D6/ditch	0.2850	0.289	0.190	0.119	0.055	11.400	6.333	2.850	0.029	0.073	0.7373	0.004
R1/pond	0.02309	0.023	0.015	0.010	0.004	0.924	0.513	0.231	0.002	0.006	0.3083	0.002
R1/stream	0.1858	0.189	0.124	0.077	0.036	7.432	4.129	1.858	0.019	0.048	0.3327	0.002
R3/stream	0.2607	0.265	0.174	0.109	0.050	10.428	5.793	2.607	0.026	0.067	0.2954	0.001
R4/stream	0.1843	0.187	0.123	0.077	0.035	7.372	4.096	1.843	0.018	0.047	0.2866	0.001

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 diflufenican for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in potato

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
Test species		<i>Cyprinus carpio</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>			<i>Chironomus riparius</i>	<i>Lemna gibba</i>	Test species	<i>Chironomus riparius</i>
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	E _r C ₅₀	NOEC	NOEC	E _b C ₅₀	Endpoint (µg/kg)	NOEC
AF		98.5	15	240	52	0.25	0.45	0.1	100	39		2000
RAC (µg/L)		100	10	100	10	10	10	1	10	10	AF	10
		0.985	1.5	2.40	5.2	0.025	0.045	0.1	10	3.9	RAC (µg/kg)	200
FOCUS Scenario	PEC _{gl-max} (µg/L)										PEC _{gl-max} (µg/kg)	
Step 1												
	3.33	3.381	2.220	1.388	0.640	133.200	74.000	33.300	0.333	0.854	92.25	0.461
Step 2												
N-Europe	0.68	0.690	0.453	0.283	0.131	27.200	15.111	6.800	0.068	0.174	20.01	0.100
S-Europe	1.25	1.269	0.833	0.521	0.240	50.000	27.778	12.500	0.125	0.321	37.58	0.188
Step 3												
D3/ditch	0.2339	0.237	0.156	0.097	0.045	9.356	5.198	2.339	0.023	0.060	0.1688	0.001
D4/pond	0.009431	0.010	0.006	0.004	0.002	0.377	0.210	0.094	0.001	0.002	0.1086	0.001
D4/stream	0.1931	0.196	0.129	0.080	0.037	7.724	4.291	1.931	0.019	0.050	0.03350	< 0.001
D6/ditch, 1st	0.2314	0.235	0.154	0.096	0.045	9.256	5.142	2.314	0.023	0.059	0.08475	< 0.001
D6/ditch, 2nd	0.2352	0.239	0.157	0.098	0.045	9.408	5.227	2.352	0.024	0.060	0.4032	0.002

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
R1/pond	0.02007	0.020	0.013	0.008	0.004	0.803	0.446	0.201	0.002	0.005	0.4716	0.002
R1/stream	0.1616	0.164	0.108	0.067	0.031	6.464	3.591	1.616	0.016	0.041	0.5027	0.003
R2/stream	0.2140	0.217	0.143	0.089	0.041	8.560	4.756	2.140	0.021	0.055	5.307	0.027
R4/stream	0.2282	0.232	0.152	0.095	0.044	9.128	5.071	2.282	0.023	0.059	0.4481	0.002

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 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for diflufenican for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in sunflower

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
Test species		<i>Cyprinus carpio</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>			<i>Chironomus riparius</i>	<i>Lemna gibba</i>	Test species	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	E _r C ₅₀	NOEC	NOEC	E _b C ₅₀	Endpoint (µg/kg)	NOEC
(µg/L)		98.5	15	240	52	0.25	0.45	0.1	100	39		2000
AF		100	10	100	10	10	10	1	10	10	AF	10
RAC (µg/L)		0.985	1.5	2.40	5.2	0.025	0.045	0.1	10	3.9	RAC (µg/kg)	200
FOCUS Scenario	PEC _{gl-max} (µg/L)										PEC _{gl-max} (µg/kg)	
Step 1												
	3.33	3.381	2.220	1.388	0.640	133.200	74.000	33.300	0.333	0.854	92.25	0.461
Step 2												
N-Europe	0.68	0.690	0.453	0.283	0.131	27.200	15.111	6.800	0.068	0.174	20.01	0.100
S-Europe	1.25	1.269	0.833	0.521	0.240	50.000	27.778	12.500	0.125	0.321	37.58	0.188

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
Step 3												
D3/ditch	0.2339	0.2375	0.1559	0.0975	0.0450	9.3560	5.1978	2.3390	0.0234	0.0600	0.1706	< 0.001
D4/pond	0.009433	0.0096	0.0063	0.0039	0.0018	0.3773	0.2096	0.0943	0.0009	0.0024	0.08915	< 0.001
D4/stream	0.2003	0.2034	0.1335	0.0835	0.0385	8.0120	4.4511	2.0030	0.0200	0.0514	0.01657	< 0.001
D5/pond	0.009528	0.010	0.006	0.004	0.002	0.381	0.212	0.095	0.001	0.002	0.08192	< 0.001
D5/stream	0.2114	0.215	0.141	0.088	0.041	8.456	4.698	2.114	0.021	0.054	0.01180	< 0.001
R1/pond	0.02187	0.022	0.015	0.009	0.004	0.875	0.486	0.219	0.002	0.006	0.5152	0.003
R1/stream	0.1615	0.164	0.108	0.067	0.031	6.460	3.589	1.615	0.016	0.041	0.5866	0.003
R3/stream	0.2281	0.232	0.152	0.095	0.044	9.124	5.069	2.281	0.023	0.058	0.5206	0.003
R4/stream	0.1974	0.200	0.132	0.082	0.038	7.896	4.387	1.974	0.020	0.051	0.8154	0.004

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

Review Comments:

For the intended use, calculated PEC/RAC ratios for diflufenican did not indicate an acceptable risk for algae as characterised by RAC values for *Scenedesmus subspicatus* of 0.045 µg/L or 0.1 µg/L. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies. The risk assessment is continued from page 100.

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE0542991 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in winter cereals (pre- and post-emergence)

Group		Algae	Inverteb. acute
Test species		<i>Pseudokirchneriella subcapitata</i>	<i>Daphnia magna</i>
Endpoint (µg/L)		E _b C ₅₀ 36000	EC ₅ 10000

Group		Algae	Inverteb. acute
AF		10	100
RAC (µg/L)		3600	100
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
	2.40	0.001	0.024
Step 2			
N-Europe	1.06	< 0.001	0.011
S-Europe	0.85	< 0.001	0.009

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 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE0542991 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in potato and sunflower

Group		Algae	Inverteb. acute
Test species		<i>Pseudokirchneriella subcapitata</i>	<i>Daphnia magna</i>
Endpoint (µg/L)		E _b C ₅₀ 36000	EC ₅ 10000
AF		10	100
RAC (µg/L)		3600	100
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			

Group		Algae	Inverteb. acute
	2.40	0.001	0.024
Step 2			
N-Europe	0.42	< 0.001	0.004
S-Europe	0.85	< 0.001	0.009

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 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE B107137 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in winter cereals (pre- and post-emergence)

Group		Fish acute	Algae	Aquatic invertebrates
Test species		<i>Oncorhynchus mykiss</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Daphnia magna</i>
Endpoint (µg/L)		LC ₅₀ 17300	E _b C ₅₀ 20400	EC ₅ 20400
AF		100	10	100
RAC (µg/L)		173	2040	204
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	5.66	0.033	0.003	0.028
Step 2				
N-Europe	2.63	0.015	0.001	0.013
S-Europe	2.12	0.012	0.001	0.010

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 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE B107137 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1912H in potato and sunflower

Group		Fish acute	Algae	Aquatic invertebrates
Test species		<i>Oncorhynchus mykiss</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Daphnia magna</i>
Endpoint (µg/L)		LC ₅₀ 17300	E _b C ₅₀ 20400	EC ₅ 20400
AF		100	10	100
RAC (µg/L)		173	2040	204
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	5.66	0.033	0.003	0.028
Step 2				
N-Europe	1.11	0.006	0.001	0.005
S-Europe	2.12	0.012	0.001	0.010

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

Formulation GLOB1912H

The Regulation (EC) No 1107/2009, in Article 29, requires that ‘interaction between the a.s., safeners, synergists and co-formulants shall be taken into account’ in the evaluation and authorisation. Guidance is provided in EFSA (2013¹) to perform the risk assessment for formulations containing more than one active substance.

The first step is to check if measured data on the product exist for the given endpoint (**Step1**). If yes, comparison between product data and active ingredient data will be possible. For GLOB1912H, we have data on the formulation for Daphnia, algae, Lemna and Myriophyllum. As there are no active ingredient data on Myriophyllum for prosulfocarb and diflufenican, the comparison is not possible, so the below scheme will be followed for Daphnia, algae and Lemna.

¹ Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Panel on Plant Protection Products and their Residues (PPR). Scientific opinion, EFSA Journal 2013;11(7):3290.

For fish, only data on the active ingredients are available. Since there is no evidence of synergistic interaction between mixture components (**Step 7**), mixture toxicity calculations according to Step 8 are possible and are shown below.

Concentration addition model (MDR) (step 2)

Therefore the LD₅₀ of the formulated product is compared to the predicted mixture toxicity assuming concentration additivity according to the concentration addition model (CA model). The CA model is based on the following equation^[1], for deriving a predicted ECx or NOEC value for a mixture of (active) substances with known toxicity (ECx_{mix-CA} or NOEC_{mix-CA}), assuming concentration additivity:

Equation 13:
$$ECx_{mix-CA} = \left(\sum_{i=1}^n \frac{p_i}{ECx_i} \right)^{-1}$$

where:

- n: number of mixture components
- i: index from 1...n mixture components
- p_i: the ith component as a relative fraction of the mixture composition (note: Σ p_i must be 1)
- ECx_i: concentration of component i provoking x % effect (pragmatically, NOEC_i may be inserted, too).

When the formulation is more toxic than that predicted from the toxicity of the individual compounds, the use of the endpoint of the formulation is recommended for the first-tier assessment because it cannot be excluded that such effects would also occur after exposure of the aquatic organism to residues in the environment.

Review Comments:

In the mixture toxicity evaluation the lowest endpoints values (E_bC₅₀ or E_yC₅₀) were used.

Table 9.5-19: Comparison of the toxicity of GLOB1912H to the predicted one based on the active ingredients

Aquatic organisms	Fraction of Prosulfocarb in mixture	Fraction of Diflufenican in mixture	Prosulfocarb EC50 (mg a.s./L)	Diflufenican EC50 (mg a.s./L)	EC _{xmix-CA} . Predicted EC50 of GLOB1912H based on the a.s. toxicity (mg as/L)	EC _{xppp} . EC50 of GLOB1912H from the studies (mg a.s./L)	EC _{xppp} . EC ₅₀ of GLOB1817H from the studies (mg sum of a.s./L)	MDR (model deviation ratio)	Comparison toxicity of the formulation and the predicted one
Daphnia	0.98	0.02	0.510	0.240	0.4988	0.954	0.6434	0.77	MDR= 0.2-5
Algae	0.98	0.02	0.113 0.112	0.00025	0.0113 0.011	0.0597 0.0310*	0.0403	0.27 0.52	MDR= 0.2-5
Lemna	0.98	0.02	0.690	0.039	0.5173	0.3352	0.2261	2.27	MDR= 0.2-5
Myriophyllum	0.98	0.02	-	-	-	0.075	-	-	-

*Endpoint based on nominal values is used for the test item even if the recovery of halauxifen-methyl in the spent solutions is too low, which can be expected based on its degradation characteristics in water. Based on the recoveries in the fresh solutions, it can be demonstrated that the exposure was sufficient. Given that diflufenican is driving the toxicity to algae and that halauxifen-methyl is not present in GLOB1912H, the nominal endpoint can be regarded as reflecting the toxicity of the formulation.

The predicted toxicity endpoint has been compared to the formulated product endpoint to derive a MDR by the formula ($MDR = EC_{xmix-CA} / EC_{xppp}$). If MDR is between 0.2 and 5, the observed and calculates toxicities are considered in agreement. If MDR is > 5, the observed toxicity of mixture is higher than that calculated assuming dose additivity. If MDR is < 0.2, the mixture is less toxic than expected.

The MDR for Daphnia, algae and Lemna are between 0.2 and 5, thus the measured and calculated toxicity are in agreement. It means that the toxicity of GLOB1912H is not higher than the predicted one. In this case, EFSA (2013) recommends that the measured toxicity of the mixture be considered in the aquatic risk assessment (see below).

For Myriophyllum, no data is available for the active substances prosulfocarb and diflufenican, and thus the comparison cannot be made. The risk assessment for Myriophyllum will be performed with the product in absence of any other supportive data.

Mixture composition in the formulation versus mixture composition at PECmix (step 3)

The aim of this step is to check whether the mixture composition in the formulation study giving the measured mixture toxicity (EC_{xPPP}) in terms of the relative proportions of the individual active substances is similar to the mixture composition at the PECmix (proportion of each active in the environment (part of the PEC)). The same equation (equation 13) as for step 2 is used, with the difference that here the pi is PEC_i/PEC_{mix}. PEC_{mix} is simply the sum the each PEC_i.

Using the same ECxmix-CA (a.s. in PPP) as the one used in step 2 for MDR, the new ECxmix-CA (a.s. in PECmix) (representing the mixture as it is in the environment) is calculated.

The following results were obtained for Step 1, 2 and 3 (from the AGD_Aquamix_v1.15).

Winter cereals, pre-emergence:

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.99
Step 2	
N-Europe	1.00
S-Europe	1.00
Step 3	
D1 Ditch	1.00
D1 Stream	1.00
D2 Ditch	1.00
D2 Stream	1.00
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D5 Pond	1.00
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	1.00
R1 Stream	1.00
R2 Stream	
R3 Stream	1.00
R4 Stream	1.00

Algae	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.73
Step 2	
N-Europe	0.83
S-Europe	0.83
Step 3	
D1 Ditch	1.00
D1 Stream	1.00
D2 Ditch	1.08
D2 Stream	1.02
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D5 Pond	1.00
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	0.85
R1 Stream	0.88
R2 Stream	
R3 Stream	0.90
R4 Stream	1.00

Macrophytes	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.92
Step 2	
N-Europe	0.95
S-Europe	0.95
Step 3	
D1 Ditch	1.00
D1 Stream	1.00
D2 Ditch	1.02
D2 Stream	1.01
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D5 Pond	1.00
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	0.96
R1 Stream	0.97
R2 Stream	
R3 Stream	0.97
R4 Stream	1.00

Winter cereals, post-emergence:

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.99
Step 2	
N-Europe	1.00
S-Europe	1.00
Step 3	
D1 Ditch	1.00
D1 Stream	1.00
D2 Ditch	1.00
D2 Stream	1.00
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D5 Pond	1.00
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	1.00
R1 Stream	1.00
R2 Stream	
R3 Stream	1.00
R4 Stream	0.99

Algae	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.73
Step 2	
N-Europe	0.83
S-Europe	0.83
Step 3	
D1 Ditch	1.00
D1 Stream	1.00
D2 Ditch	1.09
D2 Stream	1.05
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D5 Pond	1.00
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	0.86
R1 Stream	0.88
R2 Stream	
R3 Stream	0.96
R4 Stream	0.63

Macrophytes	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.92
Step 2	
N-Europe	0.95
S-Europe	0.95
Step 3	
D1 Ditch	1.00
D1 Stream	1.00
D2 Ditch	1.02
D2 Stream	1.01
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D5 Pond	1.00
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	0.96
R1 Stream	0.97
R2 Stream	
R3 Stream	0.99
R4 Stream	0.90

Potato:

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.99
Step 2	
N-Europe	1.00
S-Europe	1.00
Step 3	
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D6 ditch, 1st	1.00
D6 Ditch, 2nd	1.00
R1 Pond	1.01
R1 Stream	1.00
R2 Stream	1.00
R3 Stream	1.00

Algae	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.73
Step 2	
N-Europe	0.82
S-Europe	0.83
Step 3	
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D6 ditch, 1st	1.00
D6 Ditch, 2nd	1.00
R1 Pond	1.22
R1 Stream	1.00
R2 Stream	1.00
R3 Stream	1.00

Macrophytes	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.92
Step 2	
N-Europe	0.95
S-Europe	0.95
Step 3	
D3 Ditch	1.00
D4 Pond	1.00
D4 Stream	1.00
D6 ditch, 1st	1.00
D6 Ditch, 2nd	1.00
R1 Pond	1.06
R1 Stream	1.00
R2 Stream	1.00
R3 Stream	1.00

Sunflower:

Invertebrates		Algae		Macrophytes	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)		ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)		ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.99	Step 1	0.73	Step 1	0.92
Step 2		Step 2		Step 2	
N-Europe	1.00	N-Europe	0.82	N-Europe	0.95
S-Europe	1.00	S-Europe	0.83	S-Europe	0.95
Step 3		Step 3		Step 3	
D3 Ditch	1.00	D3 Ditch	1.00	D3 Ditch	1.00
D4 Pond	1.00	D4 Pond	1.00	D4 Pond	1.00
D4 Stream	1.00	D4 Stream	1.00	D4 Stream	1.00
D5 Pond	1.00	D5 Pond	1.01	D5 Pond	1.00
D5 Stream	1.00	D5 Stream	1.00	D5 Stream	1.00
R1 Pond	1.00	R1 Pond	1.06	R1 Pond	1.02
R1 Stream	1.00	R1 Stream	1.00	R1 Stream	1.00
R3 Stream	1.00	R3 Stream	1.00	R3 Stream	1.00
R4 Stream	0.99	R4 Stream	0.64	R4 Stream	0.90

In general, the ECxmix-CA (a.s. in PPP)/ECxmix-CA (a.s. in PECmix) = 0.8–1.2 (mixture similar), thus measured data can be used in the risk assessment. For algae, in a few cases the mixture is not regarded as similar.

In the next step, a check of a single driver for the toxicity was done.

Driver of toxicity (Step 5)

Following the EFSA Aquatic guidance document^[1], the check of a single drive for the toxicity was made according to the following formula^[1]:

^[1] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290)

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

in which TU is the ratio between the concentration (i.e. c_i) of a mixture component and its toxicological acute (e.g. EC50) or chronic (e.g. long-term NOEC) endpoint.

The other calculations are:

Toxicity of the sum of active ingredients ($TOX_{sum(ai)} = 1/(TU(ai1) + TU(ai2))$)

Contribution to toxicity = $TOX_{sum(ai)} * TU(ai) * 100$

Table 9.5-20: Contribution to toxicity of GLOB1912H by prosulfocarb and diflufenican

Organism	Active substance	EC ₅₀ (mg/L)	Fraction in mixture	Toxic unit	Tox of the sum ai	Contribution to toxicity (%)
Daphnia	Prosulfocarb	0.510	0.98	0.5204	0.4988	95.84
	Diflufenican	0.240	0.02	12.00		4.16
Algae	Prosulfocarb	0.113 0.112	0.98	0.1153 0.114	0.0113 0.011	9.78 9.6
	Diflufenican	0.00025	0.02	0.0125		90.22 90.4
Lemna	Prosulfocarb	0.690	0.98	0.7041	0.5173	73.47
	Diflufenican	0.039	0.02	1.9500		26.53

For Daphnia the toxicity is driven by prosulfocarb (contribution $\geq 90\%$). Therefore, in accordance with the EFSA guidance document^[1] the risk assessment for Daphnia can be based on single-substance toxicity data (EC₅₀s.) for the identified ‘driver’ of mixture toxicity, which is in this case prosulfocarb. Therefore, reference is made to the risk assessment performed with prosulfocarb.

For algae the toxicity is driven by diflufenican (contribution $\geq 90\%$). Therefore, in accordance with the EFSA guidance document^[1] the risk assessment for algae can be based on single-substance toxicity data (ECx a.s.) for the identified ‘driver’ of mixture toxicity, which is in this case diflufenican. Therefore, reference is made to the risk assessment performed with diflufenican.

Toxicity to Lemna is not driven by one single active substance. Since the observed and calculated toxicities are considered in agreement; the measured mixture toxicity can be used for the risk assessment. Taking into account that different assessment factors and additional data are available, a refined risk assessment using the RQ_{mix} (Step 8b) is performed.

For Myriophyllum, no data is available for the active substances prosulfocarb and diflufenican, and thus the calculation cannot be made. Therefore, the measured mixture toxicity is used for the risk assessment and compared to the PEC_{mix}.

Refined risk assessment for Lemna using RQ_{mix} (Step 8b)

The calculation of the mixture toxicity is based on the regulatory acceptable concentration of the individual a.s. (RAC_i) using the following formula yielding a risk quotient for the mixture:

Equation 21:

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

For prosulfocarb, the ETO-RAC from the mesocosm study in combination with the assessment factor of 1 is used, leading to a RAC of 15 µg/L. For diflufenican, the Tier 1 endpoint is used leading to a RAC of 3.9 µg/L.

When considering the PEC_{sw} obtained in STEP 2, the RQ_{mix} is above 1 and the risk is not considered acceptable:

Winter cereals: $RQ_{mix} = (90.77/15) + (1.54/3.9) = 6.45$

Potato: $RQ_{mix} = (74.13/15) + (1.25/3.9) = 5.26$

Sunflower: $RQ_{mix} = (74.13/15) + (1.25/3.9) = 5.26$

However the calculation can be refined using the highest PEC_{sw} value obtained in STEP 3 for prosulfocarb and diflufenican. Values obtained in FOCUS scenario D2 are excluded since the risk remains unresolved in this scenario for the active substance diflufenican. The RQ_{mix} is below 1 for potato, at 1 for the pre-emergence use in winter cereals and just slightly above 1 for the post-emergence use in winter cereals as well as the use in sunflower. However, the risk can be considered acceptable for all uses, especially when taking into account that the calculation could be refined even further by using the PEC_{sw} values obtained in STEP 4 for prosulfocarb and diflufenican. Therefore, it can be concluded that the mitigation measures needed to protect aquatic organisms based on the risk assessment of the individual active substances will be sufficient to protect Lemna from exposure to the mixture.

Winter cereals, pre-emergence: $RQ_{mix} = (13.92/15) + (0.3069/3.9) = 1.0$

Winter cereals, post-emergence: $RQ_{mix} = (14.95/15) + (0.3130/3.9) = 1.1$

Potato: $RQ_{mix} = (11.24/15) + (0.2352/3.9) = 0.81$

Sunflower: $RQ_{mix} = (15.72/15) + (0.2339/3.9) = 1.1$

Risk assessment for Myriophyllum

The RAC of 7.5 µg/L, based on measured mixture toxicity, is compared to the PEC_{mix}, which is calculated as the sum of PEC_{sw} of the individual active substances. The individual PEC_{sw}, the PEC_{mix} and the PEC/RAC ratio can be found in the table below.

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1912H for each organism group based on PEC_{mix} calculations – Step 1-2-3

Use	FOCUS scenario	PEC _{sw}		PEC _{mix}	PEC/RAC
		Prosulfocarb	Diflufenican		
Winter cereals, pre-emergence	Step 1	228.92	3.33	232.25	30.97
	Step 2				
	N-Europe	90.77	1.54	92.31	12.31
	S-Europe	74.13	1.25	75.38	10.05
	Step 3				
	D1 Ditch	13.66	0.2879	13.9479	1.86
	D1 Stream	11.95	0.2501	12.2001	1.63
	D2 Ditch	13.68	0.3069	13.9869	1.86
	D2 Stream	12.17	0.2618	12.4318	1.66
	D3 Ditch	13.46	0.2818	13.7418	1.83
	D4 Pond	0.4659	0.009742	0.475642	0.06

	D4 Stream	11.68	0.2444	11.9244	1.59
	D5 Pond	0.4668	0.009792	0.476592	0.06
	D5 Stream	12.60	0.2637	12.8637	1.72
	D6 Ditch	13.62	0.2850	13.905	1.85
	R1 Pond	1.309	0.02288	1.33188	0.18
	R1 Stream	10.28	0.1858	10.4658	1.40
	R3 Stream	13.92	0.2580		
	R4 Stream	8.931	0.1869	9.1179	1.22
Winter cereals, post-emergence	Step 1	228.92	3.33	232.25	30.97
	Step 2				
	N-Europe	90.77	1.54	92.31	12.31
	S-Europe	74.13	1.25	75.38	10.05
	Step 3				
	D1 Ditch	13.66	0.2876	13.9476	1.86
	D1 Stream	11.95	0.2501	12.2001	1.63
	D2 Ditch	13.58	0.3130	13.893	1.85
	D2 Stream	11.02	0.2437	11.2637	1.50
	D3 Ditch	13.46	0.2817	13.7417	1.83
	D4 Pond	0.4659	0.009742	0.475642	0.06
	D4 Stream	11.68	0.2444	11.9244	1.59
	D5 Pond	0.4673	0.009782	0.477082	0.06
	D5 Stream	12.60	0.2637	12.8637	1.72
	D6 Ditch	13.62	0.2850	13.905	1.85
	R1 Pond	1.302	0.02309	1.32509	0.18
	R1 Stream	10.20	0.1858	10.3858	1.38
	R3 Stream	12.97	0.2607	13.2307	1.76
	R4 Stream	14.95	0.1843	15.1343	2.02
Potato	Step 1	228.92	3.33	232.25	30.97
	Step 2				
	N-Europe	40.84	0.68	41.52	5.54
	S-Europe	74.13	1.25	75.38	10.05

	Step 3				
	D3 ditch	11.17	0.2339	11.4039	1.52
	D4 Pond	0.4510	0.009431	0.460431	0.06
	D4 Stream	9.224	0.1931	9.4171	1.26
	D6 Ditch, 1st	11.05	0.2314	11.2814	1.50
	D6 Ditch, 2nd	11.24	0.2352	11.4752	1.53
	R1 Pond	0.7623	0.02007	0.78237	0.10
	R1 Stream	7.721	0.1616	7.8826	1.05
	R2 Stream	10.22	0.2140	10.434	1.39
	R4 Stream	10.90	0.2282	11.1282	1.48
Sunflower	Step 1	228.92	3.33	232.25	30.97
	Step 2				
	N-Europe	40.8	0.68	41.48	5.53
	S-Europe	74.13	1.25	75.38	10.05
	Step 3				
	D3 Ditch	11.18	0.2339	11.4139	1.52
	D4 Pond	0.4511	0.009433	0.46053	0.06
	D4 Stream	9.571	0.2003	9.7713	1.30
	D5 Pond	0.4512	0.009528	0.460728	0.06
	D5 Stream	10.10	0.2114	10.3114	1.37
	R1 Pond	0.7971	0.02187	0.81897	0.11
	R1 Stream	7.714	0.1615	7.8755	1.05
	R3 Stream	10.90	0.2281	11.1281	1.48
	R4 Stream	15.72	0.1974	15.9174	2.12

As can be seen from the table above, the risk is not acceptable in all scenarios. Therefore, the PEC_{sw} values for prosulfocarb and diflufenican obtained in Step 4 using a buffer zone of 5 m (D scenarios) or a buffer zone of 10 m including a 10 m vegetated filter strip (R scenarios) were used to refine the calculations. The resulting PEC/RAC ratios are all below 1, so the risk is considered to be acceptable.

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1912H for each organism group based on PECmix calculations – Step 4

Use	FOCUS scenario	PEC _{sw}		PECMix	PEC/RAC
		Prosulfocarb	Di flufenican		
Winter cereals, pre-emergence	D1 Ditch	3.799	0.07945	3.87845	0.52
	D1 Stream	4.37	0.09137	4.46137	0.59
	D2 Ditch	3.807	0.1575	3.9645	0.53
	D2 Stream	4.451	0.1001	4.5511	0.61
	D3 Ditch	3.65	0.07639	3.72639	0.50
	D4 Stream	4.284	0.08927	4.37327	0.58
	D5 Stream	4.608	0.09631	4.70431	0.63
	D6 Ditch	7.401	0.1051	7.5061	1.00
	R1 Stream	4.6	0.05324	4.65324	0.62
	R3 Stream	6.354	0.0622	6.4162	0.86
	R4 Stream	3.64	0.07642	3.71642	0.50
Winter cereals, post-emergence	D1 Ditch	3.8	0.07914	3.87914	0.52
	D1 Stream	4.37	0.09137	4.46137	0.59
	D2 Ditch	3.683	0.1564	3.8394	0.51
	D2 Stream	4.09	0.0987	4.1887	0.56
	D3 Ditch	3.648	0.07635	3.72435	0.50
	D4 Stream	4.284	0.08927	4.37327	0.58
	D5 Stream	4.608	0.09631	4.70431	0.63
	D6 Ditch	7.401	0.1087	7.5097	1.00
	R1 Stream	4.564	0.05374	4.61774	0.62
	R3 Stream	5.84	0.05535	5.89535	0.79
	R4 Stream	6.748	0.07866	6.82666	0.91
Potato	D3 Ditch	3.662	0.07664	3.73864	0.50
	D4 Stream	3.937	0.08127	4.01827	0.54
	D6 Ditch, 1 st	3.622	0.07588	3.69788	0.49
	D6 Ditch, 2nd	3.712	0.1002	3.8122	0.51
	R1 Stream	2.543	0.05011	2.59311	0.35

Sunflower	R2 Stream	2.345	0.0248	2.3698	0.32
	R4 Stream	3.671	0.02917	3.70017	0.49
	D3 Ditch	3.663	0.07666	3.73966	0.50
	D4 Stream	4.091	0.08432	4.17532	0.56
	D5 Stream	4.316	0.08897	4.40497	0.59
	R1 Stream	2.574	0.05201	2.62601	0.35
	R3 Stream	4.643	0.05852	4.70152	0.63
	R4 Stream	7.148	0.08974	7.23774	0.97

Risk assessment for fish (Step 8)

Taking into account that additional data are available for prosulfocarb, a refined risk assessment using the RQmix (Step 8b) is performed. The calculation of the mixture toxicity is based on the regulatory acceptable concentration of the individual a.s. (RAC_i) using the following formula yielding a risk quotient for the mixture:

Equation 21:

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

For prosulfocarb, a geomean LC₅₀ is used, leading to a RAC of 14.2 µg/L. For diflufenican, the Tier 1 endpoint is used leading to a RAC of 0.985 µg/L.

When considering the PEC_{sw} obtained in STEP 2, the RQ_{mix} is above 1 and the risk is not considered acceptable:

Winter cereals: RQ_{mix} = (90.77/14.2) + (1.54/0.985) = 7.96

Potato: RQ_{mix} = (74.13/14.2) + (1.25/0.985) = 6.49

Sunflower: RQ_{mix} = (74.13/14.2) + (1.25/0.985) = 6.49

However the calculation can be refined using the highest PEC_{sw} value obtained in STEP 3 for prosulfocarb and diflufenican. Values obtained in FOCUS scenario D2 are excluded since the risk remains unresolved in this scenario for the active substance diflufenican.

Winter cereals, pre-emergence: RQ_{mix} = (13.92/14.2) + (0.3069/0.985) = 1.29

Winter cereals, post-emergence: RQ_{mix} = (14.95/14.2) + (0.3130/0.985) = 1.37

Potato: RQ_{mix} = (11.24/14.2) + (0.2352/0.985) = 1.03

Sunflower: RQ_{mix} = (15.72/14.2) + (0.2339/0.985) = 1.34

The RQmix is close to 1, but the risk can be further refined by using the PECsw values obtained in STEP 4 for prosulfocarb and diflufenican using a buffer zone of 5 m (D scenarios) or a buffer zone of 10 m including a 10 m vegetated filter strip (R scenarios).

Table 9.5-23: Aquatic organisms: acceptability of risk for GLOB1912H for each organism group based on RQmix – Step 4

Use	FOCUS scenario	PECsw		RQmix
		Prosulfocarb	Diflufenican	
Winter cereals, pre-emergence	D1 Ditch	3.799	0.07945	0.3482
	D1 Stream	4.37	0.09137	0.4005
	D2 Ditch	3.807	0.1575	0.4280
	D2 Stream	4.451	0.1001	0.4151
	D3 Ditch	3.65	0.07639	0.3346
	D4 Stream	4.284	0.08927	0.3923
	D5 Stream	4.608	0.09631	0.4223
	D6 Ditch	7.401	0.1051	0.6279
	R1 Stream	4.6	0.05324	0.3780
	R3 Stream	6.354	0.0622	0.5106
Winter cereals, post-emergence	R4 Stream	3.64	0.07642	0.3339
	D1 Ditch	3.8	0.07914	0.3480
	D1 Stream	4.37	0.09137	0.4005
	D2 Ditch	3.683	0.1564	0.4181
	D2 Stream	4.09	0.0987	0.3882
	D3 Ditch	3.648	0.07635	0.3344
	D4 Stream	4.284	0.08927	0.3923
	D5 Stream	4.608	0.09631	0.4223
	D6 Ditch	7.401	0.1087	0.6316
	R1 Stream	4.564	0.05374	0.3760
Potato	R3 Stream	5.84	0.05535	0.4675
	R4 Stream	6.748	0.07866	0.5551
	D3 Ditch	3.662	0.07664	0.3357
	D4 Stream	3.937	0.08127	0.3598

	D6 Ditch, 1 st	3.622	0.07588	0.3321
	D6 Ditch, 2nd	3.712	0.1002	0.3631
	R1 Stream	2.543	0.05011	0.2300
	R2 Stream	2.345	0.0248	0.1903
	R4 Stream	3.671	0.02917	0.2881
Sunflower	D3 Ditch	3.663	0.07666	0.3358
	D4 Stream	4.091	0.08432	0.3737
	D5 Stream	4.316	0.08897	0.3943
	R1 Stream	2.574	0.05201	0.2341
	R3 Stream	4.643	0.05852	0.3864
	R4 Stream	7.148	0.08974	0.5945

The resulting RQmix are all below 1, so the risk is considered to be acceptable. Therefore, it can be concluded that the mitigation measures needed to protect aquatic organisms based on the risk assessment of the individual active substances will be sufficient to protect fish from exposure to the mixture.

PECsw from FOCUS Drift Swash Tool

For completeness, the endpoints for those organisms where no driver of toxicity was detected (*Lemna* and *Myriophyllum*) were also compared to the PECsw of the formulation GLOB1912H calculated using the Drift Swash Calculator. This model takes into account spray drift as the only contamination route to the surface water for the formulation. These PECsw were calculated for the ditch, pond and stream scenarios (see Table 8.9-44 in dRR Part B8). The PEC/RAC ratios for aquatic organisms are shown in the table below.

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1912H for each organism group based on FOCUS Drift Swash Tool calculations for the use in winter cereals, potato and sunflowers

Group	Aquatic plants	
Test species	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)	EC ₅₀ 335.2	EC ₅₀ 75
AF	10	10

Group		Aquatic plants	
RAC (µg/L)		33.52	7.5
FOCUS Scenario	PEC _{gl-max} (µg/L)		
1 m			
	24.9096	0.743	3.321
5 m			
	6.7519	-	0.900

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 LC_{50} 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.

Review Comments:

Due to the numerous changes in the risk assessment introduced by zRMS, the PEC/RAC ratio for prosulfocarb has been added directly to each of the tables above.

Table 9.5-25: ~~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 GLOB1912H in winter cereals (post-emergence)~~

Intended-use		Winter cereals (post-emergence)		
Active substance		Prosulfocarb		
Application rate (g/ha)		1 × 2134		
Nozzle reduction	No-spray buffer (m)	5	10	10
	Vegetated filter-strip (m)	None	None	10
None	R4 stream	14.95	14.95	6.748
50 %		14.95	-	-
75 %		-	-	-
90 %		-	-	-
RAC (µg/L)				
14.20		PEC/RAC ratio		
None	R4 stream	1.053	1.053	0.475
50 %		1.053	-	-
75 %		-	-	-
90 %		-	-	-

Table 9.5-26: ~~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 GLOB1912H in sunflower~~

Intended use		Sunflower		
Active substance		Prosulfocarb		
Application rate (g/ha)		1 × 2134		
Nozzle reduction	No-spray buffer (m)	5	10	10
	Vegetated filter-strip (m)	None	None	10

None	R4 stream	15.72	15.72	7.148
50 %		15.72	-	-
75 %		-	-	-
90 %		-	-	-
RAC (µg/L)				
14.20		PEC/RAC ratio		
None	R4 stream	1.107	1.107	0.503
50 %		1.107	-	-
75 %		-	-	-
90 %		-	-	-

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 8.9-22 to 8.9-25 in dRR Section B8). As shown in the table below, an acceptable risk can be demonstrated already in Step 3 and no mitigation measures are necessary.

Table 9.5-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism-group based on Tier 2 calculations for the use of GLOB1912H in winter cereals (pre-emergence)

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	ErC ₅₀	HC ₅	NOEC _{community}
AF		13	4.3	4.84	30
RAC (µg/L)		10	10	3	2
FOCUS Scenario	PEC _{gl-max} (µg/L)	1.3	0.43	1.61	15
D1/ditch	2.239	1.722	5.207	1.391	0.149
D1/stream	1.417	1.090	3.295	0.880	0.094
D2/ditch	9.868	7.591	22.949	6.129	0.658
D2/stream	6.322	4.863	14.702	3.927	0.421
D3/ditch	< 0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	0.009321	0.007	0.022	0.006	0.001
D4/stream	0.01667	0.013	0.039	0.010	0.001
D5/pond	0.03390	0.026	0.079	0.021	0.002
D5/stream	0.2084	0.160	0.485	0.129	0.014

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D6/ditch	2.182	1.678	5.074	1.355	0.145
R1/pond	0.07442	0.057	0.173	0.046	0.005
R1/stream	6.959	5.353	16.184	4.322	0.464
R3/stream	9.508	7.314	22.112	5.906	0.634
R4/stream	1.536	1.182	3.572	0.954	0.102

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 9.5-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1912H in winter cereals (post-emergence)

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	ErC ₅₀	HC ₅	NOEC _{community}
AF		13	4.3	4.84	30
RAC (µg/L)		10	10	3	2
FOCUS Scenario	PEC _{gl-max} (µg/L)	1.3	0.43	1.61	15
D1/ditch	8.048	6.191	18.716	4.999	0.537
D1/stream	5.290	4.069	12.302	3.286	0.353
D2/ditch	6.645	5.112	15.453	4.127	0.443
D2/stream	4.294	3.303	9.986	2.667	0.286
D3/ditch	< 0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	0.2150	0.165	0.500	0.134	0.014
D4/stream	0.3837	0.295	0.892	0.238	0.026
D5/pond	0.03620	0.028	0.084	0.022	0.002
D5/stream	0.2228	0.171	0.518	0.138	0.015
D6/ditch	0.7463	0.574	1.736	0.464	0.050
R1/pond	0.001055	0.001	0.002	0.001	0.000
R1/stream	0.9097	0.700	2.116	0.565	0.061
R3/stream	7.643	5.879	17.774	4.747	0.510
R4/stream	1.536	1.182	3.572	0.954	0.102

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 9.5-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1912H 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	E _r C ₅₀	HC ₅	NOEC _{community}
AF		13	4.3	4.84	30
RAC (µg/L)		10	10	3	2
FOCUS Scenario		1.3	0.43	1.61	15
PEC _{gl-max} (µg/L)					
D3/ditch	< 0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	0.000007	<0.001	<0.001	<0.001	<0.001
D4/stream	0.000020	<0.001	<0.001	<0.001	<0.001
D6/ditch, 1st	1.116	0.858	2.595	0.693	0.074
D6/ditch, 2nd	0.02710	0.021	0.063	0.017	0.002
R1/pond	0.1137	0.087	0.264	0.071	0.008
R1/stream	2.791	2.147	6.491	1.734	0.186
R2/stream	0.5735	0.441	1.334	0.356	0.038
R4/stream	0.004172	0.003	0.010	0.003	0.000

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 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1912H in sunflower

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	E _r C ₅₀	HC ₅	NOEC _{community}
AF		13	4.3	4.84	30
RAC (µg/L)		10	10	3	2
FOCUS Scenario		1.3	0.43	1.61	15
PEC _{gl-max} (µg/L)					

Group		Aquatic plants	Algae	Primary producers	Mesocosm
FOCUS Scenario	PEC _{gl-max} (µg/L)				
D3/ditch	0.000001	<0.001	<0.001	<0.001	<0.001
D4/pond	0.000001	<0.001	<0.001	<0.001	<0.001
D4/stream	0.000002	<0.001	<0.001	<0.001	<0.001
D5/pond	<0.000001	<0.001	<0.001	<0.001	<0.001
D5/stream	<0.000001	<0.001	<0.001	<0.001	<0.001
R1/pond	0.03055	0.024	0.071	0.019	0.002
R1/stream	1.186	0.912	2.758	0.737	0.079
R3/stream	0.03671	0.028	0.085	0.023	0.002
R4/stream	2.223	1.710	5.170	1.381	0.148

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 diflufenican did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for algae as characterised by an EC₅₀ and NOEC for *Scenedesmus subspicatus* of 0.45 µg/L or 0.1 µg/L in connection with an assessment factor of 10 or 1, respectively) 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 9.5-31: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data for algae with mitigation of spray drift and run-off for the use of GLOB1912H in winter cereals (pre-emergence)

Intended use		Winter cereals (pre-emergence)				
Active substance		Diflufenican				
Application rate (g/ha)		1 × 44.8				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D1 ditch	0.07945	0.07028	0.07028	-	-
50 %		0.07028	0.07028	-	-	-
75 %		0.07027	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	0.09137	0.04846	0.04421	-	-
50 %		0.04567	0.04421	-	-	-
75 %		0.04421	-	-	-	-
90 %		-	-	-	-	-

None	D2 ditch	0.1575	0.1575	0.1575	-	-
50 %		0.1575	0.1575	-	-	-
75 %		0.1575	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	0.1001	0.09928	0.09928	-	-
50 %		0.09928	0.09928	-	-	-
75 %		0.09928	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	0.07639	0.04048	0.02103	-	-
50 %		0.03816	0.02024	-	-	-
75 %		0.01908	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.08927	0.04734	0.03271	-	-
50 %		0.04461	0.03271	-	-	-
75 %		0.03271	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	0.09631	0.05107	0.02652	-	-
50 %		0.04812	0.02552	-	-	-
75 %		0.02408	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	0.1051	0.1051	0.1051	-	-
50 %		0.1051	0.1051	-	-	-
75 %		0.1051	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	0.1190	0.1190	0.1190	0.05324	0.02773
50 %		0.1190	0.1190	-	0.05324	-
75 %		0.1190	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	0.1363	0.1363	0.1363	0.06220	0.03262
50 %		0.1363	0.1363	-	0.06220	-
75 %		0.1363	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	0.1693	0.1693	0.1693	0.07642	0.03990
50 %		0.1693	0.1693	-	0.07642	-
75 %		0.1693	-	-	-	-
90 %		-	-	-	-	-

RAC (µg/L)		PEC/RAC ratio				
0.045						
None	D1 ditch	1.7656	1.5618	1.5618	-	-
50 %		1.5618	1.5618	-	-	-
75 %		1.5616	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	2.0304	1.0769	0.9824	-	-
50 %		1.0149	0.9824	-	-	-
75 %		0.9824	-	-	-	-
90 %		-	-	-	-	-
None	D2 ditch	3.5000	3.5000	3.5000	-	-
50 %		3.5000	3.5000	-	-	-
75 %		3.5000	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	2.2244	2.2062	2.2062	-	-
50 %		2.2062	2.2062	-	-	-
75 %		2.2062	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	1.6976	0.8996	-	-	-
50 %		0.8480	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	1.9838	1.0520	0.7269	-	-
50 %		0.9913	0.7269	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	2.1402	1.1349	0.5893	-	-
50 %		1.0693	0.5671	-	-	-
75 %		0.5351	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	2.3356	2.3356	2.3356	-	-
50 %		2.3356	2.3356	-	-	-
75 %		2.3356	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	2.6444	2.6444	2.6444	1.1831	0.6162
50 %		2.6444	2.6444	-	1.1831	-
75 %		2.6444	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	3.0289	3.0289	3.0289	1.3822	0.7249

50 %		3.0289	3.0289	-	1.3822	-
75 %		3.0289	-	-	-	-
90 %		-	-	-	-	-
None		3.7622	3.7622	3.7622	1.6982	0.8867
50 %	R4 stream	3.7622	3.7622	-	1.6982	-
75 %		3.7622	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L)						
0.1		PEC/RAC ratio				
None		0.7945	-	-	-	-
50 %	D1 ditch	-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None		0.9137	-	-	-	-
50 %	D1 stream	-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None		1.5750	1.5750	1.5750	-	-
50 %	D2 ditch	1.5750	1.5750	-	-	-
75 %		1.5750	-	-	-	-
90 %		-	-	-	-	-
None		1.0010	0.9928	-	-	-
50 %	D2 stream	0.9928	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None		0.7639	-	-	-	-
50 %	D3 ditch	-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None		0.8927	-	-	-	-
50 %	D4 stream	-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None		0.9631	-	-	-	-
50 %	D5 stream	-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None		1.0510	1.0510	1.0510	-	-
50 %	D6 ditch	1.0510	1.0510	-	-	-

75 %		1.0510	-	-	-	-
90 %		-	-	-	-	-
None		1.1900	1.1900	1.1900	0.5324	-
50 %		1.1900	1.1900	-	-	-
75 %	R1 stream	1.1900	-	-	-	-
90 %		-	-	-	-	-
None		1.3630	1.3630	1.3630	0.6220	-
50 %		1.3630	1.3630	-	-	-
75 %	R3 stream	1.3630	-	-	-	-
90 %		-	-	-	-	-
None		1.6930	1.6930	1.6930	0.7642	-
50 %		1.6930	1.6930	-	-	-
75 %	R4 stream	1.6930	-	-	-	-
90 %		-	-	-	-	-

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

Table 9.5-32: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data for algae with mitigation of spray drift and run-off for the use of GLOB1912H in winter cereals (post-emergence)

Intended use		Winter cereals (post-emergence)				
Active substance		Diflufenican				
Application rate (g/ha)		1 × 44.8				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None		0.07914	0.07245	0.07245	-	-
50 %		0.07245	0.07245	-	-	-
75 %	D1 ditch	0.07245	-	-	-	-
90 %		-	-	-	-	-
None		0.09137	0.04846	0.04561	-	-
50 %		0.04566	0.04561	-	-	-
75 %	D1 stream	0.04561	-	-	-	-
90 %		-	-	-	-	-
None		0.1564	0.1564	0.1564	-	-
50 %		0.1564	0.1564	-	-	-
75 %	D2 ditch	0.1564	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	0.0987	0.0987	0.0987	-	-

50 %		0.0987	0.0987	-	-	-
75 %		0.0987	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	0.07635	0.04046	0.02102	-	-
50 %		0.03815	0.02023	-	-	-
75 %		0.01907	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.08927	0.04734	0.03089	-	-
50 %		0.04461	0.03089	-	-	-
75 %		0.03089	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	0.09631	0.05107	0.02652	-	-
50 %		0.04812	0.02552	-	-	-
75 %		0.02406	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	0.1087	0.1087	0.1087	-	-
50 %		0.1087	0.1087	-	-	-
75 %		0.1087	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	0.1200	0.1200	0.1200	0.05374	0.02798
50 %		0.1200	0.1200	-	0.05374	-
75 %		0.1200	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	0.1229	0.1229	0.1229	0.05535	0.02892
50 %		0.1229	0.1229	-	0.05535	-
75 %		0.1229	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	0.1743	0.1743	0.1743	0.07866	0.04108
50 %		0.1743	0.1743	-	0.07866	-
75 %		0.1743	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L)						
0.045		PEC/RAC ratio				
None	D1 ditch	1.7587	1.6100	1.6100	-	-
50 %		1.6100	1.6100	-	-	-
75 %		1.6100	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	2.0304	1.0769	1.0136	-	-
50 %		1.0147	1.0136	-	-	-

75 %		1.0136	-	-	-	-
90 %		-	-	-	-	-
None	D2 ditch	3.4756	3.4756	3.4756	-	-
50 %		3.4756	3.4756		-	-
75 %		3.4756	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	2.1933	2.1933	2.1933	-	-
50 %		2.1933	2.1933	-	-	-
75 %		2.1933	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	1.6967	0.8991	0.4671	-	-
50 %		0.8478	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	1.9838	1.0520	0.6864	-	-
50 %		0.9913	0.6864	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	2.1402	1.1349	0.5893	-	-
50 %		1.0693	0.5671	-	-	-
75 %		0.5347	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	2.4156	2.4156	2.4156	-	-
50 %		2.4156	2.4156	-	-	-
75 %		2.4156	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	2.6667	2.6667	2.6667	1.1942	0.6218
50 %		2.6667	2.6667	-	1.1942	-
75 %		2.6667	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	2.7311	2.7311	2.7311	1.2300	0.6427
50 %		2.7311	2.7311	-	1.2300	-
75 %		2.7311	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	3.8733	3.8733	3.8733	1.7480	0.9129
50 %		3.8733	3.8733	-	1.7480	-
75 %		3.8733	-	-	-	-
90 %		-	-	-	-	-

RAC (µg/L)		PEC/RAC ratio				
0.1						
None	D1 ditch	0.7914	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	0.9137	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D2 ditch	1.5640	1.5640	1.5640	-	-
50 %		1.5640	1.5640	-	-	-
75 %		1.5640	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	0.9870	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	0.7635	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.8927	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	0.9631	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	1.0870	1.0870	1.0870	-	-
50 %		1.0870	1.0870	-	-	-
75 %		1.0870	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	1.2000	1.2000	1.2000	0.5374	-
50 %		1.2000	1.2000	-	-	-
75 %		1.2000	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	1.2290	1.2290	1.2290	0.5535	-

50 %		1.2290	1.2290	-	-	-
75 %		1.2290	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	1.7430	1.7430	1.7430	0.7866	-
50 %		1.7430	1.7430	-	-	-
75 %		1.7430	-	-	-	-
90 %		-	-	-	-	-

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

Table 9.5-33: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data for algae with mitigation of spray drift and run-off for the use of GLOB1912H in potato

Intended use		Potato				
Active substance		Diflufenican				
Application rate (g/ha)		1 × 44.8				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D3 ditch	0.07664	0.04062	0.02110	-	-
50 %		0.03829	0.03829	-	-	-
75 %		0.01914	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.08127	0.04310	0.03138	-	-
50 %		0.04061	0.04061	-	-	-
75 %		0.03138	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch, 1st	0.07588	0.04822	0.04822	-	-
50 %		0.04822	0.04822	-	-	-
75 %		0.04822	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch, 2nd	0.1002	0.1002	0.1002	-	-
50 %		0.1002	0.1002	-	-	-
75 %		0.1002	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	0.1107	0.1107	0.1107	0.05011	0.02619
50 %		0.1107	0.1107	-	0.05011	-
75 %		0.1107	-	-	-	-
90 %		-	-	-	-	-

None	R2 stream	0.09009	0.04777	0.04141	0.04777	0.02480
50 %		0.04501	0.04501	-	0.02387	-
75 %		0.04141	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	0.1220	0.1220	0.1220	0.05563	0.02917
50 %		0.1220	0.1220	-	0.05563	-
75 %		0.1220	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L)						
0.045		PEC/RAC ratio				
None	D3 ditch	1.7031	0.9027	-	-	-
50 %		0.8509	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	1.8060	0.9578	-	-	-
50 %		0.9024	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch, 1st	1.6862	1.0716	1.0716	-	-
50 %		1.0716	1.0716	-	-	-
75 %		1.0716	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch, 2nd	2.2267	2.2267	2.2267	-	-
50 %		2.2267	2.2267	-	-	-
75 %		2.2267	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	2.4600	2.4600	2.4600	1.1136	0.5820
50 %		2.4600	2.4600	-	1.1136	-
75 %		2.4600	-	-	-	-
90 %		-	-	-	-	-
None	R2 stream	2.0020	1.0616	0.9202	1.0616	0.5511
50 %		1.0002	1.0002	-	0.5304	-
75 %		0.92022	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	2.7111	2.7111	2.7111	1.2362	0.6482
50 %		2.7111	2.7111	-	1.2362	-
75 %		2.7111	-	-	-	-
90 %		-	-	-	-	-

RAC (µg/L)		PEC/RAC ratio				
0.1						
None	D3 ditch	0.7664	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.8127	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch, 1st	0.7588	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch, 2nd	1.0020	1.0020	1.0020	-	-
50 %		1.0020	1.0020	-	-	-
75 %		1.0020	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	1.1070	1.1070	1.1070	0.5011	-
50 %		1.1070	1.1070	-	-	-
75 %		1.1070	-	-	-	-
90 %		-	-	-	-	-
None	R2 stream	0.9009	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	1.2200	1.2200	1.2200	0.5563	-
50 %		1.2200	1.2200	-	-	-
75 %		1.2200	-	-	-	-
90 %		-	-	-	-	-

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

Table 9.5-34: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data for algae with mitigation of spray drift and run-off for the use of GLOB1912H in sunflower

Intended use		Sunflower				
Active substance		Diflufenican				
Application rate (g/ha)		1 × 44.8				
Nozzle reduction	No-spray buffer (m)	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D3 ditch	0.07666	0.4062	-	-	-
50 %		0.03830	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.08432	0.04472	-	-	-
50 %		0.04213	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	0.08897	0.04718	0.02450	-	-
50 %		0.04446	0.02358	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	0.1148	0.1148	0.1148	0.05201	0.02721
50 %		0.1148	0.1148	-	0.05201	0.02721
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	0.1284	0.1284	0.1284	0.05852	0.03069
50 %		0.1284	0.1284	-	0.05852	0.03069
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	0.1974	0.1974	0.1974	0.08974	0.04702
50 %		0.1974	0.1974	-	0.08974	0.04702
75 %		-	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L)						
0.045		PEC/RAC ratio				
None	D3 ditch	1.7036	9.0267	-	-	-
50 %		0.8511	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-

None	D4 stream	1.8738	0.9938	-	-	-
50 %		0.9362	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	1.9771	1.0484	0.5444	-	-
50 %		0.9880	0.5240	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	2.5511	2.5511	2.5511	1.1558	0.6047
50 %		2.5511	2.5511	-	1.1558	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	2.8533	2.8533	2.8533	1.3004	0.6820
50 %		2.8533	2.8533	-	1.3004	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	4.3867	4.3867	4.3867	1.9942	1.0449
50 %		4.3867	4.3867	-	1.9942	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L)						
0.1		PEC/RAC ratio				
None	D5 stream	0.8897	-	-	-	-
50 %		-	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	1.1480	1.1480	1.1480	0.5201	-
50 %		1.1480	1.1480	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	1.2840	1.2840	1.2840	0.5852	-
50 %		1.2840	1.2840	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	1.9740	1.9740	1.9740	0.8974	-
50 %		1.9740	1.9740	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-

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

The endpoints used for the algae risk assessment are based on the standard OECD 201 study design where the algae are continually exposed to diflufenican for at least 72 hours. The Aquatic Guidance document (2013) section 9.2.1 states that 'RAs (risk assessments) based on laboratory tests performed under constant exposure conditions may over estimate potential risk. In cases where the predicted (modelled) field exposure profiles differ considerably from exposure regimes in standard toxicity studies it may be appropriate to design a higher-tier laboratory toxicity tests that more closely resemble modelled exposure scenarios'.

In the test of Odin-Feurtet M. (1998), which is reported in detail in the DAR, it was shown that *Scenedesmus subspicatus* which was the most sensitive algae species can recover within 3 days when transferred to fresh growing media after 3 days of exposure to 4.2 µg diflufenican/L. In order to cover effects on less sensitive but slower reproducing algal species the safety factor of 10 was maintained in the risk assessment.

It was decided at the EU level that the risk may be considered acceptable provided that:

- The peak exposure is below 0.42 µg diflufenican /L
- The other exposure peaks do not exceed the overall NOEC for all species tested, 0.1 µg/L within 3 days.
- The exposure does not persist for > 3 days (the duration of exposure in the study on which these assumptions were based). The exposure above the overall NOEC of 0.1 µg/L should be then ≤ 3 days.

Scenarios with a maximum PEC_{sw} below 0.1 µg/L automatically fulfil these conditions. The risk is acceptable in STEP 3 for the following scenario's:

- Winter cereals, pre-emergence: D4 pond, D5 pond, R1 pond
- Winter cereals, post-emergence: D4 pond, D5 pond, R1 pond
- Potatoes: D4 pond, R1 pond
- Sunflower: D4 pond, R1 pond

From the STEP 4 PEC_{sw} values, it can be concluded that the risk is acceptable using a 5 m no spray buffer zone in the following scenarios, since the maximum PEC_{sw} is below 0.1 µg/L:

- Winter cereals, pre-emergence: D1 ditch, D1 stream, D3 ditch, D4 stream, D5 stream
- Winter cereals, post-emergence: D1 ditch, D1 stream, D2 stream, D3 ditch, D4 stream, D5 stream
- Potatoes: D3 ditch, D4 stream, D6 ditch 1st, R2 stream
- Sunflower: D3 ditch, D4 stream, D5 stream

For the remaining scenarios, the FOCUS profiles were analysed with EPAT v1.2 (only in case the maximum PEC_{sw} was below 0.42 µg/L) in order to check if they fulfil the conditions specified above. From table 9.5-35 below, it can be concluded that the conditions are fulfilled and thus the risk is acceptable using a 5 m no spray buffer zone for the following scenarios:

- Winter cereals, pre-emergence: D2 ditch, D2 stream, D6 ditch, R1 stream, R3 stream, R4 stream
- Winter cereals, post-emergence: D2 ditch, D6 ditch, R1 stream, R3 stream, R4 stream
- Potatoes: D6 ditch 2nd, R1 stream, R3 stream
- Sunflower: R1 stream, R3 stream, R4 stream

Table 9.5-35: Analysis of FOCUS profiles with a maximum PEC_{sw} above 0.1 µg/L but below 0.42 µg/L using EPAT v1.2 using a 5 m bufferzone

Use	Scenario		Peaks above 0.1 µg/L	Max. peak concentration if above 0.1 µg/L	Interval between peaks above 0.1 µg/L (days)	Duration of peak above 0.1 µg/L (days)	Total duration of peaks above 0.1 µg/L within one exposure-recovery time frame (days)
Winter cereals, pre-emergence	STEP 4 – 5m	D2 ditch	1	0.1254	-	0.167	0.167
			2	0.1130	5.833	0.084	0.458
			3	0.1142	0.875	0.166	
			4	0.1239	0.834	0.208	
			5	0.1221	18.833	0.167	0.167
			6	0.1067	34.875	0.083	0.083
			7	0.1148	18.917	0.125	0.125
			8	0.1263	3.875	0.167	0.167
			9	0.1196	17.791	0.167	0.292
			10	0.1175	0.833	0.125	
			11	0.1245	3.875	0.167	0.167
			12	0.1333	24.917	0.416	0.416
			13	0.1183	4.542	0.125	0.125
			14	0.1028	98.25	0.167	0.167
			15	0.1089	18.583	0.167	0.167
			16	0.1083	35.75	0.291	0.458
			17	0.1145	1.667	0.167	
			18	0.1219	9.791	0.209	0.209
			19	0.1104	8.791	0.125	0.125
			20	0.1129	3.875	0.125	0.334
			21	0.1302	0.875	0.209	
			22	0.1116	3.791	0.084	0.834
			23	0.1405	1.916	0.292	
			24	0.1374	1.667	0.291	
			25	0.1219	2.75	0.167	
			26	0.1230	16.792	0.166	0.457
			27	0.1375	2.834	0.291	
			28	0.1401	15.709	0.291	0.291
			29	0.1055	32.792	0.042	0.042
			30	0.1031	23.958	0.083	0.083
			31	0.1196	7.875	0.167	0.376
			32	0.1315	0.833	0.209	
			33	0.1448	18.791	0.334	0.334
			34	0.1575	8.666	0.417	0.667
			35	0.1452	2.583	0.25	
		D2 stream	1	0.1001	-	0.042	0.042

		D6 ditch	1	0.1051	-	0.083	0.083
		R1 stream	1	0.1190	-	0.334	0.334
			2	0.1050	35.625	0.583	0.583
		R3 stream	1	0.1363	-	0.458	1.832
			2	0.1292	0.542	0.916	
			3	0.1132	0.125	0.125	
			4	0.1229	0.834	0.333	
			5	0.1096	16.708	0.334	0.334
			6	0.1054	9.666	0.334	0.334
		R4 stream	1	0.1693	-	0.541	0.958
			2	0.1558	0.500	0.417	
			3	0.1169	87.542	0.625	0.625
Winter cereals, post-emergence	STEP 4 – 5m	D2 ditch	1	0.1261	-	0.25	0.25
			2	0.1158	5.791	0.125	0.499
			3	0.1167	0.834	0.166	
			4	0.1242	0.834	0.208	
			5	0.1218	18.833	0.167	0.167
			6	0.1083	34.875	0.125	0.125
			7	0.1143	18.875	0.125	0.333
			8	0.1227	3.875	0.208	
			9	0.1161	17.75	0.167	0.292
			10	0.1142	0.833	0.125	
			11	0.1190	3.875	0.167	0.167
			12	0.1243	24.917	0.416	0.416
			13	0.1119	4.542	0.125	0.125
			14	0.1031	154.917	0.083	0.083
			15	0.1101	9.875	0.125	0.125
			16	0.1057	3	0.292	0.292
			17	0.1017	9.583	0.042	0.209
			18	0.1191	0.916	0.167	
			19	0.1009	3.833	0.042	0.626
			20	0.1310	1.958	0.25	
			21	0.1276	1.75	0.209	
			22	0.1112	2.791	0.125	
			23	0.1131	16.875	0.125	0.334
			24	0.1291	2.875	0.209	
			25	0.1331	15.791	0.209	0.209
			26	0.1132	64.791	0.125	0.292
			27	0.1262	0.875	0.167	
			28	0.1418	18.875	0.292	0.292
			29	0.1564	8.666	0.375	0.625
			30	0.1434	2.625	0.25	
		D6 ditch	1	0.1087	-	0.083	0.083
		R1 stream	1	0.1200	-	0.334	0.334
			2	0.1061	35.625	0.583	0.583
		R3 stream	1	0.1229	-	0.417	0.417
			2	0.1186	9.583	0.375	0.375
		R4	1	0.1743	-	0.541	0.958

		stream	2	0.1626	0.500	0.417	
			3	0.1219	87.542	0.625	0.625
Potatoes	STEP 4 – 5m	D6 ditch 2 nd	1	0.1002	-	0.042	0.042
		R1 stream	1	0.1107	-	0.458	0.458
			2	0.1008	9.583	0.417	0.417
		R3 stream	1	0.1220	-	0.791	0.791
Sunflower	STEP 4 – 5m	R1 stream	1	0.1148	-	0.458	0.458
			2	0.1014	5.583	0.250	0.250
			3	0.1044	3.709	0.458	0.458
		R3 stream	1	0.1284	-	0.791	0.791
		R4 stream	1	0.1974	-	0.791	0.791
			2	0.1681	12.209	0.708	0.708
			3	0.1441	9.292	0.583	2.124
			4	0.1444	2.417	0.583	
			5	0.1363	0.417	0.958	
			6	0.1138	4.083	0.417	0.751
			7	0.1075	0.583	0.334	

Following the analysis of the FOCUS profiles, it can be concluded that the risk is acceptable in all scenarios and for all crops using a 5 m no spray buffer zone.

Review Comments:

For diflufenican FOCUS profiles of scenarios with a maximum PEC_{sw} above 0.1 µg/L (but below 0.42 µg/L) were analysed using EPAT v1.2. Acceptability of this approach should be consider at MSs level. For Poland EPAT is not accepted. Thus, the mitigation measures will be based on the RAC of 0.1 µg a.s./L.

9.5.3 Overall conclusions

~~An acceptable risk is concluded for prosulfocarb at Step 3, except in the R4 scenario in winter cereals (post-emergence use only) and sunflower, 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.~~

~~An acceptable risk is concluded for diflufenican using a 5-m no-spray buffer zone.~~

~~The risk for the metabolites of diflufenican is acceptable at Step 1-2.~~

~~An acceptable risk for the formulation GLOB1912H following spray drift is concluded using a 5-m no-spray buffer zone.~~

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

Member State	Relevant scenarios	Use	Mitigation measure
Poland	D3, D4, R1	Winter cereals (pre-emergence)	5-m no-spray buffer zone
		Winter cereals (post-emergence)	

		Potato	
		Sunflower	
Czech Republic	D4, R1	Winter cereals (pre-emergence)	5 m no spray buffer zone
		Winter cereals (post-emergence)	
		Potato	
		Sunflower	
Belgium	D3, D4, R1	Winter cereals (pre-emergence)	5 m no spray buffer zone
		Winter cereals (post-emergence)	
		Potato	
Hungary	D3, D5, R1, R3, R4	Winter cereals (pre-emergence)	5 m no spray buffer zone
		Winter cereals (post-emergence)	10 m no spray buffer zone including a 10 m vegetated buffer strip
		Potato	5 m no spray buffer zone
		Sunflower	10 m no spray buffer zone including a 10 m vegetated buffer strip
Germany	Reference is made to the national addendum		

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing.

For active substances and relevant metabolites PEC_{sw} calculations were performed with FOCUS STEPS 1-2 (active substances and metabolites) and FOCUS STEP 3 - 4 (prosulfocarb and diflufenican). Additionally for diflufenican FOCUS profiles of scenarios with a maximum PEC_{sw} above 0.1 µg/L (but below 0.42 µg/L) were analysed using EPAT v1.2. Acceptability of this approach should be consider at MSs level. For Poland EPAT is not accepted.

For both active substances the R scenarios require the widest zones to confirm the safe use of GLOB1912H:

- prosulfocarb (RAC of 7.5 µg a.s./L) - 10 m no spray buffer zone including a 10 m vegetated buffer strip
- diflufenican (RAC of 0.045 µg a.s./L) - 20 m no spray buffer zone including a 20 m vegetated buffer strip; only for R4 scenario, use in sunflower, PEC/RAC is 1.0449
- diflufenican (RAC of 0.1 µg a.s./L) - 10 m no spray buffer zone including a 10 m vegetated buffer strip

Based on the mixture toxicity assessment, it can be concluded that the mitigation measures based on the risk assessment of the individual active substances will be sufficient to protect aquatic organisms.

The D2 ditch and D6 ditch scenarios are not relevant for Central Zone, thus were not taken to consideration in overall conclusions.

GLOB1912H applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures (10 m no spray buffer zone including a 10 m vegetated buffer strip).

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, diflufenican 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 and diflufenican. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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>	Diflufenican	Oral, acute	LD ₅₀ > 112.3 µg/bee	EFSA, 2007
<i>Apis mellifera</i>	Diflufenican	Contact, acute	LD ₅₀ > 100 µg/bee	EFSA, 2007
<i>Apis mellifera</i>	Diflufenican	Adult, chronic	NOEDD = 24.13 µg/bee/d LDD ₅₀ > 24.13 µg/bee/d	Ansaloni T., 2016a
<i>Apis mellifera</i>	Diflufenican	Larvae, chronic	NOED = 85.184 µg/larva	Ansaloni T., 2016b
<i>Apis mellifera</i>	GLOB1817H	Oral, acute, 48 h	LD ₅₀ = 310 µg/bee	Franke M., 2020
<i>Apis mellifera</i>	GLOB1817H	Contact, acute, 96 h	LD ₅₀ = 444 µg/bee	Franke M., 2020
<i>Bombus terrestris</i>	GLOB1817H	Oral, acute	LD ₅₀ > 563.8 µg/bee NOED ≥ 563.8 µg/bee	Amsel K., 2021
<i>Bombus terrestris</i>	GLOB1817H	Contact, acute	LD ₅₀ > 590 µg/bee NOED ≥ 590 µg/bee	Amsel K., 2021
<i>Apis mellifera</i>	GLOB1817H	Adult, chronic	NOEDD = 10.9 µg/bee/d LDD ₅₀ = 24.5 µg/bee/d	Ruhland S., 2021
<i>Apis mellifera</i>	GLOB1817H	Larvae, chronic	NOED = 5.7 µg/larva	Schmidt K., 2021

Species	Substance	Exposure System	Results	Reference
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 (SANCO/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 1 also covers the risk for bees from all other intended uses in groups 2, 3 and 4 (see 9.1.2). The risk assessment was conducted at the highest application rate (use group 5) covering the intended uses in use group 6.

Table 9.6-2: First-tier assessment of the risk for bees due to the pre-emergence use of GLOB1912H in winter cereals, potatoes and sunflower and the post-emergence use in winter cereals

Intended use	Winter cereals (pre-+post-emergence), potatoes, sunflower		
Active substance	Prosulfocarb		
Application rate (g/ha)	1 × 2134		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	103.4	2134	20.64
Contact toxicity	79.3		26.91
Active substance	Diflufenican		
Application rate (g/ha)	1 × 44.8		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	112.3	44.8	0.40
Contact toxicity	100		0.45
Product	GLOB1912H		
Application rate (g/ha)	1 × 3231		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	310	3231	10.42
Contact toxicity	444		7.28

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)

The chronic risk assessments were only performed using the endpoints of the studies with the formulated product, since these are worst case compared to the endpoints of the studies with the active substances.

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:

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

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: $3.231 \times 2.9 \times 198/1000 = 1.8552 \text{ } \mu\text{g/larva}$

Pollen dose: $3.231 \times 6.1 \times 2/1000 = 0.0394 \text{ } \mu\text{g/larva}$

Total exposure ETE = 1.8947 $\mu\text{g/larvae}$ (as a default worst-case residue at 3.231 kg/ha)

This can be compared to the larval NOED of 5.7 $\mu\text{g/larva}$.

$$\text{TER} = \text{NOEDD } (\mu\text{g/larva}) / \text{ETE } (\mu\text{g/larva}) = 5.7/1.8947 = 3.01$$

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 3.01, the proposed uses of GLOB1912H pose an acceptable risk to bee larval development.

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

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:

$$ETR = AR \cdot SV / NOEL = 3.231 \cdot 4.4 / 5.7 = 2.49$$

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

Cereals & Potatoes, BBCH < 10:

Treated crop:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 0.002 \cdot 0.85 / 5.7 = 0.0010$$

Adjacent crop:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 0.0033 \cdot 4.4 \cdot 0.85 / 5.7 = 0.01$$

Weeds in the treated field:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 2.2 \cdot 0.85 / 5.7 = 1.06$$

Plants in the field margin:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 0.0092 \cdot 2.2 \cdot 0.85 / 5.7 = 0.01$$

Succeeding crops:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 0.4 \cdot 0.85 / 5.7 = 0.19$$

Sunflower, BBCH < 10:

Treated crop:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 0.4 \cdot 0.85 / 5.7 = 0.19$$

Adjacent crop:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 0.0033 \cdot 4.4 \cdot 0.85 / 5.7 = 0.01$$

Weeds in the treated field:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 2.2 \cdot 0.85 / 5.7 = 1.06$$

Plants in the field margin:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 0.0092 \cdot 2.2 \cdot 0.85 / 5.7 = 0.01$$

Succeeding crops:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 0.4 \cdot 0.85 / 5.7 = 0.19$$

Cereals, BBCH 10-29:

Treated crop:

$$ETR_{\text{larvae}} = AR \cdot Ef \cdot SV \cdot TWA / NOEL_{\text{larvae}} = 3.231 \cdot 1 \cdot 0.15 \cdot 0.85 / 5.7 = 0.07$$

Adjacent crop:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 3.231 * 0.0033 * 4.4 * 0.85 / 5.7 = 0.01$$

Weeds in the treated field:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 3.231 * 1 * 2.2 * 0.85 / 5.7 = 1.06$$

Plants in the field margin:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 3.231 * 0.0092 * 2.2 * 0.85 / 5.7 = 0.01$$

Succeeding crops:

$$ETR_{\text{larvae}} = AR * Ef * SV * TWA / NOEL_{\text{larvae}} = 3.231 * 1 * 0.4 * 0.85 / 5.7 = 0.19$$

The protection goal is met as the calculated value is below the trigger value of 0.2, except for weeds in the treated field.

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.

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

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:

$$\text{Daily dose } (\mu\text{g a.i./bee}) = A.R. \times [128 \text{ mg}/(1000 \times 0.3)] \times RUD = 3.231 \times [128/(1000 \times 0.3)] \times 2.9 = 3.9978 \mu\text{g/bee}$$

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).

$$TER = NOEDD / \text{daily dose} = 10.9 / 3.9978 = 2.73$$

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.

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 2.73, the proposed uses of GLOB1912H 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:

$$ETR = AR \cdot SV / 10d \text{ LDD50} = 3.231 \cdot 7.6 / 24.5 = 1.002$$

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:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 1 \cdot 0.012 \cdot 0.72 / 24.5 = 0.001$$

Adjacent crop:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 0.0033 \cdot 5.8 \cdot 0.72 / 24.5 = 0.002$$

Weeds in the treated field:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 1 \cdot 2.9 \cdot 0.72 / 24.5 = 0.275$$

Plants in the field margin:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 0.0092 \cdot 2.9 \cdot 0.72 / 24.5 = 0.003$$

Succeeding crops:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 1 \cdot 0.54 \cdot 0.72 / 24.5 = 0.051$$

Sunflower, BBCH < 10:

Treated crop:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 1 \cdot 0.54 \cdot 0.72 / 24.5 = 0.051$$

Adjacent crop:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 0.0033 \cdot 5.8 \cdot 0.72 / 24.5 = 0.002$$

Weeds in the treated field:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 1 \cdot 2.9 \cdot 0.72 / 24.5 = 0.275$$

Plants in the field margin:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 0.0092 \cdot 2.9 \cdot 0.72 / 24.5 = 0.003$$

Succeeding crops:

$$ETR_{\text{chronic adult oral}} = AR \cdot Ef \cdot SV \cdot TWA / LD_{50\text{oral}} = 3.231 \cdot 1 \cdot 0.54 \cdot 0.72 / 24.5 = 0.051$$

Cereals, BBCH 10-29:

Treated crop:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 3.231 * 1 * 0.92 * 0.72 / 24.5 = 0.087$$

Adjacent crop:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 3.231 * 0.0033 * 5.8 * 0.72 / 24.5 = 0.002$$

Weeds in the treated field:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 3.231 * 1 * 2.9 * 0.72 / 24.5 = 0.275$$

Plants in the field margin:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 3.231 * 0.0092 * 2.9 * 0.72 / 24.5 = 0.003$$

Succeeding crops:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * TWA / LD_{50\text{oral}} = 3.231 * 1 * 0.54 * 0.72 / 24.5 = 0.051$$

The protection goal is met for adjacent crops and plants in the field margin as the calculated value is below the trigger value of 0.03.

The risk from foraging on the treated crop, on weeds in the treated field and on succeeding crops is not acceptable as the ETR is above the trigger value of 0.03. However, the EFSA bee guidance (2013) is based on extremely conservative assumptions. Therefore, reference is made to the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028) as presented above, which is providing a realistic and workable risk assessment while providing a comparable level of protection to the EFSA approach.

In addition, the treated crops winter cereals and potatoes, are not attractive to bees for collecting nectar, however, exposure via pollen needs to be considered according to Appendix D in the draft EFSA guidance document (2013). As GLOB1912H will be applied before the flowering period in cereals (autumn to winter application) and pre-emergence in potato, the limited number of bees that will forage pollen will therefore not be exposed.

The treated crop sunflower is attractive to bees for both nectar and pollen according to Appendix D in the draft EFSA guidance document (2013). As GLOB1912H will be applied pre-emergence in sunflower, bees that will forage nectar and pollen will therefore not be exposed.

Moreover, 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 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.

Prosulfocarb has a single first-order (SFO) DT₅₀ of 12.1 days in soil. The metabolism of prosulfocarb in rotational crops was not investigated in the framework of the peer review because the DT₉₀ of prosulfocarb and its relevant soil metabolites were below the trigger of 100 days. According to the environmental fate profile of prosulfocarb, no residues are expected in rotational crops and it is unlikely that the active substance would pose risk to bees in the succeeding crop scenario.

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, Julius-Kühn-Archiv, 450, 2015.

Based on the metabolism study in rotational crops with diflufenican, the only compound of concern was the metabolite AE B107137, for which a plant-back interval of 150 days was proposed. Taking into account the early application timing of GLOB1912H, a long interval before planting subsequent crops can be expected. Therefore, no unacceptable risk to bees is expected in the succeeding crop scenario.

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 GLOB1912H 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:

$$HQ_{\text{contact}} = AR/LD_{50\text{contact}} = 3231/590 = 5.5$$

The protection goal is met as the calculated value is below the trigger value of 7.

Oral exposure assessment for spray applications

Screening step assessment for spray applications:

$$ETR_{\text{acute adult oral}} = AR \cdot SV/LD_{50\text{oral}} = 3231 \cdot 11.2/563.8 = 0.06$$

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:

$$ETR_{\text{acute adult oral}} = AR \cdot Ef \cdot SV/LD_{50\text{oral}} = 3231 \cdot 1 \cdot 0.03/563.8 = 0.0002$$

Adjacent crop:

$$ETR_{\text{acute adult oral}} = AR \cdot Ef \cdot SV/LD_{50\text{oral}} = 3231 \cdot 0.0033 \cdot 11.2/563.8 = 0.0002$$

Weeds in the treated field:

$$ETR_{\text{acute adult oral}} = AR \cdot Ef \cdot SV/LD_{50\text{oral}} = 3231 \cdot 1 \cdot 6.5/563.8 = 0.0372$$

Plants in the field margin:

$$ETR_{\text{acute adult oral}} = AR \cdot Ef \cdot SV/LD_{50\text{oral}} = 3231 \cdot 0.0092 \cdot 6.5/563.8 = 0.0003$$

Succeeding crops:

$$ETR_{\text{acute adult oral}} = AR \cdot Ef \cdot SV/LD_{50\text{oral}} = 3231 \cdot 1 \cdot 0.9/563.8 = 0.00522$$

The protection goal is met for all scenarios as the calculated value is always below the trigger value of 0.036, except for weeds in the treated field where there is a very slight exceedance of the trigger.

Sunflower, BBCH < 10:

Treated crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 1 * 0.9 / 563.8 = 0.0052$$

Adjacent crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 0.0033 * 11.2 / 563.8 = 0.0002$$

Weeds in the treated field:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 1 * 6.5 / 563.8 = 0.0372$$

Plants in the field margin:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 0.0092 * 6.5 / 563.8 = 0.0003$$

Succeeding crops:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 1 * 0.9 / 563.8 = 0.0052$$

The protection goal is met for all scenarios as the calculated value is always below the trigger value of 0.036, except for weeds in the treated field where there is a very slight exceedance of the trigger.

Cereals, BBCH 10-21:

Treated crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 1 * 2.3 / 563.8 = 0.0132$$

Adjacent crop:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 0.0033 * 11.2 / 563.8 = 0.0002$$

Weeds in the treated field:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 1 * 6.5 / 563.8 = 0.0372$$

Plants in the field margin:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 0.0092 * 6.5 / 563.8 = 0.0003$$

Succeeding crops:

$$ETR_{\text{acute adult oral}} = AR * Ef * SV / LD_{50\text{oral}} = 3231 * 1 * 0.9 / 563.8 = 0.0052$$

The protection goal is met for all scenarios as the calculated value is always below the trigger value of 0.036, except for weeds in the treated field where there is a very slight exceedance of the trigger.

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.

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, Julius-Kühn-Archiv, 450, 2015.

Finally, 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

A low risk to bees is expected when applying GLOB1912H according to the intended uses.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* and *Bombus terrestris* to GLOB1912H.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

The risk assessment based on the EFSA Guidance (2013) is not yet approved and certain parts are currently under revision. Additionally, the Applicant performed the risk assessment following the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028).

There is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EFSA Guidance (2013) or the modified EPPO 2010 approach at national level.

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, diflufenican 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 and diflufenican. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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)	GLOB1817H	Extended laboratory test Bean leaf discs (2D)	LR ₅₀ = 1.368 L/ha ER ₅₀ > 0.75 L/ha NOER _{mortality} = 0.75 L/ha NOER _{reproduction} = 0.375 L/ha	Röhlig U., 2020a
<i>Aphidius rhopalosiphi</i> (adults)	GLOB1817H	Extended laboratory test Barley plants (3D)	LR ₅₀ = 2.176 L/ha ER ₅₀ > 1.5 L/ha NOER _{mortality} = 1.5 L/ha NOER _{reproduction} ≥ 1.5 L/ha	Röhlig U., 2020b
<i>Aleochara bilineata</i>	GLOB1817H	Extended laboratory test Sandy soil (2D)	LR ₅₀ > 6 L/ha ER ₅₀ > 6 L/ha NOER _{reproduction} ≥ 6 L/ha	Röhlig U., 2020c
<i>Poecilus cupreus</i>	GLOB1817H	Extended laboratory test Sandy soil (2D)	LR ₅₀ > 6 L/ha ER ₅₀ > 6 L/ha NOER _{mortality} ≥ 6 L/ha	Röhlig U., 2020d
Field or semi-field tests				
-				

9.7.1.1 Justification for new endpoints

-

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 1 also covers the risk for non-target arthropods from all other intended uses in groups 2, 3 and 4 (see 9.1.2). The risk assessment was conducted at the highest application rate (use group 5) covering the intended uses in use group 6.

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 GLOB1912H in winter cereals, potato and sunflower and the post-emergence use in winter cereals

Intended use	Winter cereals (pre-+post-emergence), potato, sunflower			
Active substance/product	GLOB1912H			
Application rate (L/ha)	1 × 3.2			
MAF	/			
Test species Higher-tier	LR₅₀ / ER₅₀ (lab.) (L/ha)	PER_{in-field} (L/ha)	HQ_{in-field} criterion: HQ ≤ 1	

<i>Typhlodromus pyri</i>	1.368 / > 0.75	3.2	2.34/ < 4.27
<i>Aphidius rhopalosiphi</i>	2.176 / > 1.5		1.47/ < 2.18
<i>Aleochara bilineata</i>	6		0.53
<i>Poecilus cupreus</i>	6		0.53

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

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

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the pre-emergence use of GLOB1912H in winter cereals, potato and sunflower and the post-emergence use in winter cereals

Intended use		Winter cereals (pre-+post-emergence), potato, sunflower				
Active substance/product		GLOB1912H				
Application rate (L/ha)		1 × 3.2				
MAF		/				
vdf		5 (Higher-tier)*				
Test species	LR₅₀ / ER₅₀ (lab.) (L/ha)	Drift rate	PER_{off-field} (L/ha)	CF	HQ_{off-field} criterion: HQ ≤ 1	
<i>Typhlodromus pyri</i>	1.368 / > 0.75	0.0277	0.01773	5**	0.13	0.065/<0.118
<i>Aphidius rhopalosiphi</i>	2.176 / > 1.5				0.081	0.041/<0.059
<i>Aleochara bilineata</i>	6				0.030	0.015
<i>Poecilus cupreus</i>	6				0.030	0.015

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).

**a 5-fold correction factor for extended laboratory studies according to ESCORT 2

9.7.2.3 Additional higher-tier risk assessment

The above calculations demonstrate that there is a potential in-field risk for *T. pyri* and *A. rhopalosiphi*. Therefore higher-tier assessment is required.

The ESCORT 2 guidance document recommends that any initial effects are acceptable if the potential for recovery within one year can be demonstrated. No detrimental effects on arthropod populations are predicted to occur in off-field areas (see above). Consequently, off-field areas can act as a source of individuals for recolonization of treated crop areas. It is possible to model the dissipation of residues over time using simple first order kinetics and determine the time after the treatment application that the foliar residues would drop to a level that demonstrate an acceptable risk to non-target arthropods. In modelling the foliar decline GLOB1912H is treated as a single entity, whilst in reality it is a complex mixture containing many individual ingredients. As a point of reference, no unacceptable effects would be predicted once the PER drops below 0.375 L/ha (or 378 g/ha taking into account the density of the formulation of 1.0085 g/mL), that being the most sensitive endpoint in the tier II studies (NOER_{reproduction} in *T. pyri*). Modelling the foliar residue decline after one application of GLOB1912H with a default foliar DT₅₀ of 10 days (according to EFSA Guidance Document on the Risk Assessment of Birds and Mammals, 2009), the PER will drop below 378 g/ha within 30.6 days (see table below), indicating that

potential recovery of in-field populations by arthropod immigration from the off-field habitat can occur well within 1 season and in less than 1 year, as required by ESCORT 2.

Table 9.7-4: Time dependent dissipation of GLOB1912H in the in-field habitat

DT50 (d)	Critical endpoint (g/ha)	Time to critical endpoint from initial residue level (rate 3.2 L/ha or 3231 g/ha*)
10	378	30.6
2.2	378	6.7

*taking into account the density of the formulation of 1.0097 g/mL

Prosulfocarb, the active ingredient with the highest content in the formulation, has a foliar DT₅₀ of 2.2 days (see section 9.3.2.2). Using the foliar DT₅₀ of 2.2 days instead of 10 days, the PER will drop to a level of 378 g/ha within 6.7 days.

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 30.6 days (default foliar DT₅₀) or within 6.7 days (empirical DT₅₀) is well within this timeframe.

It should also be noted that the laboratory tests on the sensitive standard species present an extreme worst-case exposure. For pre-emergence applications, only ground-dwelling arthropods are likely to be exposed. But also for the early post-emergence applications, the number of foliar-dwelling arthropods are likely to be low.

Review Comments:

According to the ESCORT 2 when the acceptable in-field risk cannot be confirmed based on extended laboratory studies, than age residue test for the most sensitive species is require. This study has not been presented.

Nevertheless, in zRMS opinion, the in-field risk for NTA is acceptable based on following assumptions:

1. for main active substance in GLOB1912H – prosulfocarb, DT₅₀ in plants and soil (based on field studies) are 1.83 and 13 days, respectively. Therefore, recolonization can take place in short time after application due to rapid decrease in toxicity;
2. the GLOB1912H, based on tests performed on *T. pyri* and *A. rhopalosiphi* indicated only acute toxicity (mortality), without effects on reproduction (in the tested doses). For pesticides sublethal effects on arthropod physiology and behavior must be considered for a complete analysis of their impact;
3. the GLOB1912H is a selective herbicide, applied foliar, which actively acts on weeds during their germination and causes the lack of their emergence or the production of deformed sprouts or young seedlings that quickly die. Thus, the exposure of foliar-dwelling arthropods is likely to be very limited.
4. the GLOB1912H, is a herbicide and therefore has indirect negative impact on folivorous insects population by removing arable weeds. This effect is unrelated to the toxicity of the product itself. It should be highlighted that there are few examples of direct toxic effects of herbicides on invertebrates (including GLOB1912H), with many of those only being demonstrated in the laboratory bioassays and high application rates. Most effects of herbicides are through the indirect effects on the host plants which not germinate or will be destroyed within few days after product application. Thus, effects mediated via plant food resources or habitat modification will result in negative impact on arthropods population, especially of foliar species, regardless of the toxicity of the product. This is an effect emphasized in the context of biodiversity,
5. the GLOB1912H has a low toxicity to spiders and ground dwelling beetles which recolonization takes more time compering to foliar species;

6. only one application per season is recommended;

7. no adverse effects on off-field arthropods are likely and therefore rapid recolonization can take place.

Moreover, additional data for prosulfocarb toxicity to several species of NTA are available in LoEP (laboratory, extended laboratory and semi-field studies), which can be used to confirm acceptable in-field risk.

In conclusion, in zRMS opinion based on WoE approach, the acceptable in-field risk can be concluded. Nevertheless, as is not standard assessment, thus acceptability of this statement should be taken at MSs level.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

No unacceptable risk to non-target arthropods is expected when GLOB1912H is applied according to the intended use.

Review Comments:

Based on the results of the conducted higher-tier risk assessment (extended laboratory studies for four species and WoE approach) it can be concluded that no long term in-field risk for non-target arthropods is expected from use of GLOB1912H. The low risk was concluded for off-field habitats. No mitigation measures are required.

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, diflufenican 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 GLOB1912H were not evaluated as part of the EU assessment of prosulfocarb and diflufenican. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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 Prosulfocarb 800 EC)	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 22.2 mg/kg dw NOEC _{corr} = 11.1 mg/kg dw*	Servajean E., 2012
<i>Eisenia fetida</i>	Prosulfocarb sulfoxide	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 2.22 mg/kg dw	Worst case assumption: 10x more toxic than parent.
<i>Eisenia fetida</i>	Diflufenican	56 d, chronic	NOEC = 1000 mg/kg dw NOEC _{corr} = 500 mg/kg dw*	EFSA, 2007
<i>Eisenia fetida</i>	AE B107137	56 d, chronic	NOEC _{corr} = 50 mg/kg dw*	Worst case assumption: 10x more toxic than parent.
<i>Eisenia fetida</i>	AE 0542291	56 d, chronic	NOEC _{corr} = 50 mg/kg dw*	Worst case assumption: 10x more toxic than parent.
<i>Folsomia candida</i>	Prosulfocarb (Based on GLOB1817H)	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 27.2 mg a.s./kg dw NOEC _{corr} = 13.6 mg/kg dw*	Friedrich S., 2020
<i>Folsomia candida</i>	Prosulfocarb sulfoxide	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 2.72 mg a.s./kg dw	Worst case assumption: 10x more toxic than parent.
<i>Folsomia candida</i>	Diflufenican (based on Diflufenican 500 SC)	Mixed into substrate Chronic	NOEC = 438 mg/kg dw	EFSA, 2007
<i>Folsomia candida</i>	AE B107137	Mixed into substrate Chronic	NOEC = 43.8 mg/kg dw	Worst case assumption: 10x more toxic than parent.
<i>Folsomia candida</i>	AE 0542291	Mixed into substrate Chronic	NOEC = 43.8 mg/kg dw	Worst case assumption: 10x more toxic than parent.
<i>Hypoaspis aculeifer</i>	Prosulfocarb (Based on Prosulfocarb 800 EC)	Mixed into substrate 14 d, chronic 5% peat content	NOEC = 126.65 mg a.s./kg dw NOEC _{corr} = 63.25 mg/kg dw* EC ₁₀ = 38.39 mg/kg dw EC _{10corr} = 19.19 mg/kg dw*	Lauvaux S., 2016
<i>Hypoaspis aculeifer</i>	Prosulfocarb sulfoxide	Mixed into substrate 14 d, chronic 5% peat content	NOEC = 12.665 mg a.s./kg dw EC ₁₀ = 3.84 mg/kg dw	Worst case assumption: 10x more toxic than parent.
<i>Hypoaspis aculeifer</i>	Diflufenican (based on Diflufenican 500	Mixed into substrate 14 d, chronic	NOEC = 1000 mg a.s./kg dw	Taylor K., 2016

Species	Substance	Exposure System	Results	Reference
	SC)	5 % peat content	NOEC _{corr} = 500 mg/kg dw*	
<i>Hypoaspis aculeifer</i>	AE B107137	14 d, chronic 5 % peat content	NOEC _{corr} = 50 mg/kg dw*	Worst case assumption: 10x more toxic than parent.
<i>Hypoaspis aculeifer</i>	AE 0542291	14 d, chronic 5 % peat content	NOEC _{corr} = 50 mg/kg dw*	Worst case assumption: 10x more toxic than parent.
<i>Eisenia fetida</i>	GLOB1817H	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 41 mg/kg dw NOEC _{corr} = 20.5 mg/kg dw* EC ₁₀ = 45 mg/kg dw (95% CI : 27-75) EC ₂₀ = 95 mg/kg dw (95% CI: 70-129) EC ₅₀ > 268 mg/kg dw	Friedrich S., 2020
<i>Folsomia candida</i>	GLOB1817H	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 41 mg/kg dw NOEC _{corr} = 20.5 mg/kg dw* EC ₁₀ = 40 mg/kg dw (95% CI : 26-63) EC ₂₀ = 48 mg/kg dw (95% CI: 36-64) EC ₅₀ = 66 mg/kg dw (95% CI: 61-79)	Friedrich S., 2020
<i>Hypoaspis aculeifer</i>	GLOB1817H	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 43 mg/kg dw NOEC _{corr} = 21.5 mg/kg dw* EC ₁₀ = 66.9 mg/kg dw (95% CI : 53.6-83.4) EC ₂₀ = 133.2 mg/kg dw (95% CI: 117.1-151.6) EC ₅₀ > 387 mg/kg dw	Schulz L., 2020

Field studies

In an earthworm field study with Prosulfocarb 800 EC, no adverse effects were observed at 4000 g prosulfocarb/ha on bare soil.

Litter bag test

Diflufenican: Acceptable effects after an application of 187.5 and 562.5 g a.i./ha while the litter bags were still on the soil surface.

* 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 GLOB1817H and Prosulfocarb 800 EC (containing 800 g/L prosulfocarb) were converted to active ingredient.

As there is no chronic endpoint for *Hypoaspis aculeifer* based on a study with the active substance diflufenican in the EFSA review report of diflufenican, the endpoint of the formulated product Diflufenican 500 SC (containing 500 g/L diflufenican) was converted to active ingredient.

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

Review Comments:

The endpoints from GLOB1912H (as GLOB1817H) studies to earthworms and other soil meso-, macro-organisms were accepted for evaluation purposes. Additionally, zRMS agree with the Applicant that results from tests with other formulation (Prosulfocarb 800 EC, Diflufenican 500 SC) are needed to properly assess the risk to soil organisms. The evaluation of effects to soil organisms will be based on tests with GLOB1817H, Prosulfocarb 800 EC, Diflufenican 500 SC and endpoints from LoEP of both substances.

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 GLOB1912H in winter cereals, potato and sunflower and the post-emergence use in winter cereals

Intended use	Winter cereals (pre-+ post-emergence), potato, sunflower; 3.2 L/ha		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prosulfocarb	11.1	2.8453	3.90
Prosulfocarb sulfoxide	2.22	0.2056	10.8
Diflufenican	500	0.1510	3311
AE B107137	50	0.0215 0.0544	2326 919
AE 0542291	50	0.0336 0.0850	1488 588
GLOB1912H	20.5	4.3080	4.75

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)
<i>Folsomia candida</i>			
Prosulfocarb	13.6	2.8453	4.78
Prosulfocarb sulfoxide	2.72	0.2056	13.2
Diflufenican	438	0.1510	2901
AE B107137	43.8	0.0215 0.0544	2037 805
AE 0542291	43.8	0.0336 0.0850	1304 515
GLOB1912H	20.5	4.3080	4.75
<i>Hypoaspis aculeifer</i>			
Prosulfocarb	63.25 19.19	2.8453	22.2 6.7
Prosulfocarb sulfoxide	12.665 3.84	0.2056	61.6 18.7
Diflufenican	500	0.1510	3311
AE B107137	50	0.0215 0.0544	2326 919
AE 0542291	50	0.0336 0.0850	1488 588
GLOB1912H	21.5	4.3080	5.0
Intended use	Winter cereals (pre-+ post-emergence), potato, sunflower; 3.0 L/ha		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Prosulfocarb	11.1	2.6680	4.16
Prosulfocarb sulfoxide	2.22	0.1928	11.5
Diflufenican	500	0.1417	3529
AE B107137	50	0.0202 0.0511	2475 978
AE 0542291	50	0.0315 0.0797	1587 627
GLOB1912H	20.5	4.0387	5.08
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)
<i>Folsomia candida</i>			
Prosulfocarb	13.6	2.6680	5.1
Prosulfocarb sulfoxide	2.72	0.1928	14.1
Diflufenican	438	0.1417	3091
AE B107137	43.8	0.0202 0.0511	2168 857
AE 0542291	43.8	0.0315 0.0797	1390 550
GLOB1912H	20.5	4.0387	5.08

<i>Hypoaspis aculeifer</i>			
Prosulfocarb	63.25 19.19	2.6680	23.7 7.2
Prosulfocarb sulfoxide	12.665 3.84	0.1928	65.7 19.9
Diiflufenican	500	0.1417	3528
AE B107137	50	0.0202 0.0511	2475 978
AE 0542291	50	0.0315 0.0797	1587 627
GLOB1912H	21.5	4.0387	5.32

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

The TER_{it} for earthworms due to exposure to prosulfocarb (at both dose rates) and GLOB1912H (at the highest dose rate only) are below the trigger of 5. However, 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 TER_{it} for Collembola due to exposure to prosulfocarb and GLOB1912H at the highest dose rate are slightly below the trigger of 5. However, the risk assessment performed above is very worst-case taking into account that the study was performed with GLOB1817H, a formulation containing an additional active substance and a safener in comparison to GLOB1912H. Moreover, the endpoint of the study was corrected by a factor 2 because of the log Pow of prosulfocarb and diiflufenican, although the study was already performed on a soil with only 5% peat content. Therefore, the risk to Collembola from exposure to GLOB1912H is regarded as acceptable.

In addition, the long-term risk for earthworms and Collembola 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). GLOB1912H is only applied once per season, so prolonged exposure of soil organisms to prosulfocarb is highly unlikely.

Review Comments:

Based on risk assessment performed for dose rate of 3 L/ha acceptable risk was indicated for formulation, prosulfocarb sulfoxide, diiflufenican and its metabolites: AE B107137, AE 0542291. Only for earthworms for prosulfocarb the TER value was below trigger of 5. Nevertheless, safe use can be indicated based on an earthworm field study with Prosulfocarb 800 EC, where no adverse effects at an application rate of 4000 g prosulfocarb/ha on bare soil were observed.

For dose rate of 3.2 L/ha, the TER values for earthworms and collembola due to exposure to prosulfocarb and formulation were below the trigger of 5. An acceptable risk for earthworms can be concluded based on field study with prosulfocarb 800 EC. For collembola exposed to prosulfocarb (endpoint derived from product study - GLOB1817H), earthworms and collembola exposed to formulation, the risk is unresolved based on standard assessment. In zRMS opinion, the acceptable risk for soil organisms can be concluded based on WoE approach.

- The TER values are only slightly below the trigger of 5;
- Prosulfocarb DT₅₀ in soil (based on field studies) is 13 days;
- Only one application per season is recommended;
- In PEC_{soil} calculation 0% interception factor was considered, based on crop growth stage. Nevertheless, it should be noted that the GLOB1912H is a selective herbicide, applied foliar. Thus, the arable weed community gives some, additional reduction of soil organisms exposure.

9.8.3 Overall conclusions

The risk to soil organisms is acceptable when applying GLOB1912H according to the intended use.

Review Comments:

The long-term risks of GLOB1912H to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil} . The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).

Based on the results of the conducted first and higher-tier risk assessment it can be concluded that no adverse effects on soil organisms is expected from recommended uses of GLOB1912H.

Nevertheless, as it is not standard assessment, the acceptability of this statement should be taken at MSs level.

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

Effects on soil microorganisms of GLOB1912H were not evaluated as part of the EU assessment of prosulfocarb and diflufenican. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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	Diflufenican	14 d, aerobic clay-loam	No effects > 25% at 2500 g/ha (= 3.33	EFSA, 2007

Endpoint	Substance	Exposure System	Results	Reference
			mg/kg d.w. soil)*	
N-mineralisation	AE B107137	28 d, aerobic	No effects > 25% at 269.32 g/ha (= 0.359 mg/kg d.w. soil)*	EFSA, 2007
N-mineralisation	AE 0542291	28 d, aerobic	No effects > 25% at 268.41 g/ha (= 0.358 mg/kg d.w. soil)*	EFSA, 2007
N-mineralisation	GLOB1817H	28 d, aerobic loamy sand	No effects > 25% at 40 mg/kg d.w. soil	Schulz L., 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 1 also covers the risk for soil organisms from all other intended uses in groups 2, 3 and 4 (see 9.1.2). The risk assessment was conducted at the highest application rate (use group 5) covering the intended uses in use group 6 (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 GLOB1912H in winter cereals, potato and sunflower and the post-emergence use in winter cereals

Intended use	Winter cereals (pre + post-emergence), potato, sunflower		
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)	2.8453	yes
Prosulfocarb sulfoxide	5.33 (at 42 d)	0.2056	yes
Diflufenican	3.33 (at 14 d)	0.1510	yes
AE B107137	0.359 (at 28 d)	0.0215 0.0544	yes
AE 0542291	0.358 (at 28 d)	0.0336 0.0850	yes
GLOB1912H	40 (at 28 d)	4.3080	yes

9.9.3 Overall conclusions

There is no unacceptable risk for soil micro-organisms when applying GLOB1913H according to the intended uses.

Review Comments:

The submitted risk assessment has been accepted.

Based on the results of the conducted risk assessment it can be concluded that no adverse effects on soil microorganisms is expected from recommended uses pattern of GLOB1912H.

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, diflufenican 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 GLOB1912H were not evaluated as part of the EU assessment of prosulfocarb and diflufenican. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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>Allium cepa</i> (onion) _m ¹⁾ <i>Avena sativa</i> (oat) _m ²⁾ <i>Brassica napus</i> (oilseed rape) _d ³⁾ <i>Lycopersicon esculentum</i> (tomato) _d ⁴⁾ <i>Daucus carota</i> (carrot) _d ⁵⁾ <i>Glycine max</i> (soybean) _d ⁶⁾	GLOB1817H	21 d Seedling emergence	¹⁾ ER ₅₀ = 576.54 mL/ha ²⁾ ER ₅₀ = 478.76 mL/ha ³⁾ ER ₅₀ = 1091.16 mL/ha ⁴⁾ ER ₅₀ > 1662 mL/ha ⁵⁾ ER ₅₀ = 335.65 mL/ha ⁶⁾ ER ₅₀ > 1662 mL/ha	Stead A., 2021
<i>Allium cepa</i> (onion) _m ¹⁾ <i>Avena sativa</i> (oat) _m ²⁾ <i>Brassica napus</i> (oilseed rape) _d ³⁾ <i>Lycopersicon esculentum</i> (tomato) _d ⁴⁾ <i>Daucus carota</i> (carrot) _d ⁵⁾	GLOB1817H	21 d Vegetative vigour	¹⁾ ER ₅₀ = 962.19 mL/ha ²⁾ ER ₅₀ > 1662 mL/ha ³⁾ ER ₅₀ > 1662 mL/ha ⁴⁾ ER₅₀ = 75.93 mL/ha ⁵⁾ ER ₅₀ > 1662 mL/ha ⁶⁾ ER ₅₀ > 1662 mL/ha	Lewington-Gower M., 2021

Species	Substance	Exposure System	Results	Reference
<i>Glycine max (soybean)</i> _d ⁶⁾				

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”, (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for soil organisms from all other intended uses in groups 2, 3 and 4 (see 9.1.2).

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 2001 g a.s./ha	Arable crops/0%	0-24	0.1470	0.1321*	0.1183	0.0686	0.0522
1 x 2314 g a.s./ha	Arable crops/0%	0-24	0.1568	0.1408*	0.1261	0.0961	0.0557

*intrapolated

Table 9.10-3: Assessment of the risk for non-target plants due to the use of GLOB1912H in winter cereals, potato and sunflower

Intended use		Winter cereals (pre + post-emergence), potato, sunflower		
Active substance/product		GLOB1912H		
Application rate (mL/ha)		1 × 3200		
MAF		-		
Test species	ER₅₀ (mL/ha)	Drift rate	PER_{off-field}* (mL/ha)	TER criterion: TER ≥ 5
<i>Lycopersicon esculentum</i>	75.93	2.77%	88.88 (= 88.64 + 0.24)	0.85
Intended use		Winter cereals (pre + post-emergence), potato, sunflower		
Active substance/product		GLOB1912H		
Application rate (mL/ha)		1 × 3000		
MAF		-		
Test species	ER₅₀ (mL/ha)	Drift rate	PER_{off-field}* (mL/ha)	TER criterion: TER ≥ 5
<i>Lycopersicon esculentum</i>	75.93	2.77%	83.32 (= 83.1 + 0.22)	0.91

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 GLOB1912H in winter cereals, potato and sunflower considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter cereals (pre + post-emergence), potato, sunflower			
Active substance/product		GLOB1912H			
Application rate (mL/ha)		1 × 3200			
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	88.88 (= 88.64 + 0.24)	44.44	22.22	8.89
3	1	32.21 (= 32.0 + 0.21)	16.11	8.05	3.22
5	0.57	18.43 (= 18.24 + 0.19)	9.22	4.61	1.84
10	0.29	9.42 (= 9.28 + 0.14)	4.71	2.36	0.94
Toxicity value		TER			
ER ₅₀ = 75.93 mL/ha		criterion: TER ≥ 5			
1		0.85	1.71	3.42	8.54
3		2.36	4.71	9.43	-
5		4.12	8.24	-	-
10		8.06	-	-	-
Intended use		Winter cereals			
Active substance/product		GLOB1912H			
Application rate (mL/ha)		1 × 3000			
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	83.32 (= 83.1 + 0.22)	41.66	20.83	8.33
3	1	30.20 (= 30.0 + 0.20)	15.10	7.55	3.02
5	0.57	17.28 (= 17.1 + 0.18)	8.64	4.32	1.73
10	0.29	8.8 (= 8.7 + 0.10)	4.4	2.2	0.88
Toxicity value		TER			
ER ₅₀ = 75.93 mL/ha		criterion: TER ≥ 5			
1		0.91	1.82	3.65	9.11
3		2.51	5.03	-	-
5		4.39	8.79	-	-

10	8.63	-	-	-
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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, ~~a buffer zone of 3 m in combination with 75% drift reducing techniques~~, 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 GLOB1912H at the dose rate of 3.2 L/ha.

A buffer zone of 1 m in combination with 90% drift reducing techniques, a buffer zone of ~~3~~ **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 GLOB1912H at the dose rate of 3.0 L/ha.

Review Comments:

The UBA tool EVA 3.0 rev2h is not accepted in all MSs. Nevertheless, the risk assessment performed by the Applicant was accepted as represent worst case exposure scenario and inclusion of dry deposition has no impact for overall conclusion. The 3 m buffer zone was crossed out. For field crops standard buffers zones in meters are 1, 5, 10 etc.

Based on the risk assessment it can be concluded that the proposed use of GLOB1912H poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from GLOB1912H applications are required for the protection of terrestrial non-target plants.

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 GLOB1912H was performed according to the EU Regulation 1272/2008 (CLP labelling).

Acute toxicity tests were performed with the formulation GLOB1817H. The formulation endpoints for GLOB1912H are obtained by bridging with the formulation GLOB1817H. This formulation has the same composition as GLOB1912H, apart from an additional active substance and a safener, and thus the endpoint obtained in studies with GLOB1817H can be regarded as worst-case for GLOB1912H. More information on the composition of GLOB1912H and GLOB1817H can be found in Part C.

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 GLOB1817H. No chronic toxicity data with the formulation is available. As all EC₅₀ values were ≤ 1 mg/L, GLOB1912H must be classified as Acute Aquatic Toxicity Category 1; H400.

For chronic classification, the summation method was applied. The product GLOB1912H should be classified as category 2 for chronic aquatic toxicity; H411. For more details, reference is made to the Part C.

Review Comments:

On the May 28, 2021, the European Commission released the 17th Adaptation to Technical Progress (ATP) to the Classification, Labelling and Packaging (CLP) Regulation. The ATP is another update to the CLP Annex VI Harmonised Chemical Classification List. One of the substances listed in the 17th ATP (CLP00/ATP17) is diflufenican for which is proposed new, stricter hazard class:

- Aquatic Acute 1 (H400) with M-factor of 10 000
- Aquatic Chronic 1 (H410) with **M-factor of 1 000**

This regulation will apply starting Dec. 17, 2022.

As GLOB1912H is a new product and to avoid reclassifying and relabelling, the zRMS proposed following classification:

- **Aquatic Acute 1 (H400)**
- **Aquatic Chronic 1 (H410)**

Labelling:

Code(s) for hazard pictogram(s): GHS 09

Signal word: Warning

Hazard statement(s):

H410: Very toxic to aquatic life with long lasting effects

Precautionary statement:

P391 and P501

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. (PL, CZ, BE)~~

~~SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 5 m to surface water bodies for the use in winter cereals (pre-emergence) and potato. (HU)~~

SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m to surface water bodies including a 10 m vegetated filter strip

~~for the use in winter cereals (post-emergence) and sunflower. (HU)~~
SPe3: To protect non-target plants respect an unsprayed buffer zone of 10 m or an unsprayed buffer zone of 5 m in combination with 50% drift reducing nozzles ~~or an unsprayed buffer zone of 3 m in combination with 75% drift reducing nozzles~~ or an unsprayed buffer zone of 1 m in combination with 90% drift reducing nozzles to non-agricultural land. ~~(3.2 L/ha)~~

~~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 50% drift reducing nozzles or an unsprayed buffer zone of 1 m in combination with 90% drift reducing nozzles to non-agricultural land. (3.0 L/ha)~~

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.	2008	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	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 GLP Unpublished	N	Globachem NV
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	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		
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 8.3.1.2	Ansaloni, T.	2016a	Chronic toxicity of Diflufenican technical on honeybees (<i>Apis mellifera</i> L.) TRC16-019BA Trialeamp GLP Unpublished	N	Sapac-Agro S.A. and Globachem NV
KCA 8.3.1.3	Ansaloni, T.	2016b	Toxicity of Diflufenican technical on honey bee larvae (<i>Apis mellifera</i> L.) after repeated exposure under laboratory conditions TRC16-018BA Trialeamp GLP Unpublished	N	Sapac-Agro S.A. and Globachem NV
KCP 10.2.1	Juckeland, D.	2021a	Acute toxicity of GLOB1817H to <i>Daphnia magna</i> in a 48-hour semi-static test 20-48-ADL-0015 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Juckeland, D.	2021b	Effects of GLOB1817H on <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test 20-48-AAL-0019	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
			Biochem Agrar GmbH GLP Unpublished		
KCP 10.2.1	Juckeland, D.	2021c	Effects of GLOB1817H on <i>Lemna gibba</i> in a growth inhibition test under semi-static test conditions 20 48 ALE 0017 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Juckeland, D.	2021d	Effect of GLOB1817H on <i>Myriophyllum spicatum</i> in a semi-static water-sediment system 20 48 AMS 0010 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1	Franke, M.	2020	Acute toxicity of GLOB1817H to the honeybee <i>Apis mellifera</i> L. under laboratory conditions. 20 48 BAA 0130 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1.1	Amsel, K.	2021	Acute toxicity of GLOB1817H to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions 20 48 BBA 0029 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.2	Ruhland, S.	2021	Chronic toxicity of GLOB1817H to the honey bee <i>Apis mellifera</i> L. under laboratory conditions 20 48 BAC 0071 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP	Schmidt, K.	2021	GLOB1817H – Repeated exposure of the honey bee (<i>Apis mellifera</i> L.) larvae under laboratory conditions	N	Globachem

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
10.3.1.3			20 48 BLC 0052 Biochem Agrar GmbH GLP Unpublished		NV
KCP 10.3.2.2	Röhlig, U.	2020a	Effects of GLOB1817H on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Destefani-Perez) in an extended laboratory test. 20 48 NAE 0018 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Röhlig, U.	2020b	Effects of GLOB1817H on the predatory mite <i>Typhlodromus Pyri</i> Scheuten in an extended laboratory test. 20 48 NTE 0013 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Röhlig, U.	2020c	Effects of GLOB1817H on the rove beetle <i>Aleochara bilineata</i> Gyll. in an extended laboratory test. 20 48 NKE 0010 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	Röhlig, U.	2020d	Effects of GLOB1817H on the carabid beetle <i>Poecilus cupreus</i> L. in an extended laboratory test. 20 48 NLE 0007 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.1.1	Servajean, E.	2012	Earthworm reproduction test with prosulfocarb 800 g/L EC (OECD 222, April 2004). 12-99-012-ES Phytosafe s.a.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 10.4.1.1	Friedrich, S.	2020	Effects of GLOB1817H on the reproduction of the earthworm <i>Eisenia fetida</i> . 20 48 TEC 0054 Biochem Agrar 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	Lauvaux, S.	2016	A dose response study to assess the NOEC, EC ₁₀₋₂₀₋₅₀ on reproduction and LR ₁₀₋₂₀₋₅₀ on mortality of Prosulfocarb 800 EC of the predatory mite <i>Hypoaspis aculeifer</i> on artificial soil in the laboratory. HA04/2016 Walloon Agricultural Research Centre GLP Unpublished	N	Globachem NV
KCP 10.4.2.1	Taylor, K.	2016	Diflufenican 500 g/L SC: Predatory mite (<i>Hypoaspis aculeifer</i>) reproduction test in soil. DF50GM Envigo CRS Limited GLP Unpublished	N	Globachem NV & Sapeco Agro S.A.
KCP 10.4.2.1	Schulz, L.	2020	Effects of GLOB1817H on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> . 20 48 THC 0043 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2.1	Friedrich, S.	2020	Effects of GLOB1817H on the reproduction of the collembolan <i>Folsomia candida</i> 20 48 TCC 0059 Biochem Agrar 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	Schulz, L.	2020	Effect of GLOB1817H on the activity of soil microflora (Nitrogen transformation test) 20 48 SMN 0052 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.6	Stead, A.	2021	GLOB1817H: terrestrial plant test: seedling emergence and seedling growth test STC/20/E1410 Stockbridge Technology Center Ltd GLP Unpublished	N	Globachem NV
KCP 10.6	Lewington-Gower, M.	2021	GLOB1817H: terrestrial plant test: vegetative vigour test STC/20/E1409 Stockbridge Technology Center Ltd 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
KCA 8.1.3	Sacker, D.	2008	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	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 GLP Unpublished	N	Globachem NV
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	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 8.3.1.2	Ansaloni, T.	2016a	Chronic toxicity of Diflufenican technical on honeybees (<i>Apis mellifera</i> L.) TRC16-019BA Trialcamp GLP Unpublished	N	Sapex Agro S.A. and Globachem NV
KCA 8.3.1.3	Ansaloni, T.	2016b	Toxicity of Diflufenican technical on honey bee larvae (<i>Apis mellifera</i> L.) after repeated exposure under laboratory conditions TRC16-018BA Trialcamp GLP Unpublished	N	Sapex Agro S.A. and Globachem NV

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 study was not accepted. The risk assessment was based on experimental data from Bätischer (2006), summarised in the Addendum to the Draft Assessment Report (July 2007).
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Reference: KCA 8.1.3

Report The bioaccumulation potential of prosulfocarb in earthworm (*Eisenia foetida*), Sacker D., 2008, 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: No

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
Lighting:	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 (k₁) and depuration (k₂) 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 (k₁) and depuration (k₂) 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.

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 was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment. The results refer to nominal concentrations.
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Reference:	KCP 10.2.1
Report	Acute toxicity of GLOB1817H to <i>Daphnia magna</i> in a 48 hour semi-static test, Juckeland D., 2021a, 20 48 ADL 0015
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

The purpose of the study was to determine possible effects of the test item under semi-static exposure on *Daphnia magna* 24 and 48 hours after test item application and to estimate the concentration, which immobilizes 10, 20 and 50% of the daphnids (EC₁₀, EC₂₀ and EC₅₀ values at 24 and 48 hours). The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) was determined. A LOEC of 0.889 mg/L test item was determined. The corresponding NOEC was 0.593 mg/L test item. The EC₅₀ for immobility was 0.954 mg/L test item at 48 hours.

Materials and Methods

Test item:	GLOB1817H Batch no.: KS010420
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	Content of active substances (analysed)
	Prosulfocarb: 672.8 g/L
	Diflufenican: 14.20 g/L
	Halauxifen-Methyl: 1.323 g/L
	Cloquintocet-mexyl (Safener): 1.349 g/L
Test species:	<i>Daphnia magna</i> STRAUS
Test system:	Exposure of <i>Daphnia</i> to the test item applied in test medium (dilution water)

Test conditions

Temperature	20.6 – 20.8 °C
Photoperiod:	none, complete darkness
Treatments:	Control (untreated test medium) test item (GLOB1817H)
Number of test vessels/concentration:	4
Number of <i>Daphnia</i> /concentration:	20
Test concentration (nominal)*:	0.593, 0.889, 1.33, 2.00, 3.00 mg/L test item equivalent to 395.6, 593.4, 889.4, 1334.8, 2002 µg/L Prosulfocarb 8.35, 12.5, 18.8, 28.2, 42.2 µg/L Diflufenican 0.78, 1.17, 1.75, 2.62, 3.94 µg/L Halauxifen-Methyl 0.79, 1.19, 1.78, 2.68, 4.01 µg/L Cloquintocet-mexyl
* nominal test concentrations based on the weighed amount of test item (mean values of 0 and 24 hours)	
Exposure time:	48 hours (semi-static test procedure)
Biological observations:	Number of immobilised <i>Daphnia</i> : after 3, 24 and 48 h
Statistics:	Step-down Cochran-Armitage Test Procedure for statistical significance of immobility ($p \leq 0.05$, one- sided) Probit analysis for calculation of the EC_x for immobility (with 95% confidence limits) Statistical program: ToxRat Professional Version 3.3; 20.10.2018 (RATTE)
<u>Dates of work:</u>	
Biological phase:	experimental start date: 16.02.2021 experimental completion date: 18.02.2021
Analytical phase:	experimental start: 20.04.2021 experimental completion: 21.04.2021

Results and Discussion

The measured concentrations of Prosulfocarb were within ranges of 95.8 – 105.2% of nominal concentrations in the freshly prepared test solutions at the start of the test and at renewal after 24 hours and within a range of 94.7 – 103.6% in the spent solutions at the renewal of the test solutions after 24 hours and at the test end (48 hours) based on nominal values.

Measured concentrations of Diflufenican in test solutions were within ranges of 86.1 – 94.3% of nominal concentrations in the freshly prepared test solutions at the start of the test and at renewal after 24 hours and within a range of 81.9 – 92.0% in the spent solutions at the renewal of the test solutions after 24 hours and at the test end (48 hours) based on nominal values.

Measured concentrations of Halauxifen-Methyl in test solutions were within ranges of 91.9 – 98.3% of nominal concentrations in the freshly prepared test solutions at the start of the test and at renewal after 24 hours and within a range of 90.6 – 96.1% in the spent solutions at the renewal of the test solutions after 24 hours and at the test end (48 hours) based on nominal values.

Therefore, the calculated endpoints are based on the nominal concentrations for the test item and on the active substances, since the measured concentrations were within 80 to 120% of nominal.

Effects of the test item on immobility of *Daphnia magna*

Effect concentration		GLOB1817H				
		24 h		48 h		
		after application				
NOEC						
mg/L Test item, nominal		0.593			0.593	
µg/L Prosulfocarb, nominal		395.6			395.6	
µg/L Diflufenican, nominal		8.35			8.35	
µg/L Halauxifen-Methyl, nominal		0.78			0.78	
LOEC						
mg/L Test item, nominal		0.889			0.889	
µg/L Prosulfocarb, nominal		593.4			593.4	
µg/L Diflufenican, nominal		12.5			12.5	
µg/L Halauxifen-Methyl, nominal		1.17			1.17	
EC	EC ₁₀	EC ₂₀	EC ₅₀	EC ₁₀	EC ₂₀	EC ₅₀
and 95% CI (lower – upper)						
mg/L Test item, nominal	0.925 (0.730 – 1.06)	1.04 (0.868 – 1.17)	1.31 (1.17 – 1.47)	0.658 (0.508 – 0.758)	0.747 (0.612 – 0.844)	0.954 (0.845 – 1.08)
µg/L Prosulfocarb, nominal	617.1 (487.0 – 705.2)	695.8 (579.1 – 781.2)	875.3 (779.2 – 983.3)	439.0 (338.9 – 505.7)	498.3 (408.3 – 563.1)	636.4 (563.7 – 717.2)
µg/L Diflufenican, nominal	13.0 (10.3 – 14.9)	14.7 (12.2 – 16.5)	18.5 (16.4 – 20.8)	9.26 (7.15 – 10.7)8.6	10.5 8.62 – 11.9	13.4 (11.9 – 15.1)
µg/L Halauxifen-Methyl, nominal	1.21 (0.96 – 1.39)	1.37 (1.14 – 1.54)	1.72 (1.53 – 1.93)	0.86 (0.67 – 0.99)	0.98 (0.80 – 1.11)	1.25 (1.11 – 1.41)

Calculations were done with unrounded values, CI – confidence intervals

¹ calculations based on geometric mean of measured concentrations over 0-24 hours

² calculations based on geometric mean of measured concentrations over 0-48 hours

Observations

Time after application	Test concentration mg/L test item nominal					
	Control	0.593	0.889	1.33	2.00	3.00
	Test concentration µg/L Prosulfocarb, nominal					
	Control	395.6	593.4	889.4	1334.8	2002
	Test concentration µg/L Diflufenican, nominal					
	Control	8.35	12.5	18.8	28.2	42.4
	Test concentration µg/L Halauxifen-Methyl, nominal					
	Control	0.78	1.17	1.75	2.62	3.94
	Immobility (%)					
3 h	0.0	0.0	0.0	0.0	0.0	0.0
24 h	0.0	0.0	15.0 +	35.0 +	100.0 +	100.0 +
48 h	0.0	0.0	55.0 +	80.0 +	100.0 +	100.0 +

* significantly different from the control
(Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater)

Validity criteria

The validity criteria were achieved:

Number of immobilised daphnids: $\leq 10\%$ (observed: 0% in the control)

Dissolved oxygen concentration at the end of the test: ≥ 3 mg/L in control and test vessels (measured, lowest value 8.15 mg/L)

Daphnids in the control group must not have been trapped at the surface of the water (observed: none).

Conclusion

An acute immobilisation test was performed to assess the effects of the test item GLOB1817H on *Daphnia magna* during 48 hours of semi-static exposure.

Significant effects on immobility were found using Step-down Cochran-Armitage Test ($\alpha = 0.050$, one-sided greater) at the nominal test concentrations ≥ 0.889 mg/L test item at 48 hours.

As a result, a LOEC of 0.889 mg/L test item was determined. The corresponding NOEC was 0.593 mg/L test item. The EC₅₀ for immobility was 0.954 mg/L test item at 48 hours.

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment. The results refer to nominal concentrations and mean measured concentrations.
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Reference: KCP 10.2.1

Report Effects of GLOB1817H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test, Juckeland D., 2021b, 20 48 AAL 0019

Guideline(s): Yes, OECD 201 (2011)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

The purpose of this study was to evaluate effects of the test item on growth of the freshwater green algae *Pseudokirchneriella subcapitata* under static conditions. Exponentially growing cultures of the algal species were exposed to different concentrations of the test item under defined conditions. The aim of the test was to estimate effect concentrations of E_rC₁₀, E_rC₂₀, E_rC₅₀ values (average specific growth rate), E_yC₁₀, E_yC₂₀, E_yC₅₀ values (yield), and LOEC/NOEC values related to growth inhibition and yield over a period of 72 hours.

The 72 h E_rC₅₀ (growth rate) was 59.7 µg/L test item and the 72 h E_yC₅₀ (yield) was 31.0 µg/L test item, based on nominal concentrations.

Materials and Methods

Test item: GLOB1817H
Batch no.: KS010420

Content of active substances (analysed): Prosulfocarb: 672.8 g/L

Test species:	Diflufenican: 14.20 g/L Halauxifen-Methyl: 1.323 g/L Cloquintocet-mexyl (Safener): 1.349 g/L Freshwater green alga – <i>Pseudokirchneriella subcapitata</i> KORSHIKOV
Test system:	Exposure of <i>Pseudokirchneriella subcapitata</i> to the test item applied once in test medium (static conditions)
Test conditions:	Temperature: 22.8 – 22.9°C Continuous light: (on average 75 $\mu\text{E m}^{-2} \text{s}^{-1}$)
Treatments:	Control (untreated test medium) GLOB1817H
Test concentration (nominal):	20.4, 34.6, 58.8, 100.0, 170.0 $\mu\text{g/L}$ test item equivalent to 13.6, 23.1, 39.2, 66.7, 113.4 $\mu\text{g/L}$ Prosulfocarb 0.29, 0.49, 0.83, 1.41, 2.39 $\mu\text{g/L}$ Diflufenican 0.027, 0.045, 0.077, 0.131, 0.223 $\mu\text{g/L}$ Halauxifen-Methyl 0.027, 0.046, 0.079, 0.134, 0.227 $\mu\text{g/L}$ Cloquintocet-mexyl
Test concentration (mean measured):	12.32, 19.85, 36.34, 57.81, 102.68 $\mu\text{g/L}$ Prosulfocarb 0.251, 0.396, 0.714, 1.17, 2.01 $\mu\text{g/L}$ Diflufenican 0.012, 0.021, 0.043, 0.065, 0.118 $\mu\text{g/L}$ Halauxifen-Methyl
Exposure time:	72 hours (static test procedure)
Biological observations:	Number of cells: after 24, 48 and 72 hours
Statistics:	EC _x -values: linear regression: probit analysis LOEC/NOEC: Welch-t-test; Williams t-test, alpha = 0.05, one-sided smaller Statistical program: ToxRat Professional Version 3.3 (20.10.2018)
Dates of work:	Biological phase: experimental start date: 23.02.2021 experimental completion date: 26.02.2021 Analytical phase: experimental start: 10.05.2021 experimental completion: 11.05.2021

Results and Discussion

Measured concentrations of Prosulfocarb in test solutions were within a range of 97.0 to 99.2% of nominal values at the test start and after 72 hours the concentrations ranged from 76.2 to 88.4% of nominal in spent test solutions.

Measured concentrations of Diflufenican in test solutions were within a range of 84.5 to 88.0% of nominal values at the test start and after 72 hours the concentrations ranged from 78.2 to 88.4% of nominal in spent test solutions.

Measured concentrations of Halauxifen-Methyl in test solutions were within a range of 90.4 to 93.8% of nominal values at the test start and after 72 hours the concentrations ranged from 20.6 to 32.5% of nominal in spent test solutions.

Therefore, the calculated endpoints are based on the nominal concentrations for the test item and mean measured concentrations for Prosulfocarb, Diflufenican and Halauxifen-Methyl, since the measured concentrations were not within 80 to 120% of nominal.

Effects on growth rate and yield of *Pseudokirchneriella subcapitata*

Effect concentration	GLOB1817H, µg/L	
	Average specific growth rate inhibition	Yield inhibition
	0 – 72 h after application	
NOEC		
Test item, nominal	20.4	20.4
Prosulfocarb, nominal	13.6	13.6
Diflufenican, nominal	0.29	0.29
Halauxifen-Methyl, nominal	0.03	0.03
Prosulfocarb, mean measured	12.32	12.32
Diflufenican, mean measured	0.251	0.251
Halauxifen-Methyl, mean measured	0.012	0.012
LOEC		
Test item, nominal	34.6	34.6
Prosulfocarb, nominal	23.1	23.1
Diflufenican, nominal	0.49	0.49
Halauxifen-Methyl, nominal	0.05	0.05
Prosulfocarb, mean measured	19.85	19.85
Diflufenican, mean measured	0.396	0.396
Halauxifen-Methyl, mean measured	0.021	0.021
EC10 and 95% confidence intervals (lower – upper)	ErC10	EyC10
Test item, nominal	18.4 (11.9 – 24.2)	23.8 (17.2 – 26.8)
Prosulfocarb, nominal	12.3 (7.94 – 16.1)	15.9 (11.5 – 17.9)
Diflufenican, nominal	0.26 (0.17 – 0.34)	0.34 (0.24 – 0.38)
Halauxifen-Methyl, nominal	0.024 (0.016 – 0.032)	0.03 (0.02 – 0.04)
Prosulfocarb, mean measured	10.98 (7.13 - 14.40)	14.33 (9.74 – 16.09)
Diflufenican, mean measured	0.220 (0.142 – 0.289)	0.290 (0.200 – 0.324)
Halauxifen-Methyl, mean measured	0.011 (0.008 – 0.015)	0.014 (0.009 – 0.016)
EC20 and 95% confidence intervals (lower – upper)	ErC20	EyC20
Test item, nominal	27.5 (20.1 – 33.9)	26.0 (20.4 – 28.6)
Prosulfocarb, nominal	18.3 (13.4 – 22.6)	17.3 (13.6 – 19.1)
Diflufenican, nominal	0.39 (0.28 – 0.48)	0.37 (0.29 – 0.40)
Halauxifen-Methyl, nominal	0.036 (0.026 – 0.044)	0.034 (0.027 – 0.038)
Prosulfocarb, mean measured	16.43 (12.05 – 20.16)	15.51 (11.58 – 16.95)
Diflufenican, mean measured	0.329	0.313

Halauxifen-Methyl, mean measured	(0.240 – 0.404) 0.017 (0.013 – 0.021)	(0.236 – 0.341) 0.016 (0.011 – 0.017)
EC50 and 95% confidence intervals (lower – upper) Test item, nominal	ErC50 59.7 (51.2 – 69.6)	EyC50 31.0 (28.1 – 32.4)
Prosulfocarb, nominal	39.8 (34.2 – 46.4)	20.7 (18.7 – 21.6)
Diflufenican, nominal	0.84 (0.72 – 0.98)	0.44 (0.40 – 0.46)
Halauxifen-Methyl, nominal	0.08 (0.07 – 0.09)	0.041 (0.037 – 0.043)
Prosulfocarb, mean measured	35.53 (30.53 – 41.35)	18.05 (16.08 – 18.80)
Diflufenican, mean measured	0.710 (0.608 – 0.829)	0.362 (0.324 – 0.376)
Halauxifen-Methyl, mean measured	0.040 (0.034 – 0.046)	0.019 (0.016 – 0.020)

Calculations were done using unrounded values

Observations

Treatment group	% Inhibition	
µg/L test item, nominal	Average specific growth rate	Yield
	0 - 72 h after application	
Control	n.r.	n.r.
20.4	-0.5 ¹	-1.9 ¹
34.6	30.0 +	70.1 +
58.8	58.6 +	91.6 +
100.0	70.6 +	95.4 +
170.0	78.3 +	97.2 +

+ significantly different from control (Williams t-test)

alpha = 0.05, one-sided smaller), n.r. – not relevant

¹ negative values in % inhibition indicate a higher growth relative to that of the control

Validity criteria

The biomass in the control cultures increased exponentially by a factor of 48.4 within the 72 hours test period (factor 16 after 72 hours is required according to guideline OECD Guideline 201 (2011)). The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 32.1% (not exceeding 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 1.4% and did not exceed 7%.

Conclusion

A growth inhibition test was performed to assess the effects of the test item GLOB1817H to a freshwater green alga (*Pseudokirchneriella subcapitata*) during 72 hours of exposure.

The analysis of the test solutions demonstrates that the organisms were exposed to the appropriate concentration of test material at study initiation. The calculated endpoints are based on the nominal test concentrations for the test item and based on mean measured concentrations for Prosulfocarb, Diflufenican and Halauxifen-Methyl since the measured concentrations were not within 80 to 120% of nominal.

In a 72-hour static test in which *Pseudokirchneriella subcapitata* were exposed to GLOB1817H, based on nominal concentrations the 72 h E_rC_{50} (growth rate) was 59.7 µg/L test item and the 72 h E_yC_{50} (yield) was 31.0 µg/L test item.

Comments of zRMS:	The study was conducted to OECD guideline 221 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment. The test item concentrations during the renewal periods, the results refer to nominal and mean measured concentrations of formulation and of active substances.
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Reference:	KCP 10.2.1
Report	Effects of GLOB1817H on <i>Lemna gibba</i> in a growth inhibition test under semi-static conditions, Juckeland D., 2021c, 20 48 ALE 0017
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

Purpose of this study was to determine effects of GLOB1817H on *Lemna gibba* (duckweed) under semi-static test conditions. As study endpoints, LOEC and NOEC values based on the inhibition of the growth of *Lemna* (for frond number, dry weight, and growth rate) over a period of 7 days were determined. Effect concentrations (EC_x values) of EC_{10} , EC_{20} and EC_{50} were determined for both growth rate and yield based on frond number and biomass.

No statistically significant effect on yield and growth rate of *Lemna* based on frond number and biomass was observed at the nominal concentrations ≤ 160.4 µg/L test item, whereas statistically significant effects ($\alpha = 0.05$) were calculated for nominal concentrations ≥ 304.7 µg/L test item. As a result, the NOEC for yield and growth rate based on frond number and biomass was determined to be 160.4 µg/L test item and the LOEC was determined to be 304.7 µg/L test item, based on nominal concentrations. The lowest EC_{50} -value (0-7 d) was 335.2 µg/L test item (nominal) for yield based on frond number.

Materials and Methods

Test item:	GLOB1817H Batch no.: KS010420 Content of active substances (analysed) Prosulfocarb: 672.8 g/L Diflufenican: 14.20 g/L Halauxifen-Methyl: 1.323 g/L Cloquintocet-mexyl (Safener): 1.349 g/L
Test species:	Duckweed – <i>Lemna gibba</i> L.

Test system: Exposure of *Lemna gibba* to the test item applied in test medium (semi-static conditions)

Test conditions

Temperature: 22.7 – 23.6°C (recorded in the water bath)
Lighting: continuous illumination (on average $125 \mu\text{E} \times \text{m}^{-2} \times \text{s}^{-1}$)
Treatments: control, untreated test medium, test item (GLOB1817H)
Test concentration (nominal)*: 84.4, 160.4, 304.7, 579.0, 1100.0 $\mu\text{g/L}$ test item equivalent to
56.3, 107.0, 203.3, 386.2, 733.8 $\mu\text{g/L}$ Prosulfocarb
1.19, 2.26, 4.29, 8.15, 15.5 $\mu\text{g/L}$ Diflufenican
0.11, 0.21, 0.40, 0.76, 1.44 $\mu\text{g/L}$ Halauxifen-Methyl
0.11, 0.21, 0.41, 0.77, 1.47 $\mu\text{g/L}$ Cloquintocet-mexyl

* nominal test concentrations based on the weighed amount of test item (mean values of day 0, day 3 and day 5)

Test concentrations

(geometrical mean measured a.i.): 0.08, 0.14, 0.28, 0.53, 0.91 $\mu\text{g/L}$ Halauxifen-Methyl

Exposure time: 7 days (semi-static test procedure)

Biological observations: Frond number: day 0, 3, 5 and 7
Changes in plant development: day 0, 3, 5 and 7
Dry weight: day 0 and 7

Statistics: LOEC/NOEC:
Williams t-test, ($\alpha = 0.05$, one-sided smaller)
EC_x: Probit analysis using linear max. likelihood regression
ToxRat Professional Version 3.3 (20.10.2018)

Dates of work:

Biological phase: experimental start date: 12.02.2021
experimental completion date
(determination dry weight) : 22.02.2021

Analytical phase: experimental start: 16.04.2021
experimental completion date: 20.04.2021

Results and Discussion

The measured concentrations of Prosulfocarb remained within a range of 100.1 – 110.8% of nominal concentrations in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The Prosulfocarb concentrations in the spent test solutions were determined at 94.3 – 102.0% of nominal at each renewal and at the end of the test (day 7).

The measured concentrations of Diflufenican remained within a range of 86.6 – 101.9% of nominal concentrations in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The Diflufenican concentrations in the spent test solutions were determined at 83.4 – 100.3% of nominal at each renewal and at the end of the test (day 7).

The measured concentrations of Halauxifen-Methyl remained within a range of 88.6 – 96.3% of nominal concentrations in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The Halauxifen-Methyl concentrations in the spent test solutions were determined at 33.8 – 66.4% of nominal at each renewal and at the end of the test (day 7).

Therefore, the calculated study endpoints are based on nominal, geometric mean measured concentrations for the test substance Halauxifen-Methyl.

LOEC, NOEC and effect concentrations EC_x of GLOB1817H for growth rate and yield based on frond number and biomass for *Lemna gibba* at day 7 d

Effect concentration	GLOB1817H, µg/L			
	average specific growth rate inhibition		yield inhibition	
	Frond number	Biomass	Frond number	Biomass
NOEC				
Test item, nominal	160.4	160.4	160.4	160.4
Prosulfocarb, nominal	107.0	107.0	107.0	107.0
Di flufenican, nominal	2.26	2.26	2.26	2.26
Halauxifen-Methyl, nominal	0.21	0.21	0.21	0.21
Halauxifen-Methyl, mean measured	0.14	0.14	0.14	0.14
LOEC				
Test item, nominal	304.7	304.7	304.7	304.7
Prosulfocarb, nominal	203.3	203.3	203.3	203.3
Di flufenican, nominal	4.29	4.29	4.29	4.29
Halauxifen-Methyl, nominal	0.40	0.40	0.40	0.40
Halauxifen-Methyl, mean measured	0.28	0.28	0.28	0.28
EC₁₀	E_rC₁₀	E_rC₁₀	E_yC₁₀	E_yC₁₀
(CI, lower - upper)				
Test item, nominal	197.9 (169.6 – 223.7)	261.9 (181.7 – 330.0)	167.5 (141.2 – 189.7)	196.3 (142.9 – 241.6)
Prosulfocarb, nominal	132.0 (113.1 – 149.2)	174.7 (121.2 – 220.2)	111.7 (94.2 – 126.6)	131.0 (95.3 – 161.2)
Di flufenican, nominal	2.79 (2.39 – 3.15)	3.69 (2.56 – 4.65)	2.36 (1.99 – 2.67)	2.76 (2.01 – 3.40)
Halauxifen-Methyl, nominal	0.26 (0.22 – 0.29)	0.34 (0.24 – 0.43)	0.22 (0.19 – 0.25)	0.26 (0.19 – 0.32)
Halauxifen-Methyl, mean measured	0.19 (0.17-0.20)	0.25 (0.18-0.30)	0.15 (0.13-0.17)	0.19 (0.14-0.22)
EC₂₀	E_rC₂₀	E_rC₂₀	E_yC₂₀	E_yC₂₀
(CI, lower - upper)				
Test item, nominal	275.0 (245.7 – 301.6)	425.3 (339.5 – 497.5)	212.6 (187.4 – 233.7)	272.0 (216.2 – 318.7)
Prosulfocarb, nominal	183.5 (163.9 – 201.2)	283.7 (226.5 – 331.9)	141.8 (125.0 – 155.9)	181.5 (144.2 – 212.6)
Di flufenican, nominal	3.87 (3.46 – 4.25)	5.99 (4.78 – 7.00)	2.99 (2.64 – 3.29)	3.83 (3.04 – 4.49)
Halauxifen-Methyl, nominal	0.36 (0.32 – 0.40)	0.56 (0.45 – 0.65)	0.28 (0.25 – 0.31)	0.36 (0.28 – 0.42)
Halauxifen-Methyl, mean measured	0.25 (0.23-0.27)	0.39 (0.32-0.44)	0.19 (0.17-0.21)	0.25 (0.21-0.29)
EC₅₀	E_rC₅₀	E_rC₅₀	E_yC₅₀	E_yC₅₀
(CI, lower - upper)				
Test item, nominal	515.9 (483.6 – 550.9)	1075.0 (926.7 – 1322.0)	335.2 (312.5 – 359.5)	507.6 (449.9 – 574.0)
Prosulfocarb, nominal	344.2 (322.6 – 367.5)	717.2 (618.2 – 881.9)	223.6 (208.5 – 239.8)	338.6 (300.1 – 382.9)
Di flufenican, nominal	7.26 (6.81 – 7.76)	15.1 (13.1 – 18.6)	4.72 (4.40 – 5.06)	7.15 (6.33 – 8.08)
Halauxifen-Methyl, nominal	0.68 (0.63 – 0.72)	1.41 (1.22 – 1.73)	0.44 (0.41 – 0.47)	0.67 (0.59 – 0.75)
Halauxifen-Methyl, mean measured	0.46 (0.44-0.48)	0.90 (0.80-1.06)	0.31 (0.29-0.33)	0.46 (0.41-0.50)

CI - confidence interval

Calculations performed using unrounded values

Effects of GLOB1817H on growth rate and yield for *Lemna gibba*

Treatment group	Final frond number replicate mean	Biomass (dry weight) replicate mean	% Inhibition	
			Average specific growth rate (% I _r)	yield (% I _y)

µg/L test item nominal	day 7	day 7 (mg)	frond number	biomass	frond number	biomass
Control	83.5	12.9	-	-	-	-
84.4	86.0	13.9	-1.3*	-3.0*	-3.4*	-8.8*
160.4	82.3	13.6	0.7	-1.9*	1.6	-5.7*
304.7	48.7	10.3	24.2 +	8.5 +	46.8 +	21.4 +
579.0	22.0	5.0	60.0 +	36.9 +	82.6 +	66.4 +
1100.0	14.0	3.8	80.2 +	46.9 +	93.3 +	75.9 +

* negative values mean a higher growth compared to the control

+ significantly different to the control (Williams t-test; alpha = 0.05, one-sided)

Validity criteria

According to the guideline, the doubling time of the frond number in the control must be less than 2.5 d (60 h), corresponding to approximately a 7-fold increase in biomass in 7 days and an average specific growth rate of 0.275 d⁻¹. The measured doubling time of the frond numbers in the control was on average 2.18 days (1.87 days for dry weight), corresponding to a 9.3-fold increase in frond number over the 7-day study period (mean of 9 to 83.5 fronds in the control vessels) and a 13.3-fold increase in dry weight (0.967 mg to 12.9 mg dry weight). The average specific growth rate in the control was 0.318 d⁻¹ for frond number and 0.370 d⁻¹ for dry weight.

The E_rC₅₀ (growth rate based on frond number) value for the reference item (toxic standard) 3,5-dichlorophenol was 3.27 mg/L. This value is included in the range 2.2 - 3.8 mg/L 3,5-dichlorophenol as stated in Guideline ISO 20079, demonstrating that the test system was sensitive.

Conclusion

A *Lemna* growth inhibition test was performed to assess the effects of the test item GLOB1817H (active substances: Prosulfocarb, Diflufenican and Halauxifen-methyl) to *Lemna gibba* (duckweed) during 7 days of exposure in a semi-static test design.

No statistically significant effect on yield and growth rate of *Lemna* based on frond number and biomass was observed at the nominal concentrations ≤ 160.4 µg/L test item, whereas statistically significant effects (alpha = 0.05) were calculated for nominal concentrations ≥ 304.7 µg/L test item. As a result, the NOEC for yield and growth rate based on frond number and biomass was determined to be 160.4 µg/L test item and the LOEC was determined to be 304.7 µg/L test item, based on nominal concentrations.

The lowest EC₅₀-value (0-7 d) was 335.2 µg/L test item (nominal) for yield based on frond number.

Comments of zRMS:	The study was conducted to OECD guideline 239 and according to the principles of GLP. All 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 Effects of GLOB1817H on *Myriophyllum spicatum* in a semi-static water-sediment system, Juckeland D., 2021d, 20 48 AMS 0010

Guideline(s): Yes, OECD 239 (2014)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

The objective of this study was to assess test item related effects on vegetative growth of the submersed aquatic dicotyledon *Myriophyllum spicatum* (water milfoils family) in a water-sediment system based on assessments of selected measurement variables under semi-static test conditions. Growths of shoots and lateral branches as well as shoot fresh and dry weight were the measured variables.

The EC_x, LOEC and NOEC for these endpoints based on the inhibition of *Myriophyllum* growth over a period of 14 days were determined.

The lowest EC₅₀ value based on growth rate was 0.075 mg/L test item calculated for total shoot length. The lowest EC₅₀ value based on yield was 0.040 mg/L test item calculated for total shoot length.

Materials and Methods

Test item:	GLOB1817H Batch no.: KS010420 Content of active substances (analysed) <u>Prosulfocarb</u> : 672.8 g/L <u>Diflufenican</u> : 14.20 g/L <u>Halauxifen-Methyl</u> : 1.323 g/L <u>Cloquintocet-mexyl (Safener)</u> : 1.349 g/L
Test species:	<i>Myriophyllum spicatum</i> L.
Test system:	exposure of <i>Myriophyllum spicatum</i> to the test item applied in test medium (semi-static conditions), no vehicle was used
Test conditions:	20.1 – 20.4 °C
Lighting:	16/8 light/dark phases (on average 138 µE *m ⁻² *s ⁻¹)
Treatments:	control (untreated test medium) test item
Test concentration (nominal)*:	0.009, 0.029, 0.092, 0.293, 0.937, 3.00 mg/L test item equivalent to 5.962, 19.09, 61.09, 195.5, 625.4, 2001.4 µg/L Prosulfocarb 0.13, 0.40, 1.29, 4.13, 13.2, 42.2 µg/L Diflufenican 0.01, 0.04, 0.12, 0.38, 1.23, 3.94 µg/L Halauxifen-Methyl 0.01, 0.04, 0.12, 0.39, 1.25, 4.01 µg/L Cloquintocet- mexyl
* nominal test concentrations based on the weighed amount of test item (mean values of day 0, 2, 4, 6, 8, 10 and 12)	
Exposure time:	14 days (semi-static test procedure)
Biological observations:	<u>day 0 and 14:</u> main shoot length, length and number of lateral branches <u>day 8 and 14:</u> changes in plant development <u>day 0 and 14:</u> fresh and dry weight <u>day 0, 14:</u> observation root development
Statistics:	NOEC/LOEC: Williams t-test; Welch's t-test (alpha = 0.05, one-sided smaller) ECx: Probit analysis using linear max. likelihood regression Statistical program: ToxRat Professional Version 3.3 (20.10.2018)
Dates of work:	biological phase (exposure time):

experimental start date: 16.02.2021
experimental completion date
(biological, determination dry weight): 02.03.2021
analytical phase:
experimental start: 23.04.2021
experimental completion date: 04.05.2021

Results and Discussion

The test was valid based on doubling of total shoot length and fresh weight in control plants: required factor: 2, achieved: factor 3.2 for total shoot length and factor 2.7 for fresh weight. Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants. No observations of the latter were made at the surface of the sediment and in test medium. The mean coefficient of variation for yield, based on measurements of shoot fresh weight in control cultures, does not exceed 35% (achieved: 11.7%).

The measured concentrations of Prosulfocarb were within ranges of 91.0 – 114.3% of nominal concentrations in the freshly prepared test solutions at the start of the test and at each renewal and within a range of 87.5 – 107.4% in the spent solutions at each renewal of the test solutions and at the test end (day 14) based on nominal values.

The measured concentrations of Diflufenican were within ranges of 82.0 – 126.7 of nominal concentrations in the freshly prepared test solutions at the start of the test and at each renewal and within a range of 81.7 – 115.2% in the spent solutions at each renewal of the test solutions and at the test end (day 14) based on nominal values.

The measured concentrations of Halauxifen-Methyl were within ranges of 86.0 – 106.0% of nominal concentrations in the freshly prepared test solutions at the start of the test and at each renewal and within a range of 71.6 – 107.9% in the spent solutions at each renewal of the test solutions and at the test end (day 14) based on nominal values.

Therefore, the calculated endpoints are based on nominal concentrations for the test item and active substances Prosulfocarb, Diflufenican and Halauxifen-Methyl, since the decrease was below 20%.

Effects of GLOB1817H on yield and growth of *Myriophyllum spicatum* for biomass (fresh and dry weight)

Treatment Group mg/L test item, nominal	Biomass (fresh weight) replicate mean day 14 (mg)	Biomass (dry weight) replicate mean day 14 (mg)	% Inhibition			
			Average specific growth rate (% I _r)		Yield (% I _y)	
			Biomass (fresh weight)	Biomass (dry weight)	Biomass (fresh weight)	Biomass (dry weight)
Control	453.3	26.4	n.r.	n.r.	n.r.	n.r.
0.009	467.0	29.3	-2.9 ¹	-14.8 ¹	-4.8 ¹	-21.5 ¹
0.029	407.4	25.4	11.1 +	5.3	16.0 +	7.0
0.092	367.3	24.5	21.1 +	9.6	30.0 +	13.5
0.293	299.3	22.5	41.6 +	21.6 +	53.8 +	28.2 +
0.938	276.9	20.3	49.8 +	35.8 +	61.6 +	44.1 +
3.00	230.3	18.1	67.9 +	51.2 +	77.8 +	60.1 +

n.r. – not relevant

+ significantly different to the control
(Williams t-test, alpha = 0.05, one-sided smaller)

¹ negative values indicate higher growth compared to the control

Effects of GLOB1817H on growth rate and yield of *Myriophyllum spicatum* for main shoot length

Treatment group mg/L test item, (nominal)	Main shoot length replicate mean day 14 (cm)	% Inhibition	
		Average specific growth rate (% I _r)	Yield (% I _y)
Control	23.4	n.r.	n.r.
0.009	24.3	3.6	-1.4 ¹
0.029	18.5	33.9 +	40.8 +
0.092	17.4	53.2 ²	57.1 +
0.293	14.9	57.7 +	66.4 +
0.938	14.4	71.1 +	76.0 +
3.00	13.6	75.4 +	81.1 +

+ Significantly different to the control (Welch's-t-test; alpha = 0.05, one-sided smaller)

n.r. – not relevant

¹ negative values indicate higher growth compared to control

² for the test concentration of 0.092 mg/L test item a significant difference compared to the control could not be determined by the statistical program and therefore a NOEC cannot be determined for growth rate based on main shoot length, nevertheless the NOEC seems to be 0.009 mg/L test item

Effects of GLOB1817H on growth rate and yield of *Myriophyllum spicatum* for total shoot length

Treatment group mg/L test item, (nominal)	Total shoot length replicate mean day 14 (cm)	% Inhibition	
		Average specific growth rate (% I _r)	Yield (% I _y)
Control	29.9	n.r.	n.r.
0.009	27.4	13.2 +	15.2 +
0.029	19.4	43.3 +	54.8 +
0.092	17.5	62.2 +	69.8 +
0.293	14.9	66.5 +	77.0 +
0.938	14.4	77.1 +	83.5 +
3.00	13.6	80.5 +	87.0+

+ significantly different to the control (Welch's-t-test for growth rate, Williams t-test for yield; alpha = 0.05, one-sided smaller)

¹ negative values indicate higher growth compared to control

n.r. – not relevant

EC_x-values, LOEC and NOEC values of GLOB1817H for growth rate and yield based on biomass (fresh and dry weight) of *Myriophyllum spicatum* at test end after 14 days

Effect concentration	GLOB1817H			
	Average specific growth rate		Yield	
	biomass (fresh weight)	biomass (dry weight)	biomass (fresh weight)	biomass (dry weight)
NOEC				
mg/L Test item, nominal	0.009	0.092	0.009	0.092
µg/L Prosulfocarb, nominal	5.962	61.09	5.962	61.09
µg/L Diflufenican, nominal	0.13	1.30	0.13	1.30
µg/L Halauxifen-Methyl, nominal	0.01	0.12	0.01	0.12
LOEC				
mg/L Test item, nominal	0.029	0.293	0.029	0.293

µg/L Prosulfocarb, nominal	19.09	195.5	19.09	195.5
µg/L Diflufenican, nominal	0.41	4.13	0.41	4.13
µg/L Halauxifen-Methyl, nominal	0.04	0.38	0.04	0.38
EC₁₀	ErC₁₀	ErC₁₀	EyC₁₀	EyC₁₀
95 % confidence limits (lower – upper) mg/L test item	0.021 (0.009 – 0.039)	0.074 (0.021 – 0.150)	0.012 (0.005 – 0.022)	0.044 (0.011 – 0.095)
µg/L Prosulfocarb, nominal	14.01 (5.962 – 26.02)	49.37 (14.01 – 100.07)	8.006 (3.336 – 14.68)	29.35 (7.338 – 63.38)
µg/L Diflufenican, nominal	0.30 (0.13 – 0.55)	1.04 (0.30 – 2.11)	0.17 (0.07 – 0.31)	0.62 (0.15 – 1.34)
µg/L Halauxifen-Methyl, nominal	0.03 (0.01 – 0.05)	0.10 (0.03 – 0.20)	0.02 (0.01 – 0.03)	0.06 (0.01 – 0.12)
EC₂₀	ErC₂₀	ErC₂₀	EyC₂₀	EyC₂₀
95 % confidence limits (lower – upper) mg/L test item	0.073 (0.041 – 0.112)	0.255 (0.119 – 0.412)	0.038 (0.020 – 0.060)	0.147 (0.061 – 0.251)
µg/L Prosulfocarb, nominal	48.70 (27.35 – 74.72)	170.12 (79.39 – 274.86)	25.35 (13.34 – 40.03)	98.07 (40.69 – 167.45)
µg/L Diflufenican, nominal	1.03 (0.58 – 1.58)	3.59 (1.68 – 5.80)	0.54 (0.28 – 0.84)	2.07 (0.86 – 3.53)
µg/L Halauxifen-Methyl, nominal	0.10 (0.05 – 0.15)	0.33 (0.16 – 0.54)	0.05 (0.03 – 0.08)	0.19 (0.08 – 0.33)
EC₅₀	ErC₅₀	ErC₅₀	EyC₅₀	EyC₅₀
95 % confidence limits (lower – upper) mg/L test item	0.784 (0.571 – 1.13)	2.71 (1.67 – 5.82)	0.344 (0.248 – 0.487)	1.45 (0.922 – 2.75)
µg/L Prosulfocarb, nominal	523.0 (380.9 – 755.2)	1805.9 (1116.8 – 3882.0)	229.49 (165.45 – 324.89)	968.67 (615.09 – 1836.61)
µg/L Diflufenican, nominal	11.0 (8.04 – 16.0)	38.1 (23.6 – 81.9)	4.84 (3.49 – 6.86)	20.4 (13.0 – 38.8)
µg/L Halauxifen-Methyl, nominal	1.03 (0.75 – 1.49)	3.55 (2.20 – 7.63)	0.45 (0.33 – 0.64)	1.90 (1.21 – 3.61)

Calculations were conducted using unrounded values

EC_x-values, LOEC and NOEC values of GLOB1817H for growth rate and yield based on main and total shoot length of *Myriophyllum spicatum* at test end after 14 days

Effect concentration	GLOB1817H			
	Average specific growth rate		Yield	
	main shoot length	total shoot length	main shoot length	total shoot length
NOEC				
mg/L Test item, nominal	0.009	< 0.009	0.009	< 0.009
µg/L Prosulfocarb, nominal	5.962	< 5.962	5.962	5.962
µg/L Diflufenican, nominal	0.13	< 0.13	0.13	< 0.13
µg/L Halauxifen-Methyl, nominal	0.01	< 0.01	0.01	< 0.01
LOEC				
mg/L Test item, nominal	0.029	0.009	0.029	0.009
µg/L Prosulfocarb, nominal	19.09	5.962	19.09	5.962
µg/L Diflufenican, nominal	0.41	0.13	0.41	0.13
µg/L Halauxifen-Methyl, nominal	0.04	0.01	0.04	0.01
EC₁₀	ErC₁₀	ErC₁₀	EyC₁₀	EyC₁₀
95 % confidence limits				

(lower – upper) mg/L test item	0.003 (0.0001 – 0.011)	n.d.	0.003 (0.0003 – 0.009)	n.d.
µg/L Prosulfocarb, nominal	2.001 (0.067 – 7.338)	n.d.	2.001 (0.200 – 5.962)	n.d.
µg/L Diflufenican, nominal	0.04 (0.0014 – 0.15)	n.d.	0.04 (0.004 – 0.13)	n.d.
µg/L Halauxifen-Methyl, nominal	0.004 (0.00013 – 0.014)	n.d.	0.004 (0.0004 – 0.014)	n.d.
EC20	ErC20	ErC20	EyC20	EyC20
95 % confidence limits (lower – upper) mg/L test item	0.011 (0.001 – 0.031)	0.005 (0.001 – 0.014)	0.010 (0.002 – 0.023)	0.005 (0.001 – 0.010)
µg/L Prosulfocarb, nominal	7.338 (0.667 – 20.68)	3.336 (0.667 – 9.340)	6.671 (1.334 – 15.34)	3.336 (0.667 – 6.671)
µg/L Diflufenican, nominal	0.15 (0.01 – 0.44)	0.07 (0.01 – 0.20)	0.14 (0.03 – 0.32)	0.07 (0.01 – 0.14)
µg/L Halauxifen-Methyl, nominal	0.01 (0.001 – 0.04)	0.01 (0.001 – 0.02)	0.01 (0.003 – 0.03)	0.01 (0.001 – 0.01)
EC50	ErC50	ErC50	EyC50	EyC50
95 % confidence limits (lower – upper) mg/L test item	0.160 (0.073 – 0.346)	0.075 (0.037 – 0.140)	0.101 (0.052 – 0.189)	0.040 (0.022 – 0.065)
µg/L Prosulfocarb, nominal	106.74 (48.70 – 230.83)	50.04 (24.68 – 93.40)	67.38 (34.69 – 126.09)	26.69 (14.68 – 43.36)
µg/L Diflufenican, nominal	2.25 (1.03 – 4.87)	1.06 (0.52 – 1.97)	1.42 (0.73 – 2.66)	0.56 (0.31 – 0.92)
µg/L Halauxifen-Methyl, nominal	0.21 (0.10 – 0.45)	0.10 (0.05 – 0.18)	0.13 (0.07 – 0.25)	0.05 (0.03 – 0.09)

Calculations were conducted using unrounded values

n.d. - not determined due to mathematical reasons or inappropriate data

Validity of the study

The test was valid based on doubling of total shoot length and fresh weight in control plants: required factor: 2, achieved: factor 3.2 for total shoot length and factor 2.7 for fresh weight. Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants. No observations of the latter were made at the surface of the sediment and in test medium. The mean coefficient of variation for yield, based on measurements of shoot fresh weight in control cultures, does not exceed 35% (achieved: 11.7%).

Conclusion

A growth inhibition test was performed to assess the effects of the test item GLOB1817H to the rooted aquatic macrophyte *Myriophyllum spicatum* during 14 days of exposure.

The calculated study endpoints are based on nominal concentrations for test item and the active substances Prosulfocarb, Diflufenican and Halauxifen-Methyl.

The lowest EC₅₀ value based on growth rate was 0.075 mg/L test item calculated for total shoot length.

The lowest EC₅₀ value based on yield was 0.040 mg/L test item calculated for total shoot length.

Comments of zRMS:	Study not evaluated.
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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

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_rC₅₀ was 281.6 µg prosulfocarb sulfoxide/L and the E_yC₅₀ 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 µE/m ² .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 (µg/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 (µg/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 (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	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 (µg prosulfocarb sulfoxide/L)		After 72 h (µg 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:	Study not evaluated.
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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 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: 8.01 – 8.05 Test end: 8.29 – 9.09
Lighting:	Continuous fluorescent illumination at an average of 113 µE/m ² .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_rC₅₀ and E_yC₅₀ 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 ≤ 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 (x 10 ⁴ cell/mL) 0-48 h	Percentage inhibition	Mean yield (x 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:	Study not evaluated.
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Reference:	KCA 8.2.6.2
Report	Effects of Prosulfocarb sulfoxide on <i>Anabaena flos-aquae</i> 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*
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_rC₅₀ was 42.5 mg prosulfocarb sulfoxide/L and the E_yC₅₀ 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:	Study not evaluated.
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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_rC₅₀ was 7.97 mg prosulfocarb sulfoxide/L and the E_yC₅₀ 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 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 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 µg 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_tC_{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:	Study not evaluated.
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Reference: KCA 8.2.6.2

Report: Effects of Prosulfocarb sulfoxide on *Skeletonema costatum* 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
Aeriation	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 E₁C₅₀ 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.

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:	Report not evaluated.
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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:	Report not evaluated.
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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_{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.

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: zooplankton	Natural populations of phytoplankton, periphyton, macrophytes and
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

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)
<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:	Report not evaluated.
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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 effects 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 phytoplankton 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 conducted to OECD guidelines 213 and 214 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 10.3.1.1
Report	Acute toxicity of GLOB1817H to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke M., 2020, 20 48 BAA 0130
Guideline(s):	Yes, OECD 213 (1998) and OECD 214 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

The purpose of this study was to determine the acute toxicity of GLOB1817H 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 LD₅₀ (48 h) was 496 µg GLOB1817H/bee (corresponding to 339 µg total a.s./bee) and the LD₅₀ (96 h) was 444 µg GLOB1817H/bee (corresponding to 304 µg total a.s./bee). The oral LD₅₀ (48 h) was 310 µg GLOB1817H/bee that is corresponding to 212 µg total a.s./bee.

Materials and Methods

Test item:	GLOB1817H; Batch No.: KS010420
Content of active substance (a.s.):	<u>nominal</u> <u>analysed</u>

	Prosulfocarb:	667 g/L	672.8 g/L
	Diflufenican:	14.0 g/L	14.20 g/L
	Halauxifen-Methyl:	1.33 g/L	1.323 g/L
	Cloquintocet-Mexyl:	1.33 g/L	1.349 g/L
Reference item:	Dimethoate 400 EC was tested parallel to test item (analysed content of 411.20 ± 3.47 g/L)		
Test species:	Honeybee – <i>Apis mellifera</i> L. subspecies Buckfast (Hymenoptera, Apoidea): worker bees of a healthy and queen-right colony; female, adult worker bees (forager bees) were collected in the morning before use; apiary: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany		
Test design:	<u>Contact test:</u>	96-h; 2 control groups of deionised water, 1 % v/v tween solution; 5 dose rates of test item; 4 dose rates of the reference item; comprising 3 replicates per dose rate each of 10 bees, application volume: 2 µL/bee	
	<u>Oral test:</u>	48-h; 1 control group of 50 % w/v sucrose solution; 5 dose rates of test item; 4 dose rates of the reference item; comprising 3 replicates per dose rate each of 10 bees; application volume: 200 µL/cage by group feeding of 10 bees (corresponding to 20 µL/bee)	
	The mortality and the behavior were assessed 4, 24, 48, 72, 96 hours after application for the contact and 4, 24, 48 hours for the oral test		
Endpoints:	Mortality, behavioral impairments		
Dose rates [product/bee]	<u>Test item:</u>		
	Contact test:	1000, 600, 360, 216, 130 µg product/bee	
	Oral test (offered):	1200, 600, 300, 150, 75.0 µg product/bee	
	Oral test (consumed):	1101, 561, 288, 147, 73.2 µg product/bee*	
Dose rates [total a.s./bee] based on sum of analysed content of a.s.	<u>Test item</u>		
	Contact test:	684, 410, 246, 148, 88.6 µg a.s./bee	
	Oral test (offered):	821, 410, 205, 103, 51.3 µg a.s./bee	
	Oral test (consumed):	753, 384, 197, 100, 50.1 µg a.s./bee*	
	* based on the actual food uptake		
Test conditions:	Temperature:	23.8 – 24.9 °C (contact test); 24.0 – 24.9 °C (oral test)	
	Relative humidity:	49 - 69 % (contact and oral)	
	Illumination:	constant darkness throughout the test (diffuse artificial light only during handling and assessments)	
	Food:	50 % (w/v) sucrose solution (after application <i>ad libitum</i>)	
Statistics:	Statistical program used: ToxRat Professional 3.3.0 (2018)		
	<u>Calculation of LD₅₀ values:</u>		
	<u>Test item:</u>	Contact: Probit analysis (linear maximum likelihood regression) Oral: Weibull analysis (linear maximum likelihood regression)	
	<u>Reference item:</u>	Contact: Probit analysis (linear maximum likelihood regression) Oral: Probit analysis (linear maximum likelihood regression)	
	<u>Statistical significance of mortality values:</u>		
	Test item: Fisher's Exact Binomial Test with Bonferroni Correction (α = 0.05)		

Reference item: Fisher's Exact Binominal Test with Bonferroni Correction ($\alpha = 0.05$)

Validity criteria Control mortality (48 h): $\leq 10\%$

LD₅₀ – value of the reference (24 h): 0.10 – 0.30 µg a.s./bee (contact)
0.10 – 0.35 µg a.s./bee (oral)

Results and Discussion

Experimental dates: 29 September 2020 to 3 October 2020

Contact test

After 48 hours, the control groups treated with deionised water or 1 % tween solution showed no bee mortality. In the test item treatment group, statistically significant mortality of 96.7, 66.7 and 20.0 % was observed after thoracic application of 1000, 600 and 360 µg GLOB1817H/bee, respectively, after 48 hours. Due to a significant increase of the bee mortality between the 24-h and 48-h assessments, the contact test was extended up to 96 hours. After 96 hours, the control group treated with deionised water demonstrated 3.3 % mortality, whereas the control group treated with 1 % tween solution showed no bee mortality. In the test item treatment group, statistically significant mortality of 100.0, 76.7 and 23.3 % at the dose rates of 1000, 600 and 360 µg GLOB1817H/bee, respectively, after 96 hours.

Oral test

After 48 hours, the control group fed 50 % sucrose solution demonstrated no mortality. In the test item treatment group, statistically significant mortality of 100.0, 100.0 and 33.3 % was observed after oral consumption of 1101, 561 and 288 µg GLOB1817H/bee, respectively, after 48 hours.

LD₅₀-values of the contact and oral toxicity test

LD ₅₀ values	Contact toxicity test				Oral toxicity test ¹	
	24 h	48 h	72 h	96 h	24 h	48 h
LD ₅₀ [µg product/bee]	607 (537 – 691)	496 (437 – 564)	444 (393 – 503)	444 (393 – 503)	324 (283 - 390)	310 (266 - 355)
LD ₅₀ [µg total a.s./bee]*	415 (367 - 473)	339 (299 - 386)	304 (269 - 344)	304 (269 - 344)	222 (193 - 268)	212 (182 - 243)

¹ Oral dose rates based on actual consumed doses; * based on analysed content of a.s.

The contact and oral LD₅₀ (24 h) of the reference item was calculated to be 0.155 µg a.s./bee and 0.108 µg a.s./bee, respectively. All validity criteria have been met.

Table 4: Validity criteria of the acute honeybee study

Validity criterion		Occurred / calculated	Recommended
Control mortality (48 h/96 h)	Contact test: - deionised water - 1 % tween solution	0.0 % / 3.3 % 0.0 % / 0.0 %	≤ 10 %
	Oral test: - sucrose solution	0.0 %	≤ 10 %
LD ₅₀ value of the reference item (24 h)	Contact test	0.155 µg a.s./bee	0.10 – 0.30 µg a.s./bee
	Oral test	0.108 µg a.s./bee	0.10 – 0.35 µg a.s./bee

All validity criteria were achieved in this study.

Conclusions

The acute contact and oral toxicity of GLOB1817H was tested on honeybees under laboratory conditions over 96 hours and 48 hours, respectively. The contact LD₅₀ (48 h) was 496 µg GLOB1817H/bee (corresponding to 339 µg total a.s./bee) and the LD₅₀ (96 h) was 444 µg GLOB1817H/bee (corresponding to 304 µg total a.s./bee). The oral LD₅₀ (48 h) was 310 µg GLOB1817H/bee that is corresponding to 212 µg total a.s./bee.

Comments of zRMS:	The study was conducted to OECD guidelines 246 and 247 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 10.3.1.1.1
Report	Acute toxicity of GLOB1817H to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, Amsel K., 2021, 20 48 BBA 0029
Guideline(s):	Yes, OECD 246, OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

In the contact toxicity test, young adult worker bumblebees (*Bombus terrestris* L.) were exposed to GLOB1817H. The toxicity of the test item was determined at one dose rate of 590.0 µg product/bumblebee (equivalent to 400.0 µg a.s./bumblebee). Additionally, bumblebees were treated with Dimethoate EC 400 as reference item at a dose rate of 10.0 µg a.s./bumblebee and furthermore with deionised water and 0.5% (v/v) TritonX solution as controls.

In the oral toxicity test, young adult worker bumblebees (*Bombus terrestris* L.) were exposed to GLOB1817H. The toxicity of the test item was determined at one nominal dose of 590.4 µg product/bumblebee (equivalent to 400.2 µg total a.s./bumblebee). The resulting oral uptake was 563.8 µg product/bumblebee (equivalent to 382.2 µg total a.s./bumblebee). Additionally, bumblebees were treated with Dimethoate EC 400 as reference item at a dose rate of 1.46 µg consumed dimethoate/bumblebee and furthermore with a 50% (w/v) sucrose solution as a control.

In the acute contact toxicity test with GLOB1817H, the resulting LD₅₀ after 48 hours was > 590.0 µg product/bumblebee (equivalent to > 400.0 µg total a.s./bumblebee) and the NOED was ≥ 590.0 µg product/bumblebee (equivalent to ≥ 400.0 µg total a.s./bumblebee).

In the acute oral toxicity test with GLOB1817H, the resulting LD₅₀ after 48 hours was > 563.8 µg consumed product/bumblebee (equivalent to > 382.2 µg consumed total a.s./bumblebee) and the NOED

after 48 hours was $\geq 563.8 \mu\text{g}$ consumed product/bumblebee (equivalent to $\geq 382.2 \mu\text{g}$ consumed total a.s./bumblebee).

Materials and Methods

Test item:	GLOB1817H, batch No.: KS010420, density (at 20 °C):1.0085 g/mL Content of active substance (a.s.): <table><tr><th></th><th><u>nominal</u></th><th><u>analysed</u></th></tr><tr><td>Prosulfocarb:</td><td>667 g/L</td><td>672.8 g/L</td></tr><tr><td>Diflufenican:</td><td>14.0 g/L</td><td>14.20 g/L</td></tr><tr><td>Halauxifen-Methyl:</td><td>1.33 g/L</td><td>1.323 g/L</td></tr><tr><td>Cloquintocet-Mexyl:</td><td>1.33 g/L</td><td>1.349 g/L</td></tr></table>			<u>nominal</u>	<u>analysed</u>	Prosulfocarb:	667 g/L	672.8 g/L	Diflufenican:	14.0 g/L	14.20 g/L	Halauxifen-Methyl:	1.33 g/L	1.323 g/L	Cloquintocet-Mexyl:	1.33 g/L	1.349 g/L
	<u>nominal</u>	<u>analysed</u>															
Prosulfocarb:	667 g/L	672.8 g/L															
Diflufenican:	14.0 g/L	14.20 g/L															
Halauxifen-Methyl:	1.33 g/L	1.323 g/L															
Cloquintocet-Mexyl:	1.33 g/L	1.349 g/L															
Test species:	<i>Bombus terrestris</i> L. (bumblebee), adult worker bumblebees derived from 4 queen right bumblebee hives; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany; collected from 4 bumblebee hives under red light in the evening prior to testing with a starvation period of 4 hours before beginning of the oral test.																
Test design:	<u>Contact test:</u> In a 48 hours test, adults of <i>Bombus terrestris</i> were exposed to 1 dose rate of GLOB1817H in an appropriate carrier (0.5% (v/v) TritonX solution) placed on the dorsal bumblebee thorax. In total, 3 treatment groups were set up: 2 control groups, 1 dose rate of the test item and 1 dose rate of the reference item with 50 replicates per dose for controls and test item and 30 replicates for reference item and one bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours. <u>Oral test:</u> In a 48 hours test, adults of <i>Bombus terrestris</i> L. were exposed to 1 dose rate of GLOB1817H in treated food (50% (w/v) sucrose solution). In total, 3 treatment groups were set up: 1 control group, 1 dose rate of the test item and 1 dose rate of the reference item with 50 replicates per dose for control and test item and 30 replicates for reference item and one bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours.																
Endpoints:	Mortality, behavioural abnormalities																
Reference item:	Dimethoate EC 400 (analysed content of dimethoate: 411.20 g/L)																
Treatments:	<u>Contact test:</u> Water control (deionised water) TritonX control (0.5% (v/v) TritonX solution) Test item at a dose rate of: 590.0 μg product/bumblebee (equivalent to 400.0 μg a.s./bumblebee) Reference item at a dose rate of: 10.0 μg dimethoate/bumblebee <u>Oral test:</u> Sucrose control (50% (w/v) sucrose solution) Test item at a dose rate of: 590.4 μg product/bumblebee (equivalent to 400.2 μg total a.s./bumblebee) actual uptake: 563.8 μg product/bumblebee (equivalent to 382.2 μg total a.s./bumblebee) Reference item at a dose rate of: 1.51 μg dimethoate/bumblebee (actual uptake: 1.46 μg dimethoate/bumblebee)																
Test conditions:	<u>Contact test:</u>																

Temperature: 24.1 °C – 24.7 °C; relative humidity: 50% - 68%
Photoperiod: 24 h darkness
Food: 50% (w/v) sucrose solution
Oral test:
Temperature: 23.9 °C – 24.8 °C; relative humidity: 50% - 65%
Photoperiod: 24 h darkness
Food: 50% (w/v) sucrose solution

Results and Discussion

Experimental dates: 29 September 2020 – 02 October 2020

The validity criteria of the acute bumblebee study (contact and oral test) with GLOB1817H are given in the table below.

Validity of the acute bumblebee study

	Validity criterion	Occurred / calculated	Recommended
Control mortality (48 hours)	Contact test: - Deionised water - 0.5% (v/v) TritonX solution	2.0% 0.0%	≤ 10%
	Oral test: - Sucrose solution	0.0%	≤ 10%
Mortality reference item (48 hours)	Contact toxicity test	100.0%	≥ 50%
	Oral toxicity test	100.0%	≥ 50%

In the contact toxicity test, no mortality occurred in the control group treated with 0.5% (v/v) TritonX solution and 2% mortality occurred in the deionised water control group. In the test item treatment, no mortality occurred after thoracic application of 590.0 µg product/bumblebee within 48 hours. No behavioural effects of bumblebees were observed up to 48 hours.

For the contact toxicity test solution, the mean recoveries of prosulfocarb were 102%, the mean recoveries of diflufenican were 39% and the mean recoveries of halauxifen-methyl were 89%. No active substance was detected in the control sample.

The results of the contact test are summarised in the following tables.

Contact toxicity of GLOB1817H to *Bombus terrestris*

Treatment group [dosage unit]	Dosage applied	Mean mortality [%]	
		24 h	48 h
Control	Water	0.0	2.0
	0.5% (v/v)TritonX	0.0	0.0
GLOB1817H [µg product/bumblebee]	590.0	0.0	0.0

Calculations are performed with non-rounded values

Mortality in the reference item treatment in the contact test was 100.0% after 48 hours.

Contact toxicity of GLOB1817H to *Bombus terrestris*, LD₅₀ / NOED values

	Endpoint	24 h	48 h
GLOB1817H	LD ₅₀ [µg product/bumblebee]	> 590.0	> 590.0
	LD ₅₀ [µg total a.s./bumblebee]	> 400.0	> 400.0
	NOED [µg product/bumblebee]	≥ 590.0	≥ 590.0
	NOED [µg total a.s./bumblebee]	≥ 400.0	≥ 400.0

In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred at the dose rate of 563.8 µg consumed product/bumblebee after 48 hours. No behavioural effects of surviving bumblebees were observed during the oral toxicity test.

For the oral toxicity test solution, the recovery of prosulfocarb was 95%, the recovery of diflufenican was 106% and the recovery of halauxifen-methyl was 94%. No active substance was detected in the control sample.

The results of the oral test are summarised in the following tables.

Oral toxicity of GLOB1817H to *Bombus terrestris*

Treatment group [dosage unit]	Dosage consumed	Mean mortality [%]	
		24 hours	48 hours
Control	Sucrose solution	0.0	0.0
GLOB1817H [µg product/ bumblebee]	563.8	0.0	0.0

Mortality in the reference item treatment in the oral test was 100.0% after 48 hours.

Oral toxicity of GLOB1817H to *Bombus terrestris*, LD₅₀ / NOED values

	Endpoint ¹	24 h	48 h
GLOB1817H	LD ₅₀ [µg product/bumblebee]	> 563.8	> 563.8
	LD ₅₀ [µg total a.s./bumblebee]	> 382.2	> 382.2
	NOED [µg product/bumblebee]	≥ 563.8	≥ 563.8
	NOED [µg total a.s./bumblebee]	≥ 382.2	≥ 382.2

¹ based on consumed values

Conclusion

In the acute contact toxicity test with GLOB1817H, the resulting LD₅₀ after 48 hours was > 590.0 µg product/bumblebee (equivalent to > 400.0 µg total a.s./bumblebee) and the NOED was ≥ 590.0 µg product/bumblebee (equivalent to ≥ 400.0 µg total a.s./bumblebee).

In the acute oral toxicity test with GLOB1817H, the resulting LD₅₀ after 48 hours was > 563.8 µg consumed product/bumblebee (equivalent to > 382.2 µg consumed total a.s./bumblebee) and the NOED after 48 hours was ≥ 563.8 µg consumed product/bumblebee (equivalent to ≥ 382.2 µg consumed total a.s./bumblebee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.3.1.2
Report	Chronic toxicity of Diflufenican technical on honeybees (<i>Apis mellifera</i> L.), Ansaloni T., 2016a, TRC16-019BA
Guideline(s):	Yes, CEB (2012) method, adaptations of OECD Guidelines n° 213 (1998), publications of Decourty et al. (2005) and Suchail et al (2001), recommendations of the German ring test group (2013) and EPPO 170
Deviations:	Yes, Temperature in the climatic chamber was higher than 35 °C during one period of 24 consecutive hours and three periods of 8 consecutive hours during the test (Max = 37.09°C). Relative humidity in the climatic chamber was lower than 50% during one period of 60 consecutive hours (min = 22.96%). These deviations had no negative impact on the outcome of the study.
GLP:	Yes
Acceptability:	Yes

Materials and Methods

Test item (Common name):	Diflufenican technical
Purity:	98.7% w/v
Lot/batch no.:	20151014
Organism (Species):	Honey bee (<i>Apis mellifera</i>)
Study type:	chronic oral
Environmental conditions:	Temperature: 33 ± 2°C Relative humidity: 50 – 70% Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.
Reference substance:	Dimethoate: 0.9 mg/kg food

A single dose of 100 µg Diflufenican/bee/day was assessed. A stock solution was prepared daily by mixing a defined amount of the test item in a defined amount of acetone. The test dose was prepared daily by mixing an aliquot of the stock solution with a defined amount of a 50% w/v aqueous sucrose solution. Two control groups, one with untreated sucrose solution 50% w/v only and one with sucrose solution mixed with acetone, and the reference product Dimethoate 40% EC at a daily dose of 0.107 µg a.i./bee/day were concurrently tested. Five replicates per treatment each enclosing at least ten bees, were group fed with one feeder per cage containing 1000 µl of test solution, thus providing 100 µl of test solution per bee per day. Feeders were weighed prior to their placement in the test cages and were changed on a daily basis with new feeders containing fresh test solutions. When removed each feeder was reweighed and the mean dose consumed per bee was calculated taking in account the surviving individuals at the moment of replacement. Five additional cages with syringes with the feeding solution but no bees were maintained in the climatic chamber. Syringes of these additional cages were changed daily in concomitance with the test syringes and were weighed before and after each replacement for the calculation of sucrose solution evaporation. Daily consumption of the test solutions (control and treatments with the test and the reference products) were adjusted taking in account the daily evaporation.

Assessments:	Honeybees were observed daily at approximately the same time (when the feeders were changed) for mortality and behaviour assessments. Dead bees were removed from the test units
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Statistics:

Mean daily consumptions of the controls and of the test substance were compared amongst them by means of a non-parametric pair wise test (Mann-Whitney exact test; $\alpha = 0.05$). Cumulative mortality at 10 days observed for each control and for the treatment with the test item were compared amongst them by means of a non-parametric pair wise test (Mann-Whitney exact test; $\alpha = 0.05$).

Results and Discussion

Dates of work: 02 March 2016 – 12 March 2016

The test was considered valid as the results obtained met the set validity criteria:

- Mortality observed in control treatment was equal or less than 15% for the duration of the test (final cumulated mortality = 0.00% for the negative control and 2.00% for the solvent control).
- Mean mortality in the reference product concentration was $\geq 50\%$ at the end of the test (final cumulated mortality = 100.00%).

Mean daily consumptions in the water control and the solvent control groups were 22.90 and 23.14 $\mu\text{l}/\text{bee}$ of the offered diet, respectively. Mean daily consumption of the bees exposed to the test item was 24.13 $\mu\text{l}/\text{bee}$ of the offered diet.

Mean cumulative consumption (consumption over the ten days dosing period) was 241.33 μg diflufenican/bee. No statistical significant difference in mean daily diet consumption was observed between the control groups and between the treatment group and each of the controls.

Mean cumulative mortality in the water control and in the solvent control after the ten days of exposure were 0.00% and 2.00%, respectively. Mean cumulative mortality of the honeybees dosed orally with the test item for ten consecutive days was 4.00%. Estimated LDD50 (Lethal Dietary Dose) was higher than the mean daily consumed dose of 24.13 μg diflufenican/bee/day. Based on the mortality data, the NOEDD (No Observed Effect Dietary Dose) was determined to correspond to a daily consumed dose of 24.13 μg diflufenican/bee/day.

No symptoms of intoxication were observed throughout the test for any of the controls bees and for the bees exposed to the test substance.

Conclusion

The estimated consumed chronic LDD50-value for Diflufenican technical was higher than the mean consumed dose of 24.13 μg diflufenican/bee/day. Based on the mortality data, the NOEDD (No Observed Effect Dietary Dose) was determined to correspond to a daily consumed dose of 24.13 μg diflufenican/bee/day.

No symptoms of intoxication were observed throughout the test for any of the controls bees and for the bees exposed to the test substance.

	LDD50 ($\mu\text{g}/\text{bee}/\text{day}$)		NOEDD ($\mu\text{g}/\text{bee}/\text{day}$)	
	Test item	Diflufenican*	Test item	Diflufenican*
Endpoints	> 24.45	> 24.13	24.45	24.13

*analytical content

The results obtained with the toxic reference substance confirmed the sensitivity of the bees under the conditions of the oral test.

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.3.1.2
Report	Chronic toxicity of GLOB1817H to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Ruhland S., 2021, 20 48 BAC 0071
Guideline(s):	Yes, OECD TG 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

In a 10-day chronic toxicity feeding test, max. 2 days old worker honey bees (*Apis mellifera* L. subspecies Buckfast) were exposed to a daily application of GLOB1817H diluted in the bee food (50% (w/v) sucrose solution + 0.1% (w/v) xanthan). The chronic oral toxicity of the test item was determined at nominal doses of 150, 75.2, 37.6, 18.8 and 9.41 µg product/bee/day. The corresponding test item concentrations in the feeding solutions were 3.832, 1.916, 0.958, 0.479 and 0.240 g product/kg food. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 43.0, 29.7, 19.1, 10.9 and 7.92 µg product/bee/day.

An additional group of honey bees was exposed to a daily application of dimethoate diluted in the bee food (50% (w/v) sucrose solution) as a reference item at a nominal dose of 27.3 ng a.i./bee/day.

Untreated 50% (w/v) sucrose solution served as blank control. Untreated 50% (w/v) sucrose solution + 0.1% (w/v) xanthan served as viscosifier control.

The LDD₅₀ was calculated to be 24.5 µg consumed product/bee/day and the LC₅₀ was calculated to be 1.435 g product/kg food. The LDD₂₀ was calculated to be 18.1 µg consumed product/bee/day and the LC₂₀ was calculated to be 0.907 g product/kg food. The LDD₁₀ was calculated to be 14.8 µg consumed product/bee/day and the LC₁₀ was calculated to be 0.669 g product/kg food. The NOEDD was determined to be 10.9 µg consumed product/bee/day, corresponding to a NOEC of 0.479 g product/kg food.

Materials and Methods

Test item:	GLOB1817H, batch no.: KS010420		
	Content of active ingredients:	<u>nominal</u>	<u>analysed</u>
	Prosulfocarb:	667 g/L	672.8 g/L
	Diflufenican:	14.0 g/L	14.20 g/L
	Halauxifen-methyl:	1.33 g/L	1.323 g/L
	Cloquintocet-mexyl ⁶ :	1.33 g/L	1.349 g/L
	Density (at 20°C): 1.0085 g/mL		
Reference item:	Danadim® Progress, batch no.: 10214034		
	Content of active ingredient:	<u>nominal</u>	<u>analysed</u>
	Dimethoate:	400 g/L	411.20 g/L
	Density (at 20°C): 1.069 g/mL		
Validity criteria:	Control mortality: ≤ 15% mean mortality after 10 days of continuous exposure Reference mortality: ≥ 50% mean mortality after 10 days of continuous exposure		
Test species:	<i>Apis mellifera</i> L. subspecies Buckfast (honey bee), not older than 2 days and derived from healthy and queen-right colonies; source: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany		

⁶ serves as herbicide safener

Test design:	<p>In a 10-day chronic toxicity feeding test, young adults of <i>Apis mellifera</i> L. (not older than 2 days) were continuously exposed to GLOB1817H diluted in the bee food (50% (w/v) sucrose solution + 0.1% (w/v) xanthan).</p> <p>The following treatment groups were set up: 5 doses of the test item, 1 untreated control group AC fed with 50% (w/v) sucrose solution, 1 untreated control group BC fed with 50% (w/v) sucrose solution + 0.1% (w/v) xanthan and 1 dose of the reference item. For each treatment group, 3 replicates per dose and 10 bees per replicate were used.</p> <p>All feeding solutions were freshly prepared every day and provided <i>ad libitum</i> (minimum quantity of 2 mL). Assessments of bee mortality, food consumption and behavioural abnormalities were conducted daily.</p> <p>In the analytical phase of the study, the concentration of the active ingredients prosulfocarb, diflufenican and halauxifen-methyl in the highest and lowest test item feeding solution applied on each day of application was determined.</p>
Endpoints:	Mortality, behavioural abnormalities
Test concentrations:	<p>Control group AC: untreated food (50% (w/v) sucrose solution)</p> <p>Control group BC: untreated food (50% (w/v) sucrose solution + 0.1% (w/v) xanthan</p> <p>Test item group: treated food at nominal doses of 150, 75.2, 37.6, 18.8 and 9.41 µg product/bee/day, corresponding to concentrations of 3.832, 1.916, 0.958, 0.479 and 0.240 g product/kg food</p> <p>Effectively consumed doses: 43.0, 29.7, 19.1, 10.9 and 7.92 µg product/bee/day</p> <p>Reference item group: treated food at a nominal dose of 27.3 ng dimethoate/bee/day (corresponding to a concentration of 0.694 mg dimethoate/kg food)</p>
Test conditions:	<p>Temperature: 32.4 – 32.9°C</p> <p>Relative humidity: 54.3 – 65.3%</p> <p>Photoperiod: darkness (diffuse artificial light only during assessments and exchange of feeders)</p> <p>Food: 50% (w/v) sucrose solution</p>
Statistics:	<p>Statistical software used: ToxRat Professional 3.3.0 (2018).</p> <p>Step-down Cochran-Armitage Test Procedure for mortality data and determination of NOEDD/NOEC (one-sided greater, $\alpha = 0.05$). Weibull analysis using linear maximum likelihood regression for the calculation of LDD_x and LC_x values along with their 95% confidence limits.</p>

Results and Discussion

Experimental dates: 25 August 2020 – 04 September 2020

All validity criteria were met.

After 10 days of continuous exposure, a mean mortality of 0.0% was observed in the blank control group AC. In the viscosifier control group BC, a mean mortality of 3.3% was observed. Finally, in the reference item group, a mean mortality of 100% was recorded. Therefore, all validity criteria for the study were met.

Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 43.0, 29.7, 19.1, 10.9 and 7.92 µg product/bee/day which resulted in mortalities of 100, 76.7, 23.3, 10.0 and 3.3% after 10 days, respectively (corrected for mortality of viscosifier control group BC: 100, 75.9, 20.7, 6.9 and 0.0%). The obtained mortalities in the three highest test item doses (43.0, 29.7 and 19.1 µg consumed product/bee/day) were statistically significantly increased compared to the viscosifier control group BC (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater).

During the course of the test, behavioural abnormalities were observed in the highest test item dose (43.0 µg consumed product/bee/day). Single bees were observed as being moribund or affected (uncoordinated movements) on days 3, 7 and 8. On day 9, all bees of this treatment group were dead. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

Mean mortality and behaviour of bees in the chronic toxicity feeding test after 10 days

treatment group	treat-ment group ID	daily dose		concentration [g product/kg food]	after 10 days		
		nominal [µg product/bee/day]	consumed ¹		mean mortality absolute [%]	corrected [%]	number of bees showing behavioural abnormalities ²
blank control viscosifier control	AC	--	--	--	0.0	--	0 out of 30
	BC	--	--	--	3.3	--	0 out of 29
test item	AT	150	43.0	3.832	100*	100	--
	BT	75.2	29.7	1.916	76.7*	75.9	0 out of 7
	CT	37.6	19.1	0.958	23.3*	20.7	0 out of 23
	DT	18.8	10.9	0.479	10.0	6.9	0 out of 27
	ET	9.41	7.92	0.240	3.3	0.0	0 out of 29
		[ng a.i./bee/day]		[mg a.i./kg food]			
reference item	AR	27.3	10.5	0.694	100	--	--

Results are averages based on 3 replicates, containing 10 bees each. Calculations were performed with non-rounded values.

corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Mortality of the test item treatment group was corrected for mortality of untreated viscosifier control group BC, whereas mortality of the reference item treatment group was corrected for mortality of untreated blank control group AC. Negative values were treated as "0".

* statistically significant difference in pairwise comparison between treatment and untreated viscosifier control group BC

(Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

¹ taking into account the actual food uptake and evaporation

² number of bees showing behavioural abnormalities referring to the number of remaining bees

Toxicity of GLOB1817H in a chronic toxicity feeding test

	endpoints	after 10 days
test item doses	LDD ₅₀ [µg consumed product/bee/day] ¹ ²	24.5 (21.9 – 26.9)
	LDD ₂₀ [µg consumed product/bee/day] ¹ ²	18.1 (14.8 – 20.5)
	LDD ₁₀ [µg consumed product/bee/day] ¹ ²	14.8 (11.2 – 17.4)
	NOEDD [µg consumed product/bee/day] ¹ ³	10.9
test item concentrations	LC ₅₀ [g product/kg food] ²	1.435 (1.214 – 1.670)
	LC ₂₀ [g product/kg food] ²	0.907 (0.664 – 1.091)
	LC ₁₀ [g product/kg food] ²	0.669 (0.429 – 0.853)
	NOEC [g product/kg food] ³	0.479

Calculations were performed with non-rounded values.

¹ taking into account the actual food uptake and evaporation

² lethal dietary doses/concentrations (95%-cl lower – upper) were calculated using Weibull analysis (linear max. likelihood regression)

³ no observed effect dietary dose/concentration were determined using Step-down Cochran-Armitage Test Procedure

($\alpha = 0.05$; one-sided greater)

In the test item treatment group, the overall mean daily food consumption ranged between 11.2 and 33.0 mg feeding solution/bee/day which corresponds to 28.6% and 84.1% of the expected daily amount.

In blank control group AC, the bees consumed on average 33.3 mg feeding solution/bee/day (corresponding to 84.9% of the expected daily amount). In viscosifier control group BC, the bees consumed on average 39.0 mg feeding solution/bee/day (corresponding to 99.3% of the expected daily amount)

The daily mean evaporation of 50% (w/v) sucrose solution ranged between 45.7 and 51.7 mg per cage. The daily mean evaporation of 50% (w/v) sucrose solution + 0.1% (w/v) xanthan ranged between 44.0 and 47.7 mg per cage. The food consumption per cage was corrected by subtracting the respective mean evaporation figure of the respective day of application.

The recovery rates of the active ingredients prosulfocarb, diflufenican and halauxifen-methyl in the analysed samples of the test item feeding solutions were between $\pm 20\%$ of the nominal concentrations. Therefore, the concentrations of active ingredients in the applied test item feeding solutions were verified and endpoints have been based on nominal concentrations. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ (limit of quantification).

Conclusion

The chronic oral toxicity of GLOB1817H to young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

The LDD₅₀ was calculated to be 24.5 µg consumed product/bee/day and the LC₅₀ was calculated to be 1.435 g product/kg food. The LDD₂₀ was calculated to be 18.1 µg consumed product/bee/day and the LC₂₀ was calculated to be 0.907 g product/kg food. The LDD₁₀ was calculated to be 14.8 µg consumed product/bee/day and the LC₁₀ was calculated to be 0.669 g product/kg food. The NOEDD was determined to be 10.9 µg consumed product/bee/day, corresponding to a NOEC of 0.479 g product/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:	Study not evaluated.
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Reference:	KCA 8.3.1.3
Report	Toxicity of Diflufenican technical on honey bee larvae (<i>Apis mellifera</i> L.) after repeated exposure under laboratory conditions, Ansaloni T., 2016b, TRC16-018BA
Guideline(s):	Yes, OECD Guideline n° 237 (2013), EPPO 170
Deviations:	Yes, a first study was cancelled because the solvent control did not comply with the validity criteria (mortality across replicates > 15%). Temperature in the incubator was slightly below 34 °C (Min = 31.08 °C) during five consecutive hours (see Annex V). Other short deviations in temperature and relative humidity occurred in concomitance with the opening of the incubator for manipulation of the test system (assessments and/or diet provisioning, see Annex V). The analytical report is not annexed to this final report because it is not available. The aforementioned deviations have had no negative impact on the outcome of the study.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and Methods

Test item (Common name):	Diflufenican technical
Purity:	98.7% w/v

Lot/batch no.: 20151014
Organism (*Species*): Honey bee (*Apis mellifera*)
Study type: Chronic Larval – repeated exposure

Selection of test larvae: Queens of a minimum of three colonies were confined within an empty comb or a comb with emerging worker bees and empty cells of their own colony with an exclusion cage 3 days before the beginning of the test (D -3). At Day -2 (D -2), and within a maximum of 30 hours after confinement, the queens were released after checking the presence of fresh laid eggs. The comb with the eggs was left in the cage near the brood combs until hatching (D1), when the first instar (L1) larvae were taken from the combs and individually placed in well-plates under controlled conditions.

Test Units: Larvae were reared in sterilised crystal polystyrene grafting cells placed individually into a well of a 48 well plate, with the top maintained at the level of the plate by means of a dental roll wetted with approximately 500 µL of the sterilising solution enhanced with 15% w/v glycerol. The plates were placed into a hermetic Plexiglass. The desiccator was placed into an incubator with forced ventilation at 34-35 °C and water saturated atmosphere (95 ± 5% Relative Humidity) for the duration of the test.

Diet composition: All larvae were fed once a day with the exception of D2. Three different diets, adapted to the needs of each larval stage, were prepared during the test: Diet A (D1, 20 µL/larva): 44.25% weight of fresh royal jelly, 44.25% weight of deionized water, 0.90% weight of yeast extract, 5.30% weight of glucose and 5.30% weight of fructose. Diet B (D3, 20 µL/larva): 42.95% weight of fresh royal jelly, 42.95% weight of deionized water, 1.30% weight of yeast extract, 6.40% weight of glucose and 6.40% weight of fructose. Diet C (D4 to D6): 50% weight of fresh royal jelly, 30% weight of deionized water, 2% weight of yeast extract, 9.00% weight of glucose and 9.00% weight of fructose. The following volumes of diet were administered on days D4 to D6: D4 = 30 µL, D5 = 40 µL, D6 = 50 µL.

Application of the test substance: Five doses of the test item with a spacing factor of 2.2 were applied daily for four consecutive days (D3 to D6). Each test dose was prepared daily from a fresh stock solution obtained by mixing a defined amount of the test item with a defined amount of acetone and dilutions of this stock solution with acetone. Aliquots of each test solution needed for each test concentration were mixed with a fixed amount of the corresponding diet. The volume of each test solution corresponded to 2% of the final diet volume. The final cumulative doses (total of four applications) were of 8.0, 17.6, 38.72, 85.184 and 187.405 µg Diflufenican/larva. Two controls (pure diet and diet + 2% solvent) and a reference product (Dimethoate 40% EC) were concurrently tested. On D3, a minimum of sixteen well-fed larvae from each of the three colonies (48 larvae per treatment) were selected for each treatment and dosed with 20 µL of the corresponding diet (diet B) containing the test solution with the corresponding concentration. Administration of the selected doses of test item continued on a daily basis until day 6 with the corresponding diets. Mixing of the test solution with the diet was performed just before administration.

Assessments: Mortality was assessed and recorded at feeding time at D4, D5, D6, D7 and D8. An immobile larva or a larva that did not react to the contact with the grafting tool was noted as dead. Dead larvae were removed at each assessment and anomalies in behaviour were recorded. On D8, the presence of uneaten food was qualitatively recorded.

Toxic reference treatment: A toxic standard reference product, Dimethoate (Dimethoate 40% EC) was applied at a constant concentration of 40 mg a.i./Kg diet/day on forty eight larvae on the same days the test item was applied. Procedures followed those described above for the test item.

Statistics: For mortality data of the test item, a Fisher's exact test ($\alpha = 0.05$) was performed for the estimation of the No Observed Effect Dose (NOED). All statistics were performed using the statistical software SPSS 19; SPSS©Onc, 1989-2010.

Results and Discussion

Dates of work: 11 April 2016 – 18 April 2016

The test is considered valid as the results obtained met the set validity criteria:

- Mortality observed in control treatments was 6.25% (negative control) and 10.42% (solvent control) 120 hours after dosing.
- Corrected mortality (Schneider-Orelli, corrected with respect to the negative control) observed in the larvae exposed to the reference product was 82.22% 120 hours after dosing.

Mean mortality in the control groups was 6.25% (negative control) and 10.42% (solvent control) 120 hours after the first application (D8).

Mean mortality of honey bees' larvae dosed orally with the test item ranged between 6.25% (T1 = 8.000 µg Diflufenican/larva/developmental period) and 31.25% (T5 = 187.405 µg Diflufenican/larva/developmental period) 120 hours after the first application (D8).

The estimated ED₅₀-value was higher than the highest cumulative dose tested (187.405 µg Diflufenican/larva/developmental period).

A significant effect (mortality significantly higher than the solvent control mortality) at 120 hours after the first application (D8) was observed for treatment T5 (187.405 µg Diflufenican/larva/developmental period). Therefore, cumulative NOED corresponded to a cumulated dose of 85.184 µg Diflufenican/larva at 120 hours after the first application.

At 120 hours after the first application, no unconsumed diet and no abnormal symptoms were observed for any of the surviving larvae.

Reference treatment: Corrected mortality (negative control) observed in the larvae exposed to the reference product was 82.22% at 120 hours after the first application.

Conclusion

The estimated ED₅₀-value for Diflufenican technical was higher than the cumulative (over 4 days of application) dietary dose of 187.405 µg Diflufenican/larva 120 hours after dosing started.

Hours after the first application	ED ₅₀ (µg Diflufenican/larva/developmental period)
120	> 187.405

A cumulative dietary dose of 85.184 µg Diflufenican/larva resulted in a NOED at the end of the study (No Observed effect Dose over the 4 days of exposure, cumulative dosing, at 120 hours after the first application).

Hours after the first application	NOED (µg Diflufenican/larva/developmental period)
120	85.184

The results obtained with the toxic reference substance confirmed the sensitivity of the test system (bees' larvae) under the test conditions.

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: KCP 10.3.1.3

Report GLOB1817H – Repeated exposure to the honeybee (*Apis mellifera* L.) larvae under laboratory conditions, Schmidt K., 2021, 20 48 BLC 0052

Guideline(s): Yes, OECD 239 (2016)

Deviations: Yes, because of a malfunction of the climatic chamber, the temperature and humidity were out of range on D8 for six hours. The temperature ranged in

this time between 28.5 to 35.7°C (average 30.7°C) instead of $34.5 \pm 0.5^\circ\text{C}$. The relative humidity ranged in this time between 18.9 to 97.1% (average 28.6% instead of $80 \pm 5\%$). No impact is assumed as no effects on development of larvae in the untreated control were observed.

GLP: Yes

Acceptability: Yes

Executive summary

In a test under laboratory conditions, honey bee larvae (*Apis mellifera* L.) were repeatedly exposed to GLOB1817H. The toxicity of the test item was determined at cumulative doses of 88.7, 35.5, 14.2, 5.7 and 2.3 µg product/larva. The concentrations of test item in the diets were 560.9, 224.3, 89.7, 35.9 and 14.4 mg product/kg food. Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a total dose of 7.6 µg a.i./larva or with an untreated diet as control.

The ED₅₀ (adult emergence up to D22) was determined to be > 88.7 µg product/larva, which is equivalent to an EC₅₀ of > 560.9 mg product/kg food. The ED₂₀ (adult emergence up to D22) was determined to be 13.5 µg product/larva, which is equivalent to an EC₂₀ of 85.0 mg product/kg food. The ED₁₀ (adult emergence up to D22) was determined to be 1.9 µg product/larva, which is equivalent to an EC₁₀ of 12.1 mg product/kg food. The NOED was 5.7 µg product/larva and the corresponding NOEC was 35.9 mg product/kg food.

Materials and Methods

Test item: GLOB1817H, Batch No.: KS010420

Content of active ingredients:	<u>nominal</u>	<u>analysed</u>
Prosulfocarb:	667 g/L	672.8 g/L
Diflufenican:	14.0 g/L	14.20 g/L
Halauxifen-methyl:	1.33 g/L	1.323 g/L
Cloquintocet-mexyl ⁷ :	1.33 g/L	1.349 g/L
Density (at 20 °C):	1.0085 g/mL	

Reference item: Dimethoate tech. (analysed purity: 98.8% ± 0.5%)

Test species: *Honey bee* – *Apis mellifera* L., subspecies: Buckfast (Hymenoptera, Apoidea): First instar larvae (L1 during grafting) of queen-right colonies in good health conditions are used for the test. For each test, larvae were collected from at least three different colonies, each representing a replicate, to ensure the results are representative. source: BioChem agrar GmbH, Machern OT Gerichshain, Germany

Test design: One day old honey bee larvae (D1) of *Apis mellifera* L., subspecies: Buckfast were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment. On 4 successive days (D3 to D6) the larvae were repeatedly exposed to GLOB1817H diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place. In total, 7 treatment groups were set up: 5 doses of the test item, 1 untreated control group and 1 dose of the reference item with 3 replicates per dose and 12 larvae per replicate, each. Assessments of cumulative larval mortality were performed on D4, D5, D6, D7 and D8. Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed on D15 and emergence of adults was evaluated on D22.

In an analytical phase of the study the concentration of the active ingredients

⁷ serves as herbicide safener

	prosulfocarb, diflufenican and halauxifen-methyl in the test item stock solutions and in the control was determined.		
Endpoints:	Successful adult emergence, mortality, qualitative observations: <i>e.g.</i> body size, remaining food		
Test concentrations:	Controls:	AC	untreated diet B/C (aqueous sugar solution + royal jelly)
	Test item:	AT	treated diet B/C at a concentration of 560.9 mg product/kg food
		BT	treated diet B/C at a concentration of 224.3 mg product/kg food
		CT	treated diet B/C at a concentration of 89.7 mg product/kg food
		DT	treated diet B/C at a concentration of 35.9 mg product/kg food
		ET	treated diet B/C at a concentration of 14.4 mg product/kg food
	Reference:	AR	treated diet B/C at a concentration of 48 mg a.i./kg food
Test conditions:	Temperature:	28.5 – 35.7 °C (see deviation)	
	Relative humidity:	D1 - D8: 99.1 – 99.9%	
		D8-D15: 18.9 – 97.1% (see deviation)	
		D15-D22: 62.9 – 72.9%	
	Photoperiod:	Darkness (except during assessments)	
	Food:	aqueous sugar solution with royal jelly	
Statistics:	Descriptive statistics, Step-down Cochran-Armitage Test (one-sided greater, alpha = 0.05) for determination of NOED/NOEC, ED/EC10/20 values were determined by Probit analysis using linear weighted regression. The dataset does not allow for calculation of reliable ED50 and EC50.		

Results and Discussion

Experimental dates: 21 September 2020 – 12 October 2020

All validity criteria were met:

Mean larval mortality in the control:	0.0% (between D3 - D8)
Mean adult emergence rate in the control:	83.3% (up to D22)
Mean larval mortality in the reference item group:	94.4% (between D3 and D8)

On D8, a larval mortality of 0.0% was observed in the control (AC). Pupal mortality (between D8 and D15) was 11.1% in the control. The control group showed a total mortality of 16.7% on D22. In the test item treated groups, larval mortalities ranged between 0.0 and 13.9% on D8. Pupal mortalities (D8-D15) ranged between 11.1 and 19.4% in the test item treatment groups. Total mortalities ranged between 22.2 and 41.7% on D22. Mortality in the reference item treated group (AR) was above 50% across all replicates on D8, being 94.4%.

On D8, none of the remaining larvae treated with test item, were observed to have food left and/or a smaller body size.

In the final assessment on D22, an adult emergence rate of 83.3% was determined for the honey bees in the control group (AC). In the test item treated groups, the adult honey bees emerged at rates ranging between 58.3% and 77.8% following an application of 88.7, 35.5, 14.2, 5.7 and 2.3 μg product/larva, during the larval stages. On D22, larvae treated with 88.7, 35.5 and 14.2 μg product/larva showed emergence rates, which were statistically significantly decreased if compared to the control.

The recoveries of active ingredients prosulfocarb, diflufenican and halauxifen-methyl in the test item stock solutions A and E ranged between 97% and 117%. No test item was detected in the control specimen.

Because control mortality was $\leq 15\%$ on D8, cumulative mortality in the reference item treatment group was $\geq 50\%$ on D8 and adult emergence in the control was $\geq 70\%$ on D22, the study can be regarded as valid.

Mean larval mortality in the control:	0.0% (between D3 - D8); validity criterion was met
Mean adult emergence rate in the control:	83.3% (up to D22); validity criterion was met
Mean larval mortality in the reference item group:	94.4% (between D3 and D8); validity criterion was met

The results are summarized below.

Toxicity of GLOB1817H to larvae of *Apis mellifera* L. after repeated exposure

Treat- ment group	Treat- ment ID	Dose	Concen- tration	On D8			On D15		On D22		
				Larval mortality D3 to D8		Mean OO	Pupal mortality D8-D15		Total mortality D3-D22		Adult emer- gence rate
				[%]			[%]		[%]		
				abs.	corr.		abs.	corr.	abs.	corr.	
		[µg pro- duct/ larva]	[mg product/ kg food]								
Con- trol	AC	-	-	0.0	-	0.0	11.1	0.0	16.7	0.0	83.3
Test item	AT	88.7	560.9	13.9	-	0.0	12.8	1.9	41.7	30.0	58.3*
	BT	35.5	224.3	0.0	-	0.0	19.4	9.4	38.9	26.7	61.1*
	CT	14.2	89.7	0.0	-	0.0	19.4	9.4	38.9	26.7	61.1*
	DT	5.7	35.9	0.0	-	0.0	16.7	6.3	30.6	16.7	69.4
	ET	2.3	14.4	0.0	-	0.0	11.1	0.0	22.2	6.7	77.8
Refe- rence item	AR	[µg a.i./ larva]	[mg a.i./ kg food]								
		7.6	48	94.4	-	0.0	100.0	0.0	100.0	100.0	0.0

Treatment	Endpoint: Successful adult emergence	Up to D22
Test item doses	ED ₅₀ [µg product/larva] ²	> 88.7
	ED ₂₀ [µg product/larva] ²	13.5 (5.2 – 34.9)
	ED ₁₀ [µg product/larva] ²	1.9 (0.3 – 12.6)
	NOED [µg product/larva] ¹	5.7
Test item concentrations	EC ₅₀ [mg product/kg food] ²	> 560.9
	EC ₂₀ [mg product/kg food] ²	85.0 (32.8 – 220.8)
	EC ₁₀ [mg product/kg food] ²	12.1 (1.8 – 79.8)
	NOEC [mg product/kg food] ¹	35.9

Results are averages based on 3 replicates, containing 12 larvae each; exception: Average% of pupal mortality was calculated according to the following formula: Sum of dead larvae between D8 and Dx / Sum of living larvae on D8 x 100% (replicate wise); see Appendix 4 for details; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947) : test and reference item treated groups were corrected by AC, negative values were set to "0"; abs.: absolute; OO: Other observations (e.g. remaining food, smaller body size, discolourations); Calculations were performed with non-rounded values;

* statistically significant difference compared to the control (Step-down Cochran-Armitage Test Procedure);

¹ Step-down Cochran-Armitage Test; alpha=0.05; one-sided greater; ² effective doses/concentrations (95%-cl lower – upper) were calculated using Probit analysis using linear maximum likelihood regression

Conclusion

In a repeated exposure larval toxicity study with GLOB1817H, the ED₅₀ (adult emergence up to D22) was determined to be > 88.7 µg product/larva, which is equivalent to an EC₅₀ of > 560.9 mg product/kg food.

The ED₂₀ (adult emergence up to D22) was determined to be 13.5 µg product/larva, which is equivalent to an EC₂₀ of 85.0 mg product/kg food. The ED₁₀ (adult emergence up to D22) was determined to be 1.9 µg product/larva, which is equivalent to an EC₁₀ of 12.1 mg product/kg food. The NOED was 5.7 µg product/larva and the corresponding NOEC was 35.9 mg product/kg food.

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 conducted to the guideline and according to the principles of GLP. All validity criterions 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	Effects of GLOB1817H on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Destefani-Perez) in an extended laboratory test, Röhlig U., 2020, 20 48 NAE 0018
Guideline(s):	Yes, IOBC (Mead-Briggs <i>et al.</i> , 2009)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1817H on the parasitic wasp *Aphidius rhopalosiphi*. For determination of mortality and reproduction adult wasps were exposed to fresh, dry residues of GLOB1817H on potted barley plants. 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 7 treatment groups (5 test item rates, control, reference item), each with 6 replicates. Five females per replicate were exposed to dried residues of GLOB1817H sprayed on potted barley plants at application rates of 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha with a water volume corresponding to 400 L/ha. Additional test units were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g dimethoate/L) as the reference item. Endpoints of the study were the mortality (including determination of the LR₅₀) and additionally effects on reproduction.

In the water-treated control a mortality of 3.3% was observed. In the test item treatments mortality ranged between 3.3% and 100%. This resulted in corrected mortality rates between 0% and 100%. No statistically significant effects on mortality were determined in all test item treatments up to and including 1.5 L product/ha. The LR₅₀ for GLOB1817H was calculated to be 2.176 L product/ha in 400 L water/ha. The NOER for mortality was 1.5 L product/ha.

The mean number of mummies per female in the test item treatments was between 21.6 and 22.3, and 22.8 mummies per female in the control. No statistically significant effects on reproductive capacity were determined in the test item treatments, up to and included 1.5 L product/ha. The ER₅₀ for GLOB1817H was estimated to be > 1.5 L product/ha in 400 L water/ha. The NOER for reproduction was ≥ 1.5 L product/ha.

Materials and Methods

Test item:	GLOB1817H, batch No.: KS010420 analysed content of a.i.: Prosulfocarb: 672.8 g/L (nominal 667 g/L) Diflufenican: 14.20 g/L (nominal 14 g/L) Halauxifen-methyl: 1.323 g/L (nominal 1.33 g/L) Cloquintocet-mexyl: 1.349 g/L (nominal 1.33 g/L) Density: 1.0085g/mL
Test species:	Parasitic wasp <i>Aphidius rhopalosiphii</i> (DEStEFANI-PEREZ), adults (< 48 hours old) source (in the stage of mummies): “Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany
Test design:	Exposure of the adults was achieved via air-dried spray residues on treated, potted barley plants. Seven treatment groups (5 test item rates, water treated control, reference item) were set up with 6 replicates (consisting of 5 females) per treatment. Mortality assessments were carried out 2, 24 and 48 hours after start of exposure of the wasps. At 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 all treated groups (1 assessment, 14 days after application).
Endpoints:	Mortality: number of dead wasps, including the determination of the LR ₅₀ . Reproductive capacity: number of mummies per female, including the determination of the ER ₅₀ .
Reference item:	DANADIM PROGRESS (Dimethoate 411.2 g/L, nominal: 400 g/L)
Validity criteria:	Mortality in the control group: ≤ 10 % (48 hours)

reproduction in the control group: ≥ 5 mummies per female (only, when a reproduction test was performed with surviving wasps of the test item group)
corrected mortality in the reference item group: $> 50\%$ (48 hours)

Test rates: Control (deionised water)
Test item (GLOB1817H):
0.375 – 0.75 – 1.5 – 3 – 6 L product/ha

The reference item was applied at a rate of 10 mL/ha. All substances were applied in 400 L water/ha. The substances were sprayed on potted barley plants via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 19-22 °C
Relative humidity: 67-82 %
Light-dark-cycle: 16 hours light, 8 hours dark
Light intensity: 1150 lux (mortality phase)
5460 lx (parasitisation phase)
6820 lx (reproduction phase)
Food: 10 % w/w aqueous fructose solution

Statistics: Multiple Sequentially-rejective FISHER test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item)
FISHER's Exact Binomial test ($\alpha = 0.05$) for mortality (reference item)
Probit Analysis for LR₅₀ calculation
DUNNETT's-t-test ($\alpha = 0.05$) for repellence (test item)
WILLIAMS t-test ($\alpha = 0.05$) for reproductive capacity (test item)

Results and discussion

Experimental dates: 10 August 2020 – 24 August 2020

All validity criteria were met.

In the water-treated control a mortality of 3.3 % was observed. In the test item treatments mortality ranged between 3.3 % and 100 %. This resulted in corrected mortality rates between 0% and 100%. No statistically significant effects on mortality were determined in all test item treatments up to and including 1.5 L product/ha (Multiple Sequentially-rejective FISHER test after BONFERRONI-HOLM, $\alpha = 0.05$). The LR₅₀ for GLOB1817H was calculated to be 2.176 L product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was 1.5 L product/ha.

The mean number of mummies per female in the test item treatments was between 21.6 and 22.3, and 22.8 mummies per female in the control. No statistically significant effects on reproductive capacity were determined in the test item treatments, up to and included 1.5 L product/ha (WILLIAMS-t-test, $\alpha = 0.05$). The ER₅₀ for GLOB1817H was estimated to be > 1.5 L product/ha in 400 L water/ha. The NOER (no observed effect rate) for reproduction was ≥ 1.5 L product/ha.

The results are summarised below.

Effects on the parasitic wasp (*Aphidius rhopalosiphii*) exposed to GLOB1817H in an extended laboratory test

Treatment	Rate ¹ [L product/ha]	Mortality ² [%]	Corrected Mortality ³ [%]	Reproduction ⁴ [mean number of mummies/female]	Effects on reproduction ⁵ [%]
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Control	-	3.3	-	22.8	-
Test item	0.375	3.3 (n.s.)	0	22.3 (n.s.)	2.2
Test item	0.75	3.3 (n.s.)	0	21.7 (n.s.)	4.8
Test item	1.5	13.3 (n.s.)	10.3	21.6 (n.s.)	5.3
Test item	3	86.7*	86.2	-	-
Test item	6	100*	100	-	-
Endpoint [L product/ha]					
LR₅₀ [95 % CL]	2.176 [1.913 – 2.471]				
ER₅₀	> 1.5				

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 Multiple Sequentially-rejective FISHER test after BONFERRONI-HOLM ($\alpha = 0.05$).

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 WILLIAMS-t-test ($\alpha = 0.05$).

⁵ Change in mean number of mummies per female, relative to control. A positive value indicates a decrease relative to the control.

n.s. not statistically significant different compared to the control

* statistically significant different compared to the control

No unusual observations were noted in the control and all test item groups up to and including 6 L product/ha 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 the test item groups up to and including 6 L product/ha compared to the control (DUNNETT's-t-test, $\alpha = 0.05$).

The reference item caused a mortality of 100 % of exposed wasps, resulting in a corrected mortality of 100 %.

Conclusions

In an extended laboratory study with GLOB1817H the LR₅₀ for *Aphidius rhopalosiphi* was calculated to be 2.176 L product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was 1.5 L product/ha. The ER₅₀ for GLOB1817H was estimated to be > 1.5 L product/ha in 400 L water/ha. The NOER (no observed effect rate) for reproduction was 1.5 L product/ha.

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions 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 Effects of GLOB1817H on the predatory mite *Typhlodromus Pyri* Scheuten in an extended laboratory test, Röhlig U., 2020, 20 48 NTE 0013

Guideline(s): Yes, IOBC (Blümel *et al.* 2000), modified for the exposure on natural substrate (extended laboratory test)

Deviations: No
GLP: Yes
Acceptability: Yes

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1817H on the predatory mite *Typhlodromus pyri* SCHEUTEN. For determination of mortality and reproduction, protonymphs of the mites were exposed to fresh, dry residues of GLOB1817H on bean leaf discs over 14 days. Effects on reproduction were assessed by the number of eggs laid and number of juveniles per evaluation period.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 5 replicates. 20 protonymphs per replicate were exposed to dried residues of GLOB1817H sprayed on bean leaf discs (*Phaseolus vulgaris*) at application rates of 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha with a water volume corresponding to 200 L/ha. Additional test units were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as the reference item. Endpoints of the study were the mortality and additionally effects on reproduction.

After 7 days, in the water-treated control a mortality of 2.0% was observed. In the test item treatments mortality ranged between 2.0% and 100%. This resulted in corrected mortality rates between 0% and 100%. No statistically significant effects on mortality were determined at tested rates, up to including 0.75 L product/ha compared to the control. The LR₅₀ was calculated to be 1.368 L product/ha. The NOER for mortality was 0.75 L product/ha.

The reproductive capacity of the mites was assessed in the control group and the 0.375 and 0.75 L product/ha test item rates. The reproduction rate amounted to 6.45 eggs/female in the control treatment. The reproduction rate in the test item treated groups was 5.93 eggs /female and 5.14 eggs/female. Thus, an effect on reproduction of 8.1 % and 20.3 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction was determined at the rate of 0.375 L product/ha. The ER₅₀ could not be calculated, this was estimated to be > 0.75 L product/ha. The NOER for reproduction was 0.375 L product/ha.

Materials and Methods

Test item: GLOB1817H, batch No.: KS010420
analysed content of a.i.:
Prosulfocarb: 672.8 g/L (nominal 667 g/L)
Diflufenican: 14.20 g/L (nominal 14 g/L)
Halauxifen-methyl: 1.323 g/L (nominal 1.33 g/L)
Cloquintocet-mexyl: 1.349 g/L (nominal 1.33 g/L)
Density: 1.0085g/mL

Test species: Predatory mite *Typhlodromus pyri* SCHEUTEN, protonymphs
(< 24 hours old); source (in the stage of eggs):
“Katz Biotech AG”, An der Birkenpfehlheide 10, 15837 Baruth, Germany

Test design: Protonymphs were exposed to dried spray residues of different application rates of the test item applied on bean leaf discs (*Phaseolus vulgaris*). 7 treatment groups (5 test item rates, water treated control, reference item) were set up with 5 replicates (consisting of 20 protonymphs) per treatment. Exposure lasted until 14 days after application.

Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. In addition, for the control and the both test item treatment groups of 0.375 and 0.75 L product/ha the reproduction, i.e.

number of eggs per female, was determined (3 assessments, 9, 11 and 14 days after application).

Endpoints: Mortality after exposure over 7 days, including determination of a LR₅₀ (Lethal Rate 50 %, rate resulting in 50 % mortality)
Reproductive capacity of the surviving mites from day 7-14 including determination of an ER₅₀ (Effect Rate 50 %, rate resulting in 50 % effect on reproduction)

Reference item: DANADIM PROGRESS
(Dimethoate 411.2 g/L, nominal: 400 g/L)

Validity criteria: Mortality in the control group: ≤ 20% (dead and escaped mites) on day 7.
Corrected mortality in the reference group: 50-100% on day 7.
Reproduction in the control group: ≥ 4 eggs per female (only when a fecundity test was performed with surviving mites of the test item group).

Test rates: Control (deionised water)
Test item (GLOB1817H): 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha

The reference item was applied at a rate of 30 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed on bean via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23 °C - 25 °C
Relative humidity: 61 % - 80 %
Light-dark-cycle: 16 hours light : 8 hours dark;
Light intensity: 2040 lx
Food: pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1

Statistics: Multiple Sequentially-rejective Chi²-2x2 Table test after BONFERRONI-HOLM test (α = 0.05) for mortality (test item)
Chi² 2x2 Table test (α = 0.05) for mortality (reference item)
Spearman-Kärber procedure for LR₅₀ calculation
WILLIAMS-t-test (α = 0.05) for reproductive capacity

Results and Discussion

Experimental dates: 28 July 2020 – 11 August 2020

All validity criteria were met.

After 7 days, in the water-treated control a mortality of 2.0% was observed. In the test item treatments mortality ranged between 2.0 % and 100 %. This resulted in corrected mortality rates between 0% and 100%. No statistically significant effects on mortality were determined at tested rates, up to including 0.75 L product/ha compared to the control (Multiple Sequentially-rejective Chi²-2x2 Table test after BONFERRONI-HOLM, α = 0.05). The LR₅₀ was calculated to be 1.368 L product/ha. The NOER (no observed effect rate) for mortality was 0.75 L product/ha.

The reproductive capacity of the mites was assessed in the control group and the 0.375 and 0.75 L product/ha test item rates. The reproduction rate amounted to 6.45 eggs/female in the control treatment. The reproduction rate in the test item treated groups was 5.93 eggs /female and 5.14 eggs/female. Thus, an effect on reproduction of 8.1 % and 20.3 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction was determined at the rate of 0.375 L

product/ha (WILLIAMS-t-test, $\alpha = 0.05$). The ER_{50} could not be calculated, this was estimated to be > 0.75 L product/ha. The NOER (no observed effect rate) for reproduction was 0.375 L product/ha.

The results are summarised below.

Effects on predatory mite *Typhlodromus pyri* exposed to fresh dry residues of GLOB1817H in an extended laboratory trial

Treatment	Rate ¹ [L product/ha]	Mortality ² [%]	Corrected mortality ³ [%]	Mean number of eggs per female ⁴ [7-14 Day]	Effect on Reproduction ⁵ [%]
Control	-	2.0	-	6.45	-
Test item	0.375	3.0 (n.s.)	1.0	5.93 (n.s.)	8.1
Test item	0.75	2.0 (n.s.)	0	5.14*	20.3
Test item	1.5	63.0*	62.2	n.d.	-
Test item	3	100*	100	n.d.	-
Test item	6	100*	100	n.d.	-
Endpoint [L product/ha]					
LR₅₀ [95 % CL]	1.368 [1.277 – 1.465]				
NOER	0.75				
ER₅₀				> 0.75	
NOER				0.375	

¹ Application rate in 200 L water/ha

² Mortality after 7 days of exposure to residues on treated leaf discs. The results for mortality in individual test item treatments were compared to that in the control using the Multiple Sequentially-rejective Chi²-2x2 Table test after BONFERRONI-HOLM ($\alpha = 0.05$).

³ Corrected mortality according to ABBOTT (1925)

⁴ Results for reproduction compared by WILLIAMS-t-test ($\alpha = 0.05$)

⁵ Change in mean number of eggs per female, relative to control. A positive value indicates a decrease relative to the control.

n.s. not statistically significant different compared to the control

* statistically significant different compared to the control

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

The reference item caused a mortality of 79.0 % of exposed mites, resulting in a corrected mortality of 78.6 %.

Conclusions

In an extended laboratory study with GLOB1817H the LR_{50} for *Typhlodromus pyri* was calculated to be: $LR_{50} = 1.368$ L product/ha in 200 L water/ha. The NOER (no observed effect rate) for mortality was 0.75 L product/ha. The ER_{50} could not be calculated, this was estimated to be > 0.75 L product/ha. The NOER (no observed effect rate) for reproduction was 0.375 L product/ha.

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions 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	Effects of GLOB1817H on the rove beetle <i>Aleochara bilineata</i> Gyll. in an extended laboratory test, Röhlrig U., 2020, 20 48 NKE 0010
Guideline(s):	Yes, IOBC (Grimm <i>et al.</i> 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1817H on the rove beetle *Aleochara bilineata*. For determination of the reproductive capacity adults were exposed to different application rates of GLOB1817H sprayed onto sandy soil. Effects on reproduction were assessed by the number of emerged beetles compared to the control group.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 4 replicates. 10 females and 10 males (10 pairs) per replicate were exposed to the test item sprayed onto sandy soil at application rates of 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha. Additional test units were treated with deionised water as control or with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as reference item. The endpoint of the study was the reproductive capacity.

In the water-treated control the average number of hatched beetles of the F₁ generation was 524. In the test item treatments reproductive capacity ranged between 496 and 524. This resulted in effects on reproduction between 5.3% and -0.1% inhibition. No statistically significant differences compared to the control were observed at all rate of GLOB1817H, compared to the water-treated control. The ER₅₀ was estimated to be > 6 L product/ha. The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 6 L product/ha.

Materials and Methods

Test item:	GLOB1817H, batch No.: KS010420 analysed content of a.i.: Prosulfocarb: 672.8 g/L (nominal 667 g/L) Diflufenican: 14.20 g/L (nominal 14 g/L) Halauxifen-methyl: 1.323 g/L (nominal 1.33 g/L) Cloquintocet-mexyl: 1.349 g/L (nominal 1.33 g/L) Density: 1.0085g/mL
Test species:	Rove beetle <i>Aleochara bilineata</i> GYLL., adults (1-7 days old); source: reared in the laboratory of the test facility
Test design:	The test item rates, control and reference item were sprayed via a laboratory spray applicator (tracksprayer) on the soil surface. Exposure of the beetles was reached via air-dried residues on treated sandy soil (LUFA 2.1). Seven treatment groups (5 test item rates, water-treated control, reference item) were set up with 4 replicates (consisting of 10 females and 10 males (10 pairs) per treatment. On day 7, 14 and 21 approx. 500 pupae of <i>Delia antiqua</i> were buried in the sandy soil (LUFA 2.1) of each replicate to be parasitised by the larvae of the beetles. On day 28 the adults were separated from the soil and the sandy soil with the pupae was allowed to dry for seven days. On day 35 the pupae were removed from the soil by a sieve and transferred into a hatching unit. After

hatching, the test endpoint reproductive capacity (average number of hatched beetles of the F₁ generation) was determined (daily assessments during 5 weeks).

Endpoint: Reproductive capacity (average number of hatched beetles of the F₁ generation)

Reference item: DANADIM PROGRESS
(Dimethoate 411.2 g/L, nominal: 400 g/L)

Validity criteria: Average number of hatched beetles per replicate of the F₁-generation in the control: > 400 (i.e. parasitisation rate > 26.7 % of the 1500 introduced fly pupae per replicate should be parasitised).
Reduction of the reproductive capacity in the reference item treatment relative to control: ≥ 50 %.

Test rates: Control (deionised water)
Test item: 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha with an application volume of 400 L/ha

The reference item was applied at a rate of 1.5 L/ha. All substances were applied in 400 L water/ha. The substances were sprayed onto sandy soil via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 19 °C - 22 °C;
Relative humidity: 63 % - 82 %
Light-dark-cycle: 16 hours light : 8 hours dark;
Light intensity: 1940 lx
Food: *Chironomus* spp. larvae (thawed)

Statistics: WILLIAMS-t-test ($\alpha = 0.05$) for reproductive capacity (test item)
STUDENT-t-test ($\alpha = 0.05$) for reproductive capacity (reference item)

Results and Discussion

Experimental dates: 16 July 2020 – 21 September 2020

All validity criteria were met.

In the water-treated control the average number of hatched beetles of the F₁ generation was 524. In the test item treatments reproductive capacity ranged between 496 and 524.

This resulted in effects on reproduction between 5.3% and -0.1% inhibition. No statistically significant differences compared to the control were observed at all rate of GLOB1817H (WILLIAMS-t-test, $\alpha = 0.05$), compared to the water-treated control. The ER₅₀ was estimated to be > 6 L product/ha. The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 6 L product/ha.

The results are summarised below.

Effects on reproductive capacity of the rove beetle (*Aleochara bilineata* GYLL.) exposed to GLOB1817H in an extended laboratory test

Treatment	Rate ¹ [L product/ha]	Reproduction [mean number of emerged beetles per replicate]	Reproduction [absolute number of emerged beetles per treatment group]	Effect on Reproduction ² [%]
Control	-	524	2095	-

Test item	0.375	498 (n.s.)	1993	4.9
Test item	0.75	521 (n.s.)	2083	0.6
Test item	1.5	524 (n.s.)	2097	-0.1
Test item	3	504 (n.s.)	2015	3.8
Test item	6	496 (n.s.)	1983	5.3
Reference item	1.5 L product/ha	155*	618	70.5

¹ Application rate in 400 L water/ha

² Effect on reproduction according to the following formula: $(1 - Pt/Pc) * 100\%$ calculated on the absolute number of emerged beetles (positive values represent a decreased and negative values indicates an increased reproduction compared to the control)

n.s. statistically significantly different compared to the control: WILLIAMS-t-test, $\alpha = 0.05$ (test item)

* statistically significantly different compared to the control: STUDENT-t- test, $\alpha = 0.05$ (reference item)

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusions

In an extended laboratory study, the ER₅₀ for *Aleochara bilineata* was estimated to be > 6 L product/ha. The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 6 L product/ha.

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions 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 Effects of GLOB1817H on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test, Röhlig U., 2020, 20 48 NLE 0007

Guideline(s): Yes, IOBC (Heimbach *et al.* 2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1817H on the carabid beetle *Poecilus cupreus* L. For determination of the mortality adult beetles were exposed to fresh dried spray residues of the test item applied onto sandy soil. Effects on mortality were assessed by the number of surviving beetles, additionally behavioural impacts (food uptake) were assessed.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 5 replicates. Three females and three males per replicate were exposed to dried residues of GLOB1817H sprayed onto sandy soil at rates of 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha in 400 L/ha. Additional test units were treated with deionised water as control and with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as reference item. Endpoints of the study were mortality and additionally effects on the food uptake.

After 14 days, in the water-treated control a mortality of 3.3 % was observed. In the test item treatments mortality was between 0 % and 3.3 %. This resulted in corrected mortality rates of -3.4 % and 0 %. No statistically significant effects on mortality were observed at all tested rates. The NOER for mortality was ≥ 6 L product/ha.

The food uptake (mean number of consumed fly pupae per surviving beetle during the total study period) ranged between 8.43 and 9.13 fly pupae in the test item treatment groups, in comparison to the control with 8.43 fly pupae. No statistically significant effects on food uptake were determined at all tested rates. The NOER for food uptake was ≥ 6 L product/ha.

Materials and Methods

Test item:	GLOB1817H, batch No.: KS010420 analysed content of a.i.: Prosulfocarb: 672.8 g/L (nominal 667 g/L) Diflufenican: 14.20 g/L (nominal 14 g/L) Halauxifen-methyl: 1.323 g/L (nominal 1.33 g/L) Cloquintocet-mexyl: 1.349 g/L (nominal 1.33 g/L) Density: 1.0085g/mL
Test species:	Carabid beetle <i>Poecilus cupreus</i> L., adults (3-7 weeks old); source (in-house culture): in the laboratory of the test facility BioChem agrar GmbH
Test design:	Exposure of the adults was achieved via air-dried spray residues onto sandy soil (LUFA 2.1). Seven treatment groups (5 test item rates, water-treated control, reference item) were set up with 5 replicates (consisting of 3 females and 3 males) per treatment. Mortality and behavioural assessments were carried out 2 hours, 1, 2, 4, 7, 11 and 14 days after application. Assessment of food uptake, i.e. number of consumed fly pupae, was made for the control and the test item groups on 1, 2, 4, 7, 11 and 14 days after application.
Validity criteria:	Mortality in the control group (after 2 weeks): ≤ 6.7 %. Corrected mortality in the reference item group (after 2 weeks): 65 ± 35 %.
Endpoints:	Mortality: number of dead beetles, including estimation of a LR_{50} Food uptake: number of consumed fly pupae per surviving beetle, including estimation of an ER_{50}
Test rates:	Control (deionised water) Test item (GLOB1817H): 0.375 – 0.75 – 1.5 – 3 – 6 L product/ha Reference item (DANADIM PROGRESS): 2.25 L product/ha. All substances were applied in 400 L water/ha. The substances were sprayed onto sandy soil (LUFA 2.1) via laboratory spraying equipment and air dried afterwards.
Test conditions:	Temperature: 19 °C - 22 °C; Relative humidity: 63 % - 73 % Light-dark-cycle: 16 hours light : 8 hours dark; Light intensity: 1030 lx Food: defrosted pupae of onion fly <i>Delia antiqua</i>
Statistics:	Chi ² 2x2 Table Test with BONFERRONI Correction ($\alpha = 0.05$) for mortality (test item) Chi ² 2x2 Table Test ($\alpha = 0.05$) for mortality (reference item) WILLIAMS Multiple Sequential t-Test ($\alpha = 0.05$) for food uptake (test item)

STUDENT-t-test ($\alpha = 0.05$) for food uptake (reference item)

Results and Discussion

Experimental dates: 21 September 2020 – 05 October 2020

All validity criteria were met.

- mortality in the control group (after 2 weeks): $\leq 6.7\%$ (observed: 3.3 %)
- corrected mortality in the reference item group (after 2 weeks): $65 \pm 35\%$ (observed: 100 %)

The food uptake (mean number of consumed fly pupae per surviving beetle during the total study period) ranged between 8.43 and 9.13 fly pupae in the test item treatment groups, in comparison to the control with 8.43 fly pupae. No statistically significant effects on food uptake were determined (WILLIAMS Multiple Sequential t-Test, $\alpha = 0.05$) at all tested rates. The NOER (no observed effect rate) for food uptake was ≥ 6 L product/ha.

The results are summarised below.

Effects on the carabid beetle (*Poecilus cupreus*) exposed to fresh dry residues of GLOB1817H in an extended laboratory test

Treatment	Rate ¹ [L product/ha]	Mortality ² [%]	Corrected Mortality ³ [%]	Total number of consumed fly pupae	Food uptake ⁴ [mean number of consumed fly pupae/surviving beetle]		Effect on food uptake ⁵ [%]
					during the total study period	per assessment day	
Control	-	3.3	-	253	8.43	1.41	-
Test item	0.375	0 (n.s.)	-3.4	259	8.63	1.44 (n.s.)	-2.1
Test item	0.75	3.3 (n.s.)	0	256	8.53	1.42 (n.s.)	-0.7
Test item	1.5	0 (n.s.)	-3.4	257	8.57	1.43 (n.s.)	-1.4
Test item	3	0 (n.s.)	-3.4	274	9.13	1.52 (n.s.)	-7.8
Test item	6	0 (n.s.)	-3.4	253	8.43	1.41 (n.s.)	0
	Endpoint [L product/ha]						
LR ₅₀	> 6						
ER ₅₀				> 6			
Reference item DANADIM PROGRESS	2.25	100*	100	19	0.63	0.32*	77.3

¹ Application rate in 400 L water/ha

² Mortality after 14 days of exposure to residues on sandy soil. The results for mortality in individual treatments were compared to that in the control Chi² 2x2 Table Test with BONFERRONI Correction ($\alpha = 0.05$) for the test item and Chi² 2x2 Table Test ($\alpha = 0.05$) for the reference item

³ Corrected mortality according to ABBOTT (1925)

⁴ Food uptake: mean number of consumed fly pupae/surviving beetle. The results for the test item treatments and control and the reference item treatment and control were compared by WILLIAMS Multiple Sequential t-Test (test item) and STUDENT-t-test, respectively ($\alpha = 0.05$).

⁵ Change in mean number of consumed fly pupae per treatment group, relative to control. A negative value indicates an increase, relative to the control.

(n.s.) not statistically significant different compared to the control

* statistically significant different compared to the control

The reference item caused a mortality of 100 % of exposed beetles, resulting in a corrected mortality of 100 %.

Conclusions

In an extended laboratory study with GLOB1817H the LR₅₀ for *Poecilus cupreus* was estimated to be > 6 L product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was estimated to be ≥ 6 L product/ha in 400 L water/ha. The ER₅₀ for GLOB1817H was estimated to be > 6 L product/ha in 400 L water/ha. The NOER (no observed effect rate) for food uptake was estimated to be ≥ 6 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 to the OECD guideline 222 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
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The following study on earthworms was provided in support of the assessment.

Reference:	KCP 10.4.1.1
Report	Earthworm reproduction test with Prosulfocarb 800 g/L EC (OECD 222, April 2004), Servajean E., Phytosafe, 12-99-012-ES
Guideline(s):	Yes, OECD 222
Deviations:	Adults were fed on Day 0 instead of Day 1. The bio-availability of carbendazim was increased because sphagnum peat was reduced from 10% to 5% in the artificial soil, and the observed EC ₅₀ for reproduction was not within 1-5 mg/kg as it is classically observed. The initial pH of the control soil was not 6.0 ± 0.5 because the amount of CaCO ₃ which is classically added was not convenient when sphagnum peat was 5% instead of 10%. The above changes did not adversely affect the quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

Executive Summary

This study aimed to determine the effect of Prosulfocarb 800 g/L EC on the reproduction of *Eisenia fetida* under laboratory conditions on an artificial substrate previously amended with the test item at different concentrations. The definitive test was performed using four replicate units each containing 10 worms for each of eight test item treatments, and eight replicate units for the water control. The adults were maintained in the artificial soil substrate for 4 weeks. Percent mortality and mean weight of the survivals was assessed. The adults were discarded and the rest units maintained in the climatic chamber for 4 additional weeks. At the end of the period, the number of juveniles was determined. Carbendazim was used as the reference item to confirm the function of the test system. The NOEC (reproduction) was determined to be 22.2 mg test item/kg soil dry weight. The EC₅₀ (reproduction) was calculated to be 47.4 mg test item/kg soil d.w.

Materials

Test Material	Prosulfocarb 800 g/L EC
Lot/Batch #:	1910121008
Actual content of active ingredients:	Prosulfocarb: 799.8 g/L (analysed)
Description:	Transparent orange
Treatments	
Test rates:	15.4, 22.2, 31.9, 45.4, 65.7, 93.7, 135.2, 193.1 mg test item/kg soil dry weight
Control:	Water
Toxic standard:	Carbendazim
Application method:	Mixed with artificial soil
Test organisms	
Species:	<i>Eisenia fetida</i>
Age:	Adult (2 months – 1 year)
Source:	Culture maintained at test facility
Feeding:	5 g of food moistened with water on days 0, 7, 14, 21 and 28.
Test design	
Arenas:	1.5 to 2 L glass containers
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 75% fine sand (50% particles between 0.05 mm and 0.2 mm) and calcium carbonate to give pH of 6.0 ± 0.5 . 500 g dry weight per test vessel, moistened to 45-55% of WHC.
Replication:	Control: 8 Treated: 4
No./arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18.5 – 20.5°C
Photoperiod:	16 h light (400-800 lux)/8 h dark

Study Design and Methods

Experimental dates: 11 May 2012 – 9 July 2012

The test was performed using four replicate units each containing 10 worms for each of eight test item treatments as a geometrical series between approximately 20 and 250 mg/kg (15 and 195 mg a.i./kg dry soil). These concentrations were chosen in the light of the results of the acute toxicity test with the test substance which showed that NOEC for mortality = 306 mg/kg and NOEC biomass < 108 mg/kg.

Carbendazim was used as the reference item to confirm the function of the test system.

	Nature	Preparation		Volume per test unit	Concentration
		Quantity	Solvent		
Solution 8	Test item	6.2052 g	q.s.p 500 mL	10.0 mL	193.1 mg a.i./kg
Solution 7	Solution 8	-	-	7.0 mL	135.2 mg a.i./kg
Solution 6	Solution 8	-	-	4.9 mL	93.7 mg a.i./kg
Solution 5	Solution 8	-	-	3.4 mL	65.7 mg a.i./kg
Solution 4	Solution 8	-	-	2.4 mL	45.4 mg a.i./kg
Solution 3	Solution 8	-	-	1.7 mL	31.9 mg a.i./kg
Solution 2	Solution 8	-	-	1.2 mL	22.2 mg a.i./kg
Solution 1	Solution 8	-	-	0.8 mL	15.4 mg a.i./kg

Results and Discussion

Effects of Prosulfocarb 800 g/L EC on mortality and reproduction of *Eisenia fetida*

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	15.4	22.2	31.9	45.4	65.7	93.7	135.2	193.1
% Mortality	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	2.5
Gain of adult biomass (%)	+62.7	+63.9	+56.3	+45.5	+52.6	+43.0	+46.7	+36.1	+17.7
Number of juveniles	235.3	243.5	203.5	140.0	97.5	71.8	29.5	33.3	7.5
SD	38.3	31.5	20.9	45.3	13.6	15.7	16.1	19.1	9.0
NOEC (mortality)	> 193.1								
NOEC (reproduction)	22.2								
NOEC (biomass)	45.4								
EC ₅₀ (reproduction)	47.4 (95 % confidence limits 26.2 – 85.6)								

Validity criteria

The validity criteria are as follows:

- Mean percent mortality in the control $\leq 10\%$ of the initial population
- Production of juveniles in the control ≥ 30 per unit
- The coefficient of variation of reproduction in the control $\leq 30\%$

Conclusions

The NOEC for mortality was determined to be > 193.1 mg test item/kg soil dry weight. The NOEC for adult biomass was determined to be 45.4 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 22.2 mg test item/kg soil dry weight. The EC_{50} (based on reproduction) was calculated to be 47.4 mg test item/kg soil dry weight.

Comments of zRMS:	The study was conducted to the OECD guideline 222 and according to the principles of GLP. All validity criterions 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 Effects of GLOB1817H on the reproduction of the earthworm *Eisenia fetida*, Friedrich S., 2020, 20 48 TEC 0054

Guideline(s): Yes, OECD 222 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

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 a 56-day earthworm reproduction study with GLOB1817H, no statistically significant effect on survival of the adult earthworms and no statistically significant effects on biomass of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 268 mg test item/kg soil dry weight, i.e. the highest concentration tested. The NOEC for mortality and change of biomass was determined to be 268 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 41 mg test item/kg soil dry weight. The EC_{10} , EC_{20} and EC_{50} values for reproduction were calculated to be 45 , 95 and > 268 mg test item/kg soil dry weight.

Materials and Methods

Test item: GLOB1817H

Batch No.: KS010420

Active ingredient/ content:	Prosulfocarb:	<u>nominal</u> 667 g/L	<u>analysed</u> 672.8 g/L
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	<p>Di flufenican: 14.0 g/L 14.20 g/L</p> <p>Halauxifen- methyl: 1.33 g/L 1.323 g/L</p> <p>Cloquintocet-mexyl: 1.33 g/L 1.349 g/L</p>
Test species:	earthworm <i>Eisenia fetida</i> (Savigny, 1826)
Test design:	<p><u>Effects on earthworms</u>: 56 days;</p> <p>8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment, 10 worms per replicate; assessment of adult worm mortality, behavioural effects and biomass development after 28 days, reproduction rate after an additional 28 days (assessed 56 days after application)</p>
Test system:	Exposure of worms to different concentrations of the test item mixed into artificial soil substrate (with 10 % peat)
Reference item:	<p>Maypon Flow (Carbendazim, SC 500)</p> <p>The effects of the reference item were investigated in a separate study.</p>
Test conditions:	<p>Temperature: 19.0 - 21.6 °C</p> <p>Light intensity: 630 lux</p> <p>Photoperiod: light : dark = 16 h : 8 h</p>
Treatments:	Control (untreated), test item (GLOB1817H)
Test concentrations:	10, 16, 26, 41, 65, 105, 168, 268 mg test item/kg soil dry weight (spacing factor: 1.6)
Dates of work:	<p>Experimental start date: 17 September 2020</p> <p>Experimental completion date: 12 November 2020</p>
Statistics:	<p>Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality,</p> <p>($\alpha = 0.05$, one-sided greater), Williams t-test for biomass change and reproduction ($\alpha = 0.05$, one-sided smaller),</p> <p>Probit analysis for calculation of for calculation of EC_x;</p> <p>Statistical program: ToxRat Professional 3.3.0 (2018)</p>

Results and Discussion

The test item caused no statistically significant effect (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater) on mortality and no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams t-test, $\alpha = 0.05$, one-sided smaller). Statistically significant effects (Williams t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the control group were recorded at concentrations of 65, 105, 168 and 268 mg test item/kg soil d.w.

Effects of GLOB1817H on *Eisenia fetida* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	10	16	26	41	65	105	168	268
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	2.5	2.5	0.0	0.0	0.0
Mean biomass change after 4 weeks (%)	26.9	26.4	27.9	29.1	26.7	28.3	25.2	27.3	23.9
Mean number of juveniles after 8 weeks	255.9	261.0	245.0	262.3	225.0	206.5*	202.3*	193.0*	143.3*

Reduction of reproduction compared to control (%)	-	-2.0	4.3	-2.5	12.1	19.3	21.0	24.6	44.0
Endpoint (mg test item/kg soil d.w.)									
NOEC (mortality)	268								
NOEC (biomass)	268								
NOEC (reproduction)	41								
LC ₅₀ (mortality) ¹	> 268								
EC ₁₀ (reproduction) ²	45 (95 % confidence limits 27 - 75)								
EC ₂₀ (reproduction) ²	95 (95 % confidence limits 70 – 129)								
EC ₅₀ (reproduction) ²	> 268								

Not statistically significantly different to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater) and biomass (Williams t-test, $\alpha = 0.05$, one-sided smaller)

* statistically significantly different compared to control regarding reproduction (Williams t-test, $\alpha = 0.05$, one-sided smaller)

Negative values = increase, relative to control

¹ based on estimation of the data, ² Probit analysis

The validity criteria for the control group were met:

- Adult mortality: $\leq 10\%$ (being 0.0 % after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being 205 to 317)
- Coefficient of variation of reproduction: $\leq 30\%$ (being 14.4 %)

Conclusions

In a 56-day earthworm reproduction study with GLOB1817H, no statistically significant effect on survival of the adult earthworms and no statistically significant effects on biomass of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 268 mg test item/kg soil dry weight, i.e. the highest concentration tested.

The NOEC for mortality and change of biomass was determined to be 268 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 41 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 45, 95 and > 268 mg test item/kg soil dry weight.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Comments of zRMS:	<p>The current study meets all criteria required for a valid earthworm field study as requested by the available ISO guidelines.</p> <p>The objective of this field study was to investigate potential effects and/or recovery of field populations of earthworms after the application of the test item Prosulfocarb 800 g/L EC. The trial was placed on arable land near Machern in Germany.</p> <p>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. The mean recovery was in the recommended range of 50 - 150 %. 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.</p> <p>The toxic reference item Nutdazim 50 FLOW reduced total earthworm abundance by 22.0 % at 1st sampling, 0.2 % at 2nd sampling and 20.2 % at 3rd sampling. <i>L. terrestris</i> was the most sensitive species and was significantly reduced in total abundance by 65.3 %, 56.6 % and 63.0 % on these sampling dates.</p> <p>The total earthworm biomass was significantly reduced by the reference item by</p>
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	<p>50.3 % at 1st sampling, 16.9 % at 2nd sampling and 17.4 % at 3rd sampling. The validity of the test system was confirmed by significant reduction in total earthworm biomass at 1st sampling (about 1 month after test item application).</p> <p>The earthworm community included five species at pre-sampling, i.e. <i>A. chlorotica</i> (on average 12.2 %), <i>A. caliginosa</i> (on average 45.0 %), <i>A. rosea</i> (on average 3.7 %), <i>A. longa</i> (on average 3.3 %) and <i>L. terrestris</i> (on average 28.6 %). Endogeic species comprised 66.4 % and anecic species comprised 31.9 % of the total earthworm population.</p> <p>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. Only for the total biomass of the earthworm species <i>L. terrestris</i> a statistically significant reduction of 27.7 % could be observed about 12 months after Prosulfocarb 800 g/L EC application. This can be the natural variability of earthworm populations, as no effects on total biomass of <i>L. terrestris</i> could be observed about 1 and 6 months.</p> <p>The study is considered to be reliable and suitable for the risk assessment.</p>
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The following field study on earthworms was provided in support of the assessment.

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
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 earthworms	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:	<p>The study was conducted to the OECD guideline 232 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.</p> <p>The NOEC value of 41 mg test item/kg soil dry weight instead calculated EC₁₀ value of 40 test item/kg soil dry weight will be used in the risk assessment. This is due to the fact that at dose 41 mg test item/kg soil dry weight the effect on reproduction was -3.7%. No effects were seen in doses 10, 16 and 26 mg test item/kg soil, too. This confirm that NOEC is the reasonable endpoint for the risk assessment.</p>
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Reference: KCP 10.4.2.1

Report Effects of GLOB1817H on the reproduction of the collembolan *Folsomia candida*, Friedrich S., 2020, 20 48 TCC 0059

Guideline(s): Yes, OECD 232 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

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 for mortality of the parental collembolans was determined to be 41 mg test item/kg soil dry weight. The LC₅₀ value for mortality was calculated to be 118 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 41 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 40, 48 and 66 mg test item/kg soil d.w.

Materials and Methods

Test item: GLOB1817H

Batch No.: KS010420

Active ingredient/ content:	<u>nominal</u>	<u>analysed</u>
Prosulfocarb:	667 g/L	672.8 g/L
Diiflufenican:	14.0 g/L	14.20 g/L
Halaluxifen- methyl:	1.33 g/L	1.323 g/L
Cloquintocet-mexyl:	1.33 g/L	1.349 g/L

Test species: Collembola (*Folsomia candida*), age: 9 - 12 days; source: in-house culture.

Test design: Effects on *Folsomia candida*: 28 days;
8 test item treatment groups and an untreated control group,
8 replicates in the control group and 4 replicates in the test item treatment
groups, each containing 10 collembolans; assessments of adult mortality and
reproduction 28 days after application

Endpoints: Mortality and reproduction after 28 days

Test system: Exposure of collembolans to different concentrations of the test item
mixed into the substrate (artificial soil with 5 % peat)

Reference item: Boric acid
The effects of the reference item were investigated in a separate study.

Test conditions: Temperature: 18.1 - 20.4 °C
Light intensity: 580 lux
Photoperiod: light : dark = 16 h : 8 h

Treatments: Control (untreated), test item (GLOB1817H)

Test concentrations: 10, 16, 26, 41, 65, 105, 168, 268 mg test item/kg soil dry weight
(spacing factor: 1.6)

Dates of work: Experimental start date: 07 October 2020
Experimental completion date: 04 November 2020

Statistics: Step-down Cochran-Armitage test, ($\alpha = 0.05$, one-sided greater),
Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller),

Probit analysis for mortality
4-parametric logistic cumulative distribution function (CDF) for reproduction,
Statistical program: ToxRat Professional 3.3.0 (2018)

Results and Discussion

Statistically significant effects on parental mortality (Step-down Cochran-Armitage test, $\alpha = 0.05$, one-sided greater) and on the number of juveniles (Williams-t-test, $\alpha = 0.05$, one-sided smaller) compared to the control group were recorded at concentrations of 65, 105, 168 and 268 mg test item/kg soil d.w. Results are summarised in the table below.

Chronic effects of GLOB1817H on *Folsomia candida* in a 28-day reproduction study

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	10	16	26	41	65	105	168	268
Mean adult mortality [%]	2.5	2.5	2.5	2.5	5.0	27.5*	37.5*	67.5*	95.0*
Mean number of juveniles	702	696	717	687	728	427*	311*	282*	126*
Reduction of reproduction [%] compared to control	-	0.8	-2.1	2.1	-3.7	39.2	55.8	59.8	82.0
Endpoints [mg test item/kg soil dry weight]									
NOEC (mortality)	41								
NOEC (reproduction)	41								
LC ₅₀ (mortality) ¹	118 (95 % confidence limits 104 – 134)								
EC ₁₀ (reproduction) ²	40 (95 % confidence limits 26 – 63)								
EC ₂₀ (reproduction) ²	48 (95 % confidence limits 36 – 64)								
EC ₅₀ (reproduction) ²	66 (95 % confidence limits 61 – 79)								

* statistically significant different compared to the control (Step-down Cochran-Armitage test for mortality, $\alpha = 0.05$, one-sided greater and Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)

Calculations were done using unrounded values

Negative values = increase, relative to control

¹ based on Probit analysis

² based on 4-parametric logistic cumulative distribution function (CDF)

In a separate study, the EC₅₀ (reproduction) of the reference item boric acid was calculated to be 107 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system

The validity criteria for the control group were met:

- Mean adult mortality: ≤ 20 % (observed: 2.5 %)
- Mean number of juveniles per test vessel: ≥ 100 (observed: average of 702/vessel)
- Coefficient of variation for the mean number of juveniles: < 30 % (observed: 6.9 %)

Conclusions

In a 28-day *Folsomia candida* reproduction study with GLOB1817H, the NOEC for mortality of the parental collembolans was determined to be 41 mg test item/kg soil dry weight. The LC₅₀ value for mortality was calculated to be 118 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 41 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 40, 48 and 66 mg test item/kg soil d.w.

Comments of zRMS:	<p>The study was conducted to the OECD guideline 226 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.</p> <p>NOEC of 165 and EC₁₀ of 50.0 mg test item/kg soil will be used in the risk assessment.</p> <p>Taking to consideration analysed concentration of 786 prosulfocarb/L and density 1.024 kg/L the following endpoints were recalculated to active substance:</p> <p>NOEC is 126.65 mg a.s./kg soil</p> <p>EC₁₀ is 38.38 mg a.s/kg soil and this value will be use in the risk assessment.</p>
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The following toxicity study on *Hypoaspis aculeifer* was provided in support of the assessment.

Reference:	KCP 10.4.2.1
Report	A dose response study to assess the NOEC, EC ₁₀₋₂₀₋₅₀ on reproduction and LR ₁₀₋₂₀₋₅₀ on mortality of Prosulfocarb 800 EC of the predatory mite <i>Hypoaspis aculeifer</i> on artificial soil in the laboratory, Lauvaux S., Walloon Agricultural Research Centre, HA04/2016
Guideline(s):	Yes, OECD 226
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

Executive summary

This study was carried out to determine the NOEC of Prosulfocarb 800 EC on reproduction capacity of the predatory mite *Hypoaspis aculeifer* on artificial soil in the laboratory. The test product was diluted in water and then added to the artificial soil to obtain a final concentration of 82.5, 165, 330, 660 and 1320 ppm of formulated product. Boric acid was used as toxic standard and deionised water as control. Ten mature females were introduced in the units at day 0, at the same time as flour mites as food. The units were kept 14 days in the laboratory. Adult and juvenile mites were counted using the Berlese extraction methods. After 14 days of exposure, the reproduction rates reached 51.13 juveniles/unit in the control, 50.75, 34.00, 22.50, 23.50 and 24.50 juveniles/unit with Prosulfocarb 800 EC at 82.5, 165, 330, 660 and 1320 ppm and 0.00 juveniles/units in the toxic standard. The NOEC based on fertility was estimated at 165 ppm. The EC₁₀₋₂₀₋₅₀ were calculated to be 50.0095, 100.5784 and 520.301 ppm, respectively.

Materials

Test Material	Prosulfocarb 800 EC
Lot/Batch #:	1058314
Actual content of active ingredients:	Prosulfocarb 800 g/L (nominal), 786 g/L (analysed)
Stability of test compound:	Stable under standard conditions.
Density:	1024 kg/m ³
Treatments	
Test rates:	82.5, 165, 330, 660, 1320 ppm
Control:	Deionised water

Toxic standard:	boric acid
Application method:	Mixed with artificial sediment
Test organisms	
Species:	<i>Hypoaspis aculeifer</i>
Age:	28-35 day old females
Source:	Commercial supplier (Katz Biotech, Germany)
Feeding:	Flour mites
Test design	
Arenas:	plastic vessel with a pierced lid (80-100 mL)
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7 % quartz sand and 0.3% calcium carbonate. 30 g dry weight per test vessel.
Replication:	Control: 8 Treated: 4
No./arena :	10
Duration of test:	2 weeks
Environmental test conditions	
Temperature:	20 ± 2°C
Water content of soil:	60% of WHC
Photoperiod:	16 h day (400-800 lux)/8 h night

Study Design and Methods

Experimental dates: 26 April 2016 – 17 June 2016

After mixing of product and substrate, 10 mated females were released in the units with the wetted substrate. Food (flour mite and dry baker yeast) was added ad lib at the same time. The units were closed with a cotton cloth and a lid above to avoid escaped females. At day 14, units were dismantled to count adult survival and juvenile progeny with the Berlese method.

Mortality of adults was corrected using the formula by Abbott (1925). Number of predatory mite progeny was analysed with an ANOVA test at $p = 0.05$ level. If no differences between objects appeared, the NOEC was expressed as \geq highest tested rate. If differences between objects appeared, each concentration of test item was compared to the control with a Dunnett test ($p = 0.05$) and the NOEC expressed as the highest concentration not statistically different from the control. The ECx values were calculated by non-linear regression.

Results and discussions

Mortality and fecundity are summarised in the table below.

Effects of residues of Prosulfocarb 800 EC on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg test item/kg soil d.w.)					
	Control	82.5	165	330	660	1320
% Mortality	5.0	20.0	20.0	15.0	5.0	32.5
% corrected mortality (Abbott) ^a	-	15.8	15.8	10.5	0.0	28.9
Mean number of juveniles	51.13	50.75	34.00	22.50*	23.50*	24.50*
NOEC (reproduction)	165					
EC ₁₀	50.0095 (95% confidence limits -30.5276 – 130.5465)					
EC ₂₀	100.5784 (95% confidence limits -20.9469 – 222.1037)					
EC ₅₀	520.301 (95% confidence limits 12.9569 – 1027.6451)					

* statistically significantly different compared to control ($p \leq 0.05$)

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 3.8 % (must be < 20%)
- Average reproduction rate in the control was 859 (must be > 50 juveniles/unit)
- The coefficient of variation of reproduction in the control was 15.2% (must not be > 30%)
- Average reproduction rate in the toxic standard (must be < 50% of the control)

Conclusion

The NOEC based on fertility was estimated at 165 ppm. The EC₁₀₋₂₀₋₅₀ were calculated to be 50.0095, 100.5784 and 520.301 ppm, respectively.

Comments of zRMS:	The study was conducted to the OECD guideline 226 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
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The following toxicity study on *Hypoaspis aculeifer* was provided in support of the assessment.

Reference:	KCP 10.4/03
Report	Diufenican 500 g/L SC: Predatory Mite (<i>Hypoaspis aculeifer</i>) Reproduction Test in Soil, Taylor K., 2016, Envigo CRS Limited, DF50GM
Guideline(s):	Yes, OECD 226
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

Executive Summary

The purpose of this study was to investigate the effects of DFF500SC on the mortality and reproduction of the predatory mite *Hypoaspis aculeifer*. This study was designed to comply with OECD 226.

14-day test in treated artificial soil prepared according to OECD 226; different concentrations of the test item were incorporated into the soil; 3 treatment groups (1 test item concentration, negative control, positive control: Dimethoate, 400 g/L); 8 replicates for all treatment groups with 10 worms each.

Assessment of mortality was carried out after 14 days exposure in treated artificial soil. Reproduction rate (number of offspring) was assessed after an additional 14 days.

The NOEC for mortality and reproduction activity was determined to be > 1000 mg test item/kg soil. The 14-day LC₅₀ and 14-day EC₅₀ were estimated to be >1000 mg a.i./kg dry soil.

Materials and methods

1. Test Material: Diflufenican 500 g/L SC
Lot/batch: BF-CXA
Concentration/Purity: 500 g/L
Stability of test compound: March 2017
2. Vehicle and/or control: Water control
Toxic reference: Danadim Progress (Dimethoate 400 g/L);
Batch No: 0001073094
3. Test animals (Species): *Hypoaspis aculeifer* Canestrini (Acari:Laelapidae).
Source: Refer to raw data, to maintain supplier confidentiality
Feeding: Three times a week addition of *Folsomia candida* as a food source

Animals per test concentration: 10 per unit
Number of replicates: 3 treatment groups (1 test item concentrations, water control, reference control); 8 replicates with 10 organisms each.

Artificial soil components: According to OECD 226:
 - 5% Sphagnum-peat
 - 20% Kaolin clay
 - 74% fine quartz-sandThe pH was adjusted on preparation to 6.0 ± 0.5 .

Test unit: Vessels (5.2 cm internal diameter and depth of 5.6 cm x 6 cm) *Hypoaspis aculeifer* were introduced into the test chambers using a small paint brush. A lid was placed on each chamber after infestation. A weight of soil equivalent to 300 g dry soil was prepared for each application rate. After treatment a weight equivalent to 20 g of dry soil was weighed.

Untreated variant: Water control
Reference standard: Danadim® Progress (Dimethoate 400 g/L)
4. Environmental conditions
Temperature: Within the range of 17.53°C to 23.19°C
pH: Were determined using a Hanna pHep pH meter.
At test start: 6.2 to 6.3
At test end: 6.1 to 6.3

Humidity (Moisture content of the soil): At test start: 53% to 55% of the maximum water holding capacity, *i.e.* within the recommended range 40-60 % of the total water holding capacity
At test end: 52% to 55% of the maximum water holding

	capacity
Photoperiod:	16 h light : 8 h dark
Light intensity:	Within the range of 405 lux to 520 lux

Study design and methods

1. In-life dates: 06.01 – 14.03.2016 (experimental phase)
2. Experimental design:

An initial range finding test was performed using rates of 0.1, 1, 10, 100 and 1000 mg a.i./kg dry soil. Prior to treatment the maximum water holding capacity (MWHC) of the soil was determined and 55% capacity calculated as 0.243g water/g dry artificial soil which provided a suitable soil structure for the *Hypoaspis aculeifer* development. A weight of soil equivalent to 300 g dry soil was prepared for each application rate. A 34.36 mL aliquot of the prepared test solution was applied to the soil, to achieve a final moisture content equivalent to 55% MWHC. The moist soil was mixed using a hand held electric mixer.

The treatments were applied in the order of water control, Diflufenican 500 g/L SC at 1000 mg a.i./kg dry soil and dimethoate at 10.0 mg a.i./kg dry soil. The blades of the mixer were washed with reverse osmosis water between each application and with reverse osmosis water and acetone between the test item treatment and the toxic reference.

After treatment the pH of each soil treatment was recorded using a Hanna pHep pH meter. The water content of the soil was also recorded.

Test concentrations: Control, 1000 mg diflufenican/kg soil, 10 mg dimethoate/kg soil

Test duration: 14 days
3. Observations: Assessment of adult worm mortality and reproduction rate (number of second-generation juvenile) was assessed after 14 days.
4. Statistics: Statistical analysis was not carried out as a limit study was performed.

Results and discussion

The study was considered valid because less than 20% mortality was observed in the control group, the coefficient of variation of reproduction in the water control did not exceed 30% and the mean number of juveniles in each replicate of the control treatments was at least 50. In addition application of the toxic reference substance dimethoate resulted in substantial and unequivocal effects.

No mortality was observed, the 14-day LC₅₀ value for survival was therefore estimated to be >1000 mg a.i./kg dry.

The coefficient of variation of reproduction was 4.85% in the water control. The mean number of 2nd generation juvenile *Hypoaspis aculeifer* observed for Diflufenican 500 g/L SC applied at 1000 mg a.i./kg dry soil was 152 compared to 152 in the water control. For the toxic reference substance, dimethoate,

applied at a rate of 10 mg a.i./kg dry soil, no 2nd generation juveniles were produced. The 14-day EC₅₀ value for fecundity was estimated to be >1000 mg a.i./kg dry soil.

Conclusions

The 14-day LC₅₀ and 14-day EC₅₀ were estimated to be >1000 mg a.i./kg dry soil.

The NOEC for reproduction of the *Hypoaspis aculeifer* was determined to be 1000 mg test item/kg soil.

Comments of zRMS:	The study was conducted to the OECD guideline 226 and according to the principles of GLP. All validity criterions 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 Effects of GLOB1817H on the reproduction of the predatory mite *Hypoaspis aculeifer*, Schulz L., 2020, 20 48 THC 0043

Guideline(s): Yes, OECD 226 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

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). The LC₅₀ value for mortality and the EC₅₀ for reproduction could not be calculated, but it can be concluded, that these values are higher than 387 mg test item/kg soil dry weight. The EC₁₀ and EC₂₀ values for reproduction were calculated to be 66.9 and 133.2 mg test item/kg soil dry weight, respectively. The NOEC for mortality and for reproduction was determined to be 161 and 43 mg test item/kg soil dry weight, respectively.

Materials and Methods

Test item: GLOB1817H

Batch No.: KS010420

Active ingredients/content:	Prosulfocarb	667 g/L (nominal)	672.8 g/L (analysed)
	Diiflufenican	14 g/L (nominal)	14.20 g/L (analysed)
	Halauifen-methyl	1.33 g/L (nominal)	1.323 g/L (analysed)
	Cloquintocet-mexyl	1.33 g/L (nominal)	1.349 g/L (analysed)

Test species: *Hypoaspis aculeifer* (CANESTRINI)
age: adult female mites with an age difference of 2 days
source: Katz Biotech AG, Baruth, Germany

Test system: Exposure of female mites to different concentrations of the test item mixed into artificial soil substrate

Test design:	<p>The effects of the test item on mortality and reproduction of the soil mite species <i>Hypoaspis aculeifer</i> (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226.</p> <p>Each of the eight different test item concentrations was homogeneously mixed into artificial soil and filled into glass vessels. Subsequently, the soil mites were introduced on top of the soil and the vessels were covered. Four replicates were performed for the test item groups and eight replicates for the control group; each replicate consisted of ten female soil mites. The mites were fed with <i>Tyrophagus putrescentiae</i> (SCHRANK) at the beginning and every two to three days during the whole test period.</p> <p>For the main measured variable, the number of juveniles per test vessel and additionally the mortality of the adult female mites were determined. Mortality and reproductive output of the mites exposed to the test item were compared to that of the control in order to determine the no observed effect concentration (NOEC).</p> <p>Assessment of adult mortality and reproduction effects was carried out after 14 days.</p>
Endpoints:	Mortality of adults and number of juveniles
Reference item:	<p>Dimethoate 400 EC (400 g/L, nominal).</p> <p>Test concentrations: 0.9, 1.3, 2.0, 3.0, 4.4, 6.7 10.0, 15.0 mg a.s./kg soil dry weight (d.w.) nominally equivalent to 2.3, 3.5, 5.3, 7.9, 11.9, 17.8, 26.7, 40.1 mg reference item/kg soil d.w.(spacing factor 1.5)</p> <p>The effects of the reference item were investigated in a separate study.</p>
Validity criteria:	<p>Mean mortality of adult females: $\leq 20\%$</p> <p>Mean number of juveniles per replicate: ≥ 50</p> <p>Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$</p>
Test concentrations:	18, 28, 43, 67, 104, 161, 250, 387 mg test item/kg soil dry weight (spacing factor: 1.55)
Test conditions:	Artificial soil according to OECD 226, pH 6.3 - 6.5 at test start, pH 6.3 - 6.4 at test end; water content at test start 47.81 - 49.48 % of maximum water holding capacity (WHC) and 47.72 - 49.00 % of maximum WHC at test end; temperature 19.4 - 21.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 532 lux.
Dates of work:	<p>Experimental start date: 21.09.2020</p> <p>Experimental completion date: 12.10.2020</p>
Statistics:	<p><u>Mortality</u></p> <p>Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$, one-sided greater), Logit analysis using linear max. likelihood regression</p> <p><u>Reproduction</u></p> <p>Multiple Sequential-rejective Welch-t-test After Bonferroni-Holm ($\alpha = 0.05$, one-sided smaller), Logit analysis using linear max. likelihood regression</p> <p>Statistical program: ToxRat Professional 3.3.0 (RATTE 2018)</p>

Results and Discussion

All validity criteria for the study were met.

- Mean mortality of adult females: $\leq 20\%$ (observed: 1.3 %)
- Mean number of juveniles per replicate: ≥ 50 (observed: 244.3)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (observed: 6.7 %)

Mortality rates of 0.0 - 20.0 % were recorded in the test item treatment groups. In the control group the mortality rate was 1.3 %. The observed mortality rates in the test item treatment groups compared to control were not statistically significant up to and including 161 mg test item/kg soil dry weight. However, the test item caused statistically significant effects on mortality at 250 and 387 mg test item/kg soil dry weight (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 264.5, 226.8, 225.0, 218.5, 207.0, 191.5, 160.0 and 127.8 at concentrations of 18, 28, 43, 67, 104, 161, 250 and 387 mg test item/kg soil d.w., respectively. The mean reproduction in the control reached 244.3 juveniles. The test item showed no statistically significantly adverse effects on reproduction up to and including 43 mg test item/kg soil dry weight. However, the test item caused statistically significant effects on reproduction at 67, 104, 161, 250 and 387 mg test item/kg soil dry weight (Multiple Sequential-rejective Welch-t-test After Bonferroni-Holm, $\alpha = 0.05$, one-sided smaller).

In a separate study, the EC_{50} (reproduction) of the reference item Dimethoate 400 EC (400 g/L nominal) was calculated to be 4.71 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Effects of the test item on *Hypoaspis aculeifer* mortality and reproduction (day 14)

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	18	28	43	67	104	161	250	387
Mean adult mortality [%] (day 14)	1.3	2.5	0.0	2.5	10.0	2.5	0.0	20.0*	20.0*
Mean number of juveniles (day 14)	244.3	264.5	226.8	225.8	218.5*	207.0*	191.5*	160.0*	127.8*
Coefficient of variation [%]	6.7	10.5	6.1	7.4	3.0	5.5	4.3	16.5	38.1
Reproduction in [%] of control	100	108	93	92	89	85	78	66	52
Endpoint [mg test item/kg soil dry weight]									
NOEC (mortality)	161								
NOEC (reproduction)	43								
LC_{50} (mortality) ²	> 387								
EC_{10} (reproduction) ¹	66.9 (95 % confidence limit 53.6 - 83.4)								
EC_{20} (reproduction) ¹	133.2 (95 % confidence limit 117.1 - 151.6)								
EC_{50} (reproduction) ²	> 387								

* statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater and Multiple Sequential-rejective Welch-t-test After Bonferroni-Holm for reproduction, $\alpha = 0.05$, one-sided smaller)

¹ Logit analysis using linear max. likelihood regression

² based on estimation of the data

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with GLOB1817H, the LC₅₀ value for mortality and the EC₅₀ for reproduction could not be calculated, but it can be concluded, that these values are higher than 387 mg test item/kg soil dry weight. The EC₁₀ and EC₂₀ values for reproduction were calculated to be 66.9 and 133.2 mg test item/kg soil dry weight, respectively. The NOEC for mortality and for reproduction was determined to be 161 and 43 mg test item/kg soil dry weight, respectively.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No new studies submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to the OECD guideline 216 and according to the principles of GLP. All validity criterions 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	Effects of GLOB1817H on activity of soil microflora (Nitrogen transformation test), Schulz L., 2020, 20 48 SMN 0052
Guideline(s):	Yes, OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

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 GLOB1817H (tested at 4 mg/kg soil dry weight and 40 mg/kg soil dry weight) caused no adverse effects (deviation from control <25 %) on soil nitrogen transformation (measured as NO₃-N-production) at the end of the 28-day incubation period.

Materials and Methods

Test item:	GLOB1817H		
Batch No.:	KS010420		
Formulation type:	EC		
Active ingredients/content:		<u>nominal</u>	<u>analysed</u>
	Prosulfocarb:	667 g/L	672.8 g/L
	Diiflufenican:	14.0 g/L	14.20 g/L
	Halauxifen-methyl:	1.33 g/L	1.323 g/L
	Cloquintocet-mexyl:	1.33 g/L	1.349 g/L
Test soil:	Biologically active agricultural soil: loamy sand (DIN 4220) / loam (USDA), pH 6.3, 1.42 % C _{org} , WHC: 38.20 g/100 g dry soil.		

Test design:	The test was performed in accordance with the OECD Guideline 216 (2000). Aim of the study was the determination of the nitrogen transformation (NO ₃ -nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %) by comparison of nitrogen transformation in test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH ₄ -nitrogen, NO ₃ - and NO ₂ -nitrogen were determined by using the Autoanalyzer (SEAL Analytical). Sampling scheme: 0, 7, 14 and 28 days after treatment.
Test concentrations:	Control, 4 mg test item/kg soil dry weight and 40 mg test item/kg soil dry weight. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm ³ .
Endpoints:	Effects on NO ₃ -nitrogen-production after 28 days of exposure.
Reference item:	Dinoterb (purity: 99.28 % (g/g) analysed). The reference item was tested in a separate study (20 48 SMO 0001) at concentrations of 6.80, 13.60 and 27.20 mg/kg soil dry weight.
Test conditions:	Water content: approx.. 45 % of its maximum water holding capacity; water content: 17.34 - 17.74 g/100 g dry soil; pH: 6.0 - 6.2 Soil samples were incubated at 19.7 - 20.5 °C, while stored in test vessels in the dark.
Statistics:	Calculation of mean values per treatment, standard deviations, coefficients of variation.
Dates of work:	Experimental start: 05.10.2020 Experimental end: 02.11.2020

Results and Discussion

The coefficients of variation in the control group of the nitrogen test were maximum 4.0% and thus fulfilled the validity criterion of ≤ 15%.

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (4 mg/kg soil dry weight and 40 mg/kg soil dry weight) after 28 days (time interval 14-28). The results are summarized in the table below.

Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	4 mg GLOB1817H/ kg soil dry weight		40 mg GLOB1817H/ kg soil dry weight	
	NO ₃ -N/day [mg/kg soil d.w.]	NO ₃ -N/day [mg/kg soil d.w.]	% difference to control ¹⁾	NO ₃ -N/day [mg/kg soil d.w.]	% difference to control ¹⁾
0-7	4.80	5.03	+4.9	5.47	+13.9
7-14	1.57	1.35	-13.7	2.22	+41.9
14-28	1.27	1.45	+14.0	0.97	-23.6

The calculations were performed with unrounded values

¹⁾ based on NO₃-N-production; - = inhibition; + = stimulation

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +59.9 %, +216.3 % and +238.5 % at 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Conclusions

The test item GLOB1817H (tested at 4 mg/kg soil dry weight and 40 mg/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO₃-N-production) at the end of the 28-day incubation period.

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 to the OECD guideline 208 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: KCP 10.6

Report GLOB1817H: terrestrial plant test: seedling emergence and seedling growth test, Stead A., 2021, STC/20/E1410

Guideline(s): Yes, OECD 208 (2006)

Deviations: The study plan states that the synthetic sandy loam soil mix should have a pH of 7 – 8. The pH value of the synthetic sandy loam soil mix used for all species was 8.2.
The Study Plan states that daytime relative humidity in the glasshouse should be 70% (±25%). On five occasions during the field phase of the study minimum relative humidity fell below 45% (70% - 25%).
These deviations were not to the detriment of the plants as photographs of the untreated plants taken at harvest show. These deviations will not impact on the validity of the study, as demonstrated by the control performance and the fact that the validity criteria for the study were met.

GLP: Yes

Acceptability: Yes

Executive summary

The objective of this study was to generate dose response data to assess the risk of GLOB1817H to terrestrial non-target plants. This was achieved by determining pre-emergence phytotoxicity of GLOB1817H when applied to two monocotyledon species and four dicotyledon species from six different plant families, and ascertaining ER₁₀, ER₂₅, ER₅₀ and NOEC values based on shoot fresh weight reduction and ER₅₀ based on percentage visual injury at harvest. The methodology for the study was based on OECD Guideline 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (July 2006). Based on shoot fresh weight, the most sensitive monocotyledon species to pre-emergence application of GLOB1817H was oats with an ER₂₅ value of 161.44 mL product/ha and an ER₅₀ value of 478.76 mL

product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER₂₅ value of 164.98 mL product/ha and an ER₅₀ value of 335.65 mL product/ha. Based on percentage visual injury at harvest, the most sensitive monocotyledon species to pre-emergence application of GLOB1817H was onion with an ER₅₀ value of 793.37 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER₅₀ value of 312.65 mL product/ha.

Materials and Methods

Test item:	GLOB1817H		
Batch No.:	KS010420		
Formulation type:	EC		
Active ingredients/content:		<u>nominal</u>	<u>analysed</u>
	Prosulfocarb:	667 g/L	672.8 g/L
	Di flufenican:	14.0 g/L	14.20 g/L
	Halau xifen-methyl:	1.33 g/L	1.323 g/L
	Cloquintocet-mexyl:	1.33 g/L	1.349 g/L
Test site:	Glasshouse		
Test species:	<i>Allium cepa</i> , <i>Avena sativa</i> , <i>Brassica napus</i> , <i>Solanum Lycopersicon</i> , <i>Daucus carota</i> , <i>Glycine max</i>		
Treatment rates:	0, 13.0, 26.0, 51.9, 103.9, 207.8, 415.5, 831, 1662 mL product/ha in 200 L water/ha		
Trial design:	Randomised block design with 3 pots per replicate and 5 replicates per treatment per species.		
Treatment applications:	Pre-emergence with a track sprayer		
Seeds:	Obtained from commercial seed companies and from certified seed lots.		
Soil:	Sandy loam (powdered fertilizer was added for onion, carrot, tomato and oilseed rape)		
Test conditions:	Relative humidity: 70% ± 25% Photoperiod: 16 h, min. 5000 lux Temperature: 22°C ± 10°C		
Irrigation:	Prior to treatment: overhead Following treatment: via plastic saucers, according to individual crop requirement		
Endpoints:	Number of plants alive at 14 and 21 or 22 days (harvest) after 50% of the untreated plants had emerged. Visual Injury (%) at 14 and 21 or 22 days (harvest) after 50% of the untreated plants had emerged. Number of non-emerged plants and shoot fresh weights at 21 or 22 days (harvest) after 50% of the untreated plants had emerged.		
Statistics:	Statistical regression analyses to determine ER ₁₀ , ER ₂₅ , ER ₅₀ and Dunnett's Test to determine NOEC values using the JMPv8 statistical package.		
Dates of work:	Experimental start:	25.11.2020	

Experimental end: 29.12.2020

Results and Discussion

All validity criteria were met:

- Seedling emergence in the untreated control pots is at least 70%.
- 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.
- Environmental conditions for a particular species are identical and the growing media contain the same amount of soil matrix, support media or substrate from the same source.

Analytical results

A sample of the highest spray solution (Treatment I) was analysed to determine the actual prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl (safener) concentrations of the spray solutions. The samples were analysed using an external standard gas chromatography technique. The following recovery data was obtained:

Sample	Active ingredients	Actual content (mg/L)	Theoretical content (mg/L)	Recovery (%)
Treatment I	Prosulfocarb	5356	5545	97
	Diflufenican	96.18	117.0	82
	Halauxifen-methyl	9.119	10.90	84
	Cloquintocet-mexyl	8.924	11.12	80

Visual injury

Visual injury on onion consisted of stunted growth and plant death. Emergence ranged from 100% to 97%. Survival ranged from 100% to 10%.

GLOB1817H (mL/ha)	% Visual Injury		% Emergence	% Survival
	14 days	22 days (Harvest)	22 days (Harvest)	22 days (Harvest)
Untreated	0	0	100	100
13.0	0	0	100	100
26.0	0	0	100	100
51.9	6	4	100	100
103.9	0	0	100	100
207.8	11	10	100	100
415.5	27	29	97	90
831	54	54	100	60
1662	95	99	100	10

% Visual Injury: 0 % = no injury; 100 % = dead/not emerged

Visual injury on oats consisted of severe stunted growth and some bleaching to leaves at the highest rates. There was also plant death. Some plants at the mid rates had stunted growth. Emergence ranged from 100% to 93%. Survival ranged from 100% to 50%.

GLOB1817H (mL/ha)	% Visual Injury		% Emergence	% Survival
	14 days	21 days (Harvest)	21 days (Harvest)	21 days (Harvest)

Untreated	0	0	100	100
13.0	0	0	100	100
26.0	0	0	100	100
51.9	0	0	100	100
103.9	1	2	100	100
207.8	5	6	93	96
415.5	40	42	100	100
831	42	41	100	97
1662	80	87	100	50

% Visual Injury: 0 % = no injury; 100 % = dead/not emerged

Visual injury on oilseed rape consisted of stunted growth, bleaching to the cotyledon leaves and puckering to the first leaves so leaves were in a tight ball. Mid rate plants had some puckering. Emergence ranged from 100% to 97%. Survival was not affected at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury		% Emergence	% Survival
	14 days	21 days (Harvest)	21 days (Harvest)	21 days (Harvest)
Untreated	0	0	100	100
13.0	0	0	97	100
26.0	0	0	100	100
51.9	0	0	100	100
103.9	0	0	100	100
207.8	6	4	100	100
415.5	29	31	100	100
831	44	50	100	100
1662	58	67	100	100

% Visual Injury: 0 % = no injury; 100 % = dead/not emerged

Visual injury on tomato consisted of distortion and yellowing to leaves with leaves more pointed and curled at edges. Emergence was not affected at any treatment rate. Survival was not affected at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury		% Emergence	% Survival
	14 days	21 days (Harvest)	21 days (Harvest)	21 days (Harvest)
Untreated	0	0	100	100
13.0	0	0	100	100
26.0	0	0	100	100
51.9	0	0	100	100
103.9	0	0	100	100
207.8	0	0	100	100
415.5	3	4	100	100
831	11	11	100	100
1662	26	28	100	100

% Visual Injury: 0 % = no injury; 100 % = dead/not emerged

Visual injury on carrot consisted of stunted growth with some plants still at the cotyledon stage. There was also distortion to leaves causing bending and curling over, nettle shaped leaves, paler stems and some plant death. Emergence ranged from 100% to 93%. Survival ranged from 100% to 40%.

GLOB1817H (mL/ha)	% Visual Injury		% Emergence	% Survival
	14 days	22 days (Harvest)	22 days (Harvest)	22 days (Harvest)
Untreated	0	0	93	100
13.0	0	0	100	100
26.0	0	0	100	100

51.9	0	0	100	100
103.9	4	9	100	100
207.8	27	40	100	100
415.5	62	70	100	90
831	68	80	97	72
1662	83	92	100	40

% Visual Injury: 0 % = no injury; 100 % = dead/not emerged

Visual injury on soybean consisted of stunted growth and plants had smaller leaves with some puckering. Emergence ranged from 100% to 93%. Survival ranged from 100% to 97%.

GLOB1817H (mL/ha)	% Visual Injury		% Emergence	% Survival
	14 days	21 days (Harvest)	21 days (Harvest)	21 days (Harvest)
Untreated	0	0	100	100
13.0	0	0	100	100
26.0	0	0	100	100
51.9	0	0	100	100
103.9	0	0	100	100
207.8	0	0	100	100
415.5	2	2	100	100
831	8	9	93	100
1662	14	18	97	97

% Visual Injury: 0 % = no injury; 100 % = dead/not emerged

Shoot fresh weight

Mean total shoot fresh weight as a percentage of the untreated control is presented for all species below.

GLOB1817H (mL/ha)	21 or 22 days after 50% emergence of untreated controls (Harvest) Shoot Fresh Weight (% of untreated control)					
	Onion	Oats	Oilseed rape	Tomato	Carrot	Soybean
Untreated	100.0	100.0	100.0	100.0	100.0	100.0
13.0	89.0	96.6	104.4	99.5	110.8	107.2
26.0	101.2	101.0	88.9	104.6	108.1	108.9
51.9	85.0	110.9	101.9	101.9	111.1	107.8
103.9	84.6	80.2	95.6	110.8	91.1	101.1
207.8	71.3	65.6	82.8	89.9	67.9	108.5
415.5	59.5	45.9	77.2	92.4	41.4	105.1
831	41.6	46.2	54.6	85.3	17.9	90.9
1662	3.2	10.1	33.9	72.4	7.8	87.6

Endpoints

ER₁₀, ER₂₅ and ER₅₀ values (with corresponding R-Sq. values) and NOEC values, based on shoot fresh weight reduction, are summarized below.

Species	ER ₁₀ # (mL GLOB1817H/ha)	ER ₂₅ (mL GLOB1817H/ha)	ER ₅₀ (mL GLOB1817H/ha)	R-Sq.	NOEC (mL GLOB1817H/ha)
Onion	39.60	181.91	576.54	0.87	103.9

Oats	49.10	161.44	478.76	0.86	51.9
Oilseed rape	93.92	379.44	1091.16	0.79	415.5
Tomato	387.06	1505.03	>1662	0.36	1662
Carrot	80.92	164.98	335.65	0.93	103.9
Soybean	1021.88	>1662	>1662	0.40	1662

ER₁₀ values should be treated with caution due to natural plant to plant variability.

ER₅₀ values (with corresponding R-Sq. values) and NOEC values, based on percentage visual at harvest, are summarized below.

Species	ER₅₀ (GLOB1817H mL/ha)	R-Sq.
Onion	793.37	0.92
Oats	859.24	0.90
Oilseed rape	964.42	0.92
Tomato	>1662	N/A
Carrot	312.65	0.94
Soybean	>1662	N/A

Conclusion

All species displayed visual injury.

Based on shoot fresh weight, the most sensitive monocotyledon species to pre-emergence application of GLOB1817H was oats with an ER₂₅ value of 161.44 mL product/ha and an ER₅₀ value of 478.76 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER₂₅ value of 164.98 mL product/ha and an ER₅₀ value of 335.65 mL product/ha.

Based on percentage visual injury at harvest, the most sensitive monocotyledon species to pre-emergence application of GLOB1817H was onion with an ER₅₀ value of 793.37 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER₅₀ value of 312.65 mL product/ha.

Comments of zRMS:	The study was conducted to the OECD guideline 227 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: KCP 10.6

Report GLOB1817H: terrestrial plant test: vegetative vigour test, Lewington-Gower M., 2021, STC/20/E1409

Guideline(s): Yes, OECD 227 (2006)

Deviations: pH of the soils being 8.2, rather than 7-8 as stated in the study plan.
Relative humidity falling below 45%, rather than 70% - 25% as stated in the study plan.
These deviations were not to the detriment of the plants as photographs of the untreated plants taken at harvest show. These deviations will not impact on the validity of the study, as demonstrated by the control performance and the fact that the validity criteria for the study were met.

GLP: Yes

Acceptability: Yes

Executive summary

The objective of this study was to generate dose response data to assess the risk of GLOB1817H to terrestrial non-target plants. This was achieved by determining post-emergence phytotoxicity of GLOB1817H when applied to two monocotyledon species and four dicotyledon species from six different plant families, and ascertaining ER₁₀, ER₂₅, ER₅₀ and NOEC values based on shoot fresh weight reduction, and ER₅₀ values based on percentage visual injury at harvest. The methodology for the study was based on OECD Guideline 227 (July 2006) Terrestrial Plant Test: Vegetative Vigour Test.

Based on shoot fresh weight, the most sensitive monocotyledon species to post-emergence application of GLOB1817H was oats with an ER₂₅ value of 400.69 mL product/ha and an ER₅₀ value of 962.19 mL product/ha. The most sensitive dicotyledon species to post-emergence application of GLOB1817H was tomato with an ER₂₅ value of 21.32 mL product/ha and an ER₅₀ value of 75.93 mL product/ha.

Based on percentage visual injury at harvest, the monocotyledon species were not sensitive to GLOB1817H, with both oats and onion species with ER₅₀ values of >1662 mL product /ha (the highest rate tested). The most sensitive dicotyledon species was tomato, with an ER₅₀ value of 56.25 mL product /ha.

Materials and Methods

Test item: GLOB1817H
Batch No.: KS010420
Formulation type: EC
Active ingredients/content:

	<u>nominal</u>	<u>analysed</u>
Prosulfocarb:	667 g/L	672.8 g/L
Diflufenican:	14.0 g/L	14.20 g/L
Halauxifen-methyl:	1.33 g/L	1.323 g/L
Cloquintocet-mexyl:	1.33 g/L	1.349 g/L

Test site: Glasshouse

Test species: *Allium cepa*, *Avena sativa*, *Brassica napus*, *Solanum Lycopersicon*,
Daucus carota, *Glycine max*

Treatment rates: 0, 13.0, 26.0, 51.9, 103.9, 207.8, 415.5, 831, 1662 mL product/ha in 200 L water/ha

Trial design: Randomised block design with 5 replicates for all species.

Treatment applications: At BBCH 12-14 with a track sprayer

Seeds: Obtained from commercial seed companies and from certified seed lots.

Soil: Sandy loam (powdered fertilizer was added for onion, carrot, tomato and oilseed rape)

Test conditions: Relative humidity: 70% ± 25%
Photoperiod: 16 h, min. 5000 lux
Temperature: 22°C ± 10°C

Irrigation: Prior to treatment: overhead

	Following treatment: via plastic saucers, according to individual crop requirement			
Endpoints:	Number of plants alive at 14 and 21days (harvest) after treatment application. Visual Injury (%) at 14 and 21 days (harvest) after treatment application. Number of dead plants and shoot fresh weights at 21 days (harvest) after treatment application.			
Statistics:	Statistical regression analyses to determine ER ₁₀ , ER ₂₅ , ER ₅₀ and Dunnett's Test to determine NOEC values using the JMPv8 statistical package.			
Dates of work:	Experimental start:	25.11.2020		
	Experimental end:	16.12.2020		

Results and Discussion

All validity criteria were met:

- Seedling emergence in the untreated control pots is at least 70%.
- 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.
- For a given plant species, all seedlings in a test are from the same cultivation group and source.
- Environmental conditions for a particular species are identical and the growing media contain the same amount of soil matrix, support media or substrate from the same source.

Analytical results

A sample of the highest spray solution (Treatment I) was analysed to determine the actual prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl (safener) concentrations of the spray solutions. The samples were analysed using an external standard gas chromatography technique The following recovery data was obtained:

Sample	Active ingredients	Actual content (mg/L)	Theoretical content (mg/L)	Recovery (%)
Treatment I	Prosulfocarb	5356	5545	97
	Diflufenican	96.18	117.0	82
	Halauxifen-methyl	9.119	10.90	84
	Cloquintocet-mexyl	8.924	11.12	80

Visual injury and mortality

Visual injury on oat consisted of blotching (bleaching) on leaves and some stunted growth. Oat did not suffer plant loss at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury			% Mortality
	7 DAT	14 DAT	21 DAT (Harvest)	21 DAT (Harvest)
Untreated	0	0	0	0
13.0	0	0	0	0
26.0	0	0	0	0

51.9	1.2	2.0	2.6	0
103.9	1.2	1.2	2.4	0
207.8	4.4	5.4	7.0	0
415.5	6.2	10.2	16.0	0
831	8.6	14.0	22.0	0
1662	7.6	15.0	34.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Visual injury on onion consisted of lighter colour foliage and stem, stem and leaf twisting and thinner plants. Onion did not suffer plant loss at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury			% Mortality
	7 DAT	14 DAT	21 DAT (Harvest)	21 DAT (Harvest)
Untreated	0	0	0	0
13.0	0	0	0	0
26.0	0	0	0	0
51.9	0	0	0	0
103.9	2.0	2.4	3.0	0
207.8	6.4	14.0	19.0	0
415.5	16.0	20.0	24.0	0
831	12.0	18.0	23.0	0
1662	20.0	24.0	30.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Visual injury on oilseed rape consisted of blotching and bleaching of leaves and some stunted growth. Oilseed rape did not suffer from plant loss at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury			% Mortality
	7 DAT	14 DAT	21 DAT (Harvest)	21 DAT (Harvest)
Untreated	0	0	0	0
13.0	2.6	2.6	2.6	0
26.0	3.2	5.0	6.4	0
51.9	8.0	10.0	11.0	0
103.9	10.0	12.0	15.0	0
207.8	11.0	15.0	20.0	0
415.5	15.0	16.0	22.0	0
831	15.0	18.0	22.0	0
1662	18.0	22.0	27.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Visual injury on tomato consisted of smaller sized leaves, bleaching from the central vein, stem twisting, flaccid leaves and distortion to the growing point. Tomato did not suffer plant loss at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury			% Mortality
	7 DAT	14 DAT	21 DAT (Harvest)	21 DAT (Harvest)
Untreated	0	0	0	0
13.0	12.0	19.0	31.0	0
26.0	20.0	29.0	43.0	0
51.9	30.0	38.0	50.0	0
103.9	33.0	42.0	55.0	0
207.8	37.0	45.0	64.0	0
415.5	38.0	51.0	65.0	0
831	37.0	45.0	69.0	0
1662	43.0	60.0	75.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Visual injury on carrot consisted of leaf curling, stems opening out, stem twisting and flaccid stems. Carrot did not suffer plant loss at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury			% Mortality
	7 DAT	14 DAT	21 DAT (Harvest)	21 DAT (Harvest)
Untreated	0	0	0	0
13.0	2.4	2.4	2.4	0
26.0	1.8	3.2	4.8	0
51.9	10.0	11.0	16.0	0
103.9	26.0	30.0	40.0	0
207.8	28.0	37.0	49.0	0
415.5	29.0	44.0	56.0	0
831	44.0	55.0	70.0	0
1662	46.0	57.0	71.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Visual injury on soybean consisted of stunted growth, blotching on leaves, stem twisting, flaccid leaves, growing point distortion, and stem elongation and twisting at the growing point. Soybean did not suffer plant loss at any treatment rate.

GLOB1817H (mL/ha)	% Visual Injury			% Mortality
	7 DAT	14 DAT	21 DAT (Harvest)	21 DAT (Harvest)
Untreated	0	0	0	0
13.0	3.8	5.0	6.0	0
26.0	13.0	15.0	22.0	0
51.9	21.0	29.0	48.0	0
103.9	26.0	36.0	50.0	0
207.8	33.0	42.0	58.0	0
415.5	35.0	48.0	67.0	0
831	37.0	55.0	71.0	0
1662	43.0	56.0	75.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Shoot fresh weight

Mean total shoot fresh weight as a percentage of the untreated control is presented for all species below.

GLOB1817H (mL / ha)	21 DAT (Harvest) Shoot Fresh Weight (% of Untreated Control)					
	Oat	Onion	Oilseed Rape	Tomato	Carrot	Soybean
Untreated	100	100	100	100	100	100
13.0	97.5	100.5	105.5	81.3	102.6	88.6
26.0	91.3	92.1	109.0	69.7	89.4	88.4
51.9	97.6	92.7	92.8	55.3	97.8	69.4
103.9	84.1	88.1	81.5	46.8	94.2	72.0
207.8	83.4	96.0	63.3	45.2	102.0	74.0
415.5	82.4	90.3	68.3	42.6	105.5	64.0
831	41.2	89.5	66.8	36.9	87.9	59.8
1662	23.8	53.8	59.6	40.2	82.9	59.2

Endpoints

ER₁₀, ER₂₅ and ER₅₀ values (with corresponding R-Sq. values) and NOEC values, based on shoot fresh weight reduction, are summarized below.

Species	ER ₁₀ # (mL GLOB1817H/ha)	ER ₂₅ (mL GLOB1817H/ha)	ER ₅₀ (mL GLOB1817H/ha)	R-Sq.	NOEC (mL GLOB1817H/ha)
Oat	63.79	400.69	962.19	0.81	415.5
Onion	170.65	879.80	>1662	0.48	831
Oilseed rape	61.08	123.32	>1662	0.73	103.9
Tomato	<13.0	21.32	75.93	0.65	<13.0
Carrot	487.38	>1662	>1662	0.11	1662
Soybean	<13.0	86.80	>1662	0.39	26.0

ER₁₀ values should be treated with caution due to natural plant to plant variability.

The ER₅₀ values based on percentage visual injury at harvest (with their corresponding R-Sq values) are given in the table below.

Species	ER ₅₀ (mL GLOB1817H/ha)	R Sq
Oat	>1662	N/A
Onion	>1662	N/A
Oilseed rape	>1662	N/A
Tomato	56.25	0.90
Carrot	202.57	0.86
Soybean	85.14	0.87

Conclusion

Based on shoot fresh weight, the most sensitive monocotyledon species to post-emergence application of GLOB1817H was oats with an ER₂₅ value of 400.69 mL product/ha and an ER₅₀ value of 962.19 mL product/ha. The most sensitive dicotyledon species to post-emergence application of GLOB1817H was tomato with an ER₂₅ value of 21.32 mL product/ha and an ER₅₀ value of 75.93 mL product/ha.

Based on percentage visual injury at harvest, the monocotyledon species were not sensitive to GLOB1817H, with both oats and onion species with ER₅₀ values of >1662 mL product /ha (the highest rate tested). The most sensitive dicotyledon species was tomato, with an ER₅₀ value of 56.25 mL product /ha.

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.