REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB1817H

Product name: **Eledura**

Chemical active substances:

Prosulfocarb, 667 g/L
Diflufenican, 14 g/L
Halauxifen-methyl, 1.33 g/L
Cloquintocet-mexyl, 1.33 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Globachem NV

Submission date: May 2021

MS Finalisation date: January 2022

Revision date: April 2022

Version history

When	What
May 2021	Initial dossier submission by applicant for new product authorization.
January 2022	zRMS version
April 2022	Version modified to take into account comments of cMS and the applicant

Table of Contents

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	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 arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL, DE, CZ	Winter wheat (TRZAW), Winter barley (HORVW), Winter rye (SECCW), Triticale (TTLWI)	F	Annual broad leaved weeds (BBBAN) & grasses (GGGAN)	Downward spraying	BBCH10-14, (sept)oct-dec	a) 1 b) 1	/	a) 3 b) 3	a) Prosulfocarb: 2.001 Diflufenican: 0.042 Halauxifen-methyl: 0.00399 b) Prosulfocarb: 2.001 Diflufenican: 0.042 Halauxifen-methyl: 0.00399	200-300	/	Cloquintocet-mexyl: 0.00399 kg/ha							

* 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

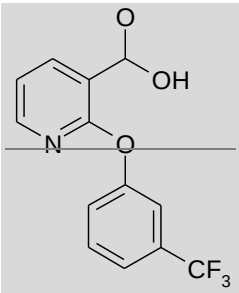
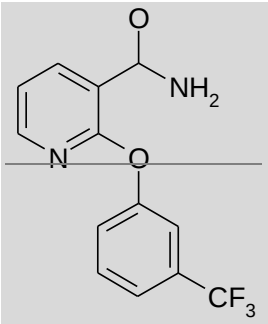
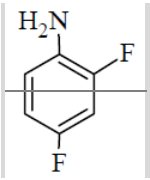
Remarks table:

(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

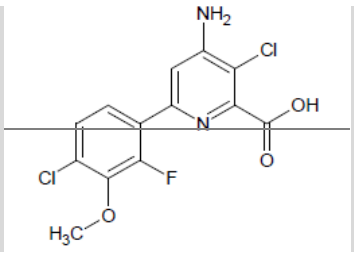
9.1.1 Overall conclusions

zRMS Comments:	The following tables considering the relevant metabolites were deleted. They are presented in Section 9.1.3.

~~9.1.1.1 Table 9.1-3 Metabolites of diflufenican~~

~~9.1.1.2 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~~

~~Table 9.1-4 Metabolites of halauxifen-methyl~~

~~Metabolite~~	~~Chemical structure~~	~~Molar mass~~	~~Maximum occurrence in compartments~~	~~Risk assessment required?~~
~~X11393729 (halauxifen)~~		~~331~~	~~Soil: 40.1% Water: 20.0% Sediment: 6.1%~~	~~Yes, soil and aquatic organisms~~

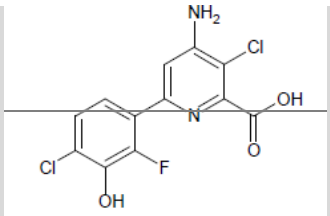
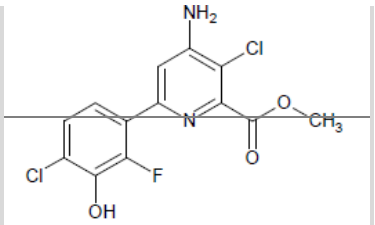
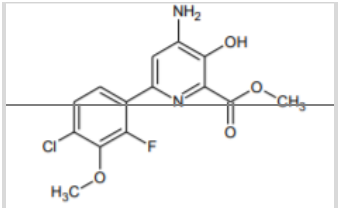
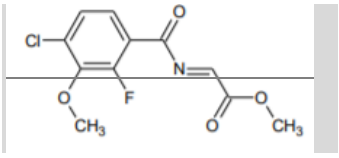
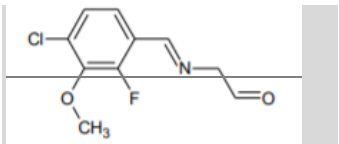
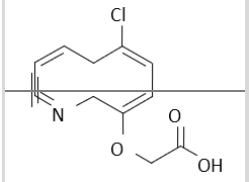
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
X11449757		317	Soil: 13.8% Water: 48.3% Sediment: 50.6%	Yes, soil and aquatic organisms
X11406790		331	Soil: 1.4% Water: 16.5% Sediment: 10.6%	Yes, aquatic organisms
Deg 10	326		Soil: – Water: 12.6%	Yes, aquatic organisms
Deg 11	273		Soil: – Water: 15.7%	Yes, aquatic organisms
Deg 14	229		Soil: – Water: 11.5%	Yes, aquatic organisms

Table 9.1-5 Metabolites of cloquintocet-mexyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA 153433	335.8		Soil: 38% Water: 38% Sediment: 27%	Yes, soil and aquatic organisms

9.1.1.3

zRMS Comments:	The cloquintocet-mexyl is used as a safener and will not be evaluated in this dossier.

9.1.1.4 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)

GLOB1817H poses a low risk to birds, mammals and other terrestrial vertebrate wildlife when applied according to the proposed use.

9.1.1.5 Effects on aquatic organisms (KCP 10.2)

A low risk to aquatic organisms is expected from the application of GLOB1817H taking into account the mitigation measures where necessary.

9.1.1.6 Effects on bees (KCP 10.3.1)

A low risk to bees is expected from the application of GLOB1817H.

9.1.1.7 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field risks of GLOB1817H for arthropods other than bees are acceptable for the intended use.

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

GLOB1817H poses low risk to earthworms and other non-target soil organisms when applied according to the proposed use rate.

There is no unacceptable risk on soil microbial activity for GLOB1817H.

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

GLOB1817H 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 50% drift reducing techniques or a buffer zone of 10 m without drift reduction.

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

Not required.

9.1.2 Grouping of intended uses for risk assessment

There was no grouping performed since there is only one intended use.

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 GLOB1817H is indicated in the

table.

Table 9.1-2 Metabolites of prosulfocarb

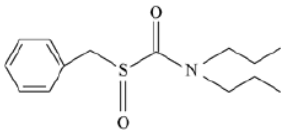
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Prosulfocarb sulfoxide		267.4	Soil: 6.8%	Yes, soil and aquatic organisms

Table 9.1-3 Metabolites of diflufenican

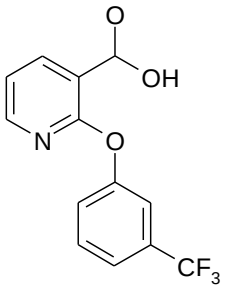
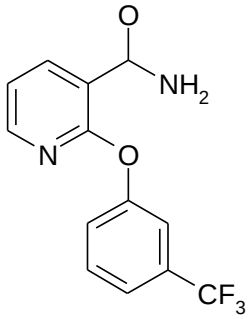
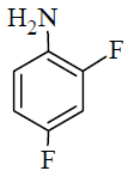
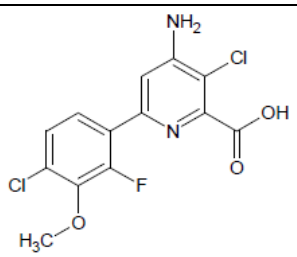
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE B107137		283	Soil: 16.8% Water: 32.6% Sediment: 13.3%	Yes, soil and aquatic organisms
AE 0542291		282	Soil: 26.3%	Yes, soil and aquatic organisms
AE C522392		129	Soil: 10.7% Water: 6.1% Sediment: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

Table 9.1-4 Metabolites of halauxifen-methyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
X11393729 (halauxifen)		331	Soil: 40.1% Water: 20.0% Sediment: 6.1%	Yes, soil and aquatic organisms

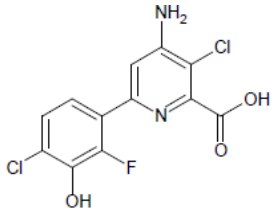
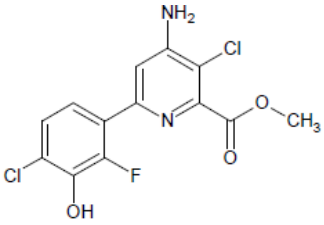
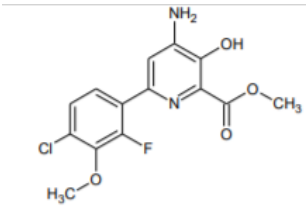
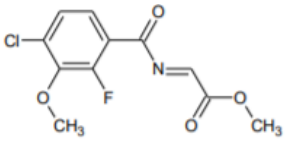
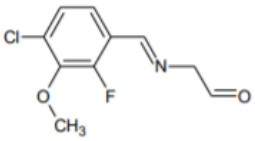
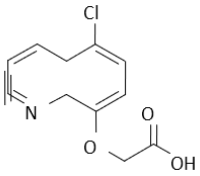
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
X11449757		317	Soil: 13.8% Water: 48.3% Sediment: 50.6%	Yes, soil and aquatic organisms
X11406790		331	Soil: 1.4% Water: 16.5% Sediment: 10.6%	Yes, aquatic organisms
Deg 10	326		Soil: - Water: 12.6%	Yes, aquatic organisms
Deg 11	273		Soil: - Water: 15.7%	Yes, aquatic organisms
Deg 14	229		Soil: - Water: 11.5%	Yes, aquatic organisms

Table 9.1-5 Metabolites of cloquintocet-mexyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA 153433	335.8		Soil: 38% Water: 38% Sediment: 27%	Yes, soil and aquatic organisms

zRMS Comments:	The cloquintocet-mexyl is used as a safener and will not be evaluated in this dossier.

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	The submitted screening step and first tier assessment of the acute and long-term risk for birds due to the use of GLOB1817H formulation in winter cereals were submitted. The used endpoints were agreed at the EU level for active substances. The risk assessment was conducted in accordance with Birds and Mammals guidance, 2009. The cloquintocet-mexyl is used as a safener and will not be evaluated in this dossier. Comparing the submitted assessment by the Applicant and recalculated risk assessment by the evaluator it can be concluded that safener does not cause any significant hazard. **Prosulfocarb, diflufenican and halauxifen-methyl.** The TER_A values are above the trigger value of 10 at first tier step indicating an acceptable acute risk for birds. **Prosulfocarb, diflufenican and halauxifen-methyl.** The TER_{LT} values for long-term risk are above the trigger value of 5 at first tier assessment indicating an acceptable long-term risk for birds. **Mixture of active substances**. Based on recalculated application rate (sum of only 3 active substances) – the difference between submitted one (with safener) and recalculated (without safener) was insignificant. The recalculated endpoints for mixture (LD_{50} mix and NOELmix) are similar to submitted. The submitted risk was corrected. It can be concluded that safener does not cause any significant hazard and do affect the final conclusion. The acute and long-term TER values are above the trigger value of 10 and 5, respectively, at first tier assessment indicating an acceptable long-term risk for birds. No further refinement is required. The **puddle scenario** was used in bird exposure assessment. The submitted assessment was accepted. **Secondary poisoning**. The risk assessment for earthworm-eating birds and fish-eating birds was accepted. No relevant metabolite was considered; the justification was accepted. The risk to birds following application of GLOB1817H formulation in accordance with the intended use is acceptable.

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prosulfocarb, diflufenican, halauxifen-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of GLOB1817H were not evaluated as part of the EU assessment of prosulfocarb, diflufenican or halauxifen-methyl.
However, the provision of further data on the GLOB1817H is not considered essential, because the risk for birds from GLOB1817H 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, *Colinus virginianus*	Prosulfocarb	Oral 1 d Acute	LD_{50} > 2250 mg/kg bw	EFSA, 2007
Mallard duck, *Anas platyrhynchos*	Prosulfocarb	Dietary 8 d Short-term	**LD_{50} > 1505.6 mg/kg bw/d**	EFSA, 2007
Mallard duck, *Anas platyrhynchos*	Prosulfocarb	Dietary Reproductive toxicity	**NOEL = 131 mg/kg bw/d**	EFSA, 2007
Bobwhite quail, *Colinus virginianus*	Diflufenican	Oral 1 d Acute	**LD_{50} > 2150 mg/kg bw**	EFSA, 2007
Bobwhite quail, *Colinus virginianus*	Diflufenican	Dietary Reproductive toxicity	**NOEL = 91.84 mg/kg bw/d**	EFSA, 2007
Bobwhite quail, *Colinus virginianus*	Halauxifen-methyl	Oral 1 d Acute	LD_{50} > 2250 mg/kg bw **LD_{50} = 4248 mg a.s./kg bw** (extrapolated)	EFSA, 2014
Zebra finch, *Taeniopygia guttata*	Halauxifen-methyl	Oral 1 d Acute	LD_{50} > 2250 mg/kg bw	EFSA, 2014
Bobwhite quail, *Colinus virginianus*	Halauxifen-methyl	Dietary 8 d Short-term	LD_{50} > 1328 mg/kg bw/d	EFSA, 2014
Mallard duck, *Anas platyrhynchos*	Halauxifen-methyl	Dietary 8 d Short-term	LD_{50} > 2088 mg/kg bw/d	EFSA, 2014
Bobwhite quail, *Colinus virginianus*	Halauxifen-methyl	Dietary Reproductive toxicity	**NOAEL = 36.9 mg/kg bw/d**	EFSA, 2014
Mallard duck, *Anas platyrhynchos*	Halauxifen-methyl	Dietary Reproductive toxicity	NOAEL = 160.5 mg/kg bw/d	EFSA, 2014
~~Bobwhite quail, *Colinus virginianus* Mallard duck, *Anas platyrhynchos*~~	~~Cloquintocet-mexyl~~	~~Oral 1 d Acute~~	~~**LD_{50} > 2000 mg/kg bw**~~	~~Safener, not reviewed at EU level~~
~~Bobwhite quail, *Colinus virginianus*~~	~~Cloquintocet-mexyl~~	~~Dietary Reproductive toxicity~~	~~**NOAEL = 47 mg/kg bw/day**, equivalent to 500 mg/kg diet~~	~~Safener, not reviewed at EU level~~

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 LD_{50} from the dietary toxicity study since this endpoint is lower than the acute LD_{50} from the acute toxicity study. The acute risk assessment of diflufenican will be performed using the LD_{50} of the acute toxicity study.

According to the EFSA Guidance Document on Birds and Mammals, it is permissible to extrapolate an

LD_{50} value upwards in cases where there is no mortality or a single mortality at a limit dose in acute avian toxicity study. The endpoint for halauxifen-methyl was extrapolated based on no mortality in the acute bird study. In the study with halauxifen-methyl, ten individuals were used per dose group in this study, so an extrapolation factor of 1.888 is appropriate and the resulting estimated acute LD_{50} is 4248 mg a.s./kg bw.

All reproductive risk assessments will be performed using the NOEL from the reproduction studies since this value is lower than the LD_{50}/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).

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 use of GLOB1817H in winter cereals

Intended use		**Winter cereals**			
Active substance/product		Prosulfocarb			
Application rate (kg/ha)		1 × 2.001			
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	317.8	**4.74**
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	30.5	1	61.0	24.7
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24.0	1	48.0	31.4
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	68.7	**1.91**
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	17.2	7.6
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	11.6	11.3
Active substance/product		Diflufenican			
Application rate (kg/ha)		1 × 0.042			

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	6.67	322
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	1.44	63.8
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	0.361	255
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.243	379
Active substance/product	Halauxifen-methyl				
Application rate (kg/ha)	1 × 0.00399				
Acute toxicity (mg/kg bw)	4248				
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	0.634	6704
Reprod. toxicity (mg/kg bw/d)	36.9				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	0.53	0.137	269
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird “goose”	16.2	0.53	0.0343	1077
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.0231	1601
~~**Active substance/product**~~	~~Cloquinctocet-mexyl~~				
~~**Application rate (kg/ha)**~~	~~1 × 0.00399~~				
~~**Acute toxicity (mg/kg bw)**~~	~~> 2000~~				
~~**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~~	~~0.634~~	~~3157~~
~~**Reprod. toxicity (mg/kg bw/d)**~~	~~47~~				
~~**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~~	~~0.137~~	~~343~~
~~Cereals, Early~~	~~Large herbivorous bird “goose”~~	~~16.2~~	~~0.53~~	~~0.0343~~	~~1372~~

~~(shoots) autumn-winter BBCH 10-29~~					
~~Cereals, BBCH 10-29~~	~~Small omnivorous bird "lark"~~	~~10.9~~	~~0.53~~	~~0.0231~~	~~2039~~

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 GLOB1817H contains 3 active ingredients and a safener, 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 GLOB1817H 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 LD_{50} 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 LD_{50} for the mixture of active substances with known toxicity assuming additivity:

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

With:
$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)
$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

~~For GLOB1817H, the LD_{50} (mix) amounts to 1518 mg/kg bw/d (=1/[(0.98/1505.6)+(0.02/2150)+(0.002/4248)+(0.002/2000)]).~~

~~Using the same approach, also a NOEL (mix) was calculated which amounts to 128.3 mg/kg bw/d (=1/[(0.98/131)+(0.02/91.84)+(0.002/36.9)+(0.002/47)]).~~

zRMS Comments:	The LD_{50} mix was recalculated only for active substances as the safener is not considered in risk assessment. The following values were obtained: LD_{50} (mix) = 1516.8 mg/kg bw/d NOEL (mix) = 129.2 mg/kg bw/d

Intended use	**Winter cereals**
Active substance/product	Mixture of active substances
Application rate (kg/ha)	1 × ~~2.05098~~ 2.04699
Acute toxicity (mg/kg bw)	LD_{50} (mix) = ~~1518~~ 1516.8
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	~~326~~ 325	**4.7**
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1	~~62.6~~ 62.4	24.3
Cereals, BBCH 10-29	Small omnivorous bird "lark"	24.0	1	~~49.2~~ 49.1	30.8
Reprod. toxicity (mg/kg bw/d)	NOEL (mix) = ~~128.~~3 129.2				
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	~~70.4~~ 70.3	**1.82**
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.53	17.6	7.29
Cereals, BBCH 10-29	Small omnivorous bird "lark"	10.9	0.53	~~11.9~~ 11.8	10.8
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 omnivorous bird	-	-	-	**1.83**
Cereals, Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	-	-	-	7.29
Cereals, BBCH 10-29	Small omnivorous bird "lark"	-	-	-	10.8

*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

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 GLOB1817H is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

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

Effective application rate (g/ha)	=	2001		
Acute toxicity (mg/kg bw)	=	1505.6	quotient	= 1.33
Reprod. toxicity (mg/kg bw/d)	=	131	quotient	= 15.3

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

Effective application rate (g/ha)	=	42		
Acute toxicity (mg/kg bw)	=	2150	quotient	= 0.020
Reprod. toxicity (mg/kg bw/d)	=	91.84	quotient	= 0.46

With a K(f)oc of 796 L/kg, halauxifen-methyl belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	3.99		
Acute toxicity (mg/kg bw)	=	4248	quotient	= 0.00094
Reprod. toxicity (mg/kg bw/d)	=	36.9	quotient	= 0.11

~~With a K(f)oc of 12850 L/kg, cloquintocet-mexyl belongs to the group of more sorptive substances.~~

~~Effective application rate (g/ha)~~	~~=~~	~~3.99~~		
~~Acute toxicity (mg/kg bw)~~	~~=~~	~~> 2000~~	~~quotient~~	~~= < 0.0020~~
~~Reprod. toxicity (mg/kg bw/d)~~	~~=~~	~~47~~	~~quotient~~	~~= 0.085~~

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.

The log P_{ow} of halauxifen-methyl amounts to 3.76 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

~~The log P_{ow} of cloquintocet-mexyl amounts to 5.20 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 9.2-3: 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

Parameter	Prosulfocarb	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	1.6051	dRR B8 Table 8.7-3
log P_{ow} / P_{ow}	4.48/30199	
Koc	1799	Geomean (n = 6)
foc	0.02	Default
BCF_{worm}	10.10	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	16.20	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	17.01	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	131	
TER_{lt}	7.70	

TER values shown in bold fall below the relevant trigger.

Table 9.2-4: 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

Parameter	Diflufenican	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1400	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}	0.433	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.454	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	91.84	
TER_{lt}	202	

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 halauxifen-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals

Parameter	Halauxifen-methyl	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.0066	dRR B8 Table 8.7-9
log P_{ow} / P_{ow}	3.76/5754	
Koc	796	Geomean (n = 9)
foc	0.02	Default
BCF_{worm}	4.39	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.029	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.030	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	36.9	
TER_{lt}	1213	

TER values shown in bold fall below the relevant trigger.

~~Table 9.2-6: Assessment of the risk for earthworm-eating birds due to exposure to cloquintocet-mexyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals~~

~~Parameter~~	~~Cloquintocet-mexyl~~	~~comments~~
~~PEC_{soil} (twa = 21 d) (mg/kg soil)~~	~~0.025~~	~~dRR B8 Table 8.7-12~~
~~log P_{ow} / P_{ow}~~	~~5.20/158489~~	
~~Koc~~	~~12850~~	~~Mean (n = 5)~~
~~foc~~	~~0.02~~	~~Default~~
~~BCF_{worm}~~	~~7.40~~	~~$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$~~
~~PEC_{worm}~~	~~0.185~~	~~$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$~~
~~Daily dietary dose (mg/kg bw/d)~~	~~0.194~~	~~$DDD = PEC_{worm} \times 1.05$~~
~~NOEL (mg/kg bw/d)~~	~~47~~	
~~TER_{lt}~~	~~242~~	

TER values shown in bold fall below the relevant trigger.

Since GLOB1817H contains 3 active ingredients and a safener, a risk assessment for the mixture was performed using the NOEL (mix) of 128.3 mg/kg bw/d.

Table 9.2-7: 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

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	~~17.678~~ 17.494	Sum of DDD in Table 9.2-3 to ~~9.2-6~~ 9.2-5
NOEL (mg/kg bw/d)	~~128.3~~ 129.2	NOEL (mix)
TER_{lt}	~~7.26~~	

Parameter	Mixture	comments
	7.38	

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} = ~~7.15~~ 7.37

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 9.2-8: 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

Parameter	Prosulfocarb	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.008448	dRR B8 Table 8.9-4
BCF_{fish}	700	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	5.9136	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.9403	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	131	
TER_{lt}	139	

TER values shown in bold fall below the relevant trigger.

Table 9.2-9: 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

Parameter	Diflufenican	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0001510	dRR B8 Table 8.9-12
BCF_{fish}	1596	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.2410	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.03832	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	91.84	
TER_{lt}	2397	

TER values shown in bold fall below the relevant trigger.

Table 9.2-10: Assessment of the risk for fish-eating birds due to exposure to halauxifen-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	Halauxifen-methyl	comments
PEC_{sw} (max) (mg/L)	0.00004836	dRR B8 Table 8.9-19
BCF_{fish}	217	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.01049	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00167	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	36.9	
TER_{lt}	22115	

TER values shown in bold fall below the relevant trigger.

~~**Table 9.2-11: Assessment of the risk for fish-eating birds due to exposure to cloquintocet-mexyl via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals**~~

~~Parameter~~	~~Cloquintocet-mexyl~~	~~comments~~
~~PEC_{sw} (max) (mg/L)~~	~~0.000172~~	~~dRR B8 Table 8.9-27~~
~~BCF_{fish}~~	~~621~~	
~~BMF~~	~~-~~	~~biomagnification factor (relevant for BCF ≥ 2000)~~
~~PEC_{fish}~~	~~0.107~~	~~$PEC_{fish} = PEC_{water} \times BCF_{fish}$~~
~~Daily dietary dose (mg/kg bw/d)~~	~~0.0170~~	~~$DDD = PEC_{fish} \times 0.159$~~
~~NOEL (mg/kg bw/d)~~	~~47~~	
~~TER_{lt}~~	~~2767~~	

TER values shown in bold fall below the relevant trigger.

Since GLOB1817H contains 3 active ingredients and a safener, a risk assessment for the mixture was performed using the NOEL (mix) of ~~128.3~~ 129.2 mg/kg bw/d.

Table 9.2-12: Assessment of the risk for earthworm-eating birds due to exposure to the mixture via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	~~0.9973~~ 0.9803	Sum of DDD in Table 9.2-8 to 9.2-11
NOEL (mg/kg bw/d)	~~128.3~~ 129.2	NOEL (mix)
TER_{lt}	~~128.6~~ 131.8	

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} = ~~124.7~~ 130.6

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

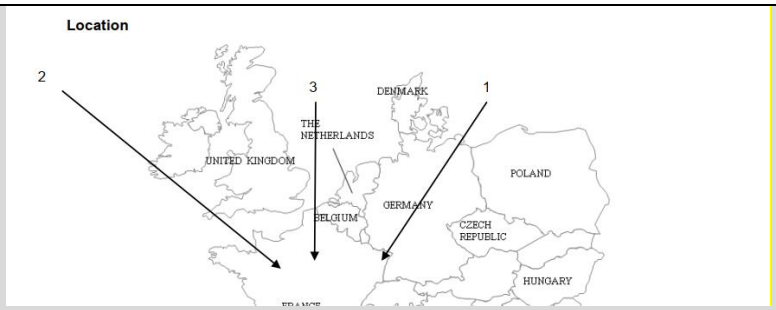
Not relevant.

9.2.4 Overall conclusions

The risk for birds is acceptable when applying GLOB1817H according to the intended use.

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

zRMS Comments:	The acute and long-term risk for mammals due to the use of GLOB1817H formulation used in winter cereals was submitted. The risk assessment was conducted in accordance with Birds and Mammals guidance, 2009. The used endpoints for all active substances were agreed at the EU level. The cloquintocet-mexyl is used as a safener and will not be evaluated in this dossier. **Prosulfocarb, diflufenican and halauxifen-methyl.** The TER_A values are above the trigger value of 10 at first tier step indicating an acceptable acute risk for mammals. ~~**Prosulfocarb,**~~ **diflufenican and halauxifen-methyl.** The TER_{LT} values for long-term risk are above the trigger value of 5 at first tier assessment indicating an acceptable long-term risk for mammals. **Prosulfocarb.** The TER_{LT} values for long-term risk are above the trigger value of 5 at higher tier assessment (refinement on the base of residue studies) indicating an acceptable long-term risk for mammals. Regarding the relevance of the studies performed in the north of France, it would be pointed out that these trials were evenly spread in the north of France as can be seen on the map here below which was copied from the study report.



In addition, the highest value of all available trials (2.2 d) was used in the risk assessment. Also in comparison with DT_{50} values available in the DAR and draft RAR of prosulfocarb, the value of 2.2 d as used by the applicant is still worst-case.

- DAR: Devine, 2004: 2 trials in winter barley in UK, resulting in DT_{50} values between 0.51 and 0.59 days.
- dRAR: North, 2015: 4 trials on spring barley in north of France and UK, resulting in DT_{50} values between 0.62 and 1.60 days.

Based on all the above arguments, the value of 2.2 days as used by the applicant is regarded as worst-case and relevant for the whole central zone.

Mixture of active substances. Based on recalculated application rate (sum of only 3 active substances) – the difference between submitted one (with safener) and recalculated (without safener) was insignificant. The recalculated endpoints for mixture (LD_{50} mix and NOELmix) are similar to submitted – the submitted risk was not updated. The submitted risk assessment for mixture was accepted and it can be concluded that safener does not cause any significant hazard and do affect the final conclusion.
The acute and long-term TER values are above the trigger value of 10 and 5, respectively, at first tier assessment indicating an acceptable long-term risk for mammals.

No further refinement is required.

The **puddle scenario** was used in mammal exposure assessment. the submitted assessment was accepted.

Secondary poisoning. The risk assessment for earthworm-eating mammals and fish-eating mammals was corrected as the study of XXXX, 2008 was not accepted at zonal evaluation (product Roxy, UK as the evaluator). The risk was not acceptable (Table 9.3-4) and further refinement was necessary. Based on the study *(Bätscher, 2006)* summarised in the Addendum to the Draft Assessment Report, July 2007, the new bioaccumulation factors were calculated for the two treatments: 0.59 and 0.77. In the refined risk assessment, presented below, the conservative approach was used (the higher value).

Parameter	Prosulfocarb	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	1.6051	dRR B8 Table 8.7-3
BCF_{worm}	0.77	$CF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	1.24	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	1.58	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	
TER_{lt}	31.6	TERlt > 5

	The risk for mammals is acceptable if application of GLOB1817H formulation is used in accordance with the intended use.

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with prosulfocarb, diflufenican, halauxifen-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GLOB1817H were not evaluated as part of the EU assessment of prosulfocarb, diflufenican and halauxifen-methyl.
However, the provision of further data on the formulation GLOB1817H is not considered essential, because the risk for mammals from GLOB1817H 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	**LD_{50} = 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	**LD_{50} > 5000 mg/kg bw**	EFSA, 2007
Rat	Diflufenican	Dietary Reproductive toxicity	**NOAEL = 35.5 mg/kg bw/d**	EFSA, 2007
Rat	Halauxifen-methyl	Oral 1 d Acute	**LD_{50} > 5000 mg/kg bw**	EFSA, 2014
Rabbit	Halauxifen-methyl	Dietary Reproductive toxicity	**NOAEL = 5.78 mg/kg bw/d**	EFSA, 2014
~~Rat~~	~~Cloquintocet-mexyl~~	~~Oral~~ ~~1 d~~ ~~Acute~~	~~**LD_{50} > 2000 mg/kg bw**~~	~~Safener, not reviewed at EU level~~
~~Rabbit~~	~~Cloquintocet-mexyl~~	~~Dietary~~ ~~Reproductive toxicity~~	~~**NOAEL = 60 mg/kg bw/d**~~	~~Safener, not reviewed at EU level~~

9.3.1.1 Justification for new endpoints

-

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: Screenig and First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB1817H in winter cereals

Intended use	Winter cereals				
Active substance/product	Prosulfocarb				
Application rate (kg/ha)	1 × 2.001				
Acute toxicity (mg/kg bw)	1820				
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 herbivorous mammal	118.4	1	237	**7.68**
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	7.6	1	15.2	120
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	42.1	1	84.2	21.6
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1	34.4	52.9
Reprod. toxicity (mg/kg bw/d)	50				
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	51.2	**0.98**
Cereals, BBCH 10-19	Small insectivorous mammal “shrew”	4.2	0.53	4.45	11.2
Cereals, early (shoots)	Large herbivorous mammal “lagomorph”	22.3	0.53	23.6	**2.12**
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	0.53	8.27	6.05
Active substance/product	Diflufenican				
Application rate (kg/ha)	1 × 0.042				
Acute toxicity (mg/kg bw)	> 5000				
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 herbivorous mammal	118.4	1	4.97	1006

Reprod. toxicity (mg/kg bw/d)	35.5				
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	1.08	32.9
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	0.0935	380
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.53	0.496	71.5
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	0.174	205
Active substance/product	Halauxifen-methyl				
Application rate (kg/ha)	1 × 0.00399				
Acute toxicity (mg/kg bw)	> 5000				
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 herbivorous mammal	118.4	1	0.472	10593
Reprod. toxicity (mg/kg bw/d)	5.78				
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	0.102	56.7
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	0.00888	651
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.53	0.0472	123
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	0.0165	350
~~**Active substance/product**~~	~~Cloquintocet-mexyl~~				
~~**Application rate (kg/ha)**~~	~~1 × 0.00399~~				
~~**Acute toxicity (mg/kg bw)**~~	~~> 2000~~				
~~**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 herbivorous mammal~~	~~118.4~~	~~1~~	~~0.472~~	~~4234~~
~~**Reprod. toxicity (mg/kg bw/d)**~~	~~60~~				
~~**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~~	~~0.102~~	~~587~~
~~Cereals, BBCH 10-19~~	~~Small insectivorous mammal "shrew"~~	~~4.2~~	~~0.53~~	~~0.00888~~	~~6755~~
~~Cereals, early~~	~~Large herbivorous mammal~~	~~22.3~~	~~0.53~~	~~0.0472~~	~~1272~~

~~(shoots)~~	~~"lagomorph"~~				
~~Cereals, BBCH 10-29~~	~~Small omnivorous mammal "mouse"~~	~~7.8~~	~~0.53~~	~~0.0165~~	~~3638~~

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 GLOB1817H contains 3 active ingredients and a safener, 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 GLOB1817H 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 LD_{50} for the mixture of active substances with known toxicity assuming additivity:

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

With:
$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)
$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

For GLOB1817H, the LD_{50} (mix) amounts to 1846.7 mg/kg bw (=1/[(0.98/1820)+(0.02/5000)+(0.002/5000)+(0.002/2000)]).

Using the same approach, also a NOEL (mix) was calculated which amounts to 48.7 mg/kg bw/d (=1/[(0.98/50)+(0.02/35.5)+(0.002/5.78)+(0.002/60)]).

zRMS Comments:	The LD_{50} mix was recalculated only for active substances as the safener is not considered in risk assessment. The following values were obtained: LD_{50} (mix) = 1841.6 mg/kg bw/d NOEL (mix) = 48.8 mg/kg bw/d As the recalculated mixture endpoints do not differ significantly (< 1%) the final TER_A and TER_{LT} values will not be essential changed. The risk assessment at screening and First-tier step was not recalculated. (For comparison, please refer to p. 9.2).

Intended use	Winter cereals
Active substance/product	Mixture of active substances
Application rate (kg/ha)	1 × ~~2.05098~~ 2.04699
Acute toxicity (mg/kg bw)	LD_{50} (mix) = ~~1846.7~~ 1841.6
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 herbivorous mammal	118.4	1	242.8	**7.6**
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	7.6	1	15.6	119
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	42.1	1	86.3	21.4
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1	35.3	52.3
Reprod. toxicity (mg/kg bw/d)	NOEL (mix) = ~~48.7~~ 48.8				
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	52.5	**0.93**
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	4.57	10.7
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.53	24.2	**2.01**
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	8.48	5.74
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	-	-	-	**0.93**
Cereals, BBCH 10-19	Small insectivorous mammal "shrew"	-	-	-	10.7
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	-	-	-	**2.02**
Cereals, BBCH 10-29	Small omnivorous mammal "mouse"	-	-	-	5.77

*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

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. Therefore, a higher-tier risk assessment is provided here.

Table 9.3-3: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of GLOB1817H in cereals – refined parameters (*) are further described and justified in the text

Intended use	Winter cereals				
Active substance/product	Prosulfocarb				
Application rate (kg/ha)	1 × 2.001				
Reprod. toxicity (mg/kg bw/d)	50				
TER criterion	5				
Crop scenario Growth stage	**Generic focal species**	**SV_m**	**MAF_m × TWA***	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.151	6.74	7.4
Intended use	Winter cereals				
Active substance/product	Mixture of active substances				
Application rate (kg/ha)	Prosulfocarb: 1 x 2.001, diflufenican: 1 x 0.042, halauxifen-methyl: 1 x 0.00399, ~~cloquintocet-mexyl: 1 x 0.00399~~				
Reprod. toxicity (mg/kg bw/d)	NOEL (mix) = ~~48.7~~ 48.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**Active substance**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals, early (shoots)	Large herbivorous mammal "lagomorph"	Prosulfocarb	22.3	0.151	6.74	-
		Diflufenican		0.53	0.696	-
		Halauxifen-methyl			0.0472	-
		Cloquintocet-mexyl			0.0472	-
		Sum	-	-	7.33	~~6.64~~ 6.66
Intended use	Winter cereals				
Active substance/product	Mixture of active substances				
Application rate (kg/ha)	1 × ~~2.05098~~ 2.04699				
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, early (shoots)	Large herbivorous mammal "lagomorph"	-	-	-	5.4

**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	

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

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

Effective application rate (g/ha)	=	2001		
Acute toxicity (mg/kg bw)	=	1820	quotient	=	1.10
Reprod. toxicity (mg/kg bw/d)	=	50	quotient	=	40

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

Effective application rate (g/ha)	=	42		
Acute toxicity (mg/kg bw)	=	> 5000	quotient	=	< 0.0084
Reprod. toxicity (mg/kg bw/d)	=	35.5	quotient	=	1.18

With a K(f)oc of 796 L/kg, halauxifen-methyl belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	3.99		
Acute toxicity (mg/kg bw)	=	> 5000	quotient	=	< 0.000798
Reprod. toxicity (mg/kg bw/d)	=	5.78	quotient	=	0.69

With a K(f)oc of 12850 L/kg, cloquintocet-mexyl belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	3.99		
Acute toxicity (mg/kg bw)	=	> 2000	quotient	=	< 0.0020
Reprod. toxicity (mg/kg bw/d)	=	60	quotient	=	0.066

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.

The log P_{ow} of halauxifen-methyl amounts to 3.76 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of cloquintocet-mexyl amounts to 5.20 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 9.3-4: 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

Parameter	Prosulfocarb	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	1.6051	dRR B8 Table 8.7-3
log P_{ow} / P_{ow}	4.48/30199	
Koc	1799	Geomean (n = 6)
foc	0.02	Default
BCF_{worm}	10.10	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	16.20	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	20.74	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	50	
TER_{lt}	**2.41**	

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 9.3-5: 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 – refined BCF

~~Parameter~~	~~Prosulfocarb~~	~~comments~~
~~PEC_{soil} (twa = 21 d) (mg/kg soil)~~	~~1.6051~~	~~dRR B8 Table 8.7-3~~
~~BCF_{worm}~~	~~1.39~~	~~XXXX D., 2008~~
~~PEC_{worm}~~	~~2.23~~	~~$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$~~
~~Daily dietary dose (mg/kg bw/d)~~	~~2.85~~	~~$DDD = PEC_{worm} \times 1.28$~~
~~NOEL (mg/kg bw/d)~~	~~50~~	
~~TER_{lt}~~	~~17.5~~	

TER values shown in bold fall below the relevant trigger.

Table 9.3-6: 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

Parameter	Diflufenican	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1400	dRR B8 Table 8.7-6
log P_{ow} / P_{ow}	4.2/15849	

Parameter	Diflufenican	comments
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}	~~0.171~~ 0.433	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	~~0.433~~ 0.554	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	35.5	
TER_{lt}	~~78.2~~ 64.1	

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 halauxifen-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals

Parameter	Halauxifen-methyl	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.0066	dRR B8 Table 8.7-9
log P_{ow} / P_{ow}	3.76/5754	
Koc	796	Geomean (n = 9)
foc	0.02	Default
BCF_{worm}	4.39	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.029	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.037	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	5.78	
TER_{lt}	156	

TER values shown in bold fall below the relevant trigger.

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

Parameter	Cloquintocet-mexyl	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.025	dRR B8 Table 8.7-12
log P_{ow} / P_{ow}	5.20/158489	
Koc	12850	Mean (n = 5)
foc	0.02	Default
BCF_{worm}	7.40	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.185	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.237	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	60	

Parameter	Cloquintocet-mexyl	comments
TER_{lt}	253	

TER values shown in bold fall below the relevant trigger.

Since GLOB1817H contains 3 active ingredients and a safener, a risk assessment for the mixture was performed using the NOEL (mix) of 48.7 mg/kg bw/d.

Table 9.3-9: 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

Parameter	Mixture	comments
Daily dietary dose (mg/kg bw/d)	3.557	Sum of DDD in Table 9.3-5 to 9.3-8
NOEL (mg/kg bw/d)	48.7	NOEL (mix)
TER_{lt}	13.7	

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} = 12.5

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 9.3-10: 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

Parameter	Prosulfocarb	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.008448	dRR B8 Table 8.9-4
BCF_{fish}	700	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	5.9136	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.8397	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	50	
TER_{lt}	59.5	

TER values shown in bold fall below the relevant trigger.

Table 9.3-11: 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

Parameter	Diflufenican	comments

PEC_{sw} (twa = 21 d) (mg/L)	0.0001510	dRR B8 Table 8.9-12
BCF_{fish}	1596	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.2410	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.03422	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	35.5	
TER_{lt}	1037	

TER values shown in bold fall below the relevant trigger.

Table 9.3-12: Assessment of the risk for fish-eating mammals due to exposure to halauxifen-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	**Halauxifen-methyl**	**comments**
PEC_{sw} (max) (mg/L)	0.00004836	dRR Table 8.9-19
BCF_{fish}	217	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.01049	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00149	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	5.78	
TER_{lt}	3879	

TER values shown in bold fall below the relevant trigger.

Table 9.3-13: Assessment of the risk for fish-eating mammals due to exposure to cloquintocet-mexyl via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	**Cloquintocet-mexyl**	**comments**
PEC_{sw} (max) (mg/L)	0.000172	dRR Table 8.9-27
BCF_{fish}	621	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.107	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0152	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	60	
TER_{lt}	3956	

TER values shown in bold fall below the relevant trigger.

Since GLOB1817H contains 3 active ingredients and a safener, a risk assessment for the mixture was performed using the NOEL (mix) of 48.7 mg/kg bw/d.

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

Parameter	**Mixture**	**comments**
Daily dietary dose (mg/kg bw/d)	0.8906	Sum of DDD in Table 9.3-10 to 9.3-13
NOEL (mg/kg bw/d)	48.7	NOEL (mix)

Parameter	Mixture	comments
TER_{lt}	54.7	

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} = 54.7

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 applying GLOB1817H according to the intended use.

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)

Evaluation Comments:	New studies were submitted and evaluated in Appendix 2. The cloquintocet-mexyl is used as a safener and was not evaluated in this dossier. **Prosulfocarb**. The endpoints agreed at the EU level and based on new studies were taken into consideration in risk assessment. The geometric mean acute fish toxicity (LC_{50}) proposed by the Applicant was not accepted for risk assessment. The following endpoints were used in risk assessment for aquatic organisms: fish: • LC_{50} = 840 µg a.s./L; EU endpoint; • NOEC = 310 µg a.s./L; EU endpoint; aquatic invertebrates • EC_{50} = 510 µg a.s./L; EU endpoint; • NOEC = 45 µg a.s./L; EU endpoint;

	aquatic insects • NOEC = 1250 µg a.s./L; EU endpoint algae • E_bC_{50} = 113 µg/L; DAR, 2006. aquatic plants • EC_{50} = 690 µg a.s./L; EU endpoint mesocosmos • NOEC = 15 µg a.s./L; EU endpoint; The mesocosm endpoint for active substance prosulfocarb with AF = 2 (proposed by the Applicant) was used in higher tier risk assessment: NOEC = 15 µg/L and RAC = 7.5 µg/L. For risk assessment the PECsw and PECsed values evaluated in Section 8 were taken into consideration. The risk for aquatic organisms is acceptable if mitigation measure of 10 m VBS and 10 NSS are implemented in winter cereals. The proper mitigation measures should be considered at MS level in accordance with the national requirements. **Metabolites of prosulfocarb.** The metabolite prosulfocarb sulfoxide was taken into consideration. The submitted risk assessment is based on PECsw i PECsed values reported in Section 8. For metabolite prosulfocarb sulfoxide the ETO-RAC = 15 µg/L was used in higher tier risk assessment. The risk assessment for metabolites prosulfocarb sulfoxide was accepted. The metabolite poses an acceptable risk. **Diflufenican.** The endpoints agreed at the EU level and based on new studies were taken into consideration in risk assessment. The following endpoints were used in risk assessment for aquatic organisms: fish: • LC_{50} = 98.5 µg a.s./L; EU endpoint; • NOEC = 15 µg a.s./L; EU endpoint; aquatic invertebrates • EC_{50} = 240 µg a.s./L; EU endpoint; • NOEC = 52 µg a.s./L; EU endpoint; algae • E_bC_{50} = 0.25 µg/L (as a lower than E_rC_{50} = 0.45 µg/L); EU endpoint • NOEC = 0.1 µg a.s./L; EU endpoint; • max concentration for possible recovery 4.2 µg a.s./L with NOEC = 0.10 µg a.s./L sediment dwelling organisms • NOEC = 100 µg a.s./L (spiked water); EU endpoint; aquatic plants • E_bC_{50} = 39 µg a.s./L; EU endpoint. Tier 1. The RAC value of 0.045 µg a.s./L. The submitted risk assessment was not solved for algae considering the Step 3 PECsw values. The Step 4 PECsw values with proposed mitigation measures were used in further risk assessment. Even if 20 m vfs and 20 m nss was considered the risk was unacceptable. Tier 2. The further refinement for algae was proposed using the new value 4.2 µg a.s./L (vide DAR, 2007). This study was evaluated in DAR and the endpoint was agreed at the EU level. The new RAC value of 0.42 µg a.s./L (based on assessment factor AF = 10) was used in risk refinement. The overall NOEC of 0.1 µg a.s./L was also used in risk refinement.

Step 4. 10 m nss (scenarios D) and 10 m nss + 10 m vfs (scenarios R)

Scenario	PECsw µg/L	RAC µg/L	PEC/RAC	RAC µg/L	PEC/RAC
D1 ditch	0.06633	0.42	0.16	0.10	0.66
D1 stream	0.04541		0.10		0.42
D2 ditch	0.1445		0.34		**1.45**
D2 stream	0.09124		0.22		0.91
D3 ditch	0.03795		0.09		0.38
D4 stream	0.04435		0.11		0.44
D5 stream	0.04785		0.11		0.48
D6 ditch	0.1006		0.24		**1.01**
R1 stream	0.05014		0.06		0.26
R3 stream	0.05160		0.06		0.27
R4 stream	0.07341		0.09		0.38

As the D2, ditch and D6 ditch scenarios are not relevant for Central Zone, the risk is acceptable if mitigation measures of 10 m no spray buffer and 10 m vegetated buffer strips are applied.

The approach concerning EPAT analysis was not evaluated. The risk assessment based on EPAT analysis was not evaluated and will be decided at Member State level.

Metabolites of diflufenican. In accordance with EFSA, 2007 the metabolites AE B107137 and AE 0542291 were taken into consideration.
The risk is acceptable for metabolites if Step 1 PECsw were used.

Halauxifen-methyl. The agreed andpoints were used in the aquatic ris assessment.
The following endpoints were used in risk assessment for aquatic organisms:
fish:

- LC_{50} > 1330 µg a.s./L; EU endpoint;
- NOEC = 11.5 µg a.s./L; EU endpoint;

aquatic invertebrates

- EC_{50} = 2120 µg a.s./L; EU endpoint;
- NOEC = 144 µg a.s./L; EU endpoint;

algae

- E_rC_{50} > 0.245 µg/L); EU endpoint

sediment dwelling organisms

- NOEC = 1260 µg a.s./L; EU endpoint;

aquatic plants

- E_bC_{50} = 0.393 µg a.s./L; EU endpoint.

The submitted risk assessment was not solved for aquatic plant considering the Step 3 PECsw values (R4 scenario). The Step 4 PECsw value with proposed mitigation measures were used in further risk assessment: PECsw = 0.02183 µg a.s./L if 10 m vfs and 10 m nss is applied. The PEC/RAC for the most sensitive aquatic organism *Myriophyllum spicatum* is of 0.56. The risk is acceptable.

The metabolites X11393729 (halauxifen), X11449757, X11406790 were considered. Additionally, the photoproducts of halauxifen-methyl were taken into consideration.
For metabolite X11393729 the risk is acceptable if Step 3 PECsw were used for all relevant scenarios in Central Zone (D2 ditch scenario is not relevant for CZ). For rest of metabolites risk

	was acceptable if Step 1 PECsw were used. **Mixture of active substances**. The mixture toxicity was corrected – the safener cloquintocet-mexyl was not taken into consideration. The recalculated mixture toxicity was added by evaluator in Table 9.5-15a. and Table 9.5-16a. The obtained results confirmed that safener does not affect the risk for aquatic organisms. Based on that, the consideration of cloquintocet-mexyl in risk assessment (RQmix) does not affect the final conclusion (Table 9.5-19 corrected). **Formulation GLOB1817H.** The submitted risk considering the drift exposure for aquatic plants and algae was corrected in accordance with PECsw presented in Section 8. The PECsw = 6.32 µg/L, which corresponds to 5 m of buffer strip as the worst case, was considered in formulation (3.0 L formulation/ha) risk assessment. The risk for aquatic organisms is acceptable if the following mitigation measures are applied: 10 m no spray buffer zone including a 10 m vegetated buffer strip. The relevant mitigation measures will be considered at the Member State level in accordance with national requirements.

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with prosulfocarb, diflufenican, halauxifen-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of GLOB1817H were not evaluated as part of the EU assessment of prosulfocarb, diflufenican and halauxifen-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Prosulfocarb, diflufenican, halauxifen-methyl, cloquintocet-mexyl and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Rainbow trout, *Oncorhynchus mykiss*	Prosulfocarb	96 h, f	**LC_{50} = 0.84 mg/L_{nom}**	EFSA, 2007
Fathead minnow, *Pimephales promelas*	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, *Oncorhynchus mykiss*	Prosulfocarb	21 d, f	**NOEC = 0.31 mg/L_{mm}**	EFSA, 2007
Common carp, *Cyprinus carpio*	Diflufenican	96 h	**LC_{50} > 98.5 µg/L**	EFSA, 2007

Species	Substance	Exposure System	Results	Reference
Rainbow trout, *Oncorhynchus mykiss*	Diflufenican	35 d	**NOEC = 15 µg/L**	EFSA, 2007
Rainbow trout, *Oncorhynchus mykiss*	AE B107137	96 h	**LC_{50} > 17300 µg/L**	EFSA, 2007
Rainbow trout, *Oncorhynchus mykiss*	Halauxifen-methyl	96 h, s	LC_{50} = 2.01 mg/L_{nom}	EFSA, 2014
Fathead minnow, *Pimephales promelas*	Halauxifen-methyl	96 h, s	LC_{50} > 3.22 mg/L_{mm}	EFSA, 2014
Sheepshead minnow, *Cyprinodon variegates*	Halauxifen-methyl	96 h, s	**LC_{50} > 1.33 mg/L_{mm}**	EFSA, 2014
Fathead minnow, *Pimephales promelas*	Halauxifen-methyl	28 d, ELS, ft	NOEC = 0.259 mg/L_{mm}	EFSA, 2014
Sheepshead minnow, *Cyprinodon variegates*	Halauxifen-methyl	28 d, ELS, ft	**NOEC = 0.0115 mg/L_{mm}**	EFSA, 2014
Rainbow trout, *Oncorhynchus mykiss*	X11393729 (halauxifen)	96 h, s	**LC_{50} > 107 mg/L_{mm}**	EFSA, 2014
Fathead minnow, *Pimephales promelas*	X11393729 (halauxifen)	28 d, ELS, ft	**NOEC = 11.8 mg/L_{mm}**	EFSA, 2014
Rainbow trout, *Oncorhynchus mykiss*	X11449757	96 h, s	**LC_{50} > 120 mg/L_{nom}**	EFSA, 2014
Fathead minnow, *Pimephales promelas*	X11449757	28 d, ELS, ft	**NOEC = 8.9 mg/L_{mm}**	EFSA, 2014
Rainbow trout, *Oncorhynchus mykiss*	X11406790	96 h, s	**LC_{50} > 30 mg/L_{nom}**	EFSA, 2014
Fathead minnow, *Pimephales promelas*	Halauxifen-methyl	21 d reproduction assay	NOEC = 0.078 mg/L_{mm}	EFSA, 2014
Fathead minnow, *Pimephales promelas*	X11393729 (halauxifen)	21 d reproduction assay	NOEC = 12 mg/L_{mm}	EFSA, 2014
~~Rainbow trout, *Oncorhynchus mykiss*~~	~~Cloquintocet-mexyl~~	~~96 h~~	~~**LC_{50} > 0.97 mg/L**~~	~~Safener, not reviewed on EU level~~
~~Rainbow trout, *Oncorhynchus mykiss*~~	~~CGA 153433~~	~~96 h~~	~~**LC_{50} = 89 mg/L**~~	~~Safener, not reviewed on EU level~~
~~Rainbow trout, *Oncorhynchus mykiss*~~	~~Cloquintocet-mexyl~~	~~21 d reproduction assay~~	~~**NOEC ≥ 1.26 mg/L**~~	~~Safener, not reviewed on EU level~~
Aquatic invertebrate				
Water flea, *Daphnia magna*	Prosulfocarb	48 h, s	**EC_{50} = 0.51 mg/L_{mm}**	EFSA, 2007
Chaoborus sp.			EC_{50} = 790 µg/L	DAR, 2006 (Ashwell, 2001)
Cleon sp.			EC_{50} = 1410 µg/L	
Asellus sp.			EC_{50} = 810 µg/L	
Hylalella azteca			EC_{50} = 1080 µg/L	
Aquatic invertebrates	Prosulfocarb	Acute	**EC_{50} = 869.5 µg/L**	Geomean

Species	Substance	Exposure System	Results	Reference
Water flea, *Daphnia magna*	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, *Daphnia magna*	Diflufenican	48 h	**EC_{50} > 240 µg/L**	EFSA, 2007
Water flea, *Daphnia magna*	Diflufenican	21 d	**NOEC = 52 µg/L**	EFSA, 2007
Water flea, *Daphnia magna*	AE B107137	48 h	**EC_{50} > 20400 µg/L**	EFSA, 2007
Water flea, *Daphnia magna*	AE 0542291	48 h	**EC_{50} > 10000 µg/L**	EFSA, 2007
Water flea, *Daphnia magna*	Halauxifen-methyl	48 h, s	**EC_{50} = 2.12 mg/L_{mm}**	EFSA, 2014
Water flea, *Daphnia magna*	Halauxifen-methyl	21 d, ss	**NOEC = 0.144 mg/L_{mm}**	EFSA, 2014
Water flea, *Daphnia magna*	X11393729 (halauxifen)	48 h, s	**EC_{50} > 106 mg/L_{mm}**	EFSA, 2014
Water flea, *Daphnia magna*	X11393729 (halauxifen)	21 d, ss	**NOEC = 100 mg/L_{nom}**	EFSA, 2014
Water flea, *Daphnia magna*	X11449757	48 h, s	**EC_{50} > 120 mg/L_{nom}**	EFSA, 2014
Water flea, *Daphnia magna*	X11406790	48 h, s	**EC_{50} > 30 mg/L_{nom}**	EFSA, 2014
~~Water flea, *Daphnia magna*~~	~~Cloquintocet-mexyl~~	~~48 h~~	~~**EC_{50} > 0.82 mg/L**~~	~~Safener, not reviewed on EU level~~
~~Water flea, *Daphnia magna*~~	~~CGA 153433~~	~~48 h~~	~~**EC_{50} > 9.7 mg/L**~~	~~Safener, not reviewed on EU level~~
~~Water flea, *Daphnia magna*~~	~~Cloquintocet-mexyl~~	~~21 d~~	~~**EC_{50} ≥ 0.44 mg/L**~~	~~Safener, not reviewed on EU level~~
~~Water flea, *Daphnia magna*~~	~~CGA 153433~~	~~21 d~~	~~**EC_{50} ≥ 100 mg/L**~~	~~Safener, not reviewed on EU level~~
Sediment dwelling organisms				
Freshwater midge, *Chironomus riparius*	Prosulfocarb	25 d	**NOEC = 1.25 mg/L**	EFSA, 2007
Freshwater midge, *Chironomus riparius*	Diflufenican	28 d	**NOEC = 0.1 mg/L** (spiked water)	EFSA, 2007
Freshwater midge, *Chironomus riparius*	Diflufenican	28 d	**NOEC = 2.0 mg/kg** (spiked sediment)	EFSA, 2007
Freshwater midge, *Chironomus riparius*	AE C522392	28 d	NOEC = 1.0 mg/kg	EFSA, 2007
Freshwater midge, *Chironomus riparius*	Halauxifen-methyl	28 d	**NOEC = 1.26 mg/L_{im}**	EFSA, 2014
Freshwater midge, *Chironomus riparius*	Halauxifen-methyl	28 d	**NOEC = 89.3 mg/kg** (sediment treated)	EFSA, 2014

Species	Substance	Exposure System	Results	Reference
~~Freshwater midge, *Chironomus riparius*~~	~~Cloquintocet-mexyl~~	~~28 d~~	~~**NOEC ≥ 8 mg/L**~~	~~Safener, not reviewed on EU level~~
Algae				
Pseudokirchneriella subcapitata	Prosulfocarb	72 h, s	E_bC_{50} = 49 µg/L E_rC_{50} = 120 µg/L	EFSA, 2007
Scenedesmus subspicatus	Prosulfocarb	72 h/96 h	72 h E_bC_{50} = 112 µg/L **96 h E_rC_{50} = 113 µg/L**	DAR, 2006 (Ellgehausen, 1986)
Anabaena flos-aquae	Prosulfocarb	72 h	E_bC_{50} = 3770 µg/L E_rC_{50} = 7480 µg/L	DAR, 2006 (Wallace, 2001)
Chlorella vulgaris	Prosulfocarb	72 h/96 h	72 h E_bC_{50} = 1540 µg/L 96 hE_rC_{50} = 8340 µg/L	DAR, 2006 (Wallace, 2001)
Chlamydomonas reinhardtii	Prosulfocarb	72 h/96 h	72 h E_bC_{50} = 3690 µg/L 96 hE_rC_{50} = 7720 µg/L	DAR, 2006 (Swarbrick, 2001)
Navicula pelliculosa	Prosulfocarb	72 h	E_bC_{50} = 330 µg/L E_rC_{50} = 680 µg/L	DAR, 2006 (Smyth, 1998)
Algae	Prosulfocarb	-	**EC_{50} = 680 µg/L**	Geomean
Pseudokirchneriella subcapitata	Prosulfocarb sulfoxide	72 h	E_bC_{50} = 1.28 µg/L **E_rC_{50} = 4.33 µg/L**	DAR
Desmodesmus subspicatus	Prosulfocarb sulfoxide		E_rC_{50} = 85 µg/L	DAR
Chlamydomonas reinhardtii	Prosulfocarb sulfoxide	72 h	E_bC_{50} = 97.1 µg/L E_rC_{50} = 253.9 µg/L	Juckeland D, 2012a
			E_rC_{50} = 410 µg/L	DAR
Chlorella vulgaris	Prosulfocarb sulfoxide	72 h	E_bC_{50} = 730 µg/L E_rC_{50} = 1320 µg/L	Juckeland D, 2012b
			E_rC_{50} = 2860 µg/L	DAR
Anabaena flosaquae	Prosulfocarb sulfoxide	72 h	E_bC_{50} = 19500 µg/L E_rC_{50} = 42500 µg/L	Juckeland D, 2012c
			E_rC_{50} = 43000 µg/L	DAR
Navicula pelliculosa	Prosulfocarb sulfoxide	72 h	E_bC_{50} = 1400 µg/L E_rC_{50} = 7650 µg/L	Juckeland D, 2012d
			E_rC_{50} = 2700 µg/L	DAR
Skeletonema costatum	Prosulfocarb sulfoxide	72 h	E_bC_{50} = 53.8 µg/L E_rC_{50} = 134.8 µg/L	Juckeland D, 2012e
Scenedesmus subspicatus	Diflufenican	72 h	Without sediment: **E_bC_{50} = 0.25 µg/L** **E_rC_{50} = 0.45 µg/L** **NOEC = 0.1 µg/L**	EFSA, 2007
Scenedesmus	Diflufenican	72 h	With sediment:	EFSA, 2007

Species	Substance	Exposure System	Results	Reference
subspicatus			E_bC_{50} = 2.4 µg/L E_rC_{50} = 4.7 µg/L NOEC = 0.76 µg/L	
Scenedesmus subspicatus	Diflufenican	72 h	E_bC_{50} = 0.46 µg/L E_rC_{50} = 1.22 µg/L Max conc. From which recovery possible: 4.2 µg/L NOEC = 0.15 µg/L	EFSA, 2007
Scenedesmus subspicatus	AE B107137	72 h	E_bC_{50} > 20400 µg/L **E_rC_{50} > 20400 µg/L**	EFSA, 2007
Scenedesmus subspicatus	AE 0542291	72 h	**E_bC_{50} > 36000 µg/L** E_rC_{50} > 66000 µg/L	EFSA, 2007
Pseudokirchneriella subcapitata	AE 592370	72 h	E_bC_{50} > 39000 µg/L E_rC_{50} > 58000 µg/L	EFSA, 2007
Pseudokirchneriella subcapitata	AE C522392	72 h	E_bC_{50} > 3400 µg/L E_rC_{50} > 16000 µg/L	EFSA, 2007
Pseudokirchneriella subcapitata	Halauxifen-methyl	72 h/96 h	72 h E_yC_{50} / E_rC_{50} > 0.855 mg/L_{mm} **96 h E_yC_{50} / E_rC_{50} > 0.245 mg/L_{mm}**	EFSA, 2014
Skeletonema costatum	Halauxifen-methyl	72 h/96 h	E_yC_{50} 72 h = 0.904 mg/L_{mm} E_yC_{50} 96 h > 1.07 mg/L_{mm} E_rC_{50} 72 h = 1.80 mg/L_{mm} E_rC_{50} 96 h > 1.85 mg/L_{mm}	EFSA, 2014
Anabaena flos-aqua	Halauxifen-methyl	72 h/96 h	E_yC_{50} 72 h = 1.13 mg/L_{mm} E_yC_{50} 96 h > 0.775 mg/L_{mm} E_rC_{50} 72 h = 1.13 mg/L_{mm} E_rC_{50} 96 h > 0.775 mg/L_{mm}	EFSA, 2014
Navicula pelliculosa	Halauxifen-methyl	72 h/96 h	E_yC_{50} 72 h = 0.822 mg/L_{mm} E_yC_{50} 96 h = 0.663 mg/L_{mm} E_rC_{50} 72 h = 1.50 mg/L_{mm} E_rC_{50} 96 h = 1.26 mg/L_{mm}	EFSA, 2014
Pseudokirchneriella subcapitata	X11393729 (halauxifen)	72 h	E_yC_{50} = 23 mg/L_{nom} E_rC_{50} = 63 mg/L_{nom}	EFSA, 2014
Skeletonema costatum	X11393729 (halauxifen)	72 h/96 h	E_yC_{50} 72 h = 68 mg/L_{nom}	EFSA, 2014

Species	Substance	Exposure System	Results	Reference
			E_yC_{50} 96 h = 66 mg/L_{nom} E_rC_{50} 72 h = 78 mg/L_{nom} E_rC_{50} 96 h = 77 mg/L_{nom}	
Anabaena flos-aqua	X11393729 (halauxifen)	72 h	E_yC_{50} = 49 mg/L_{nom} **E_rC_{50} = 55 mg/L_{nom}**	EFSA, 2014
Navicula pelliculosa	X11393729 (halauxifen)	72 h	E_yC_{50} = 50 mg/L_{nom} E_rC_{50} = 56 mg/L_{nom}	EFSA, 2014
Pseudokirchneriella subcapitata	X11449757	72 h	E_yC_{50} = 4.13 mg/L_{mm} **E_rC_{50} > 15.8 mg/L_{mm}**	EFSA, 2014
Pseudokirchneriella subcapitata	X11406790	72 h	E_yC_{50} = 1.8 mg/L_{mm} **E_rC_{50} > 5.7 mg/L_{mm}**	EFSA, 2014
~~*Scenedesmus subspicatus*~~	~~Cloquintocet-mexyl~~	~~96 h~~	~~**EC_{50} = 0.63 mg/L**~~	~~Safener, not reviewed on EU level~~
~~*Microcystis aeruginosa*~~	~~CGA 153433~~	~~96 h~~	~~**EC_{50} > 1.9 mg/L**~~	~~Safener, not reviewed on EU level~~
Higher plants				
Duckweed, *Lemna gibba*	Prosulfocarb	14 d	**EC_{50} = 690 µg/L**	EFSA, 2007
Duckweed, *Lemna gibba*	Prosulfocarb sulfoxide	7 d	**E_rC_{50} = 13 µg/L** E_bC_{50} = 2.8 µg/L	DAR
Duckweed, *Lemna gibba*	Diflufenican	14 d	E_bC_{50} = 56 µg/L **EC_{50} frond density = 39 µg/L**	EFSA, 2007
Duckweed, *Lemna gibba*	Halauxifen-methyl	7 d, ss	E_yC_{50} = 2.13 mg/L_{mm} E_rC_{50} > 2.27 mg/L_{mm}	EFSA, 2014
Eurasian watermilfoil, *Myriophyllum spicatum*	Halauxifen-methyl	14 d	E_yC_{50} = 0.000149 mg/L_{nom} **E_rC_{50} = 0.000393 mg/L_{nom}**	EFSA, 2014
Duckweed, *Lemna gibba*	X11393729 (halauxifen)	7 d, ss	E_yC_{50} = 15 mg/L_{mm} E_rC_{50} > 50 mg/L_{mm}	EFSA, 2014
Eurasian watermilfoil, *Myriophyllum spicatum*	X11393729 (halauxifen)	14 d	E_yC_{50} = 0.0008 mg/L_{nom} **E_rC_{50} = 0.00158 mg/L_{nom}**	EFSA, 2014
Duckweed, *Lemna gibba*	X11449757	7 d, ss	E_yC_{50} > 92.9 mg/L_{mm} E_rC_{50} > 92.9 mg/L_{mm}	EFSA, 2014
Eurasian watermilfoil, *Myriophyllum spicatum*	X11449757	14 d	E_yC_{50} > 0.1 mg/L_{nom} **E_rC_{50} > 0.1 mg/L_{nom}**	EFSA, 2014
Duckweed, *Lemna gibba*	X11406790	7 d, ss	E_yC_{50} > 12 mg/L_{mm} E_rC_{50} > 12 mg/L_{mm}	EFSA, 2014
Eurasian	X11406790	14 d	E_yC_{50} > 0.1 mg/L_{nom}	EFSA, 2014

Species	Substance	Exposure System	Results	Reference
watermilfoil, *Myriophyllum spicatum*			**E_rC_{50} > 0.1 mg/L_{nom}**	
~~Duckweed, *Lemna gibba*~~	~~Cloquintocet-mexyl~~	~~14 d~~	~~**EC_{50} > 0.42 mg/L**~~	~~Safener, not reviewed on EU level~~
~~Duckweed, *Lemna gibba*~~	~~CGA 153433~~	~~14 d~~	~~**EC_{50} > 11 mg/L**~~	~~Safener, not reviewed on EU level~~
Primary producers				
Algae & higher plants	Prosulfocarb sulfoxide	-	HC5 = 4.84 µg/L	HC5
Other aquatic organisms				
Americamysis bahia	Halauxifen-methyl	96 h, s	LC_{50} > 1.30 mg a.s./L $_{mm}$	EFSA, 2014
Crassostrea virginica	Halauxifen-methyl	96 h, s	EC_{50} > 1.21 mg a.s./L $_{mm}$	EFSA, 2014
Tadpoles, *Xenopus laevis*	Halauxifen-methyl	96 h	LC_{50} > 2 mg/L_{nom}	EFSA, 2014
Leptocheirus plumulosus	Halauxifen-methyl	10 d	LC_{50} = > 58.1 mg a.s./kg (sediment treated)	EFSA, 2014
Tadpoles, *Xenopus laevis*	Halauxifen-methyl	21 d	NOEC > 0.38 mg/L_{nom}	EFSA, 2014
Americamysis bahia	Halauxifen-methyl	28 d, ss	NOEC = 0.152 mg a.s./L $_{mm}$	EFSA, 2014
Higher-tier studies (micro- or mesocosm studies)				
Microcosm	Prosulfocarb	**NOEC = 15 µg a.i./L** => ETO-RAC = 7.5 µg/L with a safety factor 2)		EFSA 2007 DAR, 2006 (van Wijngaarden 2006) + Deneer J., Roessink I. & Rico A. (2015)
	Prosulfocarb sulfoxide	**NOEC = 30 µg/L** => ETO-RAC = 15 µg/L with a safety factor 2		EFSA 2007 DAR, 2006 (XXXX 2013) + XXXX & Dark. (2015)

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB1817H

Species	Substance	Exposure System	Results	Reference
Daphnia magna	GLOB1817H	48 h, ss	EC_{50} = 0.954 mg/L $_{nom}$	XXXX, 2021a
Pseudokirchneriella subcapitata	GLOB1817H	72 h, s	E_rC_{50} = 0.0597 mg/L $_{nom}$	XXXX, 2021b

Species	Substance	Exposure System	Results	Reference
			E_yC_{50} = 0.0310 mg/L $_{nom}$	
Lemna gibba	GLOB1817H	7 d, ss	E_rC_{50} = 0.5159 mg/L $_{nom}$ E_yC_{50} = 0.3352 mg/L $_{nom}$	XXXX, 2021c
Myriophyllum spicatum	GLOB1817H	14 d, ss	E_rC_{50} = 0.075 mg/L $_{nom}$ E_yC_{50} = 0.040 mg/L $_{nom}$	XXXX, 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 *Oncorhynchus mykiss*	Prosulfocarb	96h LC_{50}	840 µg/L	EFSA, 2007	840
Fathead minnow *Pimephales promelas*	Prosulfocarb	96h LC_{50}	2400 µg/L	DAR, 2006 (Peter P., 2001)	2400
Geometric mean					**1420**
RAC					**14.2**

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				
Daphnia magna	Prosulfocarb	48h EC_{50}	510 µg /L	EFSA, 2007
Chaoborus sp.	Prosulfocarb		790 µg/L	DAR, 2006 (Ashwell. 2001)
Cleon sp.	Prosulfocarb		1410 µg/L	
Asellus sp.	Prosulfocarb		810 µg/L	
Hylalella azteca	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).

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	*Pseudokirchneriella subcapitata*	72h E_rC_{50}	120 µg a.i./L	EFSA, 2007	941
	Scenedesmus subspicatus	96h E_rC_{50} [1]	113 µg a.i./L	DAR, 2006 (Ellgehausen. 1986)	
	Chlorella vulgaris	96h E_rC_{50} [1]	8340 µg a.i./L	DAR, 2006 (Wallace. 2001)	
	Chlamydomonas reinhardtii	96h E_rC_{50} [1]	7720 µg a.i./L	DAR, 2006 (Swarbrick. 2001)	
Blue-green algae	*Anabaena flos-aquae*	72h E_rC_{50}	7480 µg a.i./L	DAR, 2006 (Wallace. 2001)	7480
Freshwater diatom	*Navicula pelliculosa*	72h E_rC_{50}	680 µg a.i./L	DAR, 2006 (Smyth. 1998)	680
Monocot macrophyte	*Lemna gibba*	14d E_rC_{50}	690 µg a.i./L	DAR, 2006 (Smyth. 1999)	690[2]
Lowest geomean EC_{50}					**680** [3]

[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" EC_{50} 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 EC_{50}" (680 µg/L).

Primary producers - Mesocosm study

EFSA conclusion (2007)

The experts discussed the endpoints derived from the new mesocosm study. Only statistically significant effects in two consecutive sampling time points were taken into account to derive the NOEC population for zooplankton. For cladocera (*Daphnia longispina*) the NOEC population was determined as 76 µg a.s./L. The lowest NOEC population for zooplankton was 15 µg a.s./L based on effects on the rotifer *Polyarthra 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 *Tetraedon 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 NOEAC 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 ecoystems 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, zooplanckton, periphyton and Effect Class 2 for green algae, except for *T. trigonum* for which the Effect Class could be classified as 3 (reduction on day 21 and 28, but this short-term though difficult to interpret because of low abundance, also in controls), can be considered as an overall ETO-RAC and then the appropriate AF would be 2-3.

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

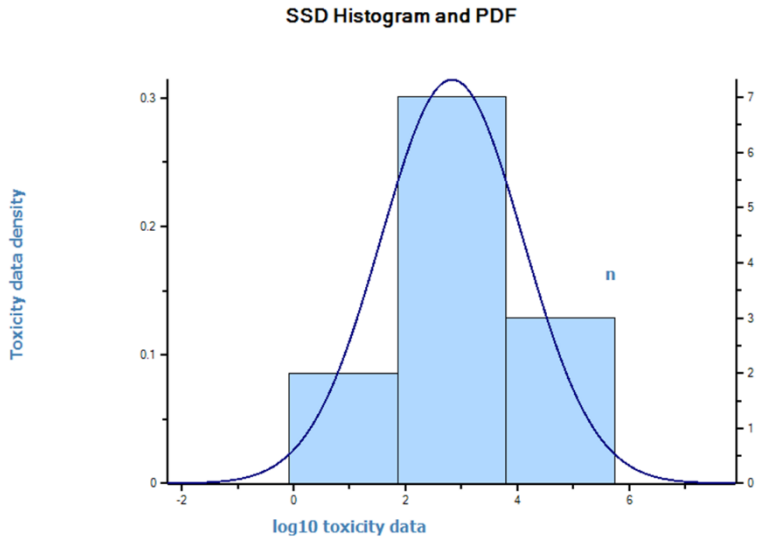
Prosulfocarb sulfoxide

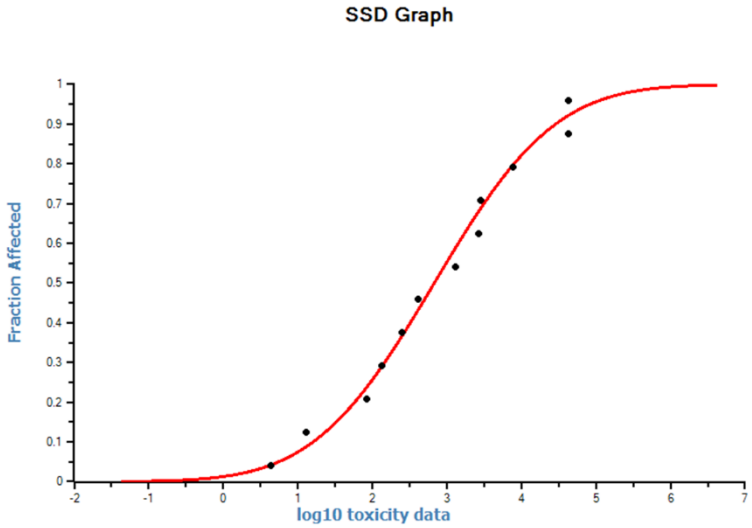
Primary producers – HC_5
A number of studies of the toxicity of the metabolite prosulfocarb sulfoxide to primary producers have been conducted and endpoints are summarised in the table below.

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	*Pseudokirchneriella subcapitata*	E_rC_{50}	4.3 µg/L	DAR
	Desmodesmus subspicatus	E_rC_{50}	85 µg/L	DAR
	Chlorella vulgaris	E_rC_{50}	2860 µg/L	DAR
		E_rC_{50}	1320 µg/L	Juckeland. 2012b
	Chlamydomonas reinhardtii	E_rC_{50}	410 µg/L	DAR
		E_rC_{50}	253.9 µg/L	Juckeland. 2012a
Blue-green algae	*Anabaena flos-aquae*	E_rC_{50}	43000 µg/L	DAR
		E_rC_{50}	42500 µg/L	Juckeland. 2012c
Freshwater diatom	*Navicula pelliculosa*	E_rC_{50}	2700 µg/L	DAR
		E_rC_{50}	7650 µg/L	Juckeland. 2012d
	Skeletonema costatum	E_rC_{50}	134.8 µg/L	Juckeland. 2012e
Monocot macrophyte	*Lemna gibba*	E_rC_{50}	13 µg/L	DAR

Anses calculated a HC_5 of **4.84 µg/L** from a SSD constructed with the 12 previous endpoints. (see below).





The median HC_5 – RAC would be 1.61 µg/L (median HC_5 / 3). Anses finally considered the RAC issued from the cosm study (see Mesocosm study).

Mesocosm study

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

Halauxifen-methyl

No acute toxicity studies were available with the aqueous photolysis metabolites Deg 10, Deg 11 and Deg 14. However, considering that all fall below 10% AR within four hours and that in the toxicity study with aquatic plants (most sensitive species) the time to onset of effects was observed after seven days, the risk from photolysis metabolites is considered addressed by the parent risk assessment (EFSA Journal 2014;12(12):3913). Photolysis was the major, and rapid, route of degradation of halauxifen-methyl in the algae tests, exposure to photolysis metabolites occurred *in situ*; consequently, any toxic contribution of

the photolysis metabolites is reflected in the reported endpoints for halauxifen-methyl. To assess the risk of photolysis metabolites to fish and invertebrates the EFSA Journal 2013;11(7):3290 risk assessment schemes on metabolites was used.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

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

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1817H in winter cereals

Group		**Fish acute**		**Fish prolonged**	**Inverteb. acute**		**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**	**Primary producers**	**Mesocosm**	
Test species		***Oncorhynchus mykiss***		***Oncorhynchus mykiss***	***Daphnia magna***		***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***	***Lemna gibba***		**Higher tier for Daphnia and primary producers**	
Endpoint		LC_{50}	~~Geomean LC_{50}~~	NOEC	EC_{50}	~~Geomean EC_{50}~~	NOEC	E_rC_{50}	NOEC	E_rC_{50}	Geomean E_rC_{50}	$NOEC_{community}$	$NOEC_{community}$
(µg/L)		840	~~1420~~	310	510	~~869.5~~	45	113	1250	690	680	15	15
AF		100	~~100~~	10	100	~~100~~	10	10	10	10	10	2	1
RAC (µg/L)		8.40	~~14.2~~	31.0	5.10	~~8.695~~	4.5	11.3	125.0	69.0	68.0	7.50	15
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**												
Step 1													
	214.66	25.55	~~15.12~~	6.92	42.09	~~24.69~~	47.70	19.00	1.72	3.11	3.16	28.62	14.31
Step 2													
N-Europe	85.11	**10.13**	~~**5.99**~~	**2.75**	**16.69**	~~**9.79**~~	**18.91**	**7.53**	**0.68**	**1.23**	**1.25**	**11.35**	**5.67**
S-Europe	69.51	**8.28**	~~**4.90**~~	**2.24**	**13.63**	~~**7.99**~~	**15.45**	**6.15**	**0.56**	**1.01**	**1.02**	**9.27**	**4.63**
Step 3													
D1/ditch	12.81	**1.525**	~~0.902~~	0.413	**2.512**	~~**1.473**~~	**2.847**	**1.134**	0.102	0.186	0.188	**1.708**	0.854
D1/stream	11.20	**1.333**	~~0.789~~	0.361	**2.196**	~~**1.288**~~	**2.489**	0.991	0.090	0.162	0.165	**1.493**	0.747
D2/ditch	12.74	**1.517**	~~0.897~~	0.411	**2.498**	~~**1.465**~~	**2.831**	**1.127**	0.102	0.185	0.187	**1.699**	0.849
D2/stream	10.34	**1.231**	~~0.728~~	0.334	**2.027**	~~**1.189**~~	**2.298**	0.915	0.083	0.150	0.152	**1.379**	0.689
D3/ditch	12.62	**1.502**	~~0.889~~	0.407	**2.475**	~~**1.451**~~	**2.804**	**1.117**	0.101	0.183	0.186	**1.683**	0.841

Group		Fish acute		Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants	Primary producers	Mesocosm	
D4/pond	0.4369	0.052	~~0.031~~	0.014	0.086	~~0.050~~	0.097	0.039	0.003	0.006	0.006	0.058	0.029
D4/stream	10.95	**1.304**	~~0.771~~	0.353	**2.147**	~~**1.259**~~	**2.433**	0.969	0.088	0.159	0.161	**1.460**	0.730
D5/pond	0.4382	0.052	~~0.031~~	0.014	0.086	~~0.050~~	0.097	0.039	0.004	0.006	0.006	0.058	0.029
D5/stream	11.81	**1.406**	~~0.832~~	0.381	**2.316**	~~**1.358**~~	**2.624**	**1.045**	0.094	0.171	0.174	**1.575**	0.787
D6/ditch	12.77	**1.520**	~~0.899~~	0.412	**2.504**	~~**1.469**~~	**2.838**	**1.130**	0.102	0.185	0.188	**1.703**	0.851
R1/pond	1.217	0.145	~~0.086~~	0.039	0.239	~~0.140~~	0.270	0.108	0.010	0.018	0.018	0.162	0.081
R1/stream	9.514	**1.133**	~~0.670~~	0.307	**1.865**	~~**1.094**~~	**2.114**	0.842	0.076	0.138	0.140	**1.269**	0.634
R3/stream	12.11	**1.442**	~~0.853~~	0.391	**2.375**	~~**1.393**~~	**2.691**	**1.072**	0.097	0.176	0.178	**1.615**	0.807
R4/stream	13.96	**1.662**	~~0.983~~	0.450	**2.737**	~~**1.606**~~	**3.102**	**1.235**	0.112	0.202	0.205	**1.861**	0.931
Step 4 5 m nonspray strip for D scenarios and 10 m nss + 10 m vfs for R scenarios													
D1/ditch	3.563	0.42			0.70		0.79	0.32				0.48	
D1/stream	4.096	0.49			0.80		0.91	0.36				0.55	
D2/ditch	3.452	0.41			0.68		0.77	0.31				0.46	
D2/stream	3.834	0.46			0.75		0.85	0.34				0.51	
D3/ditch	3.419	0.41			0.67		0.76	0.30				0.46	
D4/stream	4.015	0.48			0.79		0.89	0.36				0.54	
D5/stream	4.319	0.51			0.85		0.96	0.38				0.58	
D6/ditch	3.564	0.42			0.70		0.79	0.32				0.48	
R1/stream	4.261	0.51			0.84		0.95	0.38				0.57	
R3/stream	5.454	0.65			1.07		**1.21**	0.48				0.73	
R4/stream	6.302	0.75			1.24		**1.40**	0.56				0.84	

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 sulfoxide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1817H in winter cereals

Group		**Aquatic plants**	**Algae**	**Primary producers**	**Mesocosm**
Test species		***Lemna gibba***	***Anabaena flosaquae***		**Higher tier for primary producers**
Endpoint		E_rC_{50}	E_rC_{50}	HC_5	$NOEC_{community}$
(µg/L)		13	4.3	4.84	30
AF		10	10	3	2
RAC (µg/L)		1.3	0.43	1.61	15
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	51.68	**39.75**	**120.19**	**32.10**	**3.45**
Step 2					
N-Europe	10.11	**7.78**	**23.51**	**6.28**	0.67
S-Europe	8.11	**6.24**	**18.86**	**5.04**	0.54
Step 3					
D1/ditch	46.13	**35.485**	**107.279**	**28.652**	**3.075**
D1/stream	28.92	**22.246**	**67.256**	**17.963**	**1.928**
D2/ditch	68.65	**52.808**	**159.651**	**42.640**	**4.577**
D2/stream	43.39	**33.377**	**100.907**	**26.950**	**2.893**
D3/ditch	< 0.000001	< 0.000001	< 0.000002	< 0.000001	< 0.0000001
D4/pond	2.637	**2.028**	**6.133**	**1.638**	0.176
D4/stream	4.907	**3.775**	**11.412**	**3.048**	0.327

Group		Aquatic plants	Algae	Primary producers	Mesocosm
D5/pond	5.584	**4.295**	**12.986**	**3.468**	0.372
D5/stream	8.462	**6.509**	**19.679**	**5.256**	0.564
D6/ditch	18.23	**14.023**	**42.395**	**11.323**	**1.215**
R1/pond	0.2447	0.188	0.569	0.152	0.016
R1/stream	8.658	**6.660**	**20.135**	**5.378**	0.577
R3/stream	8.076	**6.212**	**18.781**	**5.016**	0.538
R4/stream	9.563	**7.356**	**22.240**	**5.940**	0.638

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

zRMS Comments:	The risk is not acceptable only for scenario D6 ditch. As this scenario is not relevant for Central Zone, no further action is required.

Table 9.5-5: 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 GLOB1817H in winter cereals

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**			**Sed. dwell. prolonged**	**Aquatic plants**	**Group**	**Sed. dwell. prolonged**
Test species		***Cyprinus carpio***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***			***Chironomus riparius***	***Lemna gibba***	**Test species**	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 98.5	NOEC 15	EC_{50} 240	NOEC 52	E_bC_{50} 0.25	E_rC_{50} 0.45	NOEC 0.1	NOEC 100	E_bC_{50} 39	**Endpoint (µg/kg)**	NOEC 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												
	3.12	**3.17**	**2.08**	**1.30**	0.60	**124.80**	**69.33**	**31.20**	0.31	0.80	86.49	0.43
Step 2												
N-Europe	1.44	**1.46**	0.96	0.60	0.28	**57.60**	**32.00**	**14.40**	0.14	0.37	43.63	0.22
S-Europe	1.17	**1.19**	0.78	0.49	0.23	**46.80**	**26.00**	**11.70**	0.12	0.30	35.36	0.18
Step 3												
D1/ditch	0.2699	0.274	0.180	0.112	0.052	**10.796**	**5.998**	**2.699**	0.027	0.069	1.379	0.007
D1/stream	0.2345	0.238	0.156	0.098	0.045	**9.380**	**5.211**	**2.345**	0.023	0.060	0.6183	0.0031
D2/ditch	0.2929	0.297	0.195	0.122	0.056	**11.716**	**6.509**	**2.929**	0.029	0.075	1.301	0.0065
D2/stream	0.2282	0.232	0.152	0.095	0.044	**9.128**	**5.071**	**2.282**	0.023	0.059	0.6593	0.0033
D3/ditch	0.2641	0.268	0.176	0.110	0.051	**10.564**	**5.869**	**2.641**	0.026	0.068	0.1361	0.0007
D4/pond	0.009133	0.009	0.006	0.004	0.002	0.365	0.203	0.0913	0.001	0.002	0.1160	0.0006

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	Group	Sed. dwell. prolonged
D4/stream	0.2291	0.233	0.153	0.095	0.044	**9.164**	**5.091**	**2.291**	0.023	0.059	0.04906	0.0002
D5/pond	0.009170	0.009	0.006	0.004	0.002	0.367	0.204	0.0917	0.001	0.002	0.08208	0.0004
D5/stream	0.2472	0.251	0.165	0.103	0.048	**9.888**	**5.493**	**2.472**	0.025	0.063	0.06892	0.0003
D6/ditch	0.2672	0.271	0.178	0.111	0.051	**10.688**	**5.938**	**2.672**	0.027	0.069	0.6939	0.0035
R1/pond	0.02146	0.022	0.014	0.009	0.004	0.858	0.477	0.2146	0.002	0.006	0.2998	0.0015
R1/stream	0.1742	0.177	0.116	0.073	0.034	**6.968**	**3.871**	**1.742**	0.017	0.045	0.3144	0.0016
R3/stream	0.2444	0.248	0.163	0.102	0.047	**9.776**	**5.431**	**2.444**	0.024	0.063	0.3012	0.0015
R4/stream	0.1728	0.175	0.115	0.072	0.033	**6.912**	**3.840**	**1.728**	0.017	0.044	0.2827	0.0014

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 AE0542991 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals

Group		**Algae**	**Inverteb. acute**
Test species		***Pseudokirchneriella subcapitata***	***Daphnia magna***
Endpoint (µg/L)		E_bC_{50} 36000	EC_5 10000
AF		10	100
RAC (µg/L)		3600	100
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**		
Step 1			
	2.25	0.0006	0.0225

Group		**Algae**	**Inverteb. acute**
Step 2			
N-Europe	1.00	0.003	0.0100
S-Europe	0.80	0.00022	0.0080

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 AE B107137 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals

Group		**Fish acute**	**Algae**	**Aquatic invertebrates**
Test species		***Oncorhynchus mykiss***	***Pseudokirchneriella subcapitata***	***Daphnia magna***
Endpoint		LC_{50}	E_bC_{50}	EC_5
(µg/L)		17300	20400	20400
AF		100	10	100
RAC (µg/L)		173	2040	204
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	5.30	0.306	0.0026	0.0260
Step 2				
N-Europe	2.46	0.0142	0.0012	0.0121
S-Europe	1.99	0.0115	0.00098	0.00975

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for halauxifen-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1817H in winter cereals

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**		**Sed. dwell. prolonged**
Test species		***Cyprinodon variegatus***	***Cyprinodon variegatus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***	***Myriophyllum spicatum***		***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	E_rC_{50}		NOEC
(µg/L)		> 1330	11.5	2120	144	> 245	1260	0.393		89300
AF		100	10	100	10	10	10	10		10
RAC (µg/L)		> 13.3	1.15	21.2	14.4	> 24.5	126	0.0393		8930
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**								**PECsed (µg/kg)**	
Step 1										
	1.00	0.08	0.87	0.05	0.07	0.04	0.01	**25.45**	7.53	<0.01
Step 2										
N-Europe	0.42	0.03	0.37	0.02	0.03	0.02	<0.01	**10.69**	3.37	<0.01
S-Europe	0.34	0.03	0.30	0.02	0.02	0.01	<0.01	**8.65**	2.71	<0.01
Step 3										
D1/ditch	0.03744	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.95	0.06474	<0.01
D1/stream	0.03274	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.83	0.01787	<0.01
D2/ditch	0.03722	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.95	0.04730	<0.01
D2/stream	0.03020	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.77	0.002284	<0.01
D3/ditch	0.03687	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.94	0.01684	<0.01
D4/pond	0.001276	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.002895	<0.01
D4/stream	0.03199	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.81	0.006472	<0.01

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants		Sed. dwell. prolonged
D5/pond	0.001276	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.003294	<0.01
D5/stream	0.03452	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.88	0.009048	<0.01
D6/ditch	0.03731	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.95	0.05389	<0.01
R1/pond	0.003595	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.09	0.01095	<0.01
R1/stream	0.02879	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.73	0.02587	<0.01
R3/stream	0.03644	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.93	0.02316	<0.01
R4/stream	0.04836	<0.01	0.04	<0.01	<0.01	<0.01	<0.01	**1.23**	0.03552	<0.01

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

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for X11393729 (halauxifen) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB1817H in winter cereals

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aqua***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} > 107000	NOEC 11800	EC_{50} > 106000	NOEC > 100000	E_rC_{50} 55000	E_rC_{50} 1.58
AF		100	10	100	10	10	10
RAC (µg/L)		> 1070	1180	> 1060	> 10000	5500	0.158
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	1.11	<0.01	<0.01	<0.01	<0.01	<0.01	**7.0**
Step 2							

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
N-Europe	0.41	<0.01	<0.01	<0.01	<0.01	<0.01	**2.6**
S-Europe	0.33	<0.01	<0.01	<0.01	<0.01	<0.01	**2.1**
Step 3							
D1/ditch	0.1551	<0.01	<0.01	<0.01	<0.01	<0.01	0.98
D1/stream	0.09722	<0.01	<0.01	<0.01	<0.01	<0.01	0.62
D2/ditch	0.1821	<0.01	<0.01	<0.01	<0.01	<0.01	**1.15**
D2/stream	0.1135	<0.01	<0.01	<0.01	<0.01	<0.01	0.72
D3/ditch	0.00150	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
D4/pond	0.0137	<0.01	<0.01	<0.01	<0.01	<0.01	0.09
D4/stream	0.02333	<0.01	<0.01	<0.01	<0.01	<0.01	0.15
D5/pond	0.01493	<0.01	<0.01	<0.01	<0.01	<0.01	0.09
D5/stream	0.02373	<0.01	<0.01	<0.01	<0.01	<0.01	0.15
D6/ditch	0.05376	<0.01	<0.01	<0.01	<0.01	<0.01	0.34
R1/pond	0.000706	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R1/stream	0.02153	<0.01	<0.01	<0.01	<0.01	<0.01	0.14
R3/stream	0.02194	<0.01	<0.01	<0.01	<0.01	<0.01	0.14
R4/stream	0.03723	<0.01	<0.01	<0.01	<0.01	<0.01	0.24

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

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for X11449757 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Myriophyllum spicatum***

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Endpoint (µg/L)		LC_{50} > 120000	NOEC 8900	EC_{50} > 120000	E_rC_{50} > 15800	E_rC_{50} > 100
AF		100	10	100	10	10
RAC (µg/L)		> 1200	890	> 1200	> 1580	> 10
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	1.53	<0.01	<0.01	<0.01	<0.01	0.15
Step 2						
N-Europe	0.42	<0.01	<0.01	<0.01	<0.01	0.04
S-Europe	0.37	<0.01	<0.01	<0.01	<0.01	0.03

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

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for X11406790 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} > 30000	EC_{50} > 30000	E_rC_{50} 5700	E_rC_{50} > 100
AF		100	100	10	10
RAC (µg/L)		> 300	> 300	570	> 10

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
	0.67	<0.01	<0.01	<0.01	0.06
Step 2					
N-Europe	0.16	<0.01	<0.01	<0.01	0.01
S-Europe	0.12	<0.01	<0.01	<0.01	0.01

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 the photoproducts of halauxifen-methyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals

Group		Fish acute	Inverteb. acute
Test species		***Oncorhynchus mykiss***	***Daphnia magna***
Endpoint (µg/L)		LC_{50} > 1330	EC_{50} > 2120
AF		100	100
RAC (µg/L)		> 13.3	> 21.2
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**		
Step 1			
Deg 10	0.12	0.09	0.06
Deg 11	0.12	0.09	0.06
Deg 14	0.08	0.06	0.04

Group		Fish acute	Inverteb. acute
Step 2			
N-Europe			
Deg 10	0.06	0.05	0.03
Deg 11	0.06	0.05	0.03
Deg 14	0.03	0.02	0.01
S-Europe			
Deg 10	0.04	0.03	0.02
Deg 11	0.04	0.03	0.02
Deg 14	0.02	0.02	0.01

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 cloquintocet-mexyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals**~~

~~Group~~		~~Fish acute~~	~~Fish prolonged~~	~~Inverteb. acute~~	~~Inverteb. prolonged~~	~~Algae~~	~~Sed. dwell. prolonged~~	~~Aquatic plants~~
~~Test species~~		~~*Onchorynchus mykiss*~~	~~*Onchorynchus mykiss*~~	~~*Daphnia magna*~~	~~*Daphnia magna*~~	~~*Scenedesmus subspicatus*~~	~~*Chironomus riparius*~~	~~*Lemna gibba*~~
~~Endpoint~~		~~LC_{50}~~	~~NOEC~~	~~EC_{50}~~	~~NOEC~~	~~E_rC_{50}~~	~~NOEC~~	~~E_rC_{50}~~
~~(µg/L)~~		~~> 970~~	~~≥ 1260~~	~~> 820~~	~~≥ 9700~~	~~630~~	~~≥ 8000~~	~~> 420~~
~~AF~~		~~100~~	~~10~~	~~100~~	~~10~~	~~10~~	~~10~~	~~10~~
~~RAC (µg/L)~~		~~> 9.7~~	~~≥ 126~~	~~> 8.2~~	~~≥ 970~~	~~63~~	~~≥ 800~~	~~> 42~~
~~FOCUS Scenario~~	~~PEC_{gl-max} (µg/L)~~							
~~Step 1~~								

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic plants
-	0.517	0.053	< 0.01	0.063	< 0.01	< 0.01	< 0.01	0.012
Step 2								
N-Europe	0.172	0.018	< 0.01	0.021	< 0.01	< 0.01	< 0.01	< 0.01
S-Europe	0.172	0.018	< 0.01	0.021	< 0.01	< 0.01	< 0.01	< 0.01

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 CGA 153433 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB1817H in winter cereals

Group		Fish acute	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		*Onchorynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Microcystis aeruginosa*	*Lemna gibba*
Endpoint		LC_{50}	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		89000	> 9700	≥ 100000	> 1900	> 11000
AF		100	100	10	10	10
RAC (µg/L)		890	> 97	≥ 10000	190	> 1100
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)					
Step 1						
-	0.577	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Step 2						
N-Europe	0.203	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
S-Europe	0.169	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

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 GLOB1817H

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 (2013[1])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 GLOB1817H, 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.
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)

The LD_{50} 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 ($ECx_{mix\text{-}CA}$ or $NOEC_{mix\text{-}CA}$), 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 i^{th} component as a relative fraction of the mixture composition (note: Σp_i must be 1)
ECx_i:	concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

[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.

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.

Table 9.5-15: **Comparison of the toxicity of GLOB1817H to the predicted one based on the active ingredients**

Aquatic organisms	Fraction of Prosulfocarb in mixture	Fraction of Diflufenican in mixture	Fraction of Halauxifen-methyl in mixture	Fraction of Cloquintocet-mexyl in mixture	Prosulfocarb EC_{50} (mg a.s./L)	Diflufenican EC_{50} (mg a.s./L)	Halauxifen-methyl EC_{50} (mg a.s./L)	Cloquintocet-mexyl EC_{50} (mg a.s./L)	$ECx_{mix\ CA.}$ Predicted EC_{50} of GLOB1817H based on the a.s. toxicity (mg a.s./L)	$ECx_{ppp.}$ EC_{50} of GLOB1817H from the studies (mg product/L)	$ECx_{ppp.}$ 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	0.9756	0.0205	0.0019	0.0019	0.510	0.240	2.12	0.820	0.4996	0.954	0.6467	0.77	MDR= 0.2-5
Algae	0.9756	0.0205	0.0019	0.0019	0.113	0.00025	0.245	0.630	0.0110	0.0597*	0.0405	0.27	MDR= 0.2-5
Lemna	0.9756	0.0205	0.0019	0.0019	0.690	0.039	2.13	0.420	0.5142	0.3352	0.2272	2.26	MDR= 0.2-5

*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, the nominal endpoint is still reflecting the toxicity of the formulation even with the low recovery of halauxifen-methyl in the spent solutions.

Table 9.5-16a: **Comparison of the toxicity of GLOB1817H to the predicted one based on the active substances**

Aquatic organisms	Fraction of Prosulfocarb in mixture	Fraction of Diflufenican in mixture	Fraction of Halauxifen-methyl in mixture	Prosulfocarb EC_{50} (mg a.s./L)	Diflufenican EC_{50} (mg a.s./L)	Halauxifen-methyl EC_{50} (mg a.s./L)	$ECx_{mix-CA.}$ Predicted EC_{50} of GLOB1817H based on the a.s. toxicity (mg a.s./L)	$ECx_{ppp.}$ EC_{50} of GLOB1817H from the studies (mg product/L)	$ECx_{ppp.}$ EC_{50} of GLOB1817H from the studies (mg sum of a.s./L)	MDR (model deviation ratio) (MDR = ECx mix-CA/ECx PPP)	Comparison toxicity of the formulation and the predicted one
Daphnia	0.9775	0.0205	0.0020	0.510	0.240	2.12	0.4992	0.954	0.646	0.77	MDR= 0.2-5
Algae	0.9775	0.0205	0.0020	0.113	0.00025	0.245	0.0110	0.0597	0.0405	0.27	MDR= 0.2-5
Lemna	0.9775	0.0205	0.0020	0.690	0.039	2.13	0.5145	0.3352	0.2272	2.26	MDR= 0.2-5

The predicted toxicity endpoint has been compared to the formulated product endpoint to derive a MDR by the formula (MDR = ECx_{mix-CA} /ECx_{ppp}). If MDR is between 0.2 and 5, the observed and calculated 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 GLOB1817H 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 GLOB1817H 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 (ECxPPP) 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 PECi/PECmix. PECmix is simply the sum the each PECi.
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).

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.99
Step 2	
N-Europe	0.99
S-Europe	0.99
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	0.72
Step 2	
N-Europe	0.83
S-Europe	0.82
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	0.89
R2 Stream	
R3 Stream	0.97
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

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 2 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. ci) 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 (TOXsum(ai)) = 1/(TU(ai1) + TU(ai2)+TU(ai3))+TU(ai4))
Contribution to toxicity = ~~TOXsum(ai) * TU(ai) * 100~~ [TOXsum(ai) / TU(ai)] * 100

~~Table 9.5-17: Contribution to toxicity of GLOB1817H by prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl~~

~~Organism~~	~~Active substance~~	~~EC_{50} (mg/L)~~	~~Fraction in mixture~~	~~Toxic unit~~	~~Tox of the sum ai~~	~~Contribution to toxicity (%)~~
~~Daphnia~~	~~Prosulfocarb~~	~~0.510~~	~~0.9756~~	~~0.5227~~		~~95.57~~
	~~Diflufenican~~	~~0.240~~	~~0.0205~~	~~11.7199~~	~~0.4996~~	~~4.26~~
	~~Halauxifen-methyl~~	~~2.12~~	~~0.0019~~	~~1089.7438~~		~~0.05~~
	~~Cloquintocet-mexyl~~	~~0.820~~	~~0.0019~~	~~421.5047~~		~~0.12~~
~~Algae~~	~~Prosulfocarb~~	~~0.113~~	~~0.9756~~	~~0.1158~~		~~9.53~~
	~~Diflufenican~~	~~0.00025~~	~~0.0205~~	~~0.0122~~	~~0.0110~~	~~90.45~~
	~~Halauxifen-methyl~~	~~0.245~~	~~0.0019~~	~~125.9374~~		~~0.01~~
	~~Cloquintocet-mexyl~~	~~0.630~~	~~0.0019~~	~~323.8389~~		~~0.003~~
~~Lemna~~	~~Prosulfocarb~~	~~0.690~~	~~0.9756~~	~~0.7072~~		~~72.71~~
	~~Diflufenican~~	~~0.039~~	~~0.0205~~	~~1.9045~~	~~0.5142~~	~~27.00~~
	~~Halauxifen-methyl~~	~~2.13~~	~~0.0019~~	~~1094.8841~~		~~0.05~~
	~~Cloquintocet-mexyl~~	~~0.420~~	~~0.0019~~	~~215.8926~~		~~0.24~~

Table 9.5-18a: Contribution to toxicity of GLOB1817H by prosulfocarb, diflufenican, halauxifen-methyl

Organism	Active substance	EC_{50} (mg/L)	Fraction in mixture	Toxic unit	Tox of the sum ai	Contribution to toxicity (%)
Daphnia	Prosulfocarb	0.510	0.9775	0.5217	0.4992	95.69
	Diflufenican	0.240	0.0205	11.6971		4.27
	Halauxifen-methyl	2.12	0.0020	1087.6		0.05
Algae	Prosulfocarb	0.113	0.9775	0.1156	0.0110	9.52
	Diflufenican	0.00025	0.0205	0.0122		90.16
	Halauxifen-methyl	0.245	0.0020	125.7		0.01
Lemna	Prosulfocarb	0.690	0.9775	0.7058	0.5145	72.90
	Diflufenican	0.039	0.0205	1.9008		27.07
	Halauxifen-methyl	2.13	0.0020	1092.8		0.05

For Daphnia the toxicity is driven by prosulfocarb (contribution ≥90%). Therefore, in accordance with the EFSA guidance document[1] the risk assessment for Daphnia can be based on single-substance toxicity data (ECxa.s.) 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 ≥90%). Therefore, in accordance with the EFSA guidance document[1] the risk assessment for Daphnia can be based on single-substance toxicity data (ECxa.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 RQmix (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 PECmix.

Refined risk assessment for Lemna using RQmix (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 the other active substances, the Tier 1 endpoints are used leading to a RAC of 3.9 µg/L for diflufenican, 227 µg/L for halauxifen-methyl and 42 µg/L for cloquintocet-mexyl.

When considering the PECsw obtained in STEP 2, the RQmix is above 1 and the risk is not considered acceptable:

RQmix = (85.11/15) + (1.44/3.9) + (0.42/227) + ~~(0.172/42)~~ = 6.05

However the calculation can be refined using the highest PECsw value obtained in STEP 3 for prosulfocarb, diflufenican and halauxifen-methyl. Values obtained in FOCUS scenario D2 are excluded since the risk remains unresolved in this scenario for the active substance diflufenican. For cloquintocet-mexyl, the PECsw from STEP 2 is re-used since for this active substance the risk was shown to be acceptable at STEP 2 and no further PECsw calculations were performed. The RQmix is 1 and the risk can be considered acceptable, especially when taking into account that the calculation could be refined even further by using the PECsw values obtained in STEP 4 for prosulfocarb, diflufenican and halauxifen-methyl. 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.

RQmix = (13.96/15) + (0.2699/3.9) + (0.04836/227) + ~~(0.172/42)~~ = ~~1.0~~0 0.996

Risk assessment for Myriophyllum

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

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1817H for each organism group based on PECmix calculations for the use in winter cereals – Step 1-2-3

FOCUS scenario	PECsw				PECmix	PEC/RAC
	Prosulfocarb	Diflufenican	Halauxifen-methyl	~~Cloquintocet-mexyl~~		
Step 1	214.6	3.12	1	~~0.517~~	~~219.237~~	~~**29.23**~~
Step 2						
N-Europe	85.11	1.44	0.42	~~0.172~~	~~87.142~~	~~**11.62**~~
S-Europe	69.51	1.17	0.34	~~0.172~~	~~71.192~~	~~**9.49**~~
Step 3						
D1 Ditch	12.81	0.2699	0.03753	~~Step 2 PECsw value is re-used to calculate PECmix since the risk for cloquintocet-mexyl was already acceptable at Step 2 and no further calculations were performed.~~	~~13.28943~~	~~**1.77**~~
D1 Stream	11.2	0.2345	0.03261		~~11.63911~~	~~**1.55**~~
D2 Ditch	12.74	0.2929	0.03731		~~13.24221~~	~~**1.77**~~
D2 Stream	10.34	0.2282	0.03009		~~10.77029~~	~~**1.44**~~
D3 Ditch	12.62	0.2641	0.03695		~~13.09305~~	~~**1.75**~~
D4 Pond	0.4369	0.009133	0.001294		~~0.619327~~	~~0.08~~
D4 Stream	10.95	0.2291	0.03187		~~11.38297~~	~~**1.52**~~
D5 Pond	0.4382	0.00917	0.001294		~~0.620664~~	~~0.08~~
D5 Stream	11.81	0.2472	0.03438		~~12.26358~~	~~**1.64**~~
D6 Ditch	12.77	0.2672	0.0374		~~13.2466~~	~~**1.77**~~
R1 Pond	1.217	0.02146	0.003705		~~1.414165~~	~~0.19~~
R1 Stream	9.514	0.1742	0.02918		~~9.88938~~	~~**1.32**~~
R3 Stream	12.11	0.2444	0.03558		~~12.56198~~	~~**1.67**~~
R4 Stream	13.96	0.1728	0.04474		~~14.34954~~	~~**1.91**~~

As can be seen from the table above, the risk is not acceptable in all scenarios. Therefore, the PECsw 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 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1817H for each organism group based on PECmix calculations for the use in winter cereals – Step 4

FOCUS scenario	PECsw				PECmix	PEC/RAC
	Prosulfocarb	Diflufenican	Halauxifen-methyl	Cloquintocet-mexyl		
D1 Ditch	3.563	0.07638	0.03753	Step 2 PECsw value is re-used to calculate PECmix since the risk for cloquintocet-mexyl was already acceptable at Step 2 and no further calculations were performed.	3.84891	0.51
D1 Stream	4.096	0.08565	0.03261		4.38626	0.58
D2 Ditch	3.452	0.1445	0.03731		3.80581	0.51
D2 Stream	3.834	0.09124	0.03009		4.12733	0.55
D3 Ditch	3.419	0.07155	0.03695		3.6995	0.49
D4 Stream	4.015	0.08367	0.03187		4.30254	0.57
D5 Stream	4.319	0.09028	0.03438		4.61566	0.62
D6 Ditch	3.564	0.1006	0.0374		3.874	0.52
R1 Stream	4.261	0.02611	0.02918		4.48829	0.60
R3 Stream	5.454	0.02697	0.03558		5.68855	0.76
R4 Stream	6.302	0.03833	0.04474		6.55707	0.87

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 LC_{50} is used, leading to a RAC of 14.2 µg/L. For the other active substances, the Tier 1 endpoints are used leading to a RAC of 0.985 µg/L for diflufenican, 13.3 µg/L for halauxifen-methyl and 9.7 µg/L for cloquintocet-mexyl.

When considering the PECsw obtained in STEP 2, the RQmix is above 1 and the risk is not considered acceptable:

RQmix = (85.11/14.2) + (1.44/0.985) + (0.42/13.3) + ~~(0.172/9.7)~~ = ~~7.5~~ 7.48

However the calculation can be refined using the highest PECsw value obtained in STEP 3 for prosulfocarb, diflufenican and halauxifen-methyl. Values obtained in FOCUS scenario D2 are excluded since the risk remains unresolved in this scenario for the active substance diflufenican. For cloquintocet-mexyl, the PECsw from STEP 2 is re-used since for this active substance the risk was shown to be acceptable at STEP 2 and no further PECsw calculations were performed.

RQmix = (13.96/14.2) + (0.2699/0.985) + (0.04836/13.3) + ~~(0.172/9.7)~~ = ~~1.18~~ 1.16

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) For halauxifen-methyl, STEP 3 PECsw values are re-used. For cloquintocet-mexyl, the PECsw from STEP 2 is again re-used.

Table 9.5-21: Aquatic organisms: acceptability of risk for GLOB1817H for each organism group based on RQmix calculations for the use in winter cereals – Step 4

FOCUS scenario	PECsw				RQmix
	Prosulfocarb	Diflufenican	Halauxifen-methyl	Cloquintocet-mexyl	
D1 Ditch	3.563	0.07638	0.03753	0.172	0.3490
D1 Stream	4.096	0.08565	0.03261		0.3956
D2 Ditch	3.452	0.1445	0.03731		0.4103
D2 Stream	3.834	0.09124	0.03009		0.3826
D3 Ditch	3.419	0.07155	0.03695		0.3339
D4 Stream	4.015	0.08367	0.03187		0.3878
D5 Stream	4.319	0.09028	0.03438		0.4161
D6 Ditch	3.564	0.1006	0.0374		0.3737
R1 Stream	4.261	0.02611	0.02918		0.3465
R3 Stream	5.454	0.02697	0.03558		0.4319
R4 Stream	6.302	0.03833	0.04474		0.5038

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 GLOB1817H 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 8.9-29 in dRR Part B8). The PEC/RAC ratios are shown in the table below.

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1817H for each organism group based on FOCUS Drift Swash Tool calculations for the use in winter cereals

Group		**Aquatic plants**	
Test species		***Lemna gibba***	***Myriophyllum spicatum***
Endpoint (µg/L)		EC_{50} 335.2	EC_{50} 75
AF		10	10
RAC (µg/L)		33.52	7.5
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**		
1 m			
	23.3292	0.696	**3.111**
5 m			
	5.2696	-	0.703
	6.32		0.843

For the intended use, calculated PEC/RAC ratios for prosulfocarb sulfoxide did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for primary producers as characterised by a $NOEC_{community}$ of 30 µg/L in connection with an assessment factor of 2) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on Tier 2 PECsw calculations for prosulfocarb sulfoxide (please refer to Table 8.9-9 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 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfocarb sulfoxide for each organism group based on Tier 2 calculations for the use of GLOB1817H in winter cereals

Group		**Aquatic plants**	**Algae**	**Primary producers**	**Mesocosm**
Test species		***Lemna gibba***	***Anabaena flosaquae***		**Higher tier for primary producers**
Endpoint (µg/L)		E_rC_{50} 13	E_rC_{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)**				
D1/ditch	7.483	**5.76**	**17.40**	**4.65**	0.50
D1/stream	4.917	**3.78**	**11.43**	**3.05**	0.33
D2/ditch	6.194	**4.76**	**14.40**	**3.85**	0.41
D2/stream	4.004	**3.080**	**9.312**	**2.487**	0.267
D3/ditch	< 0.000001	> 0.000001	> 0.000002	> 0.000001	> 0.0000001
D4/pond	0.2016	0.155	0.469	0.125	0.013
D4/stream	0.3594	0.276	0.836	0.223	0.024
D5/pond	0.03400	0.0262	0.0791	0.0211	0.0023
D5/stream	0.2090	0.161	0.486	0.130	0.014
D6/ditch	0.7003	0.539	**1.629**	0.435	0.047
R1/pond	0.000987	0.001	0.002	0.001	0.000
R1/stream	0.8509	0.655	**1.979**	0.529	0.057
R3/stream	7.155	**5.504**	**16.640**	**4.444**	0.477
R4/stream	1.443	**1.110**	**3.356**	0.896	0.096

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 EC_{50} for *Scenedesmus subspicatus* of 0.45 µg/L or 0.1 µg/L in connection with an assessment factor of 10) 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 9.5-24: 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 GLOB1817H in winter cereal

Intended use		Winter cereals				
Active substance		Diflufenican				
Application rate (g/ha)		1 × 42				
Nozzle reduction	**No-spray buffer (m)**	5	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	D1 ditch	0.07438	0.06633	0.06633	-	-
50 %		0.06633	0.06633	-	-	-
75 %		0.06633	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	0.08565	0.04541	0.04177	-	-
50 %		0.04284	0.04177	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D2 ditch	0.1445	0.1445	0.1445	-	-
50 %		0.1445	0.1445	-	-	-
75 %		0.1445	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	0.09124	0.09124	0.09124	-	-
50 %		0.09124	0.09124	-	-	-
75 %		0.09124	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	0.07155	0.03795	-	-	-
50 %		0.03576	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.08367	0.04435	-	-	-
50 %		0.04184	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	0.09028	0.04785	0.02486	-	-
50 %		0.04514	0.02392	-	-	-
75 %		0.02256	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	0.1006	0.1006	0.1006	-	-
50 %		0.1006	0.1006	-	-	-

75 %		0.1006	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	0.1120	0.1120	0.1120	0.05014	0.02611
50 %		0.1120	0.1120	-	-	-
75 %		0.1120	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	0.1146	0.1146	0.1146	0.05160	0.02697
50 %		0.1146	0.1146	-	-	-
75 %		0.1146	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	0.1627	0.1627	0.1627	0.07341	0.03833
50 %		0.1627	0.1627	-	-	-
75 %		0.1627	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L) 0.045		**PEC/RAC ratio**				
None	D1 ditch	**1.653**	**1.474**	**1.474**	-	-
50 %		**1.474**	**1.474**	-	-	-
75 %		**1.474**	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	**1.903**	**1.009**	0.928	-	-
50 %		0.952	0.928	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D2 ditch	**3.211**	**3.211**	**3.211**	-	-
50 %		**3.211**	**3.211**	-	-	-
75 %		**3.211**	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	**2.028**	**2.028**	**2.028**	-	-
50 %		**2.028**	**2.028**	-	-	-
75 %		**2.028**	-	-	-	-
90 %		-	-	-	-	-
None	D3 ditch	**1.590**	0.843	-	-	-
50 %		0.795	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	**1.859**	0.986	-	-	-
50 %		0.930	-	-	-	-
75 %		-	-	-	-	-

90 %		-	-	-	-	-
None	D5 stream	**2.006**	**1.063**	0.552	-	-
50 %		**1.003**	0.532	-	-	-
75 %		0.501	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	**2.236**	**2.236**	**2.236**	-	-
50 %		**2.236**	**2.236**	-	-	-
75 %		**2.236**	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	**2.489**	**2.489**	**2.489**	**1.114**	0.580
50 %		**2.489**	**2.489**	-	-	-
75 %		**2.489**	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	**2.547**	**2.547**	**2.547**	**1.147**	0.599
50 %		**2.547**	**2.547**	-	-	-
75 %		**2.547**	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	**3.616**	**3.616**	**3.616**	**1.631**	0.852
50 %		**3.616**	**3.616**	-	-	-
75 %		**3.616**	-	-	-	-
90 %		-	-	-	-	-
RAC (µg/L) 0.1		**PEC/RAC ratio**				
None	D1 ditch	0.744	0.663	0.663	-	-
50 %		0.663	0.663	-	-	-
75 %		0.663	-	-	-	-
90 %		-	-	-	-	-
None	D1 stream	0.857	0.454	0.418	-	-
50 %		0.428	0.418	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D2 ditch	**1.445**	**1.445**	**1.445**	-	-
50 %		**1.445**	**1.445**	-	-	-
75 %		**1.445**	-	-	-	-
90 %		-	-	-	-	-
None	D2 stream	0.912	0.912	0.912	-	-
50 %		0.912	0.912	-	-	-
75 %		0.912	-	-	-	-
90 %		-	-	-	-	-

None	D3 ditch	0.716	0.380	-	-	-
50 %		0.358	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D4 stream	0.837	0.444	-	-	-
50 %		0.418	-	-	-	-
75 %		-	-	-	-	-
90 %		-	-	-	-	-
None	D5 stream	0.903	0.479	0.249	-	-
50 %		0.451	0.239	-	-	-
75 %		0.226	-	-	-	-
90 %		-	-	-	-	-
None	D6 ditch	**1.006**	**1.006**	**1.006**	-	-
50 %		**1.006**	**1.006**	-	-	-
75 %		**1.006**	-	-	-	-
90 %		-	-	-	-	-
None	R1 stream	**1.120**	**1.120**	**1.120**	0.501	0.261
50 %		**1.120**	**1.120**	-	-	-
75 %		**1.120**	-	-	-	-
90 %		-	-	-	-	-
None	R3 stream	**1.146**	**1.146**	**1.146**	0.516	0.270
50 %		**1.146**	**1.146**	-	-	-
75 %		**1.146**	-	-	-	-
90 %		-	-	-	-	-
None	R4 stream	**1.627**	**1.627**	**1.627**	0.734	0.383
50 %		**1.627**	**1.627**	-	-	-
75 %		**1.627**	-	-	-	-
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 PECsw below 0.1 µg/L automatically fulfil these conditions. From table 9.5-5, it can be concluded that the risk is acceptable in STEP 3 for the following scenario's:

- D4 pond
- D5 pond
- R1 pond

From table 9.5-19, it can be concluded that the risk is acceptable using a 5 m no spray buffer zone in the following scenarios, since the maximum PECsw is below 0.1 µg/L:

- D1 ditch
- D1 stream
- D2 stream
- D3 ditch
- D4 stream
- D5 stream

~~For the remaining scenarios, the FOCUS profiles were analysed with EPAT v1.2 (only in case the maximum PECsw was below 0.42 µg/L) in order to check if they fulfil the conditions specified above. From table 9.5-20 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:~~

- ~~D6 ditch~~
- ~~R1 stream~~
- ~~R3 stream~~

~~For the scenarios D2 ditch and R4 stream, the interval between the peaks was less then 3 days when using a 5 m no spray bufferzone, and thus the risk is not considered acceptable.~~

~~Table 9.5-25: Analysis of FOCUS profiles with a maximum PECsw above 0.1 µg/L but below 0.42 µg/L using EPAT v1.2~~

~~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 (days)~~
~~STEP 4 5m~~	~~D2 ditch~~	~~1~~	~~0.117~~	~~-~~	~~0.167~~	~~3.75~~
		~~2~~	~~0.108~~	~~5.833~~	~~0.084~~	
		~~3~~	~~0.108~~	~~**0.916**~~	~~0.084~~	
		~~4~~	~~0.115~~	~~**0.875**~~	~~0.166~~	
		~~5~~	~~0.113~~	~~18.875~~	~~0.167~~	
		~~6~~	~~0.101~~	~~34.917~~	~~0.041~~	
		~~7~~	~~0.106~~	~~18.917~~	~~0.083~~	
		~~8~~	~~0.114~~	~~3.917~~	~~0.167~~	
		~~9~~	~~0.108~~	~~17.833~~	~~0.083~~	
		~~10~~	~~0.106~~	~~**0.875**~~	~~0.125~~	
		~~11~~	~~0.111~~	~~3.917~~	~~0.083~~	
		~~12~~	~~0.115~~	~~25~~	~~0.334~~	

		13	0.104	4.625	0.041	
		14	0.102	164.959	0.041	
		15	0.110	13.875	0.167	
		16	0.121	5.833	0.209	
		17	0.118	1.791	0.167	
		18	0.103	2.833	0.084	
		19	0.104	16.916	0.084	
		20	0.119	2.916	0.167	
		21	0.123	15.833	0.209	
		22	0.105	64.833	0.042	
		23	0.117	**0.916**	0.125	
		24	0.131	18.917	0.25	
		25	0.145	8.708	0.375	
		26	0.133	2.625	0.209	
	D6 ditch	1	0.101	-	0.042	0.042
	R1 stream	1	0.112	-	0.292	0.292
	R3 stream	1	0.115	-	0.375	0.709
		2	0.111	9.625	0.334	
	R4 stream	1	0.163	-	0.541	1.583
		2	0.152	**0.5**	0.417	
		3	0.114	87.542	0.625	

For the D2 ditch scenario, the FOCUS profile does not change by increasing the mitigation measures and thus the risk remains unresolved.
For the R4 stream scenario, the risk is acceptable using a 10 m no spray buffer zone including a 10 m vegetated buffer strip since the maximum PECsw is below 0.1 µg/L using these mitigation measures as can be seen from table 9.5-14.

For the intended use, calculated PEC/RAC ratios for halauxifen-methyl did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants) as characterised by an EC_{50} for *Myriophyllum spicatum* of 0.393 µg/L in connection with an assessment factor of 10) 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 9.5-26: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for halauxifen-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of GLOB1817H in winter cereal

Intended use		Winter cereals
Active substance		Halauxifen-methyl
Application rate (g/ha)		1 × 5.85
Nozzle reduction	**No-spray buffer (m)**	10
	Vegetated filter strip (m)	10
None	R4 stream	0.02183
RAC (µg/L) 0.0393		**PEC/RAC ratio**
None	R4 stream	0.56

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

9.5.3 Overall conclusions

An acceptable risk is concluded for prosulfocarb and its metabolite prosulfocarb sulfoxide at Step 3.

An acceptable risk is concluded for diflufenican using a 5 m no spray buffer zone, except in the D2 and R4 scenario. For an acceptable risk in the R4 scenario, a 10 m no spray buffer zone including a 10 m vegetated buffer strip is required. The risk in the D2 scenario remains unresolved, but it represents <1% of the drained cereal growing land in Europe and it is mainly located in areas of the UK. The D2 scenario is therefore of very limited relevance in Member States and, if relevant, can be addressed with a label restriction for heavy clay soils.
The risk for the metabolites of diflufenican is acceptable at Step 1-2.

An acceptable risk is concluded for halauxifen-methyl at Step 3, excluding the R4 scenario. For an acceptable risk in the R4 scenario, a 10 m no spray buffer zone including a 10 m vegetated buffer strip is required.
An acceptable risk is concluded for halauxifen acid at Step 3, excluding D2 ditch scenario. The D2 scenario represents <1% of the drained cereal growing land in Europe and it is mainly located in areas of the UK. The D2 scenario is therefore of very limited relevance in Member States and, if relevant, can be addressed with a label restriction for heavy clay soils.
The risk for the other halauxifen-methyl metabolites was acceptable at Step 1-2.

An acceptable risk is concluded for cloquintocet-mexyl and its metabolites at Step 1-2.

An acceptable risk for the formulation GLOB1817H 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**	**Mitigation measure**
Poland	D3, D4, R1	~~5 m no spray buffer zone~~ 10 m no spray buffer zone including a 10 m vegetated buffer strip
Czech Republic	D4, R1	5 m no spray buffer zone
Germany	Reference is made to the national addendum	

9.6 Effects on bees (KCP 10.3.1)

zRMS Comments:	The submitted risk assessment is based on SANCO guidance (2002). The EU agreed endpoints for active substances were used in risk assessment. The cloquintocet-mexyl is used as a safener and was not evaluated in this dossier. New studies for acute and chronic toxicity were submitted and accepted. The acute risk assessment performed in accordance with the SANCO guidance presented by the Applicant was accepted.

	The chronic risk assessment was also performed in accordance with EPPO scheme (2010) and accepted. The risk assessment performed in accordance with EFSA guidance (2013) was also submitted but not evaluated (marked in gray) as this guidance has not been agreed yet. Its relevance will be decided at the Member State level. The hazard quotients are below the trigger value, indicating that the active substance and formulation pose an acceptable acute and chronic risk to bees. Therefore, an acceptable risk to bees is expected from the application of GLOB1817H in accordance with proposed use pattern.

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with prosulfocarb, diflufenican, halauxifen-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of formulation were not evaluated as part of the EU assessment of prosulfocarb, diflufenican and halauxifen-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Prosulfocarb	Oral, acute	LD_{50} > 103.4 µg/bee	EFSA, 2007
Apis mellifera	Prosulfocarb	Contact, acute	LD_{50} = 79.3 µg/bee	EFSA, 2007
Apis mellifera	Diflufenican	Oral, acute	LD_{50} > 112.3 µg/bee	EFSA, 2007
Apis mellifera	Diflufenican	Contact, acute	LD_{50} > 100 µg/bee	EFSA, 2007
~~*Apis mellifera*~~	~~Diflufenican~~	~~Adult, chronic~~	~~NOEDD = 24.13 µg/bee/d~~ ~~LDD_{50} > 24.13 µg/bee/d~~	~~Ansaloni T., 2016a~~
~~*Apis mellifera*~~	~~Diflufenican~~	~~Larvae, chronic~~	~~NOED = 85.184 µg/larva~~	~~Ansaloni T., 2016b~~
Apis mellifera	Halauxifen-methyl	Oral, acute	LD_{50} > 108 µg/bee	EFSA, 2014
Apis mellifera	Halauxifen-methyl	Contact, acute	LD_{50} > 98.1 µg/bee	EFSA, 2014
~~*Apis mellifera*~~	~~Halauxifen-methyl~~	~~Adult, chronic~~	~~NOEDD ≥ 5.07 µg/bee/day~~	~~Oberrauch, 2018a~~
~~*Apis mellifera*~~	~~Halauxifen-methyl~~	~~Larvae, chronic~~	~~NOED ≥ 23.1 µg/larva~~	~~Oberrauch, 2018b~~

Species	Substance	Exposure System	Results	Reference
~~*Apis mellifera*~~	~~Cloquintocet-mexyl~~	~~Oral, acute~~	~~LD_{50} > 100 µg/bee~~	~~Safener, not reviewed on EU level~~
~~*Apis mellifera*~~	~~Cloquintocet-mexyl~~	~~Contact, acute~~	~~LD_{50} > 100 µg/bee~~	~~Safener, not reviewed on EU level~~
Apis mellifera	GLOB1817H	Oral, acute, 48 h	LD_{50} = 310 µg/bee	XXXX, 2020
Apis mellifera	GLOB1817H	Contact, acute, 96 h	LD_{50} = 444 µg/bee	XXXX, 2020
Bombus terrestris	GLOB1817H	Oral, acute, 48 h	LD_{50} > 563.8 µg/bee NOED ≥ 563.8 µg/bee	XXXX, 2021
Bombus terrestris	GLOB1817H	Contact, acute, 48 h	LD_{50} > 590 µg/bee NOED ≥ 590 µg/bee	XXXX, 2021
Apis mellifera	GLOB1817H	Adult, chronic	NOEDD = 10.9 µg/bee/d LDD_{50} = 24.5 µg/bee/d	Ruhland S., 2021
Apis mellifera	GLOB1817H	Larvae, chronic	NOED = 5.7 µg/larva	XXXX K., 2021
Higher-tier studies (tunnel test, field studies)				
-				

9.6.1.1 Justification for new endpoints

-

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of GLOB1817H in winter cereals

Intended use	Winter cereals		
Active substance	Prosulfocarb		
Application rate (g/ha)	1 × 2001		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	103.4	2001	19.4
Contact toxicity	79.3		25.2
Active substance	Diflufenican		
Application rate (g/ha)	1 × 42		

Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	112.3	42	0.37
Contact toxicity	100		0.42
Active substance	Halauxifen-methyl		
Application rate (g/ha)	1 × 3.99		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 108	3.99	0.04
Contact toxicity	> 98.1		0.04
~~**Active substance**~~	~~Cloquintocet-mexyl~~		
~~**Application rate (g/ha)**~~	~~1 × 3.99~~		
~~**Test design**~~	~~**LD_{50} (lab.) (µg/bee)**~~	~~**Single application rate (g/ha)**~~	~~**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**~~
~~Oral toxicity~~	~~> 100~~	~~3.99~~	~~0.04~~
~~Contact toxicity~~	~~> 100~~		~~0.04~~
Product	GLOB1817H		
Application rate (g/ha)	1 × 3026		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	310	3026	9.76
Contact toxicity	444		6.82

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:

[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

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 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: 3.026 x 2.9 x 198/1000 = 1.7375 µg/larva
Pollen dose: 3.026 x 6.1 x 2/1000 = 0.0369 µg/larva

Total exposure ETE = 1.7744 µg/larvae (as a default worst-case residue at 3.026 kg a.s./ha)

This can be compared to the larval NOED of 5.7 µg/larva.

TER = NOEDD (µg/larva)/ ETE (µg/larva) = 5.7/1.7744 = 3.21

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 3.21, the proposed uses of GLOB1817H pose an acceptable risk to bee larval development.

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

~~*Chronic oral exposure larvae (liquid formulations):*~~

~~Screening step assessment for spray applications:~~

~~ETR = AR*SV/NOEL = 3.026*4.4/5.7 = 2.34~~

~~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.~~

~~Treated crop:~~

~~ETR_{larvae} = AR*Ef*SV*TWA/$NOEL_{larvae}$ = 3.026*1*0.15*0.85/5.7 = 0.07~~

~~Adjacent crop:~~

~~ETR_{larvae} = AR*Ef*SV*TWA/$NOEL_{larvae}$ = 3.026*0.0033*2.2*0.85/5.7 = 0.01~~

~~Weeds in the treated field:~~

~~ETR_{larvae} = AR*Ef*SV*TWA/$NOEL_{larvae}$ = 3.026*1*2.2*0.85/5.7 = 0.99~~

~~Plants in the field margin:~~

~~ETR_{larvae} = AR*Ef*SV*TWA/$NOEL_{larvae}$ = 3.026*0.0092*2.2*0.85/5.7 = 0.01~~

~~Succeeding crops:~~

~~ETR_{larvae} = AR*Ef*SV*TWA/$NOEL_{larvae}$ = 3.026*1*0.4*0.85/5.7 = 0.18~~

~~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 in winter cereals (October – December), 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 LDD_{50} 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/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:

Daily dose (µg a.i./bee) = A.R. x [128 mg/(1000 x0.3)] x RUD = 3.026 x [128/(1000x0.3)] x 2.9 = 3.7442 µg/bee

A.R. = application rate in kg a.i./ha
RUD = residue per unit dose from the EFSA bee guidance. Mean RUDnectar = 2.9 mg a.i./kg (foliar sprays).

TER = NOEDD/daily dose = 10.9/3.7442 = 2.91

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 2.91, the proposed uses of GLOB1817H pose an acceptable chronic risk to adult bees.

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

~~*Chronic oral exposure adult bees (liquid formulations):*~~

~~Screening step assessment for spray applications:~~

~~ETR = AR*SV/10d LDD50 = 3.026*7.6/24.5 = 0.937~~

[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 pestcides to bees – 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium, September 15-17, 2014, Juliuis-Kühn-Archiv, 450, 2015.

~~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.~~

~~Treated crop:~~

~~$ETR_{chronic\ adult\ oral} = AR*Ef*SV*TWA/LD_{50oral} = 3.026*1*0.92*0.72/24.5 = 0.082$~~

~~Adjacent crop:~~

~~$ETR_{chronic\ adult\ oral} = AR*Ef*SV*TWA/LD_{50oral} = 3.026*0.0033*5.8*0.72/24.5 = 0.002$~~

~~Weeds in the treated field:~~

~~$ETR_{chronic\ adult\ oral} = AR*Ef*SV*TWA/LD_{50oral} = 3.026*1*2.9*0.72/24.5 = 0.257$~~

~~Plants in the field margin:~~

~~$ETR_{chronic\ adult\ oral} = AR*Ef*SV*TWA/LD_{50oral} = 3.026*0.0092*2.9*0.72/24.5 = 0.002$~~

~~Succeeding crops:~~

~~$ETR_{chronic\ adult\ oral} = AR*Ef*SV*TWA/LD_{50oral} = 3.026*1*0.54*0.72/24.5 = 0.048$~~

~~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 crop, winter cereals, 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 GLOB1817H will be applied before the flowering period in cereals (autumn to winter application), the limited number of bees that will forage 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) DT_{50} 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 DT_{90} 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.~~
~~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 GLOB1817H, a long interval before planting subsequent crops can~~

[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 pestcides to bees – 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium, September 15-17, 2014, Julius-Kühn-Archiv, 450, 2015.

~~be expected. Even in the case of crop failure, planting of subsequent crops is expected to occur with spring sown crops. Therefore, no unacceptable risk to bees is expected in the succeeding crop scenario. Halauxifen-methyl has a SFO DT_{50} of 1.3 days in soil and metabolism in plants has been assessed, however, due to very low residue levels (TRR < 0.01 mg a.s./kg) in all plant back intervals, a metabolic pathway was not proposed (EFSA, 2014). According to the environmental fate profile of halauxifen-methyl, it is unlikely that the active substance would pose risk to bees 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 GLOB1817H 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:~~

~~$HQ_{contact} = AR/LD_{50contact} = 3026/590 = 5.1$~~

~~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:~~

~~$ETR_{acute\ adult\ oral} = AR*SV/LD_{50oral} = 3.026*11.2/563.8 = 0.06$~~

~~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.~~

~~Treated crop:~~

~~$ETR_{acute\ adult\ oral} = AR*Ef*SV/LD_{50oral} = 3.026*1*2.3/563.8 = 0.0123$~~

~~Adjacent crop:~~

~~$ETR_{acute\ adult\ oral} = AR*Ef*SV/LD_{50oral} = 3.026*0.0033*11.2/563.8 = 0.0002$~~

~~Weeds in the treated field:~~

~~$ETR_{acute\ adult\ oral} = AR*Ef*SV/LD_{50oral} = 3.026*1*6.5/563.8 = 0.0349$~~

~~Plants in the field margin:~~

~~$ETR_{acute\ adult\ oral} = AR*Ef*SV/LD_{50oral} = 3.026*0.0092*6.5/563.8 = 0.0003$~~

~~Succeeding crops:~~

~~$ETR_{acute\ adult\ oral} = AR*Ef*SV/LD_{50oral} = 3.026*1*0.9/563.8 = 0.0048$~~

~~The protection goal is met for all scenarios as the calculated value is always below the trigger value of 0.036.~~

9.6.5 Effects on solitary bees

Not required.

9.6.6 Overall conclusions

GLOB1817H does not pose an unacceptable risk to bees when applied according to the intended use.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

zRMS Comments:	The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology" (2002) was accepted. The cloquintocet-mexyl is used as a safener and was not evaluated in this dossier. New studies for formulation were submitted. The laboratory studies 2D and 3D study were evaluated and accepted for the risk assessment. **In field risk**. The hazard quotients are below the trigger value (HQ ≤ 1) for *Aleochara bilineata* and *Poecilus cupreus*. For *Typhlodromus pyri* and *Aphidius rhopalosiphi* the HQ > 1. The higher tier assessment was required. The justification was provided: the rapid dissipation of prosulfocarb from soil and vegetation demonstrates that there is the potential for recolonisation within a one-year period. The statement is considered acceptable and the potential for the recolonisation of in-field populations has been demonstrated. **Off-field risk**. The hazard quotients are below the trigger value (HQ ≤ 1) for all species indicating that the active substance poses an acceptable risk to arthropods other than bees. The risk to arthropods other than bees is acceptable if the GLOB1817H is applied in accordance with proposed use pattern.

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with prosulfocarb, diflufenican, halauxifen-methyl 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, diflufenican and halauxifen-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	GLOB1817H	Extended laboratory test	LR_{50} = 1.368 L/ha ER_{50} > 0.75 L/ha	XXXX U., 2020a

Species	Substance	Exposure System	Results	Reference
		Bean leaf discs (2D)	$NOER_{mortality}$ = 0.75 L/ha $NOER_{reproduction}$ = 0.375 L/ha	
Aphidius rhopalosiphi (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	XXXX U., 2020b
Aleochara bilineata	GLOB1817H	Extended laboratory test Sandy soil (2D)	LR_{50} > 6 L/ha ER_{50} > 6 L/ha $NOER_{reproduction}$ ≥ 6 L/ha	XXXX U., 2020c
Poecilus cupreus	GLOB1817H	Extended laboratory test Sandy soil (2D)	LR_{50} > 6 L/ha ER_{50} > 6 L/ha $NOER_{mortality}$ ≥ 6 L/ha	XXXX 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.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GLOB1817H in winter cereals

Intended use	Winter cereals
Active substance/product	GLOB1817H
Application rate (L/ha)	1 × 3
MAF	/

Test species **Higher-tier**	**LR_{50} (lab.)** **(L/ha)**	**$PER_{in\text{-}field}$** **(L/ha)**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 1**
Typhlodromus pyri	1.368	3	**2.19**
Aphidius rhopalosiphi	2.176		**1.38**
Aleochara bilineata	6		0.5
Poecilus cupreus	6		0.5

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 LR_{50} or ER_{50} 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 use of GLOB1817H in winter cereals

Intended use	Winter cereals
Active substance/product	GLOB1817H
Application rate (L/ha)	1 × 3
MAF	/
vdf	5 (Higher-tier)*

Test species Higher-tier	**LR_{50} (lab.) (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	1.368	0.0277	0.01662	10	0.12
Aphidius rhopalosiphi	2.176				0.076
Aleochara bilineata	6				0.028
Poecilus cupreus	6				0.028

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

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

9.7.2.3 Additional higher-tier risk assessment

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 GLOB1817H 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 GLOB1817H with a default foliar DT_50 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 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 GLOB1817H in the in-field habitat

DT50 (d)	**Critical endpoint (g/ha)**	**Time to critical endpoint from initial residue level (rate 3 L/ha or 3025.5 g/ha*)**
10	378	30
2.2	378	6.6

*taking into account the density of the formulation of 1.0085 g/mL

Prosulfocarb, the active ingredient with the highest content in the formulation, has a foliar DT_{50} of 2.2 days (see section 9.3.2.2). Using the foliar DT_{50} of 2.2 days instead of 10 days, the PER will drop to a level of 378 g/ha within 6.6 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 days (default foliar DT_{50}) or within 6.6 days (empirical DT_{50}) is well within this timeframe.

It should be noted that the laboratory tests on the sensitive standard species present an extreme worst-case exposure. Foliar-dwelling arthropods are unlikely to be exposed in-field to GLOB1817H applied to the conditions of use (early post-emergence).

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

No unacceptable risk to non-target arthropods in expected when GLOB1817H is applied according to the intended use.

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

zRMS Comments:	The submitted information and justification were accepted. New studies were submitted and accepted. The cloquintocet-mexyl is used as a safener and was not evaluated in this dossier. The endpoints for active substances and their metabolites were agreed at the EU level. For metabolites the worst case assumption (the metabolite toxicity is 10x more toxic than parent) was proposed and accepted. For *Folsomia candida* the NOEC value for active substance prosulfocarb was assessd based on GLOB1817H study. This value was used in risk assessment. The max PECs values for active substances and its metabolites (PECs accum if relevant) were used for acute and long-term risk assessment. The PECs values were provided in Section 8. Fate and behavior. The NOEC values were lower than EC_{10} values and were used in further risk assessment. First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) was provided. For prosulfocarb the TERlt value of 4.16 is below the trigger of 5. Based on field study (application of 5L/ha equivalent to rate of 4000 g prosulfocarb/ha on bare soil) 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. Moreover, the short time (DT_{50}) in field studies (max DT_{50}=13 d) confirms that one application per season will not cause a long-term effect.

	An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the application of the GLOB1817H is in accordance with proposed use pattern.

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, halauxifen-methyl 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 GLOB1817H were not evaluated as part of the EU assessment of prosulfocarb, diflufenican and halauxifen-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	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*	XXXX E., 2012
Eisenia fetida	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.
Eisenia fetida	Diflufenican	56 d, chronic	NOEC = 1000 mg/kg dw $NOEC_{corr}$ = 500 mg/kg dw*	EFSA, 2007
Eisenia fetida	AE B107137	56 d, chronic	$NOEC_{corr}$ = 50 mg/kg dw*	Worst case assumption: 10x more toxic than parent.
Eisenia fetida	AE 0542291	56 d, chronic	$NOEC_{corr}$ = 50 mg/kg dw*	Worst case assumption: 10x more toxic than parent.
Eisenia fetida	Halauxifen-methyl	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 10 mg/kg dw $NOEC_{corr}$ = 5 mg/kg dw*	EFSA, 2014
Eisenia fetida	X11393729 (halauxifen)	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 10 mg/kg dw	EFSA, 2014
Eisenia fetida	X11449757	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 10 mg/kg dw	EFSA, 2014
Eisenia fetida	Non-extractable	Mixed in to substrate	NOEC = 7.10	EFSA, 2014

Species	Substance	Exposure System	Results	Reference
	residues of halauxifen-methyl	56 d, chronic 10% peat content	mg/kg dw	
~~*Eisenia fetida*~~	~~Cloquintocet-mexyl~~	~~56 d, chronic~~	~~-~~	~~Not required, a study with the formulated product is available.~~
Folsomia candida	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*	XXXX S., 2020
Folsomia candida	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.
Folsomia candida	Diflufenican (based on Diflufenican 500 SC)	Mixed into substrate Chronic	NOEC = 438 mg/kg dw	EFSA, 2007
Folsomia candida	AE B107137	NOEC = 43.8 mg/kg dw	-	Worst case assumption: 10x more toxic than parent.
Folsomia candida	AE 0542291	NOEC = 43.8 mg/kg dw	-	Worst case assumption: 10x more toxic than parent.
Folsomia candida	Halauxifen-methyl	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 1000 mg/kg dw $NOEC_{corr}$ = 500 mg/kg dw*	EFSA, 2014
Folsomia candida	X11393729 (halauxifen)	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 25 mg/kg dw	EFSA, 2014
Folsomia candida	X11449757	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 2.5 mg/kg dw	EFSA, 2014
~~*Folsomia candida*~~	~~Cloquintocet-mexyl~~	~~28 d, chronic~~	~~-~~	~~Not required, a study with the formulated product is available.~~
Hypoaspis aculeifer	Prosulfocarb (Based on Prosulfocarb 800 EC)	Mixed into substrate 14 d, chronic 5% peat content	NOEC = ~~126.65~~ 165 mg a.s./kg dw $NOEC_{corr}$ = ~~63.25~~ 82.5 mg/kg dw*	XXXX S., 2016
Hypoaspis aculeifer	Prosulfocarb sulfoxide	Mixed into substrate 14 d, chronic 5% peat content	NOEC = 12.665 mg a.s./kg dw	Worst case assumption: 10x more toxic than parent.
Hypoaspis aculeifer	Diflufenican (based on Diflufenican 500 SC)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1000 mg a.s./kg dw $NOEC_{corr}$ = 500 mg/kg dw*	XXXX K., 2016
Hypoaspis aculeifer	AE B107137	$NOEC_{corr}$ = 50 mg/kg dw*	-	Worst case assumption: 10x more toxic than parent.

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	AE 0542291	$NOEC_{corr}$ = 50 mg/kg dw*	-	Worst case assumption: 10x more toxic than parent.
Hypoaspis aculeifer	Halauxifen-methyl	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 25 mg/kg dw $NOEC_{corr}$ = 12.5 mg/kg dw*	EFSA, 2014
Hypoaspis aculeifer	X11393729 (halauxifen)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 12.5 mg/kg dw	EFSA, 2014
Hypoaspis aculeifer	X11449757	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 25 mg/kg dw	EFSA, 2014
~~*Hypoaspis aculeifer*~~	~~Cloquintocet-mexyl~~	~~14 d, chronic~~	~~-~~	~~Not required, a study with the formulated product is available.~~
Eisenia fetida	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	XXXX S., 2020
Folsomia candida	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)	XXXX S., 2020
Hypoaspis aculeifer	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	XXXX 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.				

Species	Substance	Exposure System	Results	Reference
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.

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 use of GLOB1817H in winter cereals

Intended use	Winter cereals		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prosulfocarb	11.1	2.6680	**4.16**
Prosulfocarb sulfoxide	2.22	0.1928	11.5
Diflufenican	500	0.1417	3529
AE B107137	50	~~0.0202~~ 0.05	~~2475~~ 1000
AE 0542291	50	~~0.0315~~ 0.08	~~1587~~ 625
Halauxifen-methyl	5	0.0078	641

X11393729 (halauxifen)	10	0.0035	2857
X11449757	10	~~0.0011~~ 0.0012	~~9091~~ 8333
GLOB1817H	20.5	4.0347	5.08
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Folsomia candida			
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~~ 0.05	~~2168~~ 876
AE 0542291	43.8	~~0.0315~~ 0.08	~~1390~~ 548
Halauxifen-methyl	500	0.0078	64.1
X11393729 (halauxifen)	25	0.0035	7143
X11449757	2.5	~~0.0011~~ 0.0012	~~2273~~ 2083
GLOB1817H	20.5 20 (EC_{10})	4.0347	5.08 5.0
Hypoaspis aculeifer			
Prosulfocarb	~~63.25~~ 82.5	2.6680	~~23.7~~ 30.9
Prosulfocarb sulfoxide	12.665	0.1928	65.7
Diflufenican	500	0.1417	3528
AE B107137	50	~~0.0202~~ 0.05	~~2475~~ 1000
AE 0542291	50	~~0.0315~~ 0.08	~~1587~~ 625
Halauxifen-methyl	12.5	0.0078	1603
X11393729 (halauxifen)	12.5	0.0035	3571
X11449757	25	~~0.0011~~ 0.0012	22.7 20833
GLOB1817H	21.5	4.0347	5.33

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

The TER_{lt} for earthworms due to exposure to prosulfocarb is slightly 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 results of this study, which lasted for one year, should also be considered in context of the environmental fate and behaviour properties of prosulfocarb, which has a short persistence in soil under

field conditions with DT_{50} values ranging from 6.5 to 13 days (EFSA, 2007). GLOB1817H is only applied once per season, so prolonged exposure of soil organisms, such as earthworms, to prosulfocarb is highly unlikely.

9.8.3 Overall conclusions

The risk for soil macro-organisms is acceptable when applying GLOB1817H according to the intended use.

9.9 Effects on soil microbial activity (KCP 10.5)

zRMS Comments:	The submitted information and data were accepted. The endpoints for active substances and their metabolites were agreed at the EU level. New study was submitted and accepted. The cloquintocet-mexyl is used as a safener and was not evaluated in this dossier. The max PECs values for active substances and its metabolites (PECs accum if relevant) were used for acute and long-term risk assessment. The PECs values were provided in Section 8. Fate and behavior. The worst case of PECs was used in risk assessment. An acceptable risk to soil microorganisms is expected if the GLOB1817H formulation is applied in accordance with proposed use pattern.

9.9.1 Toxicity data

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

Effects on soil microorganisms of GLOB1817H were not evaluated as part of the EU assessment of prosulfocarb, diflufenican and halauxifen-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prosulfocarb	42 d, aerobic loamy sand and clay-clay loam	No effects > 25% effect at day 42 at 5.33 and 53.3 mg/kg d.w. soil (4 and 40 kg/ha)	EFSA, 2007
N-mineralisation	Prosulfocarb	42 d, aerobic	No effects > 25%	Worst case

Endpoint	Substance	Exposure System	Results	Reference
	sulfoxide	loamy sand and clay-clay loam	effect at day 42 at 5.33 mg/kg d.w. soil	assumption: 10x more toxic than parent.
N-mineralisation	Diflufenican	14 d, aerobic clay-loam	No effects > 25% at 2500 g/ha (= 3.33 mg/kg d.w. soil)*	EFSA, 2007
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	Halauxifen-methyl	28 d, aerobic Mid loamy sand	No effects > 25% at 0.0535 mg a.s./kg d.w. soil	EFSA, 2014
N-mineralisation	X11393729 (halauxifen)	28 d, aerobic Mid loamy sand	No effects > 25% at 0.05 mg a.s./kg d.w. soil	EFSA, 2014
N-mineralisation	X11449757	28 d, aerobic Silty loamy sand	No effects > 25% at 0.052 mg a.s./kg d.w. soil	EFSA, 2014
~~N-mineralisation~~	~~Cloquintocet-mexyl~~	~~28 d, aerobic~~	~~No effects > 25% at 200 g/ha (= 0.267 mg/kg d.w. soil)*~~	~~Safener, not reviewed at EU level~~
N-mineralisation	CGA 153433	28 d, aerobic	No effects > 25% at 0.0267 mg/kg d.w. soil	Worst case assumption: 10x more toxic than parent.
N-mineralisation	GLOB1817H	28 d, aerobic loamy sand	No effects > 25% at 40 mg/kg d.w. soil	XXXX L., 2020

* Based on standard assumptions of soil bulk density 1.5 g/cm^3 and incorporation depth of 5 cm.

9.9.1.1 Justification for new endpoints

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

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).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of GLOB1817H in winter cereals

Intended use	Winter cereals

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)	2.6680	yes
Prosulfocarb sulfoxide	5.33 (at 42 d)	0.1928	yes
Diflufenican	3.33 (at 14 d)	0.1417	yes
AE B107137	0.359 (at 28 d)	~~0.0202~~ 0.05	yes
AE 0542291	0.358 (at 28 d)	~~0.0315~~ 0.08	yes
Halauxifen-methyl	0.0535 (at 28 d)	0.0078	yes
X11393729 (halauxifen)	0.05 (at 28 d)	0.0035	yes
X11449757	0.052 (at 28 d)	~~0.0011~~ 0.0012	yes
~~Cloquintocet-mexyl~~	~~0.267 (at 28 d)~~	~~0.025~~	~~yes~~
~~CGA 153433~~	~~0.0267 (at 28 d)~~	~~0.007~~	~~yes~~
~~GLOB1817H~~	~~40 (at 28 d)~~	~~4.0347~~	~~yes~~

9.9.3 Overall conclusions

The risk for soil micro-organisms is acceptable when applying GLOB1817H according to the intended use.

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

zRMS Comments:	New studies considering the formulation toxicity were submitted. Toxicity effects of formulation on the vegetative vigor and seedling emergence were tested. The new endpoints for formulation were derived: ER_{50} = 335.65 mL/ha (seedling emergence); ER_{50} = 75.93 mL/ha (vegetative vigour); and the ER_{50} for vegetative vigour, as a worse case, was used in risk assessment. An acceptable risk to non-target terrestrial plants is expected if the application of the GLOB1817H is in accordance with proposed pattern use and following mitigation measures are applied: 10 m or 3 m with use of 50% DRN or 1 m with use of 90% DRN.

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with prosulfocarb, diflufenican, halauxifen-methyl 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 GLOB1817H were not evaluated as part of the EU assessment

of prosulfocarb, diflufenican and halauxifen-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Allium cepa (onion)$_m$ [1)] *Avena sativa (oat)*$_m$ [2)] *Brassica napus (oilseed rape)*$_d$ [3)] *Lycopersicon esculentum (tomato)*$_d$ [4)] *Daucus carota (carrot)*$_d$ [5)] *Glycine max (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	XXXX., 2021
Allium cepa (onion)$_m$ [1)] *Avena sativa (oat)*$_m$ [2)] *Brassica napus (oilseed rape)*$_d$ [3)] *Lycopersicon esculentum (tomato)*$_d$ [4)] *Daucus carota (carrot)*$_d$ [5)] *Glycine max (soybean)*$_d$ [6)]	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)] **ER_{50} = 75.93 mL/ha** [5)] ER_{50} > 1662 mL/ha [6)] ER_{50} > 1662 mL/ha	XXXX M., 2021

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

-

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.

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 $PER_{off\text{-}field}$ 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, early post-emergence	Arable crops/0%	0-24	0.1470	0.1321*	0.1183	0.0686	0.0522

*intrapolated

Table 9.10-3: Assessment of the risk for non-target plants due to the use of GLOB1817H in winter cereals

Intended use	Winter cereals			
Active substance/product	GLOB1817H			
Application rate (mL/ha)	1 × 3000			
MAF	-			
Test species	**ER_{50} (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$* (mL/ha)**	**TER criterion: TER ≥ 5**
Lycopersicon esculentum	75.93	2.77%	83.32 (= 83.1 + 0.22)	**0.911**

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 GLOB1817H in winter cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter cereals			
Active substance/product		GLOB1817H			
Application rate (mL/ha)		1 × 3000			
MAF		-			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (mL /ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (mL /ha)**	**$PER_{off\text{-}field}$ 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 ER_{50} = 75.93 mL/ha		**TER** **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	-	-	-

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 50% drift reducing techniques or a buffer zone of 10 m without drift reduction is needed to protect non-target plants after application of GLOB1817H according to the intended use.

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 GLOB1817H was performed according to the EU Regulation 1272/2008 (CLP labelling).
Acute toxicity tests were performed with the formulation GLOB1817H. 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 EC_{50} values were ≤ 1 mg/L, GLOB1817H must be classified as Acute Aquatic Toxicity Category 1; H400.
For chronic classification, the summation method was applied. The product GLOB1817H should be classified as category 2 for chronic aquatic toxicity; H 410 H411. For more details, reference is made to the Part C.

SP 1: Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).

SPe3: To protect aquatic organisms respect ~~an unsprayed buffer zone of 5 m~~ 10 m no spray buffer zone including a 10 m vegetated buffer strip to surface water bodies. (*taking into account the relevant scenario's for Poland and Czech Republic*)

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.

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	xxxx	2008	The bioaccumulation potential of prosulfocarb in earthworm (*Eisenia foetida foetida*). ENV8333/040822 Chemex Environmental International Ltd GLP Unpublished	N	Globachem NV
KCA 8.2.6.1	XXXX	2012a	Effects of Prosulfocarb sulfoxide on *Chlamydomonas reinhardtii* in an algal growth inhibition test 12 10 48 057 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.6.1	XXXX	2012b	Effects of Prosulfocarb sulfoxide on *Chlorella vulgaris* in an algal growth inhibition test 12 10 48 059 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.6.2	XXXX	2012c	Effects of Prosulfocarb sulfoxide on *Anabaena flos-aquae* in an algal growth inhibition test 12 10 48 058 W Biochem Agrar GmbH 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
KCA 8.2.6.2	XXXX	2012d	Effects of Prosulfocarb sulfoxide on *Navicula pelliculosa* in an algal growth inhibition test 12 10 48 053 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCA 8.2.6.2	XXXX	2012e	Effects of Prosulfocarb sulfoxide on *Skeletonema costatum* in an algal growth inhibition test 12 10 48 060 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
~~KCA 8.3.1.2~~	~~XXXX~~	~~2018a~~	~~XDE-729 Methyl - Assessment of Effects on the Adult Honey Bee, Apis mellifera L., in a 10-Day Chronic Feeding Test under Laboratory Conditions~~ ~~S17-00191~~ ~~Eurofins Agroscience Services Ecotox GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Corteva Agriscience~~ ~~*Globachem access*~~
~~KCA 8.3.1.3~~	~~XXXX~~	~~2018b~~	~~XDE-729 methyl - Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure)~~ ~~S17-00206~~ ~~Eurofins Agroscience Services Ecotox GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Corteva Agriscience~~ ~~*Globachem access*~~
~~KCA 8.3.1.2~~	~~XXXX~~	~~2016a~~	~~Chronic toxicity of Diflufenican technical on honeybees (*Apis mellifera* L.)~~ ~~TRC16-019BA~~ ~~Trialcamp~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Sapec Agro S.A. and Globachem NV~~
~~KCA 8.3.1.3~~	~~XXXX~~	~~2016b~~	~~Toxicity of Diflufenican technical on honey bee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions~~ ~~TRC16-018BA~~ ~~Trialcamp~~	~~N~~	~~Sapec Agro S.A. and Globachem NV~~

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			~~GLP~~ ~~Unpublished~~		
KCP 10.2.1	XXXX	2021a	Acute toxicity of GLOB1817H to *Daphnia magna* in a 48-hour semi-static test 2 48 ADL 0015 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	XXXX	2021b	Effects of GLOB1817H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test 20 48 AAL 0019 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	XXXX	2021c	Effects of GLOB1817H on Lemna gibba 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	XXXX	2021d	Effect of GLOB1817H on *Myriophyllum spicatum* in a semi-static water-sediment system 20 48 AMS 0010 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1	XXXX	2020	Acute toxicity of GLOB1817H to the honeybee *Apis mellifera* L. under laboratory conditions. 20 48 BAA 0130 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1.1	XXXX.	2021	Acute toxicity of GLOB1817H to the bumblebee *Bombus terrestris* L. under laboratory conditions 20 48 BBA 0029	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 10.3.1.2	XXXX, S.	2021	Chronic toxicity of GLOB1817H to the honey bee *Apis mellifera* L. under laboratory conditions 20 48 BAC 0071 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.3	XXXX, K.	2021	GLOB1817H – Repeated exposure of the honey bee (Apis mellifera L.) larvae under laboratory conditions 20 48 BLC 0052 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	XXXX, U.	2020a	Effects of GLOB1817H on the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez) in an extended laboratory test. 20 48 NAE 0018 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	XXXX, U.	2020b	Effects of GLOB1817H on the predatory mite *Typhlodromus Pyri* Scheuten in an extended laboratory test. 20 48 NTE 0013 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2.2	XXXX, U.	2020c	Effects of GLOB1817H on the rove beetle *Aleochara bilineata* Gyll. in an extended laboratory test. 20 48 NKE 0010 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2	XXXX, U.	2020d	Effects of GLOB1817H on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test. 20 48 NLE 0007 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.1.1	XXXX, 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
KCP 10.4.1.1	XXXX, S.	2020	Effects of GLOB1817H on the reproduction of the earthworm *Eisenia fetida*. 20 48 TEC 0054 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.1.2	XXXX, 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	XXXX, S.	2016	A dose response study to assess the NOEC, $EC_{10\text{-}20\text{-}50}$ on reproduction and $LR_{10\text{-}20\text{-}50}$ on mortality of Prosulfocarb 800 EC of the predatory mite *Hypoapis aculeifer* on artificial soil in the laboratory. HA04/2016 Walloon Agricultural Research Centre GLP Unpublished	N	Globachem NV
KCP 10.4.2.1	XXXX, K.	2016	Diflufenican 500 g/L SC: Predatory mite (*Hypoaspis aculeifer*) reproduction test in soil. DF50GM Envigo CRS Limited GLP	N	Globachem NV & Sapec Agro S.A.

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 10.4.2.1	XXXX, L.	2020	Effects of GLOB1817H on the reproduction of the predatory mite Hypoaspis aculeifer. 20 48 THC 0043 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2.1	XXXX, S.	2020	Effects of GLOB1817H on the reproduction of the collembolan *Folsomia candida* 20 48 TCC 0059 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 10.5	XXXX, 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	XXXX.	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	XXXX, 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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No new studies submitted.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

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

Reference:	KCA 8.1.3
Report	The bioaccumulation potential of prosulfocarb in earthworm (*Eisenia foetida*), XXXX 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:	Yes/No/Supplementary

Executive Summary

The study was undertaken to determine the bioconcentration and subsequent depuration of prosulfocarb in earthworms (*Eisenia foetida*). Calculated bioconcentration factors (BCF) were based on analyses of sediment and worm tissues for prosulfocarb. The study was run with cocentrations 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 (Eisenia foetida foetida)
Age:	Not reported
Wet weight range at test starts:	mean wet weight: 194 mg
Source:	Obtained from Blades Biological Ltd., Kent, UK
Acclimatisation period:	6 weeks
Feeding:	Air dried, ground and sieved horse manure, weekly
Test design	
Test vessels:	2 L plastic containers, each with 750 g of wet artificial sediment
Artificial sediment:	75% quartz sand, 20% kaolin and 5% sphagnum peat moss, adjusted to pH of 5.5 to 6.5 using calcium carbonate
Replicates:	12 per treatment and control
No of worms/arena:	5
Environmental conditions	
Test temperature:	22.5 – 23.0°C
Soil pH:	6.3 - 7.3
Soil moisture content:	Not reported. Water content adjusted with deionised water
Lightning:	16 h light (daylight fluorescent tubes) and 8 h dark at approx. 400 to 800 lux
Length of the test:	Uptake: 14 days, Depuration: 14 days

Study Design and Methods

Experimental dates: 28 May 2008 to 26 June 2008

Exposure phase

The formulated sediment (according OECD 207) was prepared 8 days prior to the addition of the test material, and the test material was added 2 days before the addition of the worms.
An initial stock solution of 100 mg prosulfocarb/L was prepared in deionised water. Appropriate volumes of this solution were diluted to 1000 mL with deionised water and mixed with 7500 g dry weight of the prepared sediment to give final test concentrations of 0.75 and 7.5 mg prosulfocarb/kg. The control sediment was prepared with deionised water only.
Five earthworms were placed in each of the control and test vessels, containing 750 g of the wet artificial sediment. Observations and records of mortalities and abnormal behaviour were made on days 1, 2, 4, 7, 10 and 14, and sediment and earthworm samples were taken at the same time.

Depuration phase

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

Sampling and analysis

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

Physical and chemical parameters

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

Calculation of Bioconcentration Factors (BCF)

BCF_{ss} (steady-state)

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

BCF_{ss} was calculated from:

C_w at steady-state (mean)/C_s 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:

$$k_1 = c_w k_2/c_s \times [1-e^{(-k2t)}]$$

k_2t = depuration constant at time t

The depuration constant was calculated from:

$$k_2 = \ln(cw_1/cw_2)/t_2-t_1$$

t = time in days

Results and Discussion

The results of this study were based on the data generated in the highest test concentrations, as the concentration of prosulfocarb accumulated in the earthworms in the low test concentration were below the limit of detection (0.01 µg prosulfocarb/g) of the analytical method. No mortalities were observed in the control replicate at the end of the test period, and no abnormal behaviour was recorded in either of the test concentration or the control.
Steady-state was determined as days 4, 7 and 10. The BCF_{ss} for prosulfocarb in the high concentration was calculated to be 1.26. The BCF_k for prosulfocarb in the high concentration was calculated to be 1.39. Uptake (k_1) and depuration (k_2) rate constants for prosulfocarb in the high concentration were calculated to be 8.60 and 6.19, respectively.

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

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

Uptake and depuration of prosulfocarb in the earthworm

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

[a]Geometric means
[b]Further extract analysis not reported since concentrations of prosulfocarb had reduced to 0.00 mg/kg within 2 days

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

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

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

k_2		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 submitted study was accepted. The validity criteria were met. No deviations were noted. The following endpoints based on nominal concentration were derived: 48-h EC_{50} = 0.954 mg/L (immobility); NOEC = 0.593 mg/L LOEC = 0.889 mg/L

Reference:	KCP 10.2.1
Report	Acute toxicity of GLOB1817H to *Daphnia magna* in a 48 hour semi-static test, XXXX, 2021a, 20 48 ADL 0015
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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 (EC_{10}, EC_{20} and EC_{50} 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 EC_{50} for immobility was 0.954 mg/L test item at 48 hours.

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:	*Daphnia magna* STRAUS
Test system:	Exposure of *Daphnia* 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 *Daphnia*/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 *Daphnia*: 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)

Dates of work:

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,	**0.925**	**1.04**	**1.31**	**0.658**	**0.747**	**0.954**
nominal	(0.730 – 1.06)	(0.868 – 1.17)	(1.17 – 1.47)	(0.508 – 0.758)	(0.612 – 0.844)	(0.845 – 1.08)
µg/L Prosulfocarb,	**617.1**	**695.8**	**875.3**	**439.0**	**498.3**	**636.4**
nominal	(487.0 – 705.2)	(579.1 – 781.2)	(779.2 – 983.3)	(338.9 – 505.7)	(408.3 – 563.1)	(563.7 – 717.2)
µg/L Diflufenican,	**13.0**	**14.7**	**18.5**	**9.26**	**10.5**	**13.4**
nominal	(10.3 – 14.9)	(12.2 – 16.5)	(16.4 – 20.8)	(7.15 – 10.7)8.6	8.62 – 11.9	(11.9 – 15.1)
µg/L Halauxifen-Methyl,	**1.21**	**1.37**	**1.72**	**0.86**	**0.98**	**1.25**
nominal	(0.96 – 1.39)	(1.14 – 1.54)	(1.53 – 1.93)	(0.67 – 0.99)	(0.80 – 1.11)	(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: ≤ 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 EC_{50} for immobility was 0.954 mg/L test item at 48 hours.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No deviations were noted. The following endpoints based on nominal concentration were derived: E_rC_{50} = 0.0597 mg/L E_yC_{50} = 0.0310 mg/L NOEC = 0.0204 mg/L LOEC = 0.0346 mg/L

Reference:	KCP 10.2.1
Report	Effects of GLOB1817H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test, XXXX, 2021b, 20 48 AAL 0019
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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_rC_{10}, E_rC_{20}, E_rC_{50} values (average specific growth rate), E_yC_{10}, E_yC_{20}, E_yC_{50} values (yield), and LOEC/NOEC values related to growth inhibition and yield over a period of 72 hours.
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, based on nominal concentrations.

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:	Freshwater green alga – *Pseudokirchneriella subcapitata* KORSHIKOV
Test system:	Exposure of *Pseudokirchneriella subcapitata* to the test item applied once in test medium (static conditions)
Test conditions:	Temperature: 22.8 – 22.9°C Continuous light: (on average 75 µE m^{-2} s^{-1})
Treatments:	Control (untreated test medium) GLOB1817H
Test concentration (nominal):	20.4, 34.6, 58.8, 100.0, 170.0 µg/L test item equivalent to 13.6, 23.1, 39.2, 66.7, 113.4 µg/L Prosulfocarb 0.29, 0.49, 0.83, 1.41, 2.39 µg/L Diflufenican 0.027, 0.045, 0.077, 0.131, 0.223 µg/L Halauxifen-Methyl 0.027, 0.046, 0.079, 0.134, 0.227 µg/L Cloquintocet-mexyl
Test concentration (mean measured):	12.32, 19.85, 36.34, 57.81, 102.68 µg/L Prosulfocarb 0.251, 0.396, 0.714, 1.17, 2.01 µg/L Diflufenican 0.012, 0.021, 0.043, 0.065, 0.118 µg/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 Prosulfocarbn 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	**ErC10**	**EyC10**
and 95% confidence intervals		
(lower – upper)		
Test item, nominal	**18.4**	**23.8**
	(11.9 – 24.2)	(17.2 – 26.8)
Prosulfocarb, nominal	**12.3**	**15.9**
	(7.94 – 16.1)	(11.5 – 17.9)
Diflufenican, nominal	**0.26**	**0.34**
	(0.17 – 0.34)	(0.24 – 0.38)
Halauxifen-Methyl, nominal	**0.024**	**0.03**
	(0.016 – 0.032)	(0.02 – 0.04)
Prosulfocarb, mean measured	**10.98**	**14.33**
	(7.13 - 14.40)	(9.74 – 16.09)
Diflufenican, mean measured	**0.220**	**0.290**
	(0.142 – 0.289)	(0.200 – 0.324)
Halauxifen-Methyl, mean measured	**0.011**	**0.014**
	(0.008 – 0.015)	(0.009 – 0.016)
EC20	**ErC20**	**EyC20**
and 95% confidence intervals		
(lower – upper)		
Test item, nominal	**27.5**	**26.0**
	(20.1 – 33.9)	(20.4 – 28.6)
Prosulfocarb, nominal	**18.3**	**17.3**
	(13.4 – 22.6)	(13.6 – 19.1)
Diflufenican, nominal	**0.39**	**0.37**
	(0.28 – 0.48)	(0.29 – 0.40)
Halauxifen-Methyl, nominal	**0.036**	**0.034**
	(0.026 – 0.044)	(0.027 – 0.038)
Prosulfocarb, mean measured	**16.43**	**15.51**
	(12.05 – 20.16)	(11.58 – 16.95)
Diflufenican, mean measured	**0.329**	**0.313**

	(0.240 – 0.404)	(0.236 – 0.341)
Halauxifen-Methyl, mean measured	**0.017**	**0.016**
	(0.013 – 0.021)	(0.011 – 0.017)
EC50	**ErC50**	**EyC50**
and 95% confidence intervals		
(lower – upper)		
Test item, nominal	**59.7**	**31.0**
	(51.2 – 69.6)	(28.1 – 32.4)
Prosulfocarb, nominal	**39.8**	**20.7**
	(34.2 – 46.4)	(18.7 – 21.6)
Diflufenican, nominal	**0.84**	**0.44**
	(0.72 – 0.98)	(0.40 – 0.46)
Halauxifen-Methyl, nominal	**0.08**	**0.041**
	(0.07 – 0.09)	(0.037 – 0.043)
Prosulfocarb, mean measured	**35.53**	**18.05**
	(30.53 – 41.35)	(16.08 – 18.80)
Diflufenican, mean measured	**0.710**	**0.362**
	(0.608 – 0.829)	(0.324 – 0.376)
Halauxifen-Methyl, mean measured	**0.040**	**0.019**
	(0.034 – 0.046)	(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 submitted study was accepted. The validity criteria were met: 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. No deviations were noted. The following endpoints based on nominal concentration were derived: E_rC_{50} = 0.5159 mg/L E_yC_{50} = 0.3352 mg/L NOEC = 0.160 mg/L LOEC = 0.305 mg/L

Reference:	KCP 10.2.1
Report	Effects of GLOB1817H on *Lemna gibba* in a growth inhibition test under semi-static conditions, XXXX, 2021c, 20 48 ALE 0017
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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 – *Lemna gibba* 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 µE x m^{-2} x s^{-1})	
Treatments:	control, untreated test medium, test item (GLOB1817H)	
Test concentration (nominal)*:	84.4, 160.4, 304.7, 579.0, 1100.0 µg/L test item equivalent to 56.3, 107.0, 203.3, 386.2, 733.8 µg/L Prosulfocarb 1.19, 2.26, 4.29, 8.15, 15.5 µg/L Diflufenican 0.11, 0.21, 0.40, 0.76, 1.44 µg/L Halauxifen-Methyl 0.11, 0.21, 0.41, 0.77, 1.47 µg/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 µg/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**
Diflufenican, 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**
Diflufenican, 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**
EC_{10} (CI, lower - upper)	**E_rC_{10}**	**E_rC_{10}**	**E_yC_{10}**	**E_yC_{10}**
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)
Diflufenican, 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)
EC_{20} (CI, lower - upper)	**E_rC_{20}**	**E_rC_{20}**	**E_yC_{20}**	**E_yC_{20}**
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)
Diflufenican, 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)
EC_{50} (CI, lower - upper)	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
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)
Diflufenican, 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	Biomass (dry weight)	% Inhibition	
	replicate mean	replicate mean	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_rC_{50} (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 EC_{50}-value (0-7 d) was 335.2 µg/L test item (nominal) for yield based on frond number.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: 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 and doubling of total shoot length and fresh weight in control plants: required factor: 2, achieved: factor 4.3 for total shoot length and factor 3.8 for fresh weigh. No deviations were noted. The following endpoints based on nominal concentration were derived: E_rC_{50} = 0.075 mg/L E_yC_{50} = 0.040 mg/L NOEC = 0.009 mg/L

Reference: KCP 10.2.1

Report	Effects of GLOB1817H on *Myriophyllum spicatum* in a semi-static water-sediment system, XXXX, 2021d, 20 48 AMS 0010
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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 EC_{50} value based on growth rate was 0.075 mg/L test item calculated for total shoot length.
The lowest EC_{50} 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) 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:	*Myriophyllum spicatum* L.
Test system:	exposure of *Myriophyllum spicatum* 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:	*day 0 and 14:* main shoot length, length and number of lateral branches *day 8 and 14:* changes in plant development *day 0 and 14:* fresh and dry weight *day 0, 14:*

	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 Prosulofcarb, 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**
EC_{10}	**E_rC_{10}**	**E_rC_{10}**	**E_yC_{10}**	**E_yC_{10}**
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)
EC_{20}	**E_rC_{20}**	**E_rC_{20}**	**E_yC_{20}**	**E_yC_{20}**
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)
EC_{50}	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
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

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 EC_{50} value based on growth rate was 0.075 mg/L test item calculated for total shoot length.
The lowest EC_{50} value based on yield was 0.040 mg/L test item calculated for total shoot length.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. As since measured concentrations were not within 20% of the nominal concentrations for all test concentrations during the test duration, the relevant endpoints are based on measured concentration. The following endpoints were derived: E_rC_{50} = 0.2539 mg/L E_bC_{50} = 0.0971 mg/L NOEC = 0.0313 mg/L

Reference:	KCA 8.2.6.1
Report	Effects of Prosulfocarb sulfoxide on *Chlamydomonas reinhardtii* in an algal growth inhibition test, XXXX, 2012a, 12 10 48 057 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

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

Materials

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

	+ 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	5 x 10^3 cells/mL
Exposure regime:	Static
Aeriation	None reported
Duration:	72 h
Environmental conditions	
Temperature:	22.0 – 23.9°C
pH:	Test start: 8.05 – 8.12 Test end: 8.25 – 9.50
Lighting:	Continuous fluorescent illumination at an average of 113 $\mu E/m^{-2}.s^{-1}$

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

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

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

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

Conclusion

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

Comments of zRMS:	The submitted study was accepted. The validity criteria were met No deviations were noted. The following endpoints based on nominal concentration were derived: 72h - E_bC_{50} = 0.730 mg/L 72h - E_rC_{50} = 1.320 mg/ 72h - NOEC = 0.340 mg/L

Reference:	KCA 8.2.6.1
Report	Effects of Prosulfocarb sulfoxide on *Chlorella vulgaris* in an algal growth inhibition test, XXXX, 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:	*Chlorella vulgaris* BEIJERINCK Strain: 211-11b SAG
Source:	Laboratory cultures, originally obtained from MBM ScienceBridge Gmbh (Göttingen, Germany)
Test design	
Test vessels:	250 mL Erlenmeyer flasks with air-permeable stoppers, containing 100 mL of medium
Test medium:	OECD algal medium
Replication:	Control: 6 Treated: 3 + 1 additional vessel for analysis and retained specimen per concentration and control
Starting cell density:	5×10^3 cells/mL
Exposure regime:	Static
Aeriation	None reported
Duration:	72 h
Environmental conditions	
Temperature:	22.0 – 23.9°C
pH:	Test start: 8.01 – 8.05 Test end: 8.29 – 9.09
Lighting:	Continuous fluorescent illumination at an average of 113 $\mu E/m^{-2}.s^{-1}$

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

Growth rate

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

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

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

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

Yield

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

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

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean yield (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

	After 48 h (mg prosulfocarb sulfoxide/L)		After 72 h (mg prosulfocarb sulfoxide/L)	
Parameter	**Growth rate**	**Yield**	**Growth rate**	**Yield**
EC_{50}	1.15	0.78	1.32	0.73
95% CI	1.01-1.31	0.73-0.83	1.07-1.70	0.68-0.79
EC_{20}	0.73	0.57	0.72	0.53
95% CI	0.56-0.85	0.52-0.62	0.24-0.73	0.45-0.58
EC_{10}	0.57	0.49	0.53	0.44
95% CI	0.39-0.70	0.43-0.53	0.24-0.73	0.36-0.50
NOEC	0.34	0.34	0.34	0.34
LOEC	0.62	0.62	0.62	0.62

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

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

Conclusion

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

Comments of zRMS:	The submitted study was accepted. The validity criteria were met No deviations were noted. The following endpoints based on nominal concentration were derived: E_bC_{50} = 19.500 mg/L E_rC_{50} = 42.500 mg/L NOEC = 1.620 mg/L

Reference:	KCA 8.2.6.2
Report	Effects of Prosulfocarb sulfoxide on *Anabaena flos-aquae* in an algal growth inhibition test, XXXX, 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:	*Anabaena flos-aqua* 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 $\mu E/m^{-2}.s^{-1}$

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 (x 10^4 cell/mL) 0-48 h	Percentage inhibition	Mean yield (x 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

	After 48 h (mg prosulfocarb sulfoxide/L)		After 72 h (mg prosulfocarb sulfoxide/L)	
Parameter	**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
EC_{20}	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
EC_{10}	8.17	2.33	14.3	5.18
95% CI	5.05-11.0	1.24-3.49	11.1-17.2	2.97-7.24
NOEC	1.62	1.62	1.62	1.62
LOEC	5.17	5.17	5.17	5.17

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

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

Conclusion

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

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No deviations were noted. As since measured concentrations were not within 20% of the nominal concentrations for all test concentrations during the test duration, the relevant endpoints are based on measured concentration. The following endpoints were derived: The following endpoints based on nominal concentration were derived: E_bC_{50} = 1.400 mg/L

	E_rC_{50} = 7.650 mg/L NOEC = 0.440 mg/L

Reference:	KCA 8.2.6.2
Report	Effects of Prosulfocarb sulfoxide on *Navicula pelliculosa* in an algal growth inhibition test, XXXX, 2012d, 12 10 48 053 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

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

Materials

Test Material	Prosulfocarb sulfoxide
Lot/Batch #:	22661
Purity:	98.8%
Description:	Yellowish viscous liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	20 April 2017
Treatments	
Test rates:	Culture medium control and nominal concentrations of 0.48, 1.53, 4.88, 15.6 and 50.0 mg prosulfocarb sulfoxide/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentration:	Yes, 0 and 72 h using RP-HPLC with MS and UV detection
Test organisms	
Species:	*Navicula pelliculosa* 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_rC_{50} and E_yC_{50} values and their 95% confidence intervals were calculated by Probit analysis. (Regression analysis was performed using individual replicate responses, not treatment group means.) A Welch-t-test with Bonferroni-Holm adjustment ($p \leq 0.05$, one-sided smaller) or Williams t-test ($p \leq 0.05$, one-sided smaller) was used to identify significant differences in the calculated mean growth rate and yield of test item treatments compared to the control.

Growth rate

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

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

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

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

Yield

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

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

Nominal concentrations of prosulfocarb sulfoxide (mg/L)	Mean yield (x 10^4 cell/mL) 0-48 h	Percentage inhibition	Mean yield (x 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

	After 48 h (mg prosulfocarb sulfoxide/L)		After 72 h (mg prosulfocarb sulfoxide/L)	
Parameter	**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_rC_{50} was 7.97 mg prosulfocarb sulfoxide/L and the E_yC_{50} was 2.04 mg prosulfocarb sulfoxide/L. The LOEC at 72 hours, based on growth rate and yield, was 1.53 mg prosulfocarb sulfoxide/L. The corresponding NOEC was 0.48 mg prosulfocarb sulfoxide/L.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met No deviations were noted. The following endpoints based on nominal concentration were derived: E_bC_{50} = 53.8 µg/L E_rC_{50} = 134.8 µg/ NOEC = 21.5 µg/L

Reference:	KCA 8.2.6.2
Report	Effects of Prosulfocarb sulfoxide on *Skeletonema costatum* in an algal growth inhibition test, XXXX, 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_rC_{50} was 134.8 µg prosulfocarb sulfoxide/L and the E_yC_{50} 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	Yes, 0 and 72 h using RP-HPLC with MS and UV detection

concentration:	
Test organisms	
Species:	*Skeletonema costatum* 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 $\mu E/m^{-2}.s^{-1}$

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	% of nominal measured at 0 h*	% of nominal measured at 72 h*

prosulfocarb sulfoxide (µg/L)		
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

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

95% CI: 95% confidence interval

Validity criteria

The validity criteria were met.

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

Conclusion

Based on nominal concentrations, the 72-hour E_rC_{50} was 134.8 µg prosulfocarb sulfoxide/L and the E_yC_{50} 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.

Myriophylum-DFF-technical-4-PSG800-zu-Jun-DK

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

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guidances 213 (acute oral) and 214 (acute contact). The validity criteria were met: • oral test: the mean mortality of the control groups was 0% (recommended ≤ 10 %); • contact test: the mean mortality of the control groups was 0% and 3.3%

	in water and tween solution, respectively, (recommended ≤ 10 %); The following endpoints were derived • Oral: LD_{50} 48 h = 310 µg formulation/bee (equivalent to 212 µg total a.s./bumblebee) • Contact: LD_{50} 96 h = 444 µg formulation/bee (equivalent to 304 µg total a.s./bumblebee) The endpoints were used in the risk assessment.

Reference:	KCP 10.3.1.1
Report	Acute toxicity of GLOB1817H to the honeybee *Apis mellifera* L. under laboratory conditions, XXXX, 2020, 20 48 BAA 0130
Guideline(s):	Yes, OECD 213 (1998) and OECD 214 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine the acute toxicity of 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 LD_{50} (48 h) was 496 µg GLOB1817H/bee (corresponding to 339 µg total a.s./bee) and the LD_{50} (96 h) was 444 µg GLOB1817H/bee (corresponding to 304 µg total a.s./bee). The oral LD_{50} (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.):	nominal	analysed
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 – *Apis mellifera* 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: Contact test: 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

	Oral test:	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]	Test item:	
	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.	Test item	
	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 *ad libitum*)
Statistics:	Statistical program used: ToxRat Professional 3.3.0 (2018)	
	Calculation of LD_{50} values:	
	Test item:	Contact: Probit analysis (linear maximum likelihood regression) Oral: Weibull analysis (linear maximum likelihood regression)
	Reference item:	Contact: Probit analysis (linear maximum likelihood regression) Oral: Probit analysis (linear maximum likelihood regression)
	Statistical significance of mortality values:	
	Test item: Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$)	
	Reference item: Fisher's Exact Binominal Test with Bonferroni Correction ($\alpha = 0.05$)	
Validity criteria	Control mortality (48 h): ≤ 10 %	
	LD_{50} – 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 und 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.

LD_{50}-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 LD_{50} (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.

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 LD_{50} (48 h) was 496 µg GLOB1817H/bee (corresponding to 339 µg total a.s./bee) and the LD_{50} (96 h) was 444 µg GLOB1817H/bee (corresponding to 304 µg total a.s./bee). The oral LD_{50} (48 h) was 310 µg GLOB1817H/bee that is corresponding to 212 µg total a.s./bee.

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guidances 246 (acute oral) and 247 (acute contact). The validity criteria were met: • oral test: the mean mortality of the control groups was 0% (recommended ≤ 10 %); • contact test: the mean mortality of the control groups was 0% and 2.0% in water and tween solution, respectively, (recommended ≤ 10 %); The following endpoints were derived • Oral: 48 h LD_{50} > 563.8 µg formulation/bee (equivalent to > 382.2 µg total a.s./bumblebee) NOED ≥ 563.8 µg formulation/bee (equivalent to ≥ 382.2 µg total a.s./bumblebee) • Contact: 96 h LD_{50} = 590 µg formulation/bee (equivalent to > 400.0 µg total a.s./bumblebee NOED ≥ 590 µg formulation/bee (equivalent to ≥ 400.0 µg total a.s./bumblebee)

Reference:	KCP 10.3.1.1.1
Report	Acute toxicity of GLOB1817H to the bumblebee *Bombus terrestris* L. under laboratory conditions, XXXX, 2021, 20 48 BBA 0029
Guideline(s):	Yes, OECD 246, OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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 LD_{50} 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 LD_{50} 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).

Materials and Methods

Test item: GLOB1817H, batch No.: KS010420, density (at 20 °C):1.0085 g/mL
Content of active substance (a.s.):

	nominal	analysed
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: *Bombus terrestris* 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: Contact test: In a 48 hours test, adults of *Bombus terrestris* 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.
Oral test: In a 48 hours test, adults of *Bombus terrestris* 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:
Contact test:
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
Oral test:
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:
Contact test:
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	Contact toxicity test	100.0%	≥ 50%

hours)	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*, LD_{50} / 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*, LD_{50} / NOED values

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

[1] based on consumed values

Conclusion

In the acute contact toxicity test with GLOB1817H, the resulting LD_{50} 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 LD_{50} 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:	The study was not evaluated as it concerns the active substance. The study should be evaluated during the active substance renewal.

Reference: KCA 8.3.1.2

Report: Chronic toxicity of Diflufenican technical on honeybees (*Apis mellifera* L.), Ansaloni T., 2016a, TRC16-019BA

Guideline(s): Yes, CEB (2012) method, adaptions of OECD Guidelines nº 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 ºC during one period of 24 consecutive hours and three periods of 8 consecutive hours during the test (Max = 37.09ºC). 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/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 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
Statistics:	Mean daily consumptions of rhe 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 ≥ 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 µl/bee of the offered diet, respectively. Mean daily consumption of the bees exposed to the test item was 24.13 µl/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 (µg/bee/day)		**NOEDD (µg/bee/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 not evaluated as it concerns the active substance. The study should be evaluated during the active substance renewal.

Reference:	KCA 8.3.1.2
Report	XDE-729 Methyl - Assessment of Effects on the Adult Honey Bee, Apis mellifera L., in a 10 Day Chronic Feeding Test under Laboratory Conditions, Oberrauch S., 2018a, S17-00191
Guideline(s):	Yes, OECD Guideline proposal (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and Methods

Test item (Common name):	XDE-729 Methyl
Purity:	99.7% w/w
Description (physical state):	solid / white
Lot/batch no.:	YC2-106153-95 (TSN301574)

Organism (Species):	Honey bee (*Apis mellifera*)
Study type:	chronic oral
Study design:	Dose-response test; duration 10 days; 4 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality, diet consumption and behavioural effects daily.
Test concentrations:	0 (control, solvent control), 12.5, 25.0, 50.0, 100 and 200 mg/kg food
Information on bee colony (health etc):	The bees used in the test were from disease-free colonies. The hives had not been treated for *Varroa* mites or for disease in the last 4 weeks. The bees were maintained in a clean holding cage at a temperature of approximately 33°C and 50 to 70% humidity.
Amount of treated diet consumed:	Consumption of the treated diets ranged from 20.5 to 25.3 mg of diet. Calculated daily dosages ranged from 0.29 to 5.07 µg/bee.
Feeding method:	During holding/acclimation and after administration of the test dosages, bees were provided ad libitum a 50 % (w/v) sucrose solution in water. The bees for the definitive test were housed in cages containing pre-weighed feeders (syringes) containing approximately 3 – 5 mL of the appropriate control or treated solutions. All control and treatment feeders were exchanged daily with freshly prepared diet. Consumption of the feeding solutions was monitored by weighing the syringe before and after feeding, correcting for evaporation.
Environmental conditions:	Temperature: 32.3 – 33.0°C Relative humidity: 50.4 – 63.1% Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.
Reference substance:	Dimethoate: 0.9 mg/kg food
Solvent substance (if applicable):	5 % acetone and 0.1 % xanthan

Honey bees were exposed to a 50 % (w/v) aqueous sucrose solution containing five concentrations of XDE-729 Methyl and 5 % acetone with 0.1 % xanthan by continuous and ad libitum feeding over a period of 10 days. The control group was fed with pure 50 % (w/v) aqueous sucrose solution and the solvent control group was fed with 50 % (w/v) aqueous sucrose solution containing 5 % acetone and 0.1 % xanthan. Mortality and behavioural abnormalities were assessed daily during the 10 day exposure period. The chronic effects of XDE-729 Methyl were evaluated by comparing the results of the test item group to those of the solvent control group. Additionally, 4 test units without bees but with full diet syringes containing pure 50 % (w/v) aqueous sucrose were placed in the climatic chamber for the evaluation of the evaporation. The syringes of another 4 additional cages without bees but with full diet syringes (50 % (w/v) aqueous sucrose solution containing 5 % acetone and 0.1 % xanthan were placed in the climatic chamber for the evaluation of the evaporation.

Results and Discussion

Dates of work: 24 July 2017 – 08 January 2018

In the control groups (C and C_{sol}), after 10 days of continuous feeding 7.5 and 12.5% mortality was observed, respectively.
In the test item treatment groups no statistically significant mortality was observed after 10 days.

In the controls single affected and apathetic bees were recorded on different assessment dates throughout the test.
Behavioural abnormalities (affected, apathy, moribund) in the test item treatment groups were observed mainly between assessment one and four. Afterwards only single affected bees were recorded. The overall mean daily consumption of feeding solution over the entire test period of the control groups C and C_{sol} was 33.1 and 24.0 mg/bee/day. At the concentrations of 12.5, 25.0, 50.0, 100 and 200 mg/kg food the overall mean daily consumption of feeding solution was 23.4, 25.1, 21.5, 20.5 and 25.3 mg/bee/day, respectively. In the toxic reference item group, the overall mean daily consumption of feeding solution was 18.8 mg/bee/day.
At the end of the 10 day test period, the accumulated uptake of the test item at the concentrations of 12.5, 25.0, 50.0, 100 and 200 mg/kg was 2.94, 6.30, 10.81, 20.50 and 50.68 µg/bee, respectively. The corresponding daily mean uptake was therefore 0.29, 0.63, 1.08, 2.05 and 5.07 µg/bee/day, respectively.
The mean weight of surviving bees was 92.8 mg in the solvent control group and 95.4, 94.1, 95.6, 93.3 and 95.9 mg in the test item groups of 12.5, 25.0, 50.0, 100 and 200 mg/kg food.

Toxicity of XDE-729 Methyl to honey bees in the chronic oral toxicity test

Treatment Daily		Oral 10 day test									
Nominal mg/kg food	Measured daily mean µg/bee	Mortality (%)									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control (0)	0	0.0	0.0	2.5	2.5	2.5	5.0	5.0	7.5	7.5	7.5
C_{sol}	0	0.0	0.0	0.0	0.0	0.0	5.0	7.5	7.5	12.5	12.5
12.5	0.29	0.0	0.0	0.0	0.0	7.5	7.5	10.0	10.0	10.0	10.0
25.0	0.63	0.0	2.5	5.0	5.0	5.0	7.5	7.5	7.5	7.5	7.5
50.0	1.08	0.0	0.0	0.0	0.0	0.0	2.5	2.5	5.0	5.0	5.0
100	2.05	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5
200	5.07	0.0	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5	2.5
Reference Item		0.0	0.0	0.0	10.0	25.0	60.0	82.5	90.0	100.0	100.0
10 day LD_{50}		n.d.									
10 day NOEDD		≥ 5.07 µg/bee/day									
10 day LC_{50}		n.d.									
10 day NOEC		≥ 200 mg/kg food									

n.d. = not determined

Effect of XDE-729 Methyl on diet consumption in honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal mg/kg food	Measured daily mean µg/bee	Diet Consumption (mg/day)									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control (0)	0	20.4	23.4	27.6	30.1	33.3	34.8	29.9	48.3	40.6	43.0
C_{sol}	0	11.1	21.1	22.4	20.0	29.7	29.4	23.7	28.2	27.9	26.4
12.5	0.29	5.3	13.8	24.8	22.0	32.0	25.8	31.7	23.0	27.8	27.4
25.0	0.63	5.6	21.5	27.1	20.6	29.6	26.5	28.2	26.8	33.7	31.4
50.0	1.08	8.6	17.3	22.6	24.5	28.3	17.4	22.9	22.6	27.3	23.9
100	2.05	6.4	15.7	26.0	18.4	20.7	15.1	23.0	24.7	27.1	27.9
200	5.07	10.2	24.8	25.5	27.3	27.2	21.4	25.7	27.2	37.3	26.8
Reference Item		12.5	34.7	16.0	23.1	21.6	17.2	0.30	31.5	9.50	-
10 day NOEC, food consumption		≥ 200 mg/kg food									

- = all bees were dead

Effect of XDE-729 Methyl on weight of surviving bees in honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test			
Nominal mg/kg food	Measured daily mean µg/bee	Mean weight surviving bees (mg)			
		Replicate 1	Replicate 2	Replicate 3	Replicate 4
C_{sol}	0	87.0	98.8	92.2	93.1
12.5	0.29	92.9	93.1	101.2	93.2
25.0	0.63	93.9	92.7	93.2	96.6
50.0	1.08	96.9	92.7	99.5	92.9
100	2.05	96.5	96.7	89.4	90.8
200	5.07	97.2	88.9	98.9	97.9
10 day NOEC, weight surviving bees		≥ 200 mg/kg food			

Sublethal effects of XDE-729 Methyl to honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal mg/kg food	Measured daily mean µg/bee	Behavioural abnormalities									
		E 1	E 2	E 3	E 4	E 5	E 6	E 7	E 8	E 9	E 10
Control (0)	0	0	0	0	0	0	1a	0	0	0	0
C_{sol}	0	0	0	3ap	0	0	0	0	0	0	0
12.5	0.29	19ap	4ap	1ap	1a	0	0	1a	0	0	0
25.0	0.63	11ap, 1a	5ap	3a	2a	0	0	0	0	0	0
50.0	1.08	4ap	5ap	7ap, 2a	4a	1a	0	0	0	0	0
100	2.05	4ap, 1a	3ap, 1a	12ap, 1a, 1m	7a	1a	0	0	0	0	0
200	5.07	12ap, 1a	9ap	11ap, 1a	3a	0	0	0	0	0	0
Reference Item		0	0	5ap, 7a	10a, 1m	9a	16a	7a	4a	-	-

a = affected
ap = apathetic
m = moribund
- = all bees were dead

Conclusion

It can be concluded that the continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item XDE-729 Methyl at the treatment levels of 12.5, 25.0, 50.0, 100 and 200 mg/kg food caused no adverse effects regarding mortality, weight of surviving bees and food consumption.
The LOEC for mortality after 10 days of continuous exposure could not be statistically determined. Therefore, the NOEC was determined to be ≥ 200 mg/kg food. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be ≥ 5.07 µg/bee/day.
The LC_{10} as well as the LDD_{10} after 10 days of continuous exposure could not be determined due to the lack of a clear dose response relationship.

The LC_{50} as well as the LDD_{50} after 10 days of continuous exposure could not be determined since the observed mortalities were below 50% in all test item groups.
The LOEC based on the overall mean consumption of feeding solution after 10 days of continuous exposure could not be statistically determined. Therefore, the NOEC was determined to be ≥ 200 mg/kg food.
The LOEC based on the weight of surviving bees could not be statistically determined. Therefore, the NOEC was determined to be ≥ 200 mg/kg food.

Comments of zRMS:	The study was accepted. The validity criteria were met: Deviations from the guideline: none. The deviation to study plan concerning the analytical phase of the study was noted. There is no impact on the analytical phase of the study. The following endpoints were calculated: NOEDD = 10.9 µg consumed formulation/bee/d LDD_{50} = 24.5 µg consumed formulation/bee/d NOEC = 0.479 g formulation/kg food LC_{50} = 1.435 g formulation/kg food

Reference:	KCP 10.3.1.2
Report	Chronic toxicity of GLOB1817H to the honeybee *Apis mellifera* L. under laboratory conditions, XXXX S., 2021, 20 48 BAC 0071
Guideline(s):	Yes, OECD TG 245 (2017)
Deviations:	Yes, due to the higher abundance of the resulting ion, the mass transition m/z 345 → 250 was chosen for the quantification of halauxifen-methyl and m/z 345 → 285 was set for qualification. There is no impact on the analytical phase of the study.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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 LDD_{50} was calculated to be 24.5 µg consumed product/bee/day and the LC_{50} was calculated to be 1.435 g product/kg food. The LDD_{20} was calculated to be 18.1 µg consumed product/bee/day and the LC_{20} was calculated to be 0.907 g product/kg food. The LDD_{10} was calculated to be 14.8 µg consumed

product/bee/day and the LC_{10} 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:	nominal	analysed
	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[5]:	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:	nominal	analysed
	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:	*Apis mellifera* 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		
Test design:	In a 10-day chronic toxicity feeding test, young adults of *Apis mellifera* 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). 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. All feeding solutions were freshly prepared every day and provided *ad libitum* (minimum quantity of 2 mL). Assessments of bee mortality, food consumption and behavioural abnormalities were conducted daily. 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.		
Endpoints:	Mortality, behavioural abnormalities		
Test concentrations:	Control group AC: untreated food (50% (w/v) sucrose solution) Control group BC: untreated food (50% (w/v) sucrose solution + 0.1% (w/v) xanthan 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 Effectively consumed doses: 43.0, 29.7, 19.1, 10.9 and 7.92 µg product/bee/day		

[5] serves as herbicide safener

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)

Test conditions:	Temperature:	32.4 – 32.9°C
	Relative humidity:	54.3 – 65.3%
	Photoperiod:	darkness (diffuse artificial light only during assessments and exchange of feeders)
	Food:	50% (w/v) sucrose solution

Statistics: Statistical software used: ToxRat Professional 3.3.0 (2018).
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.

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 nominal [µg product/bee/day]	daily dose consumed[1] [µg product/bee/day]	concentration [g product/kg food]	after 10 days: mean mortality absolute [%]	after 10 days: mean mortality corrected [%]	after 10 days: number of bees showing behavioural abnormalities[2]
blank control	AC	--	--	--	0.0	--	0 out of 30
viscosifier control	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 an 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 ± 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 LDD_{50} was calculated to be 24.5 µg consumed product/bee/day and the LC_{50} was calculated to be 1.435 g product/kg food. The LDD_{20} was calculated to be 18.1 µg consumed product/bee/day and the LC_{20} was calculated to be 0.907 g product/kg food. The LDD_{10} was calculated to be 14.8 µg consumed product/bee/day and the LC_{10} 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:	The study was not evaluated as it concerns the active substance. The study should be evaluated during the active substance renewal.

Reference:	KCA 8.3.1.3
Report	Toxicity of Diflufenican technical on honey bee larvae (*Apis mellifera* 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 ºC (Min = 31.08 ºC) 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 ºC 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 ED_{50}-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 not evaluated as it concerns the active substance. The study should be evaluated during the active substance renewal.

Reference:	KCA 8.3.1.3
Report	XDE-729 Methyl - Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure), Oberrauch S., 2018b, S17-00206
Guideline(s):	Yes, OECD 239 (2016)
Deviations:	Yes, for the toxic reference item groups mortality but no other observations were assessed. No emergence boxes were used as from day 15 to enable the assignment of each emerged bee to the respective replicate.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and Methods

Test item (Common name):	XDE-729 methyl
Purity:	99.7 % w/w
Description (physical state):	solid / white
Lot/batch no.:	YC2-106153-95 (TSN301574)

Organism (*Species*):	Honey bee (*Apis mellifera*)
Study type:	Chronic Larval – repeated exposure
Study design:	Dose-response test; duration 22 days; 3 or more replicates, each starting with at least 12 synchronised 1st instar larvae per test concentration; assessment of mortality and behavioural effects daily after administration of the test item on days 3, 4, 5, and 6 and on days 7, 8, 15 and 22. Visual assessment of uneaten food on day 8 prior to transfer of plate into pupal desiccator. Monitoring of pupal development and adult emergence (eclosion) until day 22. Weighing of emerged bees on day 22.

Test concentrations:	0 (control, solvent control), 3.84, 9.60, 24.0, 60.0 and 150 mg XDE-729 methyl/kg diet equivalent to 0.591, 1.48, 3.70, 9.24 and 23.1 µg XDE-729 methyl/larva per developmental period.
Information on bee colony (health etc):	The larvae used in the test were from three disease-free colonies (one per replicate). The hive had not been treated for varroa mites or for disease for at least 4 weeks prior to study initiation.
Analytical verification:	XDE-729 methyl was analysed in the stock solution, the test item solutions and solvent control solution as well as in the test item treated larval diet and the diet of the control group and solvent control group by liquid chromatography and mass spectrometric detection (HPLC-MS/MS). Additional verification of the homogeneity (top and bottom sampling of treated diet) and stability (sampling at 24 ± 1 hours after preparation) of the test item in the larval diet. The analytical verification of XDE-729 methyl resulted in recoveries of 99 to 119% (solutions) and 91 to 106% (diet) of the nominal value. The measured concentrations in the homogeneity samples taken from the top and bottom of the treated diet of the highest and lowest test item group were equivalent to recoveries of 81 to 107%. The analytical dose verification of the aged larval diet (24 h) resulted in a recovery rate of 91% of the nominal concentration at day 4. Therefore the stability of the test item in the larval diet was proven for this period.
Feeding method:	Three different diets (A, B and C) were administered depending on the developmental stage of the larvae. The diets were based on 50 % fresh royal jelly and 50 % aqueous solution containing variable amounts of yeast extract, glucose and fructose in the three diets. The feeding solutions were prepared as needed. Diets A and B (20 µL/larvae, each) were administered on days 1 and 3, respectively. Diet C was administered once on days 4 to 6 in increasing volumes of 30 to 50 µL/larvae. The test item was administered on days 3, 4, 5 and 6 homogeneously dispersed in 20 to 50 µL/larvae of diet B or C depending upon the day of incubation.
Environmental conditions:	Temperature: 32.8 - 35.1 °C Relative Humidity: 49.9 - 100.0 % (day 1 to day 8), 56.1 - 88.8 % (day 8 to day 15), 35.7 - 70.5 % (day 15 - day 22) Photoperiod: constant darkness except during grafting, feeding and assessments.
Reference substance:	Dimethoate: 48.0 mg dimethoate/kg diet, 7.39 µg dimethoate/larva per developmental period Fenoxycarb: 0.320 mg fenoxycarb/kg diet, 0.0493 µg fenoxycarb/larva per developmental period

On day 1 synchronised honey bee larvae (first instar, L1) were taken from the combs of 3 hives and were individually transferred into well-plates, where they were fed a standardised amount of artificial diet. From day 3 until day 6 XDE-729 methyl was administered daily to the larvae in the diet in a range of

increasing concentrations, which remained constant during the application period. The presence of uneaten food was qualitatively recorded on day 8. Cumulative mortalities during the larval phase were assessed daily from day 4 until day 8. Cumulative mortalities during the pupation phase were assessed on day 15 and on day 22. The adult emergence rate was assessed on day 22. Additionally, the weight of emerged bees was assessed on day 22. Other observations and any other adverse effects were recorded in comparison to the solvent control group.

Results and Discussion

Dates of work: 03 July 2017 – 05 January 2018

On day 8 the cumulative mortality was 4.2 % in the control, 6.3 % in the solvent control, 97.9 % in the dimethoate reference item group 0.0 % in the fenoxycarb reference item group. On day 22, the adult emergence rate in the control and solvent control group was 91.7 and 77.1 %, respectively. The adult emergence rate in the fenoxycarb reference item group was 2.1 %.
Compared to the solvent control group, the adult emergence rate on day 22 was not statistically significantly different in the highest test item group of 150 mg XDE-729 methyl/kg diet (multiple Chi²-test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$). Therefore, the NOEC for adult emergence on day 22 was determined as ≥ 150 mg XDE-729 methyl/kg diet, equivalent to a NOED of ≥ 23.1 µg XDE-729 methyl/larva per developmental period.

Toxicity of XDE-729 methyl to honey bee larvae in a chronic exposure toxicity test

Nominal Treatment		Chronic larval exposure toxicity		
mg XDE-729 methyl/kg diet	µg XDE-729 methyl/larva per developmental period	Mortality (%) (Corrected Mortality (%))		Emergence (%)
		Day 8	Day 15	Day 22
Control (0)		4.2 (n.a.)	8.3 (n.a.)	91.7
Solvent Control (0)		6.3 (n.a.)	22.9 (n.a.)	77.1
3.84	0.591	8.3 (2.1)	22.9 (0.0)	75.0
9.60	1.48	4.2 (-2.2)	37.5 (18.9)	62.5
24.0	3.70	4.2 (-2.2)	27.1 (5.4)	70.8
60.0	9.24	6.3 (0.0)	31.3 (10.9)	64.6
150	23.1	2.1 (-4.5)	14.6 (-10.8)	83.3
Reference item (7.39 µg dimethoate/larva per developmental period, nominal)		97.9 (97.8)	---	---
Reference item (0.0493 µg fenoxycarb/larva per developmental period, nominal)		0.0 (-6.7)	6.3 (-21.5)	2.1
22-day NOED, nominal treatment		≥ 23.1 µg XDE-729 methyl/larva per developmental period, equivalent to ≥ 150 mg XDE-729 methyl/kg diet		

* Significantly different compared to solvent control (multiple Chi²-test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$)
n.a. not applicable

Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for XDE-729 methyl

Nominal Treatment		Chronic larval exposure toxicity		
mg XDE-729 methyl/kg diet	µg XDE-729 methyl/larva per developmental period	Uneaten food observed on day 8	Behavioural effects (day)	Developmental effects (day)
Control (0)		yes	none	none
Solvent Control (0)		yes	none	none

3.84	0.591	yes	none	none
9.60	1.48	yes	none	none
24.0	3.70	yes	none	none
60.0	9.24	yes	none	none
150	23.1	yes	none	none
Reference item (7.39 µg dimethoate/larva per developmental period)		yes	none	none
Reference item (0.0493 µg fenoxycarb/larva per developmental period)		yes	none	none

Conclusion

In a repeated exposure larval toxicity test with XDE-729 methyl and a duration of 22 days, the NOEC for adult emergence was determined as ≥ 150 mg XDE-729 methyl/kg diet, equivalent to a NOED of ≥ 23.1 µg XDE-729 methyl/larva per developmental period.

The study was deemed valid since all validity criteria were met.

Comments of zRMS:	The study was accepted. The validity criteria were met: • mean larvae mortality in the control was below 15% for larvae across all control replicates – observed 0%; • mean reference item mortality was ≥ 50 % for larvae across all reference replicates – observed 94.4%; • mean adult emergence rate in the control was ≥ 70 % – observed 83.3% Some insignificant deviations were noted; none of them affect the final conclusion. The following endpoints were calculated: NOED = 5.7 µg formulation/larva ED_{50} > 88.7 µg formulation/larva NOEC = 35.9 mg formulation/kg food EC_{50} > 560.9 mg formulation/kg food

Reference:	KCP 10.3.1.3
Report	GLOB1817H – Repeated exposure to the honeybee (*Apis mellifera* L.) larvae under laboratory conditions, XXXX 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 ± 0.5°C. The relative humidity ranged in this time between 18.9 to 97.1% (average 28.6% instead of 80 ± 5%). No impact is assumed as no effects on development of larvae in the untreated control were observed. Due to the higher abundance of the resulting ion, the mass transition m/z 345 → 250 was chosen for the quantification of halauxifen-methyl and m/z 345 → 285 was set for qualification. There is no impact on the analytical phase of the study.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

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 ED_{50} (adult emergence up to D22) was determined to be > 88.7 µg product/larva, which is equivalent to an EC_{50} of > 560.9 mg product/kg food. The ED_{20} (adult emergence up to D22) was determined to be 13.5 µg product/larva, which is equivalent to an EC_{20} of 85.0 mg product/kg food. The ED_{10} (adult emergence up to D22) was determined to be 1.9 µg product/larva, which is equivalent to an EC_{10} 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:	nominal	analysed
	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:	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 prosulfocarb, diflufenican and halauxifen-methyl in the test item stock solutions and in the control was determined.		
Endpoints:	Successful adult emergence, mortality, qualitative observations: *e.g.* 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

[6] serves as herbicide safener

		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 ≤ 15% on D8, cumulative mortality in the reference item treatment group was ≥ 50% on D8 and adult emergence in the control was ≥ 70% on D22, the study can be regarded as valid.

The results are summarized below.

Toxicity of GLOB1817H to larvae of *Apis mellifera* L. after repeated exposure

Treatment group	Treatment ID	Dose	Concentration	On D8		On D15		On D22		
				Larval mortality D3 to D8 [%]		Mean OO [%]	Pupal mortality D8-D15 [%]		Total mortality D3-D22 [%]		Adult emergence rate [%]
		[µg product/	[mg product/ kg food]	abs.	corr.		abs.	corr.	abs.	corr.	abs.

		larva]									
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] 7.6	[mg a.i./ kg food] 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 ED_{50} (adult emergence up to D22) was determined to be > 88.7 µg product/larva, which is equivalent to an EC_{50} of > 560.9 mg product/kg food. The ED_{20} (adult emergence up to D22) was determined to be 13.5 µg product/larva, which is equivalent to an EC_{20} of 85.0 mg product/kg food. The ED_{10} (adult emergence up to D22) was determined to be 1.9 µg product/larva, which is equivalent to an EC_{10} 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 accepted. Validity criteria were met. • mortality in the control group: ≤ 10 % (48 hours) (observed: 3.3 %); • reproduction in the control group: ≥ 5 mummies per female (observed: 22.8); • corrected mortality in the reference item group: > 50 % (48 hours) (observed: 100 %). The following endpoints for *Aphidius rhopalosiphi* were derived: LR_{50} = 2.176 L/ha ER_{50} > 1.5 L/ha $NOER_{mortality}$ = 1.5 L/ha $NOER_{reproduction}$ ≥ 1.5 L/ha These endpoints were used for risk assessment.

Reference:	KCP 10.3.2.2
Report	Effects of GLOB1817H on the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez) in an extended laboratory test, XXXX U., 2020b, 20 48 NAE 0018
Guideline(s):	Yes, IOBC (Mead-Briggs *et al.*, 2009)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item 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 LR_{50}) 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 LR_{50} 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 ER_{50} 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 *Aphidius rhopalosiphi* (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 (*Rhopalosiphum padi*). 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 (α = 0.05) for mortality (test item)
FISHER`s Exact Binomial test (α = 0.05) for mortality (reference item)
Probit Analysis for LR_{50} calculation
DUNNETT'S-t-test (α = 0.05) for repellence (test item)
WILLIAMS t-test (α = 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, α = 0.05). The LR_{50} 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, α = 0.05). The ER_{50} 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 rhopalosiphi*) 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] [%]
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]				
LR_{50} [95 % CL]	**2.176** [1.913 – 2.471]				
ER_{50}	**> 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 LR_{50} 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 ER_{50} 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 accepted. The validity criteria were met: • mortality in the control group: ≤ 20 % (dead and escaped mites) on day 7 (observed: 2.0 %) • corrected mortality in the reference group: 50 – 100 % on day 7 (observed: 78.6 %) • reproduction in the control group: ≥ 4 eggs per female (observed: 6.45 eggs per female) The ER_{50} could not be calculated, this was estimated to be > 0.75 L product/ha. The NOER for reproduction was 0.375 L product/ha. The LR_{50} *Typhlodromus pyri* was 1.368 L formulation/ha. This endpoint was used for risk assessment.

Reference: KCP 10.3.2.2

Report	Effects of GLOB1817H on the predatory mite *Typhlodromus Pyri* Scheuten in an extended laboratory test, XXXX U., 2020a, 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/No/Supplementary

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 LR_{50} 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 ER_{50} 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 Birkenpfuhlheide 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 LR_{50} (Lethal Rate 50 %, rate resulting in 50 % mortality)
Reproductive capacity of the surviving mites from day 7-14 including determination of an ER_{50} (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 Chi^2-2x2 Table test after BONFERRONI-HOLM test ($\alpha = 0.05$) for mortality (test item)
Chi^2 2x2 Table test ($\alpha = 0.05$) for mortality (reference item)
Spearman-Karber procedure for LR_{50} calculation
WILLIAMS-t-test ($\alpha = 0.05$) 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 Chi^2-2x2 Table test after BONFERRONI-HOLM, $\alpha = 0.05$). The LR_{50} 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]				
LR_{50} [95 % CL]	**1.368** [1.277 – 1.465]				
NOER	0.75				
ER_{50}				**> 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 Chi^2-2x2 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 accepted. The validity criteria were met. The following endpoints for *Aleochara bilineata* were derived: ER_{50} > 6.0 L/ha $NOER_{reproduction}$ ≥ 6.0 L/ha These endpoints were used for risk assessment.

Reference:	KCP 10.3.2.2
Report	Effects of GLOB1817H on the rove beetle *Aleochara bilineata* Gyll. in an extended laboratory test, XXXX U., 2020c, 20 48 NKE 0010
Guideline(s):	Yes, IOBC (Grimm *et al.* 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item 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 F_1 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 ER_{50} 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 *Aleochara bilineata* 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 *Delia antiqua* 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 F_1 generation) was determined (daily assessments during 5 weeks).
Endpoint:	Reproductive capacity (average number of hatched beetles of the F_1 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 F_1-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 F_1 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, α = 0.05), compared to the water-treated control. The ER_{50} 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 ER_{50} 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 accepted. The validity criteria were met. The following endpoints for *Aleochara bilineata* were derived: LR_{50} > 6.0 L/ha ER_{50} > 6.0 L/ha $NOER_{mortality}$ ≥ 6.0 L/ha These endpoints were used for risk assessment.

Reference:	KCP 10.3.2.2
Report	Effects of GLOB1817H on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test, XXXX U., 2020d, 20 48 NLE 0007
Guideline(s):	Yes, IOBC (Heimbach *et al.* 2000)
Deviations:	No

GLP: Yes

Acceptability: Yes/No/Supplementary

Executive summary

An extended laboratory study was carried out to determine the effects of the test item 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 *Poecilus cupreus* 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 *Delia antiqua*

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.

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 Chi^2 2x2 Table Test with BONFERRONI Correction ($\alpha = 0.05$) for the test item and Chi^2 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 LR_{50} 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 ER_{50} 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 accepted. The validity criteria were met: • adult mortality 4 weeks: less than10 % (being 0% after 4 weeks); • number of juveniles per replicate: more than 30 (being 205 to 3170; • coefficient of variation of reproduction: less than 30 % (being 14.4%). The following endpoints were derived: mortality: NOEC > 193.1 mg formulation/kg d.w. (corrected to 96.55) No LC_{50} was calclulated; reproduction: NOEC = 22.2 mg a.s./kg d.w. (corrected to 11.1) EC_{50} = 47.4 mg a.s./kg d.w. (corrected to 23.7) No LC_{50} was calculated. The study results were used in risk assessment.

Reference:	KCP 10.4.1.1
Report	Earthworm reproduction test with Prosulfocarb 800 g/L EC (OECD 222, April 2004), XXXX 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/No/Supplementary
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 EC_{50} (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:	*Eisenia fetida*
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.
The adults were maintained in the artificial soil substrate for 4 weeks. Then, the observations consisted in percent mortality and mean weight of the survivals. The adults were discarded and the test units were maintained in the climatic chamber for 4additional weeks. At the end of the period, the number of juveniles was assessed.
Carbendazim was used as the reference item to confirm the function of the test system.

F-variance analysis at 5% confidence level served to judge upon significant deviation of the number of juveniles as compared to that of the control group. The percentage of inhibition for the production of juveniles as compared to the controls was plotted against Log concentration of the corresponding value for the test item treatment as mg/kg dry soil. The regression analysis was performed using Excel spreadsheet.

Results and Discussion

Mortality and fecundity are summarized in the table below.

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 ≤ 10% of the initial population
- Production of juveniles in the control ≥ 30 per unit
- The coefficient of variation of reproduction in the control ≤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 evaluated and accepted. The validity criteria were met: • adult mortality 4 weeks: less than10 % (being 0% after 4 weeks); • number of juveniles per replicate: more than 30 (being 205 to 3170; • coefficient of variation of reproduction: less than 30 % (being 14.4%). The following endpoints for *Eisenia fetida* were derived: **mortality**: NOEC = 268 mg formulation/kg d.w. (corrected to 134) LC_{50} > 268 mg formulation/kg d.w. (corrected to 134) **reproduction**: NOEC = 41 mg formulation/kg d.w. (corrected to 20.5) EC10 = 45 mg formulation/kg d.w. (corrected to 22.5) EC_{50} > 268 mg formulation/kg d.w. (corrected to 134) LC_{50} > 268 mg formulation/kg d.w. (corrected to 134) The study results were used in risk assessment.

Reference: KCP 10.4.1.1

Report	Effects of GLOB1817H on the reproduction of the earthworm *Eisenia fetida*, XXXX S., 2020, 20 48 TEC 0054
Guideline(s):	Yes, OECD 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine potential effects of the test item on 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 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:		nominal	analysed
	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:	earthworm *Eisenia fetida* (Savigny, 1826)		
Test design:	Effects on earthworms: 56 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, 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)		
Test system:	Exposure of worms to different concentrations of the test item mixed into artificial soil substrate (with 10 % peat)		
Reference item:	Maypon Flow (Carbendazim, SC 500) The effects of the reference item were investigated in a separate study.		
Test conditions:	Temperature:	19.0 - 21.6 °C	
	Light intensity:	630 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: 17 September 2020
Experimental completion date: 12 November 2020

Statistics: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality,
(α = 0.05, one-sided greater), Williams t-test for biomass change and reproduction (α = 0.05, one-sided smaller),
Probit analysis for calculation of for calculation of EC_x;
Statistical program: ToxRat Professional 3.3.0 (2018)

Results and Discussion

The test item caused no statistically significant effect (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 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, α = 0.05, one-sided smaller). Statistically significant effects (Williams t-test, α = 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, α = 0.05, one-sided greater) and biomass (Williams t-test, α = 0.05, one-sided smaller)
* statistically significantly different compared to control regarding reproduction (Williams t-test, α = 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: ≤ 10 % (being 0.0 % after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being 205 to 317)
- Coefficient of variation of reproduction: ≤ 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 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.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. The sampling dates cover the whole study period: • pre-sampling on 31.03.2014 (about 2 weeks before test item application); • 1st sampling on 14.05.2014 (about 1 month after test item application); • 2nd sampling on 29.10.2014 (about 6 months after test item application); • 3rd sampling on 30.03.2015 (about 12 months after test item application). 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 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. 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. 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.

Reference:	KCP 10.4.1.2
Report	Effects of Prosulfoarb 800 g/L EC on earthworms under field conditions, XXXX L., Biochem Agrar, 14 10 48 008 F

Guideline(s):	Yes, ISO 11268-3 (1999), Kula *et al.*, 2006 - Technical recommendations to ISO 11268-3
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	/

Executive Summary

The objective of this field study was to investigate potential effects and the potential recovery of field populations of earthworms after the application of the test item Prosulfocarb 800 g/L EC. Therefore, a field experiment lasting about one year was performed and the effects of the test item on different earthworm species, biomass and abundance were compared to an untreated control and to a reference item.

The trial was placed on arable land near Machern in Saxony/Germany. The test item Prosulfocarb 800 g/L EC (prosulfocarb 800 g/L (nominal)) was applied once at a rate of 5 L/ha corresponding to 4 kg prosulfocarb/ha. Nutdazim 50 FLOW (carbendazim 500 g/L (nominal)) was applied once to the plots as reference item at a rate of 20 L/ha corresponding to 10 kg carbendazim/ha. Tap water was applied once as a control.

Twelve plots, each 10 m x 10 m, were arranged in a 3 x 4 formation, each plot surrounded by a 2 m wide path between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site. Defined areas were sampled to assess earthworm populations before application and three times after application, i.e. about 1, 6 and 12 months after test item application.

No measurable residues (< LOD) of prosulfocarb were determined in any of the soil samples of the control plots taken after test item application. After the application of Prosulfocarb 800 g/L EC a mean residue value of 121 % of the application rate was found in soil samples of the test item treatment group. The mean recovery was in the recommended range of 50 - 150%.

Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole.The mean earthworm abundance in the control plots was 200.5 ind./m² at pre-sampling, 93.0 ind./m² at 1st sampling, 304.0 ind./m² at 2nd sampling and 269.5 ind./m² at 3rd sampling. Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Allolobophora chlorotica* (12.2% of total earthworms), *Aporrectodea caliginosa* (45.0% of total earthworms) and *Aporrectodea rosea* (3.7% of total earthworms) as well as the anecic species *Aporrectodea longa* (3.3% of total earthworms) and *Lumbricus terrestris* (28.6% of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris* representing different ecological groups indicated the suitability of the field site.

The toxic reference item reduced total earthworm abundance and biomass by 22.0 % and 50.3 % at 1st sampling, respectively. *Lumbricus terrestris* was the most sensitive species and was reduced in total abundance and biomass by 65.3% and 66.6% on this sampling date, respectively. The statistically significant reduction in total earthworm biomass of 50.3% at 1st sampling (about 1 month after test item application) confirmed the validity of the test system.

Surface monitoring on days 1 - 3 after test item application showed that there was no acute primary effect on earthworms by Prosulfocarb 800 g/L EC. No alive, moribund or dead earthworms were found on the soil surface neither in the test item nor in the control monitoring areas.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rate of 5 L Prosulfocarb 800 g/L EC about 1, 6 and 12 months after test item application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species and ecological groups could be observed for the tested application rate of 5 L/ha about 1, 6 and 12 months after test item application. Only for the total biomass of the earthworm species *Lumbricus terrestris* a statistically significant reduction of 27.7 % could be observed about 12 months

after test item application. However, since no effects on total biomass of *Lumbricus terrestris* could be observed about 1 and 6 months after test item application and a reduction in biomass of *Lumbricus terrestris* of 27.7 % is within the range of the natural variability of earthworm populations, the statistically significant reduction in total biomass of *Lumbricus terrestris* can be considered as ecologically not relevant.

Materials and methods

Test material	Prosulfocarb 800 g/L EC
Active ingredient	Prosulfocarb, 806 g/L (analysed), 800 g/L (nominal)
Control	Tap water
Toxic reference	Nutdazim 50 FLOW (carbendazim 500 g/L, nominal)

Test site and maintenance:
The study was located near Machern in Saxony, Germany. Cultural practices performed on the test field during 2011 until 2013 followed the usual agricultural practice. The only cultivated crop within this time span was *Phacelia tanacetifolia*. Maintenance of the field during the present study was according to general agricultural practice. The application was performed on bare soil. About one month after test item application, the test field was seeded with the fodder crop "Landsberger Gemenge" (clover grass mixture) which stayed on the field until the end of the study. The test field was mulched once in autumn 2014 (see table above). No further plant protection products others than the test item and the reference item were applied on the test field. Furthermore, no mineral or organic fertilisers were applied to the test field.

Application replicates:
Application was conducted on 11 April 2014, a day with low wind an 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/m²)**				**Biomass (g/m²)**			
		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%)
A. caliginosa	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%)
A. chlorotica	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%)**
A.rosea	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%)
A. longa	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%)
L. terrestris	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/m^2 thus fulfilling the guideline recommendation of 60 ind./m^2 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./m^2), with abundances of 88.8 ind./m^2 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 XXXX (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
• ***Aporrectodea caliginosa total***	49	121	63	53	48	118	52	56
• *Aporrectodea caliginosa adults*	51	99	48	55	50	100	46	78
• *Aporrectodea caliginosa juvenile*	60	144	86	60	76	181	78	59
• ***Allolobophora chlorotica total***	61	92	78	50	61	99	81	57
• *Allolobophora chlorotica adults*	58	103	80	65	58	105	81	67
• *Allolobophora chlorotica juvenile*	158	89	96	42	218	106	125	39
Aporrectodea rosea total	74	242	92	92	79	402	85	103
Aporrectodea rosea adults	147	-	94	261	249	-	120	220
Aporrectodea rosea 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
• ***Aporrectodea longa total***	85	-	82	93	72	-	84	165
• *Aporrectodea longa adults*	88	-	90	149	82	-	89	266
• *Aporrectodea longa juvenile*	132	-	90	87	117	-	104	106
• ***Lumbricus terrestris total***	33	42	37	49	71	60	53	38
• *Lumbricus terrestris adults*	93	83	61	67	96	75	71	62
• *Lumbricus terrestris juvenile*	48	65	62	64	62	74	87	81

• Taxon is statistically evaluated in the study
MDD class 0: >100 %
MDD class I: 90-100 %
MDD class II: 70-90 %
MDD class III: 50-70 %
MDD class IV: <50 %

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. The following endpoints were derived: **mortality**: NOEC = 41 mg formulation/kg d.w. (corrected to 20.5)

	LC_{50} = 118 mg formulation/kg d.w. (corrected to 59) **reproduction**: NOEC = 41 mg formulation/kg d.w. (corrected to 20.5) EC_{50} = 66 mg formulation/kg d.w. (corrected to 33) The study results were used in risk assessment.

Reference:	KCP 10.4.2.1
Report	Effects of GLOB1817H on the reproduction of the collembolan *Folsomia candida*, XXXX S., 2020, 20 48 TCC 0059
Guideline(s):	Yes, OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine potential effects of the test item on 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 LC_{50} 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 EC_{10}, EC_{20} and EC_{50} 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:		nominal	analysed
	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:	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: Light intensity: Photoperiod:	18.1 - 20.4 °C 580 lux 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: Experimental completion date:	07 October 2020 04 November 2020
Statistics:	Step-down Cochran-Armitage test, (α = 0.05, one-sided greater), Williams-t-test for reproduction (α = 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, α = 0.05, one-sided greater) and on the number of juveniles (Williams-t-test, α = 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, α = 0.05, one-sided greater and Williams-t-test for reproduction, α = 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 EC_{50} (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 LC_{50} 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 EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 40, 48 and 66 mg test item/kg soil d.w.

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. The following endpoints were derived: **mortality**: LR_{50} = 2319 mg test item/kg soil d.w. **reproduction**: NOEC = 165 mg formulation/kg d.w. (corrected to 82.5) EC_{50} = 520 mg formulation/kg d.w. (corrected to 260) EC_{10} = 50 mg formulation/kg d.w. (corrected to 25) The study results were used in risk assessment.

Reference:	KCP 10.4.2.1
Report	A dose response study to assess the NOEC, $EC_{10\text{-}20\text{-}50}$ on reproduction and $LR_{10\text{-}20\text{-}50}$ on mortality of Prosulfocarb 800 EC of the predatory mite *Hypoaspis aculeifer* on artificial soil in the laboratory, XXXX S., Walloon Agricultural Research Centre, HA04/2016
Guideline(s):	Yes, OECD 226
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
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 $EC_{10\text{-}20\text{-}50}$ 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/m³
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:	*Hypoaspis aculeifer*
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 ≥ highest tested rate. If differences between objects appeared, each concentration of test item was compared to the control with a Dunett 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 $EC_{10\text{-}20\text{-}50}$ were calculated to be 50.0095, 100.5784 and 520.301 ppm, respectively.

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. The insignificant devaiations were noted, but they do not affect the final conclusion. The following endpoints were derived: NOEC = 1000 mg test item/kg d.w. (corrected to 500) LC_{50} > 1000 mg test item/kg d.w. (corrected to 500) EC_{50} > 1000 mg test item/kg d.w. (corrected to 500)

Reference:	KCP 10.4/03
Report	Diflufenican 500 g/L SC: Predatory Mite (*Hypoaspis aculeifer*) Reproduction Test in Soil, XXXX K., 2016, Envigo CRS Limited, DF50GM
Guideline(s):	Yes, OECD 226
Deviations:	No

GLP:	Yes
Acceptability:	Yes/No/Supplementary
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 acueleifer*. 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 LC_{50} and 14-day EC_{50} 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-sand The 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 LC_{50} 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 EC_{50} value for fecundity was estimated to be >1000 mg a.i./kg dry soil.

Conclusions

The 14-day LC_{50} and 14-day EC_{50} were estimated to be >1000 mg a.i./kg dry soil.
The NOEC for reproduction of the *Hyposaspis acueleifer* was determined to be 1000 mg test item/kg soil.

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. The following endpoints were derived: **mortality**: NOEC = 161 mg formulation/kg d.w. (corrected to 80.5) LC_{50} > 387 mg formulation/kg d.w. (corrected to 193.5) **reproduction**: NOEC = 43 mg formulation/kg d.w. (corrected to 21.5) EC_{50} > 387 mg formulation/kg d.w. (corrected to 193.5) The study results were used in risk assessment.

Reference:	KCP 10.4.2.1
Report	Effects of GLOB1817H on the reproduction of the predatory mite *Hypoaspis aculeifer*, XXXX L., 2020, 20 48 THC 0043
Guideline(s):	Yes, OECD 226 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine potential effects of the test item on mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative species of soil micro-arthropods during a test period of 14 days. The test was performed according to the OECD Guideline 226 (2016). The LC_{50} value for mortality and the EC_{50} 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 EC_{10} and EC_{20} 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)
	Diflufenican	14 g/L (nominal)	14.20 g/L (analysed)
	Halauxifen-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: The effects of the test item on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226.
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 *Tyrophagus putrescentiae* (SCHRANK) at the beginning and every two to three days during the whole test period.
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).
Assessment of adult mortality and reproduction effects was carried out after 14 days.

Endpoints: Mortality of adults and number of juveniles

Reference item: Dimethoate 400 EC (400 g/L, nominal).
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)
The effects of the reference item were investigated in a separate study.

Validity criteria: Mean mortality of adult females: $\leq 20\%$
Mean number of juveniles per replicate: ≥ 50
Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$

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: Experimental start date: 21.09.2020

Experimental completion date: 12.10.2020

Statistics:

Mortality
Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (α = 0.05, one-sided greater), Logit analysis using linear max. likelihood regression

Reproduction
Multiple Sequential-rejective Welch-t-test After Bonferroni-Holm (α = 0.05, one-sided smaller), Logit analysis using linear max. likelihood regression
Statistical program: ToxRat Professional 3.3.0 (RATTE 2018)

Results and Discussion

All validity criteria for the study were met.

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, α = 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, α = 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 LC_{50} value for mortality and the EC_{50} 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 EC_{10} and EC_{20} 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 submitted study was accepted. The validity criteria were met. No adverse effects on soil nitrogen transformation (measured as NO_3-N-production) at the end of the 28-day incubation period were observed. The effect less than 25% was observed at 4 mg/kg soil dry weight and 40 mg/kg soil dry weight

Reference:	KCP 10.3.2.2
Report	Effects of GLOB1817H on activity of soil microflora (Nitrogen transformation test), XXXX L., 2020, 20 48 SMN 0052
Guideline(s):	Yes, OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Executive summary

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation (mineralization) in a laboratory test over a period of 28 days of exposure. The test was performed in accordance with the OECD Guideline 216 (2000) by measuring the nitrogen turnover.

The test item 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 NO_3-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:		nominal	analysed
	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 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 NO_3-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 NO_3-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 submitted study was accepted. The validity criteria were met: The following endpoints based on shoot fresh weight reduction were derived: • ER_{50} = 335.65 mL formulation/ ha (dicotyledon species was carrot) and based on percentage visual injury assessment at harvest: • ER_{50} = 312.65 mL formulation/ha (dicotyledon species was carrot). The endpoint ER_{50} will be used in risk assessment.

Reference:	KCP 10.6
Report	GLOB1817H: terrestrial plant test: seedling emergence and seedling growth test, XXXX., 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/No/Supplementary

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 ER_{10}, ER_{25}, ER_{50} and NOEC values based on shoot fresh weight reduction and ER_{50} 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 ER_{25} value of 161.44 mL product/ha and an ER_{50} value of 478.76 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER_{25} value of 164.98 mL product/ha and an ER_{50} 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 ER_{50} value of 793.37 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER_{50} value of 312.65 mL product/ha.

Materials and Methods

Test item:	GLOB1817H		
Batch No.:	KS010420		
Formulation type:	EC		
Active ingredients/content:		nominal	analysed
	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 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

ER_{10}, ER_{25} and ER_{50} 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

ER_{10} values should be treated with caution due to natural plant to plant variability.

ER_{50} values (with corresponding R-Sq. values) and NOEC values, based on percentage visual at harvest, are summarized below.

Species	ER_{50} (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 ER_{25} value of 161.44 mL product/ha and an ER_{50} value of 478.76 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER_{25} value of 164.98 mL product/ha and an ER_{50} 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 ER_{50} value of 793.37 mL product/ha. The most sensitive dicotyledon species to pre-emergence application of GLOB1817H was carrot with an ER_{50} value of 312.65 mL product/ha.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: The following endpoints were derived: • ER_{50} = 75.93 mL/ha (based on shoot fresh weight, dicotyledon species was tomato) • ER_{50} = 56.25 mL/ha (based on percentage visual injury assessment at harvest, dicotyledon species was tomato) The endpoint ER_{50} will be used in risk assessment.

Reference:	KCP 10.6
Report	GLOB1817H: terrestrial plant test: vegetative vigour test, XXXX 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/No/Supplementary

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 ER_{10}, ER_{25}, ER_{50} and NOEC values based on shoot fresh weight reduction, and ER_{50} 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 ER_{25} value of 400.69 mL product/ha and an ER_{50} value of 962.19 mL product/ha. The most sensitive dicotyledon species to post-emergence application of GLOB1817H was tomato with an ER_{25} value of 21.32 mL product/ha and an ER_{50} 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 ER_{50} values of >1662 mL product /ha (the highest rate tested). The most sensitive dicotyledon species was tomato, with an ER_{50} value of 56.25 mL product /ha.

Materials and Methods

Test item: GLOB1817H

Batch No.:	KS010420		
Formulation type:	EC		
Active ingredients/content:		nominal	analysed
	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	21 DAT

			(Harvest)	(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

ER_{10}, ER_{25} and ER_{50} 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

ER_{10} values should be treated with caution due to natural plant to plant variability.

The ER_{50} 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 ER_{25} value of 400.69 mL product/ha and an ER_{50} value of 962.19 mL product/ha. The most sensitive dicotyledon species to post-emergence application of GLOB1817H was tomato with an ER_{25} value of 21.32 mL product/ha and an ER_{50} 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 ER_{50} values of >1662 mL product /ha (the highest rate tested). The most sensitive dicotyledon species was tomato, with an ER_{50} 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.